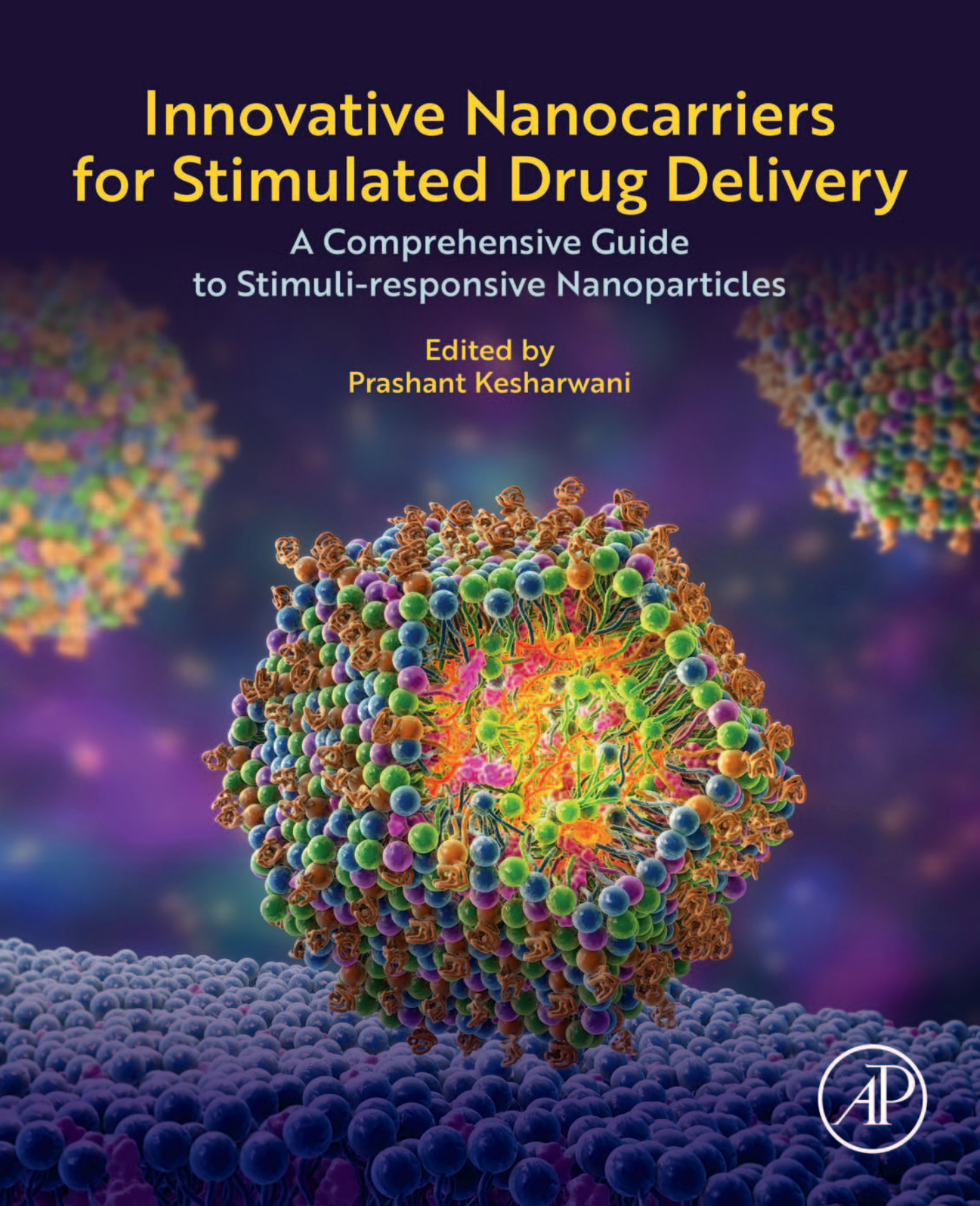
Innovative Nanocarriers for Stimulated Drug Delivery

A Comprehensive Guide to Stimuli-responsive Nanoparticles

Edited by
Prashant Kesharwani



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PART

I

Foundations of stimuli-responsive nanocarriers

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CHAPTER

Fundamental of stimuli responsive materials in drug delivery

1

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1. Introduction

The domain of drug delivery has been profoundly altered by the advent of intelligent, stimulus-responsive materials, which have surfaced as a viable alternative to the obstacles encountered in conventional therapeutic approaches [1–3]. These advanced materials are engineered to react dynamically to particular internal or external stimuli, facilitating the controlled and precise release of therapeutic substances at the intended site of action [4,5]. The demand for enhanced drug delivery systems emerges from the shortcomings of traditional methods, which frequently lead to inadequate therapeutic results due to non-specific distribution, low bioavailability, and systemic adverse effects. In cancer treatment, conventional chemotherapeutic medicines often target not just tumor cells but also adjacent healthy tissues, resulting in unpleasant side effects that can significantly diminish patient quality of life [6,7]. Employing stimuli-responsive carriers that selectively release their contents in reaction to the unique microenvironment of tumors, marked by altered pH levels, increased reactive oxygen species (ROS), and specific redox states, researchers can improve treatment efficacy while reducing harm to healthy cells. These intelligent materials can be designed to react to diverse stimuli, such as alterations in pH [8–11], temperature variations [12,13], light exposure [14], magnetic fields [15–17], and certain biomolecules [18–21]. For instance, pH-sensitive polymers can be engineered to release medications preferentially in the acidic environment characteristic of tumor tissues, whereas thermos-responsive materials can enable drug release at certain temperatures linked to pathological circumstances. Furthermore, progress in nanotechnology has resulted in the creation of many molecular assemblies, including liposomes, polymeric nanoparticles, dendrimers, and hydrogels, which function as adaptable platforms for stimuli-responsive drug delivery [22,23]. These nanocarriers can be customized for dimensions, morphology, and surface modification to attain optimal targeting and release properties. The incorporation of various stimuli-responsive mechanisms within a unified system enables precise regulation of medication release profiles customized to meet specific patient requirements [24].

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This multi-stimulus method boosts targeting precision and facilitates personalized medicine by tailoring treatment protocols to the distinct attributes of each patient's condition [25]. Moreover, the utilization of smart materials transcends medication delivery, encompassing fields such as regenerative medicine and tissue engineering [26]. Researchers hope to boost regeneration results and improve integration with host tissues by developing scaffolds that dynamically respond to physiological stimuli. Piezoelectric materials have demonstrated potential in enhancing cell development and differentiation via electrical stimulation, emulating the characteristics of real tissue [27]. The obstacles encountered in tissue engineering, including the procurement of immunologically suitable cells and the development of vascularized structures, can be mitigated through the utilization of intelligent biomaterials that adjust to their surroundings. Ongoing research on stimuli-responsive materials for drug delivery and other applications indicates that these advanced chemicals will significantly influence the future of personalized medicine [28,29]. The continued investigation into multi-stimuli responsive systems highlights the potential to develop advanced therapeutic modalities that can effectively tackle intricate medical issues with accuracy and flexibility. The introduction of stimulant-sensitive drugs is a crucial advancement in biomedical science, facilitating innovative therapy strategies that emphasize both effectiveness and safety in response to the changing requirements of patients [30].

Fig. 1.1 is selection of polymers that they are stimuli. Also Table 1.1 is a summary of articles that describe materials and substances sensitive to various stimuli for drug delivery. Fig. 1.2 shows other applications of stimuli and smart materials.

2. Light responsive material

The development of light-responsive smart biomaterials is highly attractive for constructing intricate scaffolds that guide cellular behavior for functional tissue regeneration and facilitate the release of

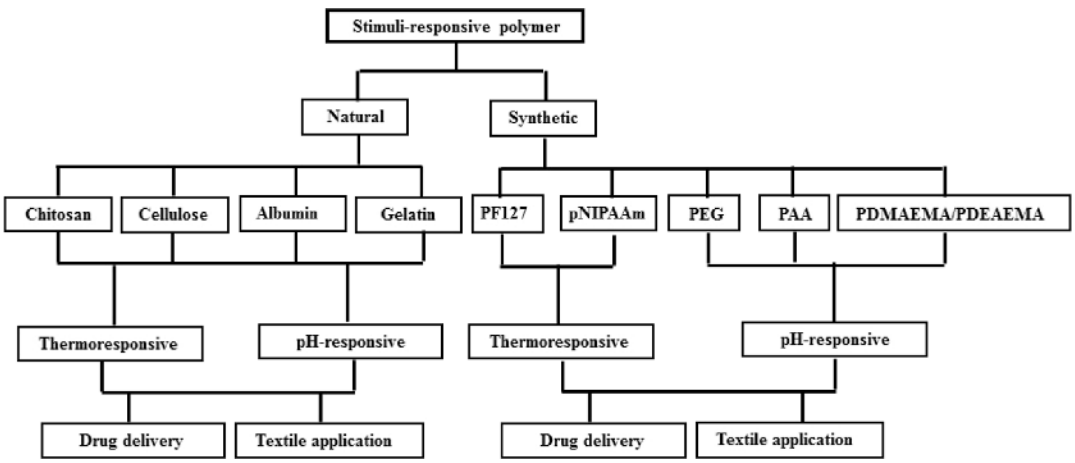


FIGURE 1.1

Some of stimuli-responsive polymers and their applications [31].

Table 1.1 Some stimuli responsive materials as carrier for drug delivery.

Material	Stimuli	Drug	Refs
Azobenzene-glycolipid into liposome	Light		[32]
Polyacrylamide-based hydrogel with azobenzene		Mesenchymal stem cells	[33]
Poly (3-hexylthiophene)			[34]
Poly (ethylene glycol) (PEG)-based hydrogel			[35]
Polyesters hydrogels			[36]
Micelle from poly(S-(o-nitrobenzyl)-L-cysteine)-b-poly (ethylene glycol) block copolymers			[37]
Liposomes with multibranched gold nanoantennas			[38]
Colloidal gold encapsulated in liposomes			[39]
Micelles of polyglycerol using spiropyran		Hydrophobic content	[40]
Liposomes formed by decyl-azobenzyl-triethylammonium and cholesterol sulfate			[41]
Iron oxide nanoparticles (USPION)	Magnetic		[42,43]
Iron oxide MNPs (magnetic nanoparticles)			[44]
Enzyme-MNP complex			[45]
Poly (N-vinyl caprolactam) (PVCL) with Clay nanocomposite	Temperature		[46]
Polypeptides (ELP)			[47]
Methyl cellulose and Collagen			[48]
Chitosan/collagen/β-glycerophosphate			[49]
Methyl cellulose			[50]
Chitosan/β-glycerophosphate/hydroxyapatite			[51]
Nanoparticles based on gelatin, poly(lactide) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) (gelatin-co-PLA-DPPE)		Doxorubicin (DOX)	[52]
poly (N-alkyl substituted acrylamide) PNIPPAm			[53]
1-ethyl-3-(3-dimethyl-aminopropyl)-1-carbodiimide hydrochloride and N-hydroxysuccinimide			[54]
Polyisocyanopeptide (PIC)			[55,56]
Hydroxypropylmethyl cellulose associated with chondroitin-6-sulfate sodium			[57]
PEG and PNIPPAm		DNA	[58]
Polyurethane dispersion (PU2)		Neural stem cells (NSCs)	[59]
Poly (polyethyleneglycol citrate-co-N-isopropylacrylamide) (PPCN) mixed with gelatin (PPCNG) and Graphene oxide		MSCs with BMP9	[60]
Hydrogels based on cytosine (C) and guanosine (G) modified hyaluronic acid (HA) via hydrogen bonding, with 1,6-hexamethylenediamine (HMDA)	pH		[61]
			[62]

Continued

Table 1.1 Some stimuli responsive materials as carrier for drug delivery.—cont'd

Material	Stimuli	Drug	Refs
HEMA (2-hydroxyethyl methacrylate)/DMAEMA (dimethylaminoethyl methacrylate			
Liposomes		Antagomir-10b y Paclitaxel (PTX)	[63,64]
Mesoporous bioglass (MBG) with hydroxyapatite (HAp)		Metformin hydrochloride (MH)	[10]
Chitosan hydrogels		Anti-inflammatory factors and antibiotics	[11]
Mesoporous ceramics with hydroxypatite (HAp)		Levofloxacin (Levo)	[65]
Keratin grafted poly (N-(2-hydroxypropy l) methacrylamide)	Redox	Doxorubicin (DOX)	[66]
Polyethylene glycol and polycaprolactone			[67]
Glycolipid, chitosan, and stearic acid			[68]
Polypyrrole (PPy)		Interleukin IL-3	[69]
Oligoviologen			[70]
Carbon nanotube (SWCNT)			[71]
Keratin nanoparticles			[72]

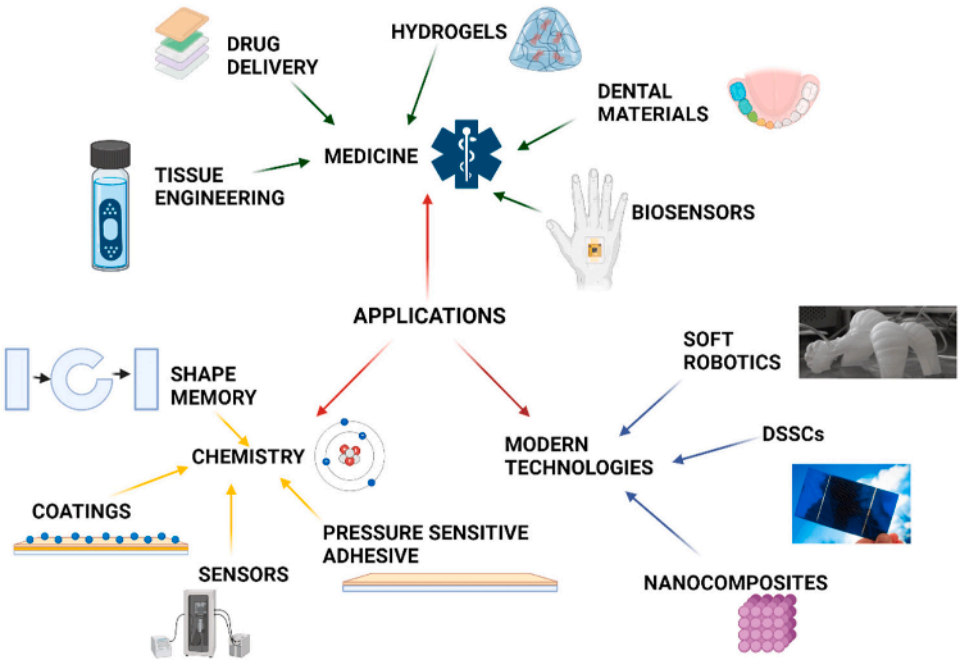


FIGURE 1.2

Application of smart polymer materials [73].

encapsulated substances [74–76]. The light stimulus possesses distinct advantages over alternative stimuli due to its rapid application and precise dosage, facilitating spatial and temporal control with reduced invasiveness [77,78].

Light serves as an efficient trigger due to its intensity and wavelength being remotely adjustable, rapidly switchable, and capable of being concentrated on specific regions with a resolution of 1 μm. Stimuli varied in wavelength from ultraviolet to near-infrared. Ultraviolet light is frequently employed in stimuli-responsive materials; nonetheless, it poses risks and lacks deep tissue penetration. Conversely, NIR appears to be more appropriate for biomedical applications due to its lower energy, reduced injury to biological cells, and enhanced tissue penetration. A compelling study examines contemporary biomedical applications of NIR-sensitive materials (Fig. 1.3) [79–81]. Also photodynamic therapy and photothermal therapy are other way to release drugs under light for treatment (Fig. 1.4) [82–84].

Photomediated isomerization is a reversible conformational alteration induced by ultraviolet and visible light exposure that does not cleave any chemical bonds. The predominant compounds utilized in photoisomerization reactions are azobenzenes and spiropyrans. Photoexcitation of azobenzenes using UV light (365 nm) induces a transformation from the trans to the cis configuration, which has been utilized to destabilize various drug delivery carriers, including micelles [81,82], dendrimers [85], and liposomes [86]. The cis conformation disrupts the packing of these assemblies due to a steric effect and heightened polarity; hence, an extended cis lifespan is essential to prolong the impact of light stimulation, facilitating the release of the encapsulated drug. Incorporating an azobenzene derivative into liposomes can induce defects in bilayers, leading to the release of the encapsulated payload [86,87]. Azobenzene-glycolipids incorporated into HSPC/DSPG/Chol liposomes were shown to maintain the stability of the entrapped medication in darkness while facilitating nearly complete cargo release upon exposure to UV light. Fluid-phase photosensitive nonphospholipid liposomes may be synthesized from decyl-azobenzyl-triethylammonium and cholesterol sulfate, presenting a viable

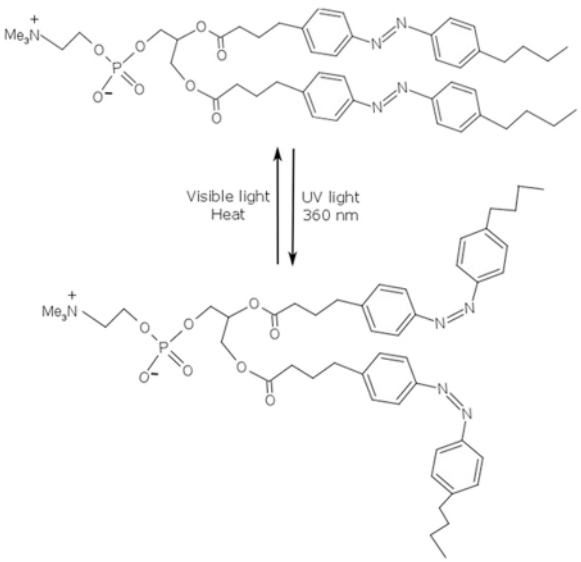


FIGURE 1.3

Example of structure changing materials (a) under UV light and (b) singlet oxygen-mediated photooxidation of plasmalogen vinyl ether linkage [79].

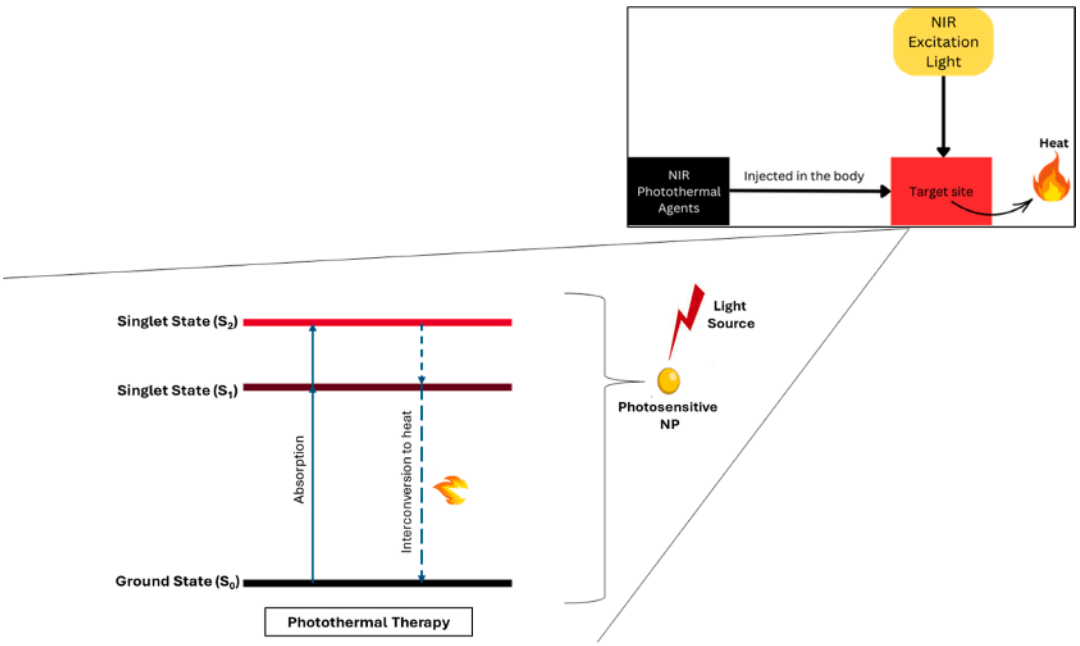


FIGURE 1.4

Mechanism of (a) photothermal therapy and (b) photodynamic therapy [82].

approach for regulating multidose release through photocycling between the cis and trans azobenzene isomers [32,41]. This distinctive composition provides these photosensitive nonphospholipid vesicles an advantage over conventional phospholipid-based liposomes, including enhanced impermeability and superior chemical resilience.

In addition to liposomes, micelles containing photoisomerizable moieties have also been synthesized [88,89]. For instance, photoresponsive micelles of polyglycerol containing spiropyran self-assemble into light-responsive micelles, wherein the hydrophobic spiropyran isomerizes to hydrophilic merocyanine upon UV irradiation, resulting in the disassembly of micelle structures and the release of hydrophobic content. Moreover, the conversion of the closed spiropyran form to the open merocyanine was successfully utilized in the formulation of an innovative drug delivery method utilizing intelligent nematic liquid crystal microspheres [40]. Spiropyran molecules linked to a particular pharmaceutical undergo photoisomerization to form merocyanine, which enables the resultant merocyanine-drug combination to traverse the liquid crystal barrier. Spiropyran-doped liquid crystal vehicles exhibit numerous enhanced optical capabilities attributable to the distinctive characteristics of liquid crystalline materials, offering an innovative method for the precise and timely administration of active therapies in response to specific stimuli.

The reversible photoisomerization properties of the aforementioned moieties have been utilized to develop advanced smart biointerfaces for functional tissue regeneration. Polymers and hydrogels are extensively utilized as biomaterials in the fabrication of medical devices and tissue engineering

scaffolds, owing to their biocompatibility, biodegradability, mechanical properties, hydrophobicity, and crystallinity, which are essential for tissue development. A new material was developed by coating strawberry-like silica Janus particles with spiropyran-containing polymer brushes to regulate cell capture and release through UV and visible light irradiation [90]. The hydrophobic coating demonstrated significant efficacy in cell capture owing to the distinctive interaction between the hydrophobic ring-closed spiropyran form and the fibronectin protein on the cell surface [91]. Due to the inadequate interaction between merocyanine and cell fibronectin protein, about 94% of the adhered cells were liberated from the coatings after exposure to UV light. This method utilizes the creation of light-responsive composite coatings as a foundation for developing intelligent biomaterials for regenerative medicine. Azobenzene-containing photoresponsive materials have been utilized as substrates for cell culture, facilitating novel investigations into the mechanisms of dynamic cell-material interactions and cell-cell signaling [92,93]. Research has extensively explored polyacrylamide hydrogels incorporating azobenzene molecules for the photoswitchable manipulation of primary human mesenchymal stem cells, with potential applications in tissue engineering and regenerative medicine. The material's promise as a minimally invasive method for investigating mechanotransduction and regulating mesenchymal stem cell activity through light-responsive modifications in substrate mechanics was established [33].

Photothermal interactions, involving the engagement of plasmonic materials with light irradiation, are extensively utilized to regulate drug distribution and promote tissue regeneration [94]. Generally, when conductive materials absorb incident photons, the energy is transformed into localized thermal energy, hence enhancing drug release. This methodology has been applied to other light-absorbing entities, including molecular dyes [95], metallic particles [96], and plasmon-resonant gold nanoparticles [97]. Colloidal gold encapsulated in liposomes is the most frequently cited hybrid method for photothermal effect-induced controlled drug delivery [39]. Gold nanoparticles are appealing because to their inertness, nontoxicity, and tunable optical and photothermal characteristics influenced by size, shape, and surface chemistry [98]. They effectively absorb photons in the Near-Infrared Radiation (NIR) spectrum, which are transformed into heat through the plasmonic effect of the nanoparticles: the absorbed photons convert into phonons, subsequently undergoing phonon-phonon relaxation, leading to an elevation in the system's temperature and, through conduction, generating localized heat.

Lajunen and colleagues utilized the photothermal properties of gold nanoparticles to develop biocompatible liposomes containing star or rod-shaped gold nanoparticles, which demonstrated regulated content release in the cell cytoplasm upon exposure to visible and near-infrared light [99]. In contrast to earlier research on UV light-activated liposomes, the lipid formulations employed enhanced safety and facilitated more effective light-induced drug release. This method is an attractive choice for addressing pathological conditions that necessitate exact regulation of the site and timing of drug administration. A different approach for light-activated medication delivery utilizing photothermal heating from near-infrared light entailed the co-delivery of low-temperature-sensitive liposomes alongside multibranched gold nanoantennas (MGNs) [38]. The co-delivery of liposomes and nanoantennas presents multiple advantages compared to the encapsulation of gold nanoantennas within vesicles: it facilitates the incorporation of a greater quantity and a wider variety of pharmaceuticals into the liposomes; the dimensions of the liposomes can be regulated; and a higher concentration of nanostructures can be precisely localized to improve photothermal transduction and drug release from liposomes. Mild hyperthermia (about 42°C) of MGNs, in conjunction with medication

delivery using liposomes, facilitates targeted treatment of several illnesses while minimizing off-target harm.

Gold nanoparticles synthesized in a collagen protein hydrogel using biomineralization-induced self-assembly yielded light-activated protein-based delivery systems [100]. The incorporation of gold nanoparticles facilitated the connection of collagen fibers, obviating the necessity for toxic cross-linkers and enhancing the hydrogel's strength and stability at elevated denaturation temperatures. This collagen-based hydrogel demonstrated efficacy as an injectable material for biomedical applications, attributed to its excellent biocompatibility and biodegradability. Its mechanical properties render it an optimal choice for tissue engineering, while its photothermal responsiveness offers potential for innovative light-activated drug delivery systems.

The photothermal stimulation technique has been employed to influence cellular mechanisms in the creation of novel platforms with prospective applications in tissue engineering. Martino et al. [34] examined the photo-modulation of Human Embryonic Kidney 293 cells cultivated on a thin layer of conjugated poly(3-hexylthiophene). NIR light absorption by the semiconducting polymer induces localized heating inside the cellular environment, hence affecting the electrical properties and conductance of the ion channels in the cell membrane. The photoexcitation of living cells by polymer absorption offers a prospective technique for light modulation in several biomedical applications.

Photocleavage is a widely employed method in light-mediated drug delivery. Photocleavage processes transpire when light irradiation disrupts a covalent bond, facilitating the liberation of encapsulated molecules. In a specific situation, drug molecules are directly attached to a carrier using photocleavable linkers, which release the drug upon cleavage by light. Alternatively, photo-responsive moieties are included into the drug carrier, and upon exposure to light stimuli, these groups are cleaved, resulting in the rupture of the drug carrier and the release of the therapeutic material. These devices generally employ ultraviolet and high-energy visible light, which possess sufficient energy per photon to shatter covalent bonds. Nonetheless, light scattering and absorbance by inherent biological chromophores inhibit the penetration of these short wavelengths into most tissues, rendering them inappropriate for in vivo applications. Consequently, many strategies have recently been employed to replace UV and visible light with NIR light, which facilitates enhanced tissue penetration [101–103].

The ortho-nitrobenzyl (o-nitrobenzyl) moiety is the most often employed photocleavable molecule [104]. For instance, micelles composed of poly (S-(o-nitrobenzyl)-L-cysteine)-b-poly (ethylene glycol) block copolymers are utilized for drug delivery [37]. Upon irradiation at 310 and 350 nm, the o-nitrobenzyl groups underwent gradual photocleavage, resulting in the shrinkage of the self-assembled micelles until complete cleavage occurred, thereby facilitating the controlled release of the encapsulated drug through modulation of the light irradiation duration. This study presents an attractive method for synthesizing photoresponsive polypeptide-based block copolymers and for developing photo-stimulated nanomedicine therapies.

The nitrobenzyl group has been integrated into liposomes for drug delivery: light irradiation induces the hydrolysis of o-nitrobenzyl, leading to the dissociation of hydrophilic and hydrophobic components from the amphiphilic phospholipid, resulting in membrane destabilization and subsequent drug release. Liposomes possessing photocleavable properties can be synthesized in situ by attaching an o-nitrobenzyl-containing azide tail precursor to an alkyne-functionalized lysolipid via the copper-catalyzed azide-alkyne cycloaddition process. The photolysis of the introduced o-nitrobenzyl group modified the molecular architecture of the photolabile phospholipids, enhancing phase transition and

lipid membrane permeability, hence compromising the liposome structure and facilitating cargo release. This in situ liposome production technique integrates precursor design with a click reaction to produce photo-responsive liposomes for drug delivery [105,106].

Light enables precise real-time spatial and temporal regulation, resulting in significant interest in photodegradable hydrogels as three-dimensional carriers for cells and medicinal agents for applications including wound healing, tissue regeneration, and disease treatment [107]. Hydrogel platforms based on coumarin are being explored as a viable alternative to previously disclosed nitrobenzyl-based systems [108]. Coumarin compounds possess numerous advantages compared to nitrobenzyl-based linkers, such as enhanced absorption efficiencies, accelerated cleavage rates, a red shift in degradation wavelengths, and a propensity for multiphoton-induced reactions [109]. Coumarin moieties can modify poly (ethylene glycol) (PEG)-based hydrogel materials at 365 and 405 nm, akin to nitrobenzyl groups, as well as at longer wavelengths ranging from 720 to 860 nm, producing biologically advantageous byproducts upon photocleavage. A brief photocage bromohydroxycoumarin crosslinker was utilized to create light-degradable hydrogels and nanogels. The researchers found that the coumarin-based crosslinker decomposes through intramolecular cyclization when exposed to UV and NIR light, facilitating the rapid degradation of polyacrylamide gels and the release of small payloads, including iron oxide nanoparticles, a model protein (BSA), and murine mesenchymal stem cells. This novel adaptive hydrogel chemistry holds potential for future tissue engineering research aimed at spatiotemporal patterning of cellular microenvironments, bioactive chemical transport in living systems, and cell delivery implants for regenerative medicine.

The in-situ fabrication of hydrogels and polymers is particularly advantageous for tissue engineering since it allows intricate shapes to adhere to and conform with tissue structures [110]. Photopolymerization is a proficient method for the in-situ synthesis of crosslinked polymers and hydrogels [111]. Photopolymerization facilitates the initiation and propagation of network polymerization in situ, yielding covalently crosslinked hydrogels, through the exposure of a photosensitive system comprising unsaturated prepolymers, photoinitiators, and additional components such as cells and therapeutic molecules to UV or visible light. Photopolymerization technique offers numerous advantages compared to conventional polymerization methods. Photopolymerization enables exact temporal and spatial regulation of the gelation process, heals rapidly at ambient or physiological temperatures, and is non-invasive. Additionally, it can be achieved within minutes utilizing cytocompatible photoinitiators and biocompatible reaction conditions [112]. This expedited approach facilitates precise regulation of the spatiotemporal formation of the gel at pertinent length scales, essential for both hydrogel crosslinking and the incorporation of certain biochemical functionalities in three-dimensional environments [113].

Photopolymerization has been employed to fabricate a biosynthetic soft tissue substitute composed of PEG and hyaluronic acid (HA) that can be injected and photocrosslinked in situ via transdermal light exposure [114]. This technique facilitates soft tissue reconstruction through implantation at the damaged site, shaping to the desired form, and subsequent in situ crosslinking with light. The authors developed a series of light-emitting diodes that can penetrate up to 4 mm of human skin without inducing any adverse effects. The implanted photocrosslinked PEG-HA in rats exhibited a dose-dependent correlation with PEG concentration and maintained near-original volume for as long as 491 days. The polymer was assessed in humans to determine the feasibility of employing transdermal photocrosslinking for implantation in abdominal soft tissues. Despite the presence of an inflammatory response surrounding the materials, this novel photocrosslinkable biosynthetic polymer and

transdermal crosslinking method exemplify a compelling paradigm for soft tissue contouring and reconstruction.

Several photocrosslinked hydrogels, besides their suitability for tissue regeneration, have potential for drug delivery, exemplified by photocrosslinkable hyperbranched polyester (HPE) hydrogels that possess sustained drug release capabilities for cellular therapies [36]. Dexamethasone acetate was encapsulated in HPE and functionalized with acrylate groups, yielding hydrogels characterized by a highly porous interconnected architecture and a robust structural network. The feasibility of utilizing these HPE networks for cellular therapies was subsequently evaluated by quantifying drug release, cell adhesion, spreading, and proliferation on the hydrogel surface. Research indicated that HPE hydrogels released encapsulated dexamethasone acetate consistently over 8 days, with the most crosslinked HPE hydrogels exhibiting enhanced cell adherence, spreading, and proliferation compared to softer, more compliant HPE hydrogels. Their findings revealed that cells swiftly adhered to and multiplied on hydrogel sheets, resulting in a uniform cell layer that encompassed the entire hydrogel surface, suggesting their potential application in cellular therapies.

3. Magnetic responsive material

Magnetic responsive nanomaterials, especially magnetic nanoparticles (MNPs), possess numerous attributes that render them attractive for diverse applications, including elevated specific surface area, chemical stability, reduced intraparticle diffusion rate, substantial loading capacity, and superparamagnetic. These nanoparticles are significant in the biomedical domain, especially for tissue regeneration, owing to their biocompatibility and enduring stability. Moreover, they possess numerous advantages regarding penetration and invasiveness, as many materials, especially biological tissues, exhibit a lower absorption capacity for magnetic fields compared to other stimuli, such as electric fields, thereby enabling remote activation of an event at a considerable distance from the magnet [115,116].

Magnetic-responsive nanoparticles are categorized into four types: oxides, coated oxides, metallic, and coated metal compounds. The initial group comprises iron oxides or ferrite nanoparticles structured in the crystalline forms of maghemite or magnetite, which have been thoroughly examined. When their size decreases below 128 nm, they shift from ferro or ferrimagnetic to superparamagnetic states, preventing self-agglomeration. This particle subclass is referred to as superparamagnetic iron oxide nanoparticles (SPION), which includes a further classification of ultrasmall superparamagnetic iron oxide nanoparticles (USPION), defined as particles smaller than 50 nm [117].

Due to the relatively inert nature of ferrite surfaces, it is necessary to enhance their reactivity with a coating, commonly silica. This surface coating involves multiple covalent modifications with diverse functional groups using organo-silane compounds. Recent findings indicated that magnetite nanoparticles (Fe3O4-MNPs) modified with silica using TEOS hydrolysis exhibited identical spherical distribution and structure to MNPS. However, augmenting the silica content in the magnetite nanoparticles enhanced the overall saturation magnetization [118,119].

Magnetic metallic nanoparticles possess a superior magnetic moment compared to oxides. Nonetheless, they are pyrophoric (able to ignite spontaneously at temperatures exceeding 55°C) and reactive with oxidizing agents, rendering them difficult to manage [117]. To mitigate the issues associated with metallic nanoparticles, their surfaces can be passivated by providing a protective

coating using several methods, including the use of surfactant agents, polymers, precious metals, or mild oxidation [115].

Top-down and bottom-up methodologies are frequently utilized to synthesize monodisperse magnetic nanoparticles characterized by good stability and size control, ranging from several to tens of nanometers [120]. These methodologies utilize three categories of techniques: physical, chemical, and biological. Chemical methodologies employing a bottom-up strategy can be utilized in both gaseous and aqueous phases. The frequently utilized methods are sol-gel, co-precipitation, hydrothermal synthesis, thermal decomposition, microemulsion, sonolysis, solvothermal, and electrochemical approaches, all of which are wet phase processes [121].

MNPs can be readily functionalized and utilized in numerous biomedical applications [122–124]. Enhanced hydrophilicity is a favorable attribute in biocompatible magnetic nanoparticles, as it augments water solubility [115]. Prior to surface functionalization, magnetic nanoparticles (MNPs) must be stabilized in nonaqueous solvents with a hydrocarbon layer, following which several methods can be employed for functionalization. The most common processes are ligand addition, ligand exchange, and hydrophilic silica coating. To enhance the water solubility of MNPs, the ligand addition method utilizes an amphiphilic molecule containing both hydrophilic and hydrophobic moieties [125]. The ligand exchange approach utilizes an innovative coordinating group that chemically adheres firmly to the surface, displacing the hydrocarbon layer. Additionally, this coordinating group is associated with a polar group, facilitating solubility in water. The third method, the previously described silica coating, is the most effective for attaining biocompatibility, stability, and hydrophilicity. The predominant approach for effectuating this modification is the sol-gel technique, which involves the hydrolysis of tetraethyl orthosilicate (TEOS) [118,126].

MNPs have been utilized across several sectors, as previously mentioned [43]. The subjects encompass small molecule drug delivery, hyperthermia treatment, gene therapy, cell tracking, and, importantly, tissue engineering. The utilization of MNPs, either independently or in conjunction with a magnetic field, significantly enhances bone regeneration by optimizing three essential elements: stem cells, growth factors, and scaffolds [127,128]. Peptide-MNP conjugates are employed for remote signaling mechano-activation. They demonstrated that employing peptide-conjugated magnetic nanoparticles to trigger signaling pathways in mesenchymal progenitor cells can remotely regulate bone tissue formation [129]. Iron oxide magnetic nanoparticles have been integrated with other materials to potentially function as a magnetically stimulated system in tissue engineering applications [44]. The amalgamation of magnetic nanoparticles with polysaccharide polymers produces magnetically responsive hydrogels that enhance cell growth and adhesion in an external magnetic field. This indicates potential for application in several tissues, including skin, cartilage, muscle, and connective tissue [130]. A study developed an enzyme-MNP complex to regulate cell proliferation using an external magnetic field [45]. This cell growth switching method may be employed to modulate cellular responses and develop innovative biomaterials for tissue regeneration.

4. Temperature responsive material

Thermoresponsive materials modify their physical properties or structure in reaction to variations in temperature. These materials are utilized in numerous biomedical applications, including as drug

delivery and tissue engineering (Fig. 1.5) [31,131–133]. The transition-state temperature is influenced by the solvent's interaction with the polymer and the hydrophilic/hydrophobic equilibrium. The thermoresponsive properties of polymers can be modified by incorporating reactants into the polymer/solvent mixture. Certain additives comprise co-polymers, surfactants, co-solvents, plasticizers, and salts. Additives can modify the quality of the solvent, hence affecting polymer-solvent interactions [60,134].

Thermoresponsive materials can be categorized according to their temperature response: polymers that exhibit insolubility above a critical temperature (LCST) and polymers that exhibit insolubility below a critical temperature (UCST) [135]. Natural thermoresponsive polymers encompass gelatin, agarose, and pectin, whereas synthetic polymers comprise poly(N-alkyl substituted acrylamides), poly(N-vinyl-alkyl-amides), poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) copolymer (PEG-PPG-PEG), and poly(ethylene glycol)-poly(d,l-lactic acid)-poly(ethylene glycol) copolymer (PEG-PLLA/PDLA-PEG), among numerous others [50].

The most recognized thermoresponsive polymers consist of PNIPAAm, a poly(N-alkyl substituted acrylamide). PNIPAAm demonstrates adjustable water solubility during thermal cycling, undergoing a reversible phase transition from a hydrophilic coil to a hydrophobic globule at approximately body temperature (LCST around 30–37°C). Polymers based on PNIPAAm are extensively utilized in biomedical applications, including drug delivery, tissue engineering, and gene therapy, exemplified by thermoresponsive polyplex micelles with PEG shells and PNIPAAm layers that safeguard DNA cores during gene therapy. Magnetic drug targeting was employed to

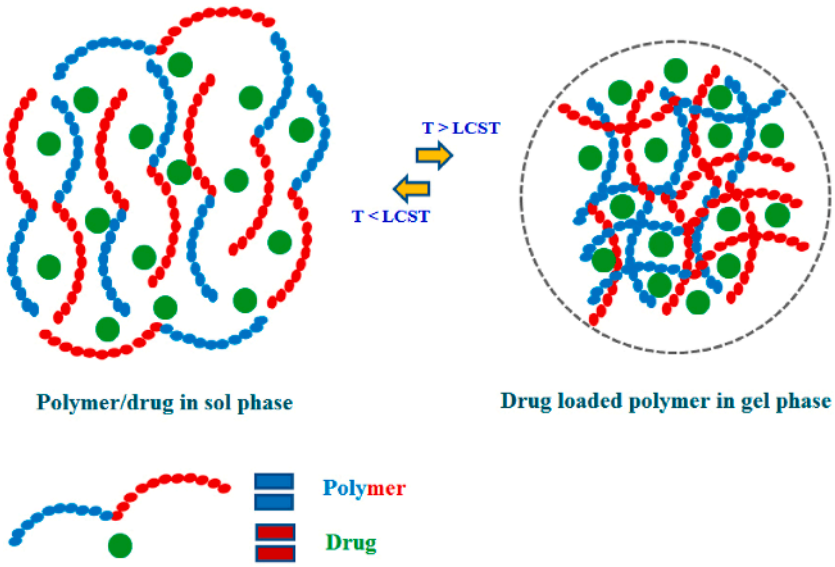


FIGURE 1.5

Schematic of teperacture responsive of polymers and materials [31].

encapsulate thermoresponsive polymer-coated magnetic nanoparticles with the anticancer agent doxorubicin, which was subsequently released concurrently via hyperthermia [53,136]. A bioink for articular cartilage was developed by merging PNIPAAm-grafted hyaluronan with methacrylated hyaluronan, yielding high-resolution layer-by-layer printed scaffolds with satisfactory viability [58]. Hydrogels have demonstrated significant potential for tissue engineering applications due to their capacity to encapsulate living cells within three-dimensional, biomimetic microenvironments. A microfibrous self-supporting hybrid scaffold was created through the electrospinning of a thermosensitive hydrogel, poly(ethylene glycol)-poly(N-isopropylacrylamide) (PEG-PNIPAAm), and a biodegradable polymer, poly(e-caprolactone) (PCL). The reverse thermosensitivity of PEG-PNIPAAm enabled it to hydrate and dissolve after cell seeding within a scaffold of PCL microfibers, while maintaining the overall hydrogel structure at ambient temperature. Cell encapsulation occurs after a subsequent temperature elevation to 37°C, resulting in the hydrogel transitioning to a gel state. The hybrid material increased chondrogenic differentiation of human mesenchymal stem cells (hMSCs) via chondrocytic gene and protein expression, leading to enhanced viscoelastic properties for the constructs. These researchers created a scaffold that facilitates a straightforward, one-step cell seeding method for inoculating cells, demonstrating potential for cartilage tissue engineering applications [137]. The encapsulation of human adipose-derived stem cells (ASCs) in hydrogels demonstrates that the AHA-g-PNIPAAm copolymers preserved the survival of the encapsulated cells, suggesting they were non-cytotoxic. The swelling equilibrium, enzymatic degradation, and cytocompatibility of copolymer hydrogels were assessed based on PNIPAAm weight ratios. Encapsulated human adipose-derived stem cells exhibited a spherical morphology, suggesting that the AHA-g-PNIPAAm copolymer hydrogel promoted cellular survival. Moreover, first in vivo investigations in mice utilizing the AHA-g-PNIPAAm copolymer hydrogel containing 53% PNIPAAm demonstrated biocompatibility. These data indicate that thermosensitive AHA-g-PNIPAAm hydrogels hold potential for adipose regeneration and various soft tissue engineering applications [138].

A hybrid natural-synthetic thermoresponsive material was created to address cartilage dysfunctions by integrating chitosan (CS) and PNIPAAm (CS-g-PNIPAAm) as a vehicle for mesenchymal stem cell proliferation and differentiation. Diverse microstrip widths were fabricated to replicate the surface region of real cartilage and evaluate cellular orientation and elongation. Following 28 days of incubation in chondrogenic media, encapsulated mesenchymal stem cells (MSCs) within the hydrogel exhibited enhanced production of glycosaminoglycan (GAG) and collagen. Histological and immunohistochemical analyses validated chondrogenic differentiation. Moreover, cells contained within 50 mm wide microstrips exhibited superior alignment compared to those on unpatterned substrates. The authors determine that the cellular morphology and architecture of the microengineered supports mirrored the surface zone of cartilage, whereas the unpatterned structures reflected the intermediate zone. A multitude of studies have employed nonlinear PEG-based thermoresponsive polymers as a substitute for poly-N-isopropyl acrylamide. The significant interest in PEG-based thermosensitive materials mostly stems from its capacity to fabricate complex structures, adjust the transition temperature, and regulate additional features such as water solubility and biocompatibility [131]. Thermosensitive hydrogels derived from polyisocyanopeptide (PIC) are noteworthy due to their liquid state below 16°C and gel formation at ambient temperature. PIC gels

exhibit mechanical properties and architecture akin to collagen and fibrin, including a stiffening response under high pressures. Their thermoresponsive action is reversible, and they exhibit biocompatibility due to enhanced cell adhesion properties. A recent study investigated full-thickness dorsal skin wounds in mice treated with PIC gel containing PIC-RGD (arginyl-glycyl-aspartic acid peptide, commonly referred to as RGD or GRGDS) after 3 and 7 days. The wound closure rates were same across all groups that did not exhibit foreign body reactions. No substantial alterations were noted in myofibroblasts, epithelial migration, macrophage levels, collagen expression, or blood vessels. The authors propose that these biomimetic PIC hydrogels could be suitable for application as wound dressings [55]. PIC materials will be tailored to meet diverse cellular needs by modifying stiffness or by conjugating the polymer with short GRGDS peptides by click chemistry. These peptides replicate the binding sites of particular integrins, rendering the hydrogels biocompatible. Upon evaluating the characteristics of the PIC polymer, they identified the optimal concentration of the GRGDS ligand conjugated with the polymer, together with the ideal stiffness of the hydrogel, for successful cellular, tissue, and organ proliferation. This work demonstrated that endothelial cells, fibroblasts, adipose-derived stem cells, and melanoma cells may survive, thrive, and differentiate within the customized PIC hydrogels. Additionally, structures resembling blood capillaries were identified in vitro [56].

Cellulose is a linear homopolymeric polysaccharide consisting of D-glucopyranose units connected by β(1→4-glycosidic) bonds. The substantial intramolecular and intermolecular hydrogen bonding in cellulose renders it insoluble in water. The etherification of cellulose's hydroxyl groups has yielded water-soluble derivatives, including methyl cellulose and hydroxypropylmethyl cellulose. Methyl cellulose, a derivative of cellulose, has been thoroughly investigated for applications in drug delivery, tissue engineering, and medicine. It possesses thermoreversible gelation properties in aqueous solutions, exhibiting a lower critical solution temperature (LCST) of 60–80°C; still, the incorporation of copolymers may diminish its efficacy. HPMC is a partly O-methylated and O-(2-hydroxypropylated) derivative of cellulose. Methoxy residues induce gelation, whereas hydroxypropyl residues have been demonstrated to alter the sol-gel transition temperature [49].

Thermoresponsive hydrogels derived from methyl cellulose for cell-sheet engineering through 3D printing can be modified to achieve lower critical solution temperatures at physiological temperature [50]. Methyl cellulose-based thermoreversible hydrogels, potentially incorporating type-I collagen to enhance cell proliferation and expansion, facilitate the adhesion and proliferation of stem cells in culture [48]. Thermosensitive and mucoadhesive eyedrops incorporating platelet lysate (a hemoderivative abundant in growth factors) for the treatment of corneal lesions utilize hydroxypropylmethyl cellulose combined with chondroitin-6-sulfate sodium, leading to a lower critical solution temperature (LCST) ranging from 32 to 35°C [57].

Elastin-like polypeptides (ELP) were designed based on elastin, particularly its precursor, tropoelastin. Tropoelastins are hydrophilic proteins that undergo crosslinking to form insoluble elastin within the extracellular matrix. Elastin topologies differ and are crucial for cell adhesion, proliferation, and/or differentiation. These materials possess two primary advantages: biocompatibility and feasible synthesis. Traditional peptide synthesis is presently prohibitively costly for large-scale production of ELPs; therefore, the most viable method is recombinant production, yielding ELPs with LCST phase behavior (below their LCST, ELPs remain soluble in aqueous solutions, while above this temperature, they form insoluble coils); additionally, the molecular weight and concentration of polypeptides can influence their LCST [47].

5. pH responsive materials

In diseased, inflamed, and infected tissues, the pH may be decreased due to dysregulated metabolism or irregular angiogenesis, which cause a rapid shortage of oxygen and nutrients, resulting in a shift toward glycolytic metabolism [139]; thus, variations of the pH in different organs, tissues, and intracellular compartments can be considered during the design of nanomaterials with the ability to release a therapeutic agent at a target site in response to pH (Fig. 1.6) [31,140].

Extracellular organelles and the bloodstream usually have a pH of 7.4, whereas the stomach has a pH of 1-3 and the duodenum and ileum have a pH of 6.6–7.5. The pH ranges for the intracellular subendosomal and lysosomal organelles are 5.5–6.8 and 4.5–5.5, respectively [141]. As a result, there is a wide variety of pH values within the human body in normal or pathological states that might be considered when designing stimuli-responsive delivery systems. Differences in pH can alter cross-linking processes (important for injectable hydrogels and self-healing materials); the protonation or deprotonation of acidic/basic groups can produce distinct interactions between a therapeutic agent and a material, potentially resulting in a defined release profile in a specific tissue or cell.

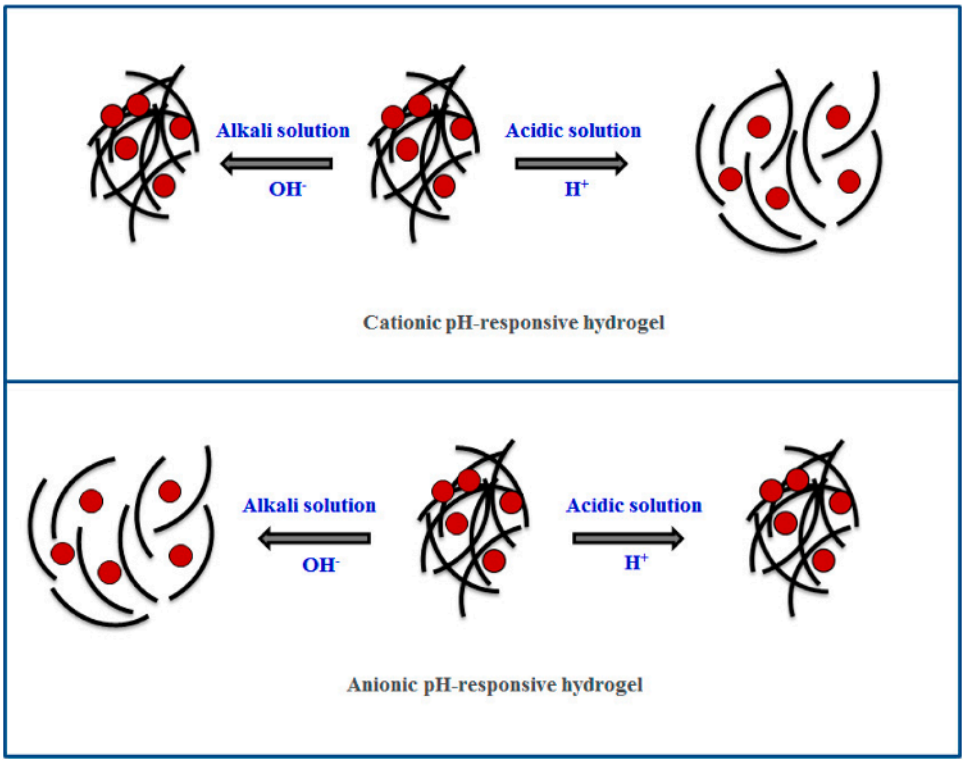


FIGURE 1.6

Schematic of pH responsive of polymers and materials [31].

Hydrogels can be tailored so that their physicochemical features, such as stiffness, degradation, and porosity, have a direct impact on the encapsulated cells' fates, including migration, proliferation, differentiation, and communication. Furthermore, stimuli-responsive hydrogels may improve in vivo therapeutic efficacy while also introducing new therapeutic pathways [142]. The transport of therapeutic compounds in nanocarriers to target cells and tissues is also critical in tissue regeneration. The transmission of genetic material has the ability to enhance the capabilities of target cells via plasmid DNA and related constructions, or to suppress functions via reduction/silencing actions. Additionally, a medicine in a nanocarrier has the ability to minimize the amount necessary for therapeutic efficacy, potentially avoiding severe side effects [143]. Furthermore, such carriers can achieve effective site-specific medication or growth factor delivery by taking use of physiological circumstances (e.g., pH) in vivo [144], boosting the possibility of enhanced regeneration or drug therapy. Infected tissues frequently have an acidic environment; however, it is important to note that the pH of cutaneous wounds is dynamic and has a good correlation with the stage of the wound healing process [145].

Balamuralidhara et al. [146] undertook a review of pH-sensitive drug delivery methods, encompassing hydrogels and liposomes. For pH-responsive drug delivery, pH-sensitive linkages, such as hydrazone bonds, are essential. The incorporation of polymers containing weakly acidic or basic groups in the backbone may induce swelling or deswelling in solution, hence affecting pH-sensitive drug release rates. Hydrogels are a significant category of materials with various uses in biomedical domains [147]. Drug molecules of varying dimensions can permeate through hydrogels, rendering both dry and swollen polymeric networks appropriate for drug delivery. Hydrogels react to stimuli by modifying their physical or chemical properties, facilitating regulated drug release.

Vashist et al. [148] emphasized the multifaceted applications of hydrogels in transforming drug delivery system research and outlined their function as drug-delivery vehicles for various disorders affecting the brain and other specific regions of the human body; the clinical applications and toxicological considerations of hydrogels were also examined. The constraints and future outlook regarding the advancement of biopolymeric hydrogels were examined. Below are selected examples of hydrogels to illustrate their applicability in pH-responsive medication delivery.

Basak et al. [149] synthesized poly (vinyl alcohol) hydrogels through crosslinking with maleic acid. They observed that the hydrogels exhibited maximal swelling in intestinal fluid (pH 7.5) and minimal swelling in simulated gastric fluid (pH 1.2). Hydrogels infused with vitamin B12 and salicylic acid demonstrated colon-specific drug release characteristics, with enhanced drug release in intestinal fluid (pH 7.5) compared to simulated gastric fluid (pH 1.2).

Bastings et al. [150] documented a pH-responsive supramolecular hydrogel utilizing fourfold hydrogen-bonding supramolecular ureidopyrimidinone (UPy) units linked to alkyl urea spacers and poly (ethylene glycol) (PEG) chains. The UPy-modified PEG hydrogels generated fibers in aqueous solution that could cross-link to create transient supramolecular networks. These distinctive UPy-transient networks exhibited pH responsiveness, facilitating a sol-to-gel transition within a narrow pH range. The hydrogel may transition to a liquid state at pH levels exceeding 8.5, exhibiting a sufficiently low viscosity to facilitate movement via a 1-meter catheter, while it promptly reverts to a hydrogel upon contact with tissue. The hydrogel demonstrated self-healing capabilities, which mitigated alterations to the injection site. Growth factors were released from the hydrogel and demonstrated a decrease in infarct scar in a porcine myocardial infarction model. The hydrogel solution, administered via catheter, converted into a localized drug release reservoir through instantaneous gelation upon contact with cardiac tissue.

Garbern et al. [151] developed a pH- and temperature-responsive injectable hydrogel to exploit the acidic microenvironment of ischemic myocardium, enhancing therapeutic angiogenesis by spatio-temporal regulation of angiogenic growth factor delivery. The pH- and temperature-sensitive random copolymer poly (N-isopropylacrylamide-co-propylacrylic acid-co-butyl acrylate) existed as a liquid at pH 7.4 and 37°C, but transitioned to a physical gel at pH 6.8 and 37°C. The retention of biotinylated basic fibroblast growth factor (bFGF) in infarcted rat myocardium was tenfold greater with hydrogel delivery compared to saline, seen between 0- and 7-days post-injection.

Kozlovskaya et al. [152] documented a unique hydrogel microparticle that exhibited significant, fast, and reversible volume alterations in reaction to pH variations. Cubic hydrogels were synthesized as interconnected poly (methacrylic acid) network copies of mesoporous manganese oxide templates through the successive infiltration of poly(methacrylic acid) and poly(N-vinylpyrrolidone), followed by the crosslinking of poly(methacrylic acid) and the dissolution of the template. The hydrogel cubes demonstrated a reversible twofold alteration in size while preserving their shape in response to pH fluctuations. The swelling characteristics of cubic and spherical hydrogel particles can be modified by altering the molecular weight of poly (methacrylic acid). They demonstrated pH-responsive loading and release of the drug DOX in cubic hydrogels. The pH-responsive release of DOX from cubic hydrogels commenced at pH 5 and culminated in the total elimination of DOX from the cubes at pH 3. The release of DOX resulted in an enhanced network size due to the disruption of electrostatic interactions between the poly (methacrylic acid) hydrogel and DOX. Following a 24-hour exposure of DOX-loaded cubes to pH levels of 7.4 and 5, the drug release was measured at (4.4 ± 0.1) % and (7.2 ± 0.1) %, respectively. Conversely, 801% of the encapsulated DOX was released from the cubes after 1 hour upon exposure to pH 3. The results indicate that DOX can be liberated from poly (methacrylic acid) cubic hydrogel particles in an acidic endosomal/lysosomal milieu subsequent to cellular absorption of the particles. The viability of A549 and HeLa cancer cells was markedly diminished following exposure to DOX-loaded cubic hydrogels.Eleven

Anirudhan et al. [153] prepared a gelatin-based pH-responsive composite hydrogel by grafting b-cyclodextrin (b-CD) to gelatin (Gel) and crosslinking with oxidized dextran (OX-Dex), and the as-prepared pH-sensitive b-CD-grafted Gel crosslinked with OX-Dex (b-CD-g-Gel/OX-Dex) or composite hydrogel (CHG) was used to investigate the colon delivery of the anticancer drug 5-fluorouracil (5-FLU). The use of b-CD improved the drug encapsulation capacity, and the drug release profile conformed to the Higuchi model. The degradation and expansion of the hydrogel were seen to facilitate drug release. The release patterns of 5-FLU were examined at stomach pH 1.2 and intestinal pH 7.4, revealing minimal drug release at pH 1.2 and significant release at pH 7.4. The drug-loaded b-CD-g-Gel/OX-Dex (CHG) demonstrated superior suppression of colon cancer cells relative to the free drug.

Gao et al. [154] developed biodegradable and pH-responsive hybrid hydrogels composed of carboxymethyl cellulose and poly (acrylic acid), which deswelled in acidic artificial gastric fluid but rapidly swelled in neutral artificial intestinal fluid. These hydrogels demonstrated selective enzymatic degradation and facilitated accelerated drug release from insulin-loaded hydrogels in artificial intestinal fluid. Oral administration of insulin-loaded hydrogels to streptozotocin-induced diabetic rats led to a continuous decline in the fasting blood glucose level within 6 h post-administration, and the relative pharmacological availability increased more than 10 times compared with oral administration of a solution of free insulin.

Chen et al. [155] described a drug-delivery microdevice that incorporated pH-responsive hydrogel nanoparticles functioning as intelligent valves; they synthesized pH-responsive hydrogel nanoparticles implanted within a composite membrane. the resultant pH-responsive composite membranes were combined with polydimethylsiloxane microreservoirs via a room-temperature transfer bonding method to create the proof-of-concept microdevice. The release rate of vitamin B12, utilized as a model medication, significantly increased when the local pH value was lowered from 7.4 to 4.

Osada et al. [156] examined their recent research on the design and synthesis of intelligent polymeric micelles derived from poly (ethylene glycol)-poly (amino acid) block copolymers, including a spherical core-shell architecture encapsulating an anticancer medication. The poly (ethylene glycol)–poly (amino acid) featuring a hydrazone linkage enables the polymeric micelles to release the medication preferentially at acidic pH levels (pH 4–6). The incorporation of folic acid onto the micelle surface may improve the efficacy of cancer-cell-specific medication delivery and facilitate pH-controlled drug release. The Schiff base, or azomethine, is regarded as the most efficient and suitable linkage for designing pH-sensitive drug release systems. The imine bond remains stable at pH 7.4 but is cleavable at pH values below 6, while also being reversible at pH 7.4. Conversely, the hydrazone bond demonstrates superior pH sensitivity and stability compared to the imine bond, with its hydrolysis rate being modifiable based on environmental conditions, hence facilitating regulated drug release.

Bae et al. [157] described a pH-sensitive polymeric micelle drug carrier synthesized via the self-assembly of an amphiphilic block copolymer, poly (ethylene glycol)-poly (aspartate hydrazone adriamycin). In this system, the anticancer agent adriamycin was conjugated to the hydrophobic segments via acid-sensitive hydrazone linkers. This drug-delivery mechanism effectively preserves the drug under physiological conditions (pH 7.4) and selectively releases it in response to the intracellular pH reduction observed in endosomes and lysosomes.

Wang et al. [158] described a poly (amino acid)-based amphiphilic copolymer drug-delivery system that demonstrated a pH-sensitive drug release profile in vitro; they observed that the cumulative release of DOX was significantly accelerated at pH 5.0 compared to pH 7.4, and the DOX-loaded drug-delivery system exhibited superior antitumor efficacy relative to free DOX.

Liu et al. [159] synthesized a poly (d,l-lactide)-block-poly (2-methacryloyloxyethyl phosphorylcholine) block copolymer and examined the loading and release of the anticancer drugs DOX and doxorubicin hydrochloride (DOX·HCl). They discovered that the release of DOX and DOX·HCl from the block copolymer exhibited a pronounced pH dependence, with a significantly accelerated release at a mildly acidic pH of 5.0 compared to physiological pH 7.4. Moreover, the DOX·HCl-loaded block copolymer demonstrated a more rapid drug release compared to the DOX-loaded block copolymer at same pH settings. The synthesized block copolymer drug-delivery system was capable of penetrating HepG2 cells, localizing within endosomes/lysosomes characterized by an acidic pH, and exhibiting improved intracellular drug release into the cytoplasm.

6. Conclusion

Stimuli-responsive materials represent a significant advancement in medication delivery methods. They offer personalized treatment solutions that surpass the limitations of conventional methods. Researchers have developed systems capable of intelligently responding to various inputs, including temperature, pH, light, redox conditions, and magnetic fields. This has led to enhanced accuracy and efficacy in pharmaceutical administration. These systems have been constructed by utilizing the distinctive properties of

their respective materials. Stimuli-responsive materials exhibit a diverse range of operational mechanisms that are highly adaptable to their surroundings. Temperature-responsive polymers can undergo phase transitions that facilitate medication release under specific heat settings. pH-sensitive systems exploit the acidic environment commonly seen in tumor tissues to selectively trigger drug release at the targeted site. Similarly, redox-responsive materials utilize the fluctuating quantities of reducing substances, such as glutathione, found within cells to initiate the degradation of drug carriers. This guarantees the intracellular release of therapeutic medications, where they are most needed. Light- and magnetic field-responsive systems offer enhanced control, allowing for the indirect manipulation of drug release kinetics from an external source. The use of stimuli-responsive materials in drug delivery offers numerous advantages over traditional approaches. The implementation of these intelligent systems can significantly diminish systemic toxicity. This is achieved by ensuring that drugs are administered exclusively to the targeted locations, hence reducing exposure to healthy tissues. Furthermore, they enable regulated release profiles that align with the pharmacokinetics of certain drugs, a crucial approach for maintaining therapeutic concentrations over prolonged durations. This feature enhances therapeutic efficacy and improves patient compliance by potentially reducing dose frequency. The integration of distinct stimuli-responsiveness into a singular system enables the advancement of intricate treatment methodologies capable of adapting to various complex biological circumstances. For example, nanoparticles engineered to respond to both pH and light provide dual control over drug release, enhancing therapeutic efficacy through enhanced targeting and timing.

Future prospects indicate that stimuli-responsive materials in drug administration possess significant potential and are poised for exploration. The key emphasis of current investigations is the creation of new materials with enhanced biocompatibility and responsiveness properties. The incorporation of advanced technologies, such as artificial intelligence, may enhance the design and customization of these systems. This would enable the personalization of drug delivery methods according to particular patient profiles determined by distinct disease characteristics. Enhancing the diversity of stimuli, including ultrasonic or electrical impulses, may lead to the creation of medication delivery systems that are more versatile and effective. Stimuli-responsive materials are poised to significantly influence the evolution of treatment paradigms across various medical fields as researchers persist in uncovering novel applications and advancing existing technology. Stimuli-responsive materials signify a significant advancement in intelligent drug delivery systems aligned with the principles of precision medicine. These materials possess the capability to deliver targeted and regulated release mechanisms that respond to endogenous signals and external stimuli, enhancing therapeutic efficacy and facilitating innovative treatment strategies that could improve patient outcomes across various disorders. The continuous advancements in this field hold significant potential for revolutionizing healthcare practices and addressing some of the most critical challenges now confronting modern medicine. This conclusion effectively summarizes the key elements of your article while emphasizing the importance of stimuli-responsive materials in drug delivery and its future potential.

AI disclosure

During the preparation of this work the author(s) used Quilbot, Grammarly and so on in order to Edit the text and search the sources. After using this tool/service, the author(s) reviewed and edited the content as needed and take (s) full responsibility for the content of the publication.

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CHAPTER

2

History, introduction, and physicochemical properties and characterization parameters of stimuli-responsive nanocarriers

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1. Introduction

Nanotechnology has become an important area of research in modern pharmaceutical and biomedical sciences, particularly in the development of advanced drug delivery systems. Among the various nanoscale delivery platforms, stimuli-responsive nanocarriers have attracted considerable attention due to their ability to release therapeutic agents in a controlled and predictable manner. These systems are often described as "smart" nanocarriers because they can respond to specific environmental or externally applied triggers, enabling more precise delivery of drugs to target sites within the body. Conventional drug delivery approaches frequently encounter challenges such as non-specific distribution, rapid drug degradation, and unwanted side effects. The emergence of nanocarrier-based delivery systems has helped address many of these limitations by improving drug stability, circulation time, and targeting capability. Stimuli-responsive nanocarriers represent a further advancement in this area, as they are designed to release their payload only when exposed to certain biological conditions, such as changes in pH, enzyme concentration, temperature, or redox environment, or external stimuli such as light or magnetic fields. This controlled response can enhance therapeutic effectiveness while reducing systemic toxicity.

The development of stimuli-responsive nanocarriers is rooted in the broader history of controlled drug delivery and functional materials research. Early efforts in drug delivery science during the mid-twentieth century focused primarily on sustained-release formulations using polymer matrices. These systems were designed to release drugs slowly over time, improving dosing convenience and therapeutic consistency. The introduction of biodegradable polymers in the 1960s and 1970s represented a major advancement, as these materials could safely break down within the body after delivering their

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therapeutic payload. Progress in materials science and nanotechnology during the 1980s and 1990s led to the creation of nanoscale delivery systems such as liposomes, polymeric nanoparticles, and micellar structures. These carriers demonstrated improved drug encapsulation efficiency and protection of sensitive molecules, including proteins and nucleic acids. During this period, researchers also began exploring polymers that could respond to environmental changes, particularly temperature-sensitive and pH-sensitive materials. These early responsive systems provided the conceptual foundation for the development of modern stimuli-responsive nanocarriers. In the early 21st century, rapid advances in nanotechnology, polymer chemistry, and biomedical engineering enabled the design of multifunctional nanocarriers with enhanced responsiveness and targeting ability. Scientists began integrating diagnostic and therapeutic functions within single nanosystems, leading to the concept of smart and programmable drug delivery platforms. Hydrogels, dendrimers, inorganic nanoparticles, and hybrid nanomaterials have since been widely investigated for stimuli-responsive applications. Today, research in stimuli-responsive nanocarriers continues to expand, driven by the need for safer and more efficient therapeutic strategies. Improvements in nanoscale characterization techniques, material synthesis, and surface engineering have contributed to the development of highly specialized delivery systems capable of functioning in complex biological environments. As understanding of disease microenvironments and material responsiveness continues to grow, stimuli-responsive nanocarriers are expected to play an increasingly important role in targeted therapy and personalized medicine.

2. Historical development of stimuli-responsive nanocarriers

The concept of drug delivery systems is not new, despite the significant progress that has recently been made in this field. For ages, contemporary drug delivery methods have been modeled, and their evolution seems to have followed humanity's. Three sections can be used to didactically split the history of medication delivery technology. The development of macroscopic delivery methods began with the pharmacy and continued through the Industrial Revolution. Since people were practicing pharmacy before the term "drug delivery approaches" was coined, it is impossible to pinpoint the exact date of its creation. The earliest known humans to use chemical processes for therapeutic purposes were the ancient populations of Mesopotamia, Egypt, China, and the indigenous groups of the Western Hemisphere, including the Aztecs, Mayans, and Incas [1].

3. Fundamentals of stimuli-responsive drug delivery system

3.1 Principles of stimulus-triggered drug release

When creating a drug delivery system, the main goal is to deliver the medication efficiently to the intended location with the fewest possible adverse effects. Low bioavailability, excessive toxicity, poor selectivity, and other related problems make it challenging to achieve the intended therapeutic effectiveness of many medications using conventional drug delivery systems [1,2]. Therefore, to assure site-specific therapeutic administration, an appropriate nanocarrier is needed [3]. Nanomedicine involves the use of nanoscale biomaterials for disease diagnosis and treatment, and it has demonstrated significant potential for delivering therapeutic agents specifically to targeted sites [4]. Numerous materials and techniques based on nanotechnology have been developed throughout the years for use in the detection and treatment of diseases. Solid lipid nanocarrier (SLN), nanostructured lipid carrier

(NLC), liposomes, dendrimers, polymeric micells, carbon nanotubes, polymeric nanoparticles, magnetic nanoparticles, and inorganic nanoparticles are a few examples of nanocarriers [5].

3.1.1 Solid lipid nanocarrier (SLN)

Solid lipids can produce SLN, which can then be stabilized by surfactants, waxes, extremely cleansed triglycerides, or complex glyceride combinations. These are more tolerable and biocompatible than liposomes, among other benefits [6].

SLNs have been widely applied in drug delivery, including enhancing the bioavailability of therapeutic agents [7]. In order to direct medications to the brain [8], When it comes to parenteral drug distribution [9], Dermal and pulmonary pathways [10]. Inside the solid hydrophobic core matrix, that is encased in a phospholipid monolayer coating, the medication may dissolve or be evenly distributed [11]. reduced size, increased stability, and the ability to administer hydrophilic and hydrophobic medications with a variety of ligands [12]. To develop SLN with more focused brain drug delivery, various drugs have been employed, including vinpocetine, camptothecin, and cotrimoxazole [13,14]. These issues mainly arise from the formation of a highly ordered crystalline structure when only solid lipids are used. SLN can also be tailored in terms of size and surface charge to enable site-specific drug delivery, allowing either immediate or sustained drug release [15].

3.1.2 Nanostructured lipid carrier (NLCs)

Nanoparticles composed of a combination of solid and liquid lipids have recently been used for therapeutic drug delivery. These lipid-based nanocarriers are known as nanostructured lipid carriers (NLCs). Since most therapeutic drugs show greater solubility in liquid lipids compared to solid lipids, NLCs are typically formulated with a higher proportion of liquid lipids. The lipid phase is initially heated above its melting temperature, and the drug is incorporated into the molten lipid. This drug-loaded lipid melt is then dispersed into the aqueous phase. The resulting hot microemulsion is homogenized to form the NLC dispersion. NLCs are typically produced using biocompatible and biodegradable lipids and surfactants, and they can deliver both hydrophobic and hydrophilic drugs to specific target sites [15]. NLCs are now a flexible platform for specialized therapeutic delivery applications due to their compact size, increased drug-loading capacity, improved stability, and ease of fabrication [16]. NLCs have proven to be more effective than other lipid-based nanocarriers at delivering medications via a variety of routes, including oral, topical, pulmonary, ophthalmic, and intranasal administration [17]. The therapeutic application of siRNA is restricted by its low stability and inefficient delivery to target tissues. To improve its therapeutic effectiveness, researchers have explored strategies such as encapsulating siRNA within NLCs. Over time, various multifunctional lipid-based NLC systems have been developed to enable the targeted delivery of drugs and siRNA [18].

3.1.3 Liposomes

When certain lipids are distributed in an aqueous medium, liposomes—nanocarriers—naturally develop [19]. They consist of two main components, an inner core and an outer phospholipid bilayer membrane [20]. They may have several lipid bilayers encircling the inner watery core, or they may have just one [21]. While hydrophobic medications are integrated into the hydrophobic portion of the outermost bilayer membrane, hydrophilic pharmaceuticals are contained within the inner watery core [22]. Traditional liposomes face several limitations, including poor stability, low drug loading

capacity, rapid drug release, and short circulation time in the bloodstream [23]. Monoclonal antibodies, carbohydrates, proteins, vitamins, peptides, antigen fragments, and glycoproteins are commonly attached to the liposome surface to enable active targeting of cancer cells [24,25]. Smart liposomes can respond to different internal and external stimuli, such as changes in pH, enzymatic activity, redox conditions, light, ultrasound, and microwave radiation [26,27]. Liposomes are widely used as model delivery systems for drugs, vaccines, cosmetic products, and nutraceuticals [28]. To increase their selectivity for target areas, liposomes are frequently coupled with ligands or antibodies [29]. In addition to being non-toxic and having good biocompatibility, liposomes can contain hydrophilic and lipophilic medications, increase drug solubility, and enhance pharmacokinetic characteristics while shielding the encapsulated medications from enzymatic breakdown. However, liposomes also have certain disadvantages, including a low drug transport rate and poor stability.

3.1.4 Dendrimers

Often branching macromolecules, dendrimers have several arms that emerge from the central core. Sugars, nucleotides, and amino acids are among the natural or synthetic components that are typically used in their production [30]. These features are most suited for drug delivery techniques because of their distinct molecular weights, increased branching, spherical shapes, and monodispersed macromolecules with 1.40–1.45 nm [31]. In recent years, dendrimers have been widely applied in biomedical fields, such as gene delivery, immunology, magnetic resonance imaging, vaccine development, and the delivery of antiviral, antibacterial, and anticancer drugs. By encasing hydrophobic medications inside their internal cavities and affixing them in their surface functional molecules for delivery, dendrimers can increase the dissolution and absorption of these medications [32]. Dendrimers are mainly composed of smaller structural units known as dendrons. Dendrons are formed by removing the core unit and are divided into three regions: the core, the interior, and the periphery. The internal void spaces of dendrons can encapsulate drug molecules, enabling improved solubility, controlled release, targeted delivery, and protection from degradation. Dendrimers offer several advantages, such as uniform particle size, the ability to attach various targeting ligands to their densely packed surface functional groups, and the presence of multiple end groups that enhance binding with different receptors [33].

Dendrimers are water-soluble and biocompatible nanocarriers with good pharmacokinetic behaviour and the ability to encapsulate and deliver a wide range of bioactive agents; however, they also exhibit disadvantages such as a poor drug release profile, rapid clearance from the body, and potential toxicity depending on their surface chemistry and generation.

3.1.5 Polymeric micelles

Polymeric micelles typically have a size below 100 nm and exhibit a narrow size distribution, which helps reduce rapid renal clearance. Due to the enhanced permeability and retention (EPR) effect, they are able to preferentially accumulate in tumour tissues [34]. These nanostructures show great potential for delivering hydrophobic drugs, as their internal core enables efficient drug encapsulation, thereby enhancing bioavailability and stability [35]. Drug targeting using a variety of polymeric micelles is based on multiple mechanisms of action, including complexation of a particular addressing ligand molecule across the micelle surface, increased permeability, and holding effect stimulation [36]. Polymeric micelles exhibit good biocompatibility and can effectively solubilize lipophilic drugs, while their surface functional groups allow conjugation with targeting ligands for site-specific drug delivery;

however, they generally suffer from low drug-loading capacity and are mainly suitable for the delivery of lipophilic drugs [37].

3.1.6 Carbon nanotubes

Carbon nanotubes were first identified by Iijima in 1991 [38]. They are nanoscale, hollow tubular structures assembled of carbon atoms. They are members of the fullerene family and are created by rolling sheets of graphene into cylindrical forms [39]. Carbon nanotubes are classified into two types: single-walled carbon nanotubes, which consist of a single graphene layer, and multi-walled carbon nanotubes, which are made up of multiple concentric graphene layers arranged in a cylindrical structure. Their unique physicochemical and biological properties make them highly effective carriers for drug delivery applications [40]. Carbon nanotubes can be modified to improve their circulation within the body through covalent or noncovalent functionalization. These modifications can either increase or decrease their circulation time as required. When functionalized to become soluble in aqueous biological fluids, they exhibit minimal toxicity and can be readily taken up by cells [41]. Carbon nanotubes are considered highly effective nanocarriers for cancer therapy. Anticancer drugs can be encapsulated within their inner cavity or attached to their surface through covalent or non-covalent interactions [42]. Carbon nanotubes can be used as functionalized platforms for applications ranging from early cancer diagnosis to gene delivery. Their strong optical absorption in the near-infrared region also makes them highly suitable for photothermal ablation of cancerous tissues [43]. Carbon nanotubes enable simple synthesis and attachment of various bioactive molecules, can carry a wide range of therapeutic agents, and protect drugs while allowing controlled and sustained release. However, they have limitations such as low water solubility, unfavourable pharmacokinetic properties, and lack of biodegradability [44].

3.1.7 Polymeric nanoparticles

Polymeric nanoparticles are solid nanosized colloidal particles made from biodegradable polymers [45]. Nanospheres and nano capsules are the two structural categories into which polymeric nanoparticles belong. Because of their matrix-type structure, the medication can dissolve, entrap, adsorbed, encapsulate, or conjugate inside a polymer scaffold of nanospheres. However, the innermost portion of nanocapsules, which are covered in a polymeric shell, contains the medication [46]. They are a good option for brain-targeted drug administration because of their low toxicity, pharmacotechnological stability, biodegradability, and the presence of surface functional groups that control the drug release rate [47]. Numerous natural and synthetic polymers that are both biocompatible and biodegradable were used to generate polymeric nanoparticles. The body breaks down these biodegradable polymers into individual monomers, which are then removed by normal metabolic processes [48]. Polymeric nanoparticles have better and more controllable physicochemical properties, longer drug circulation times, a more uniform particle size distribution, a higher drug payload, and more controlled drug delivery than polymeric micelles and liposomes [49]. Polymeric nanoparticles offer excellent biocompatibility and biodegradability, low immunogenicity, low cost, low toxicity, good stability, and ease of production, and controlled drug release; however, concerns remain regarding their potential toxicity and relatively slow degradation [50].

3.1.8 Magnetic nanoparticles

Generally, the three main ferromagnetic elements Fe, Ni, and Co can be combined to create magnetic nanoparticles that can operate as targeted carriers to deliver drugs to intra-tumoral levels or to mediate a hyperthermic action that results in tumor necrosis [51]. The size, charge, and surface chemistry of magnetic particles are especially important because they affect their blood circulation duration as well as bioavailability within the body [52]. Furthermore, The magnetic properties and internalization of magnetic particles in target tissue are significantly influenced by their size [53]. For instance, systemic administration of magnetic nanoparticles of bigger particles with a diameter in excess of 200 nm is often eliminated by the spleen, which shortens blood circulation times. Extravasation and kidney clearance rapidly remove smaller particles with an average size of below 10 nm. Because they have the longest blood circulation period, particles with sizes between 10 and 100 nm are most suited for intravenous administration [5]. Magnetic nanoparticles should be tiny enough to successfully distribute in specific tissues while avoiding the body's Reticuloendothelial System (RES) and penetrating minuscule capillaries within bodily tissues [54]. Magnetic nanoparticles exhibit excellent biodegradability, low cytotoxicity, ease of synthesis, strong stability after modification, and the ability to bind multiple targeting compounds; however, their application is limited by high synthesis cost, poor dispersion, and mobility-dependent compatibility [55].

3.1.9 Inorganic nanoparticles

Inorganic nanostructured materials have recently been found to include nanocarriers for targeted drug delivery. These consist of two parts: the shell and the core. Inorganic elements such as gold, silica, or iron oxide are found in the core, which is surrounded by an organic polymer shell that either serves as a suitable substrate for the combination of biomacromolecules or protects the core from undesirable physicochemical encounters with its surrounding biological environment [56,57]. They showed a number of advantages, including excellent biocompatibility and versatility in surface functionalization [58]. Ionic or covalent bonding and physical absorption can conjugate drugs to the surfaces of gold nanoparticles, which can then transport and regulate drug release by biological stimuli or light activation [59]. These are highly stable, hydrophilic, biocompatible, and non-toxic when compared to organic materials. Inorganic nanoparticles have drawn a lot of interest as drug or gene delivery vehicles because of their great cellular absorption capacity, non-immunogenic reaction, and low toxicity. The electromagnetic, optical, and catalytic characteristics of noble metal nanoparticles, such as gold, silver, and platinum, are known to be significantly influenced by size and shape. Metal-based nanoparticles are widely used in biomedical applications, including as probes in electron microscopy for visualizing biological structures, as carriers for delivering drugs, proteins, peptides, plasmids, and DNA, and in the detection, diagnosis, and treatment of diseases [60]. A number of other inorganic nanoparticles, such as fullerene derivatives and iron oxide nanoparticles, have been studied as drug delivery vehicles as an outcome of current developments in nanotechnology because of their nanoscale size, which allows them to pass easily through the body.The medication might be applied to the particle's outer layer or injected. The efficiency of inorganic nanoparticles has been demonstrated to be increased by cells' ability to absorb them and employ them to transfer nucleic acids into living cells. silicon dioxide, gold, ferrous oxide, manganese phosphate, and dual hydroxides are examples of inorganic substances that have been studied for DNA transport [61]. Inorganic nanoparticles exhibit efficient cellular uptake due to their small size, allowing easy biomolecule conjugation and surface functionalisation; however, they are non-biodegradable, costly for large-scale production, and prone to aggregation [62]. silicon

dioxide, gold, ferrous oxide, manganese phosphate, and dual hydroxides are examples of inorganic substances that have been studied for DNA transport. Nanocarriers like liposomes, dendrimers, or micelles do not exhibit surface plasmon resonance (SPR), a feature of metallic nanoparticles, especially silver and gold [58].

4. Classification of stimuli-responsive nanocarrier

It is commonly known that in order to increase therapeutic efficacy and minimize or prevent side effects, drugs must be delivered to target areas in a controlled manner. Stimulus-based drug delivery systems have demonstrated a great deal of promise in this regard for efficiently delivering active drug moieties. Drug delivery using thermosensitive liposomes was first implemented in 1978. The creation and broad use of stimuli-responsive nanomaterials for controlled medication delivery sparked research into stimuli-responsive polymers. Various stimuli-responsive mechanisms have been reported and are broadly classified into endogenous (internal) and exogenous (external) stimuli, enabling efficient drug release at specific target sites [63].

4.1 Endogenous stimuli-responsive drug delivery system

When normal cells and tissues and tumor cells and tissues are stimulated by diverse conditions in vivo, such as low pH, high redox potential, and high intracellular enzyme levels, this is referred to as endogenous stimulation [64].

4.1.1 pH-responsive drug delivery system

One of the most widely utilized delivery stimuli is pH, which can be applied to specific organs (such as the gastrointestinal system or vagina) or to organelles (such as lysosomes, golgi, and endosomes). Additionally, it has been employed to release the drug moieties in altered pathological situations, such as ischemia with significant pH changes, malignancy, or inflammation [65]. Blood and functioning tissue typically maintain an outside of cells pH of 7.4. Because many tumors have significant rates of glycolysis, average extracellular pH values are usually acidic [66].

In order to facilitate site-specific drug release, a low pH might be used to emphasize the tumor location. The majority of these systems use pH-responsive polymers, which can donate or take protons in pathological pH levels and permit mild conformational changes [67]. pH-responsive copolymeric systems can be created by using bonds that can be broken by acid, allowing the release of chemotherapeutics or by adding an acid-functionalized group to the polymer's backbone, which changes in conformation and solubility when the pH of the environment changes [68]. Therefore, pH variance is a key factor in the development of advanced drug delivery systems. Although pH is frequently employed in smart drug delivery, To ensure incredibly reliable and precise release at the intended areas, it should be used in conjunction with other stimuli like temperature or redox. There are drawbacks to using an acidic pH as a trigger in tumor microenvironments [69]. First, perivascular areas typically have an acidic pH that is far from blood flow, which prevents nanoparticles from responding. Furthermore, there is frequently little difference in pH between tumor and healthy tissues [70]. For extended drug delivery, Chen et al. created pH-sensitive diblock copolymer micelles integrated into biocompatible cellulose-based hydrogels [68]. In a different research, Luo et al. created self-assembling nanoparticles with paclitaxel that were coupled with Carbamethyl chitosan with amphiphilic stearic acid. This

platform facilitated the efficient death of cancer cells by pH stimulation [71]. Saha et al. created a pH-triggered auto-fluorescent polymeric nanoplatform to supply nonfluorescent aromatic nitrogen mustard chlorambucil (CBL) to cancerous tumors [72].

4.1.2 Redox-responsive drug delivery system

This excitation system has been extensively studied for internalized medication delivery systems and has garnered plenty of interest over the years in the cure of numerous ailments. Approximately 100 times more glutathione (GSH) is present in malignant tissues than in healthy physiological tissue, and 100–1000 times more than in blood [73]. Because of its intrinsic thiol group, GSH is a potent reducing agent that prevents the buildup of reactive oxidative species (ROS) in sick tissues and acts as a fascinating trigger for the administration of anticancer drugs. Additionally, it has been demonstrated that colon cancer and inflammatory tissue had ROS levels 10–100 times higher than those of normal tissue [74]. Copolymers that trigger a reaction when glutathione is present have a disulfide bond that allows micelles to form. These micelles are then broken down in vivo to disperse the targeted drug [75]. Although the redox stimulant drug delivery system showed encouraging sensitivity and precision, it is extremely challenging to obtain the necessary selectivity of the redox reaction because cancer cells are heterogeneous and the biological environment is complex [63]. Tian et al. created multipurpose mesoporous silica nanocarriers that are linked to transferrin by disulfide bonds, potentially allowing doxorubicin to be released in cancerous locations [76]. Numerous ROS-reactive drug delivery system types have been investigated for use in drug administration applications, such as amino acrylates, boron ester, peroxalate ester, thioether, selenium/tellurium, thioketal, polyproline, and polysaccharide [77].

4.1.3 Enzyme-responsive drug delivery system

As a new therapeutic area, enzyme-responsive drug delivery systems have been thoroughly investigated because of their distinct substrate specificity and selectivity [78]. Numerous enzymes have been employed to help deliver drugs to the oxidoreductase, trypsin, glycosidase, phospholipase, lipase, protease, and others [79]. Since proteases are frequently overexpressed in conditions like cancer and inflammation, they are of particular relevance among the many enzymes for the creation of innovative drug delivery systems. A vital digestive proteinase, trypsin, is essential for controlling the exocrine pancreatic secretion process, which influences the release of numerous other digestive enzymes [80].

To retrieve anticancer drugs intracellularly, for instance, Hollow nanocarriers that are activated through the trypsin/hyaluronidase enzyme were created by Radhakrishnan et al. [81] Using surface-modified antinucleosome monoclonal antibodies, Zhu et al. developed MMP2-sensitive PEG lipid linked liposomes to enhance cancer targeting [82].

4.1.4 Temperature-responsive drug delivery system

Smart DDSs that respond to temperature have been thoroughly investigated for cancer treatment [83]. The ability of temperature to regulate the release of drugs from stimulation-responsive drug delivery systems with spatiotemporal control has drawn a lot of interest [84]. Due to their hyperthermic character, the majority of inflammatory pathological sites and tumors have the ability to internally excite the body [85]. For many years, solid tumors have been treated with hyperthermia as an adjuvant therapy in clinical applications [86]. The transport of anticancer drugs to the tumor site is improved by hyperthermia because it improves the accessibility of the tumor tissue vascular [87]. The concept of

thermosensitive systems emerged from the temperature distinction among unhealthy and normal tissues. N-isopropylacrylamide, a thermoresponsive polymer, has gained interest as a smart material for biological applications because of its phase-transition behavior in reaction to temperature changes. A desirable substitute for stimuli-responsive drug delivery systems, thermo-responsive MNPs may be activated in response to external temperature variations [64]. It is possible to create stimuli-responsive delivery systems by using certain polymers that respond to temperature. As a thermally responsive drug delivery system, covalently cross-linked hydrogels are one type of thermally sensitive material that has been thoroughly researched. Poly(N-isopropylacrylamide) thermosensitive hydrogels containing chitosan were made and tested for the administration of carboplatin and cisplatin [88].

4.2 Exogenous stimuli-responsive drug delivery system

With continuous advances in medical science, several external energy-based stimuli have been utilised to activate controlled drug release from nanocarriers, enhancing targeted therapeutic delivery. Some of these extensively investigated stimuli over the past few years are discussed below.

4.2.1 Light-triggered drug delivery system

Light-triggered activation systems have exhibited promising outcomes in regulating drug release and preventing nonspecific drug dispersion at the intended site of action [89]. When exposed to radiation, photosensitive carriers in light-responsive drug delivery systems show an on/off medication dispensing mechanism. Different light wavelengths (visible, near-infrared, and ultraviolet) have been described and examined for controlled drug delivery. Because of its increased tissue penetration and safety, the NIR spectrum is regarded as the best light source for monitoring medication release, while visible and UV light were judged unsuitable for therapeutic use in vivo due to their poor penetration [90]. drug release using this method has been examined through a variety of mechanisms. The primary mechanism is the photo-thermal effect, which releases the medication by dissolving the nanocapsule and converting light to heat through a photo-thermal agent [91]. In order to improve therapeutic efficacy, photocleavage reactions (molecules having photolabile groups, such as o-nitrobenzyl or coumarin-4 yl-methyl groups) have been developed for the targeted administration of antibiotics [92]. The two-photon absorption (TPA) approach is another technique that has been employed recently. It works by using two photons of identical or distinct frequency that are simultaneously absorbed to excite the molecules from a lower energy level to a higher one. The method, which has found widespread use in biomedical imaging, such as the confocal fluorescence microscope of two photons, needs a pulsed laser source with a great deal of energy to concentrate on tiny regions in order to capture effective, immediate energy [93]. After that, photoactivatable micellar systems were created using a copolymeric system that contained either 7-diethylamino-4- (hydroxymethyl)coumarin or a 2-nitrobenzyl scaffold for the delivery of lipophilic substances [94]. The produced micelles broke down and dispersed the embedded drug into the aqueous solution when exposed to laser-based two-photon NIR irradiation [95].

4.2.2 Ultrasound-triggered drug delivery system

Due to its many advantages, such as intrinsic tissue penetration, improved spatiotemporal control, and enhanced safety, ultrasound has been employed extensively as a stimulus in clinical studies. US has been widely utilized in clinics nowadays as a detection and treatment technique [96]. Because of its

inherent tissue penetration and safety, the procedure known as "sonoporation" creates temporary or permanent openings in the blood vessel membranes, significantly enhancing the extravascular transmission of therapeutic chemicals in the area of interest [97]. Ultrasound uses microbubbles as a contrast agent [98]. Numerous vectors, including enhanced lipospheres, Acoustic contrast compounds made of polymers that can bind, and nano/micro bubble-enhanced treatment, were studied for ultrasonically-facilitated drug administration [99].

For example, Using ultrasonic tumor irradiation and a delivery method encased in a nanocarrier-DOX, Kruskal et al. were able to target tumors and distribute the drug systemically. Wang et al. developed oxyl-alkyl hydroxylamine (-oa-)linkages between amphiphile segments that are responsive to ultrasound. The hydrophobic amphiphile portion encased the hydrophobic DOX to facilitate medication administration to hepatocellular carcinoma cells [100].

4.2.3 Magnetically triggered drug delivery system

Because magnetic stimuli are freely permeable, they have been used as a non-invasive technique for regulated drug release platforms or medical imaging using MRI [101]. Hyperthermia and magnetic field-guided drug targeting are two crucial processes for regulated drug release when an external magnetic stimulation is present [102,103]. Nanocarriers produced from hypothermia have attracted a lot of interest nowadays. In a heat-sensitive PLGA matrix, Thirunavukkarasu et al. created superparamagnetic iron oxide (Fe3O4) nanoparticles (SPIONs) and loaded them with doxorubicin. SPIONs produced heat when exposed to a magnetic field, which caused the drugs to be released. Future research should examine this platform's promise chemotherapeutic qualities, which were demonstrated by its efficacy in both in vitro and in vivo investigations [102].

4.2.4 Electrically triggered drug delivery system

Following the injection of an electrosensitive drugs, applying a modest electrical field to a specific tissue location can result in programmed drug delivery by a variety of mechanisms, including redox reactions, disruption of carrier structure, and the generation of heat from electrical stimulation [104–106].

For example, To achieve regulated drug release, Neumann et al. used local pH fluctuation in electrochemical processes [107].

5. Physicochemical properties of stimuli-responsive nanocarriers

Numerous uses for nanoparticles (NPs) have been shown in the biomedical domains, such as cancer treatment, drugs transport, and bioimaging. Their unique physicochemical properties enable them to penetrate biological barriers and serve as effective delivery vehicles for targeted delivery and controlled release. For example, NPs are often used as delivery vehicles for drug delivery to enhance drug bioavailability, extend circulation time, and accurately deliver drugs to targeting sites, thereby improving drug therapeutic efficacy and reducing drug side effects. Various methods, such as coprecipitation upon solvent exchange, microfluidic technique, template-assisted synthesis, and self-assembly driven by interparticle interactions, have been developed to fabricate NPs with controllable sizes [108].

5.1 Impact of particle size

The size of NPs has a significant influence on determining the pathways of cellular uptake. Generally, large NPs (D = 250nm-5μm) enter cells through phagocytosis or macropinocytosis, while small NPs (D = 10–200 nm) enter cells through endocytosis or diffusion [109]. One of the most significant physicochemical factors to be taken into account prior to in vivo applications is cellular absorption or internalization. Among all other processes, endocytosis is the primary method by which any cell absorbs tiny chemicals and particles. The process by which materials are actively transported into the cell by being engulfed by its phospholipid bilayer while being powered by ATP is known as endocytosis. According to reports, phagocytosis and pinocytosis are the two primary endocytosis methods. Engulfing particles larger than 1 μm is the main way that phagocytic cells, including neutrophils, dendritic cells, and macrophages, internalize cells. However, another endocytosis method that involves bringing external fluids into cells is called pinocytosis. Using a tiny quantity of energy (in the form of ATP), the cell can import fluids, including dissolved solutes, by pinocytosis. It is primarily linked to the uptake of particles by cells through a variety of mechanisms, including caveolin-dependent, caveolin-independent, clathrin-mediated, and macro-pinocytosis [110].

If a nanocarrier's diameter ranges from 100–150 nm, it will be difficult for it to exit the capillaries that supply tissues like the kidney, lung, and heart [111]. Smaller particles, ranging in size from 20 to 100 nm, may, nevertheless, spread to the liver sinusoids, spleen, and bone marrow and partially exit the bloodstream through their leaky capillaries. Particles of a diameter of several micrometers can be trapped by lung alveoli, while the pulmonary capillary barrier's pore size is thought to be around 35 nm [112]. The size of these holes is two to three times smaller than the pores found in the renal capillaries' endothelium lining. The smaller pores in the kidney's glomeruli and the pancreatic islet tissues have sizes of approximately 10–15 nm [113]. Renal filtration occurs through the glomerular capillary wall for particles less than 10 nm, and they are not reabsorbed. The majority of intact nanocarriers, which vary in size from 50 to 200 nm, are unable to escape from continuous blood capillaries due to these tissue and capillary pore size ranges. However, liposomes and lipidic nanocarriers bigger than 100–150 nm can be absorbed by phagocytes or stay in these tissues for a long period after extravasating from blood vessels (mostly via discontinuous capillaries in the liver, bone marrow, spleen, and to some extent in the lungs) [114]. Most of these phagocytes gather in the spleen and liver for later removal [115]. Once in a tissue, Lipidic nanocarriers may be persisted due to the size of the capillary openings or the size of the tissue's intercellular space.

The relative size range of drug carrier systems is crucial when they are meant to be inhaled, since it affects both the carrier system and the therapeutic molecules' in vivo fate [116]. The overall aerodynamic particle dimension of an aerosol is known to affect lung deposition, which may also affect the medicinal agent's clinical efficacy [117]. About 80% of particles fewer than 3 μm will have the chance to enter the deeper airways, and between 50 and 60% of them will end up in the alveoli [118].

Therapeutic compounds are delivered transdermally when the composition is applied to intact skin, and the drug is delivered either locally or systemically at a regulated pace. Transdermal the distribution of drugs has significantly improved medical practice as a convenient method of drug administration [119]. Bioactive substances can be directed to the illness or injury site by using transdermal dose forms, and adverse effects can be minimized by preventing the drug's systemic intake [120]. It has been demonstrated that lipidic vesicle particle size significantly affects the transport of bioactive substances into the skin. Overall, the enclosed substance cannot be delivered into the innermost layers

of the skin by vesicles with a diameter of 600 nm or more [121]. To a certain degree, tiny particles with a diameter of 300 nm or less can transport their contents into the deeper layers of the skin. But still, maximum content deposition in both viable dermal and epidermal layers has been demonstrated by tiny particles with a diameter of 70 nm or less [122]. The lipidic trans epidermal pathways may take in nanoparticles smaller than 6–7 nm, while the aqueous pores can take those smaller than 36 nm on average. In contrast, particles between 10 and 210 nm in size might preferentially enter by means of the transfollicular pathway [123].

The primary cause of the inability to attain therapeutic drug dosages in the cerebral tissue has been attributed to the blood-brain barrier's (BBB) impenetrable nature. Peripheral and cerebral capillaries are distinct basically from one another [124]. Cerebral blood vessel endothelial cells are tightly linked to one another by zonulae occludentes and tight intercellular junctions, but peripheral capillaries have fenestrated gaps that can reach 50 nm in width [124]. Many pharmacological substances, including as peptides and pharmacologic macromolecules, are blocked from entering the cerebral cortex and the remaining components of the central nervous system (CNS) by the blood-brain barrier (BBB) [125].

5.2 Impact of particle shape

The NP shape is also a crucial factor influencing the cellular uptake. Benefiting from the advancements in synthesis and modification methods, NPs with various shapes, including spherical, rod-like, star-like, tube-like, cluster, flower-like, spindle-like, needle-like, cubic-like, and disc-like shapes, have been achieved [108].

The mastectomy cancer cells (BT-474, SK-BR-3, and MDA-MB-231) demonstrated a higher ambiguous cellular uptake of polystyrene (PS)-based nanospheres in comparison to their nanorod and disc counterparts. Nanorods were demonstrated to be more cellularly absorbable than spheres or discs after being coated with the monoclonal antibody trastuzumab. The greater surface area per unit volume of the nanorods was thought to be the reason for the enhanced trastuzumab adsorption. Because of their improved cellular absorption and greater antibody adsorption, rod-shaped carriers are more applicable than other morphologies. Furthermore, researchers have demonstrated that cellular internalization is influenced by the stiffness of rod and spherical structures. For PS-based devices with a rod shape, it has been confirmed that stiffness and cellular internalization are inversely correlated. Nevertheless, cellular internalization was unaffected by spherical particles' alteration in rigidity [126]. However, it has been observed that cellular intake of nanostructures varies depending on the kind of cell [127]. Agarwal et al. claim that immune cells and mammalian epithelial cells prefer disc-like nanostructures with high ARs (2–3) to those with lower ARs (~1) [128]. Consequently, when NPs transition from spherical (aspect ratio = 1) to prolonged (aspect ratio > 1) forms, AR rises [129] (Table 2.1).

5.2.1 Spherical-shaped nanocarrier

It is possible to roughly divide the kinds of nanostructures into spherical and non-spherical ones because the majority of therapeutic nanocarriers are spherical. A hollow center and two continuous layers are features of spherical nanocarriers, which can be micellar or vesicular in shape [130]. Spherical nanoparticles are widely used for targeting the liver, spleen, tumours, and the brain (with suitable ligands) due to their high stability, easy cellular uptake, and long circulation time.

Shape and cell type have an impact on how well nanostructures are absorbed by cells in various organs in vivo. In this work, cylindrical micelles (filomicelles) demonstrated more blood absorption by

Table 2.1 This table presents the suitability of different nanocarrier shapes for specific organs, along with the underlying reasons.

Organ /system	Best nanoparticle shapes	Reason
Brain (BBB)	Needle, TUBE (nanotube), small spherical	Enhanced penetration, BBB transport
Central nervous system (CNS)	Needle, tube, small spherical	Deep tissue penetration
Heart /blood vessels	Disc, rod	Strong margination, endothelial adhesion
Lung	Rod, spindle, disc	Pulmonary capillary trapping
Liver	Spherical, cubic, cluster	Kupffer cell (RES) uptake
Spleen	Cluster, spherical	Macrophage filtration
Kidney	Small spherical, short rod	Renal filtration and clearance
Gastrointestinal tract	Spherical, disc	Stability and mucosal adhesion
Skin (transdermal)	Needle, rod	Stratum corneum penetration
Lymphatic system	Rod, disc	Lymphatic uptake and retention
Immune cells (macrophages)	Cluster, flower-like	High phagocytosis
Bone	Rod, needle	Strong mineral surface binding
Eye (ocular delivery)	Spherical, disc	Low irritation, prolonged retention
Reproductive organs	Spherical, rod	Balanced circulation and penetration

monocytes, granulocytes, neutrophils, and macrophages than spherical nanostructures such as polymersomes and micelles. However, spherical nanostructures fared better than filomicelles in establishing connections with immune cells in the liver and spleen [131]. Although there is evidence that the geometrical features of nanostructures affect cellular absorption, it is important to be cautious when drawing too many broad conclusions. However, depending on the sort of cell, different morphologies would be preferable [129].

5.2.2 Rod-like nanocarrier

Rod-shaped nanocarriers have drawn interest from researchers within this range of wide and varied morphologies because of their smaller area of the cross-section, which promotes quick insertion into the cell [129]. Because of their strong margination and improved endothelium adherence, rod-shaped nanoparticles can be used to target blood arteries, tumors, the heart, and the lungs.

Curiously, According to the effect of shape and dimensions on blood half-life, rod-shaped micelles had higher tumor penetration and noticeably longer blood circulation than spherical ones [132]. The cellular uptakes of rod-like NPs with a similar diameter but different aspect ratios (ARs), e.g., spherical (D = 100 nm, L = 100 nm, AR = 1), short rod-like (D = 100 nm, L = 240 nm, AR = 2), and long rod-like (D = 100 nm, L = 450 nm, AR = 4) mesoporous SiNPs, are systematically investigated by A375 human melanoma cells. The results indicate that long rod-like SiNPs are taken up in a larger amount and exhibit a faster internalization rate as compared to spherical and short rod-like

SiNPs. This may be attributed to the large contact area of long rod-like NPs with the cell membrane than that of spherical and short rod-like NPs [108]. The most common method for creating rod-shaped GNPs is seed-assisted growth. The visible and near-infrared (NIR) regions exhibit two different transversal and longitudinal surface plasmon resonance (SPR) bands, respectively. depending on the anisotropy of certain rod-shaped GNPs. The SPR bands convert to heat as a result of the photothermal effect, creating extreme heat and permanent malignant cell damages [133]. Additionally, the fluorescence of nanorods is significantly better than that of nanosphere GNPs. This improvement results from the nanorod GNPs' capacity to adapt their localized surface plasmon resonance (LSPR) to the red/NIR dye's spectrum. To get the maximum improvement in fluorescence, this effectively boosts the fluorescence of red and NIR dyes. Advanced red/NIR emission nanorod GNPs make excellent bioimaging possibilities [132].

5.2.3 Star-like nanocarrier

In general, two traditional synthesis strategies for the introduction of star polymers: arm-first and core-first. The foundation of the core-first approach is the expansion of a hetero-multifunctional core during the polymerization process. The arm-first method involves synthesizing each polymeric arm independently before joining it to the core molecule [134]. Because of their large surface area, improved drug-loading capability, and photothermal effects, star-shaped nanocarriers are especially well-suited for focusing on tumors. Wider dimensions of spherical and rod-like GNPs have been found to show stronger cellular uptake compared to star-shaped GNPs [135]. Star polymers have improved drug loading content (DLC), drug loading efficiency (DLE), and longer circulation duration in comparison to their linear counterparts [136].

5.2.4 Tube-like nanocarrier

Covalent or non-covalent interactions, such as p–p stacking, can be used to chemically functionalize tube-shaped nanocarriers, more especially CNTs. Compared to liposomes or dendrimer drug carriers, single-walled carbon nanotubes (SWCNTs) have a better capacity for drug loading due to their large specific surface area [21]. Tube-shaped nanocarriers (nanotubes) are suitable for brain and tumour targeting because they can cross biological barriers and exhibit high penetration ability. In order to meet drug delivery demands, Halloysite nanotubes (HNTs) having an outer silicon dioxide and inner alumina layer with a diameter of 50 nm were employed by Kushwaha et al. These HNTs exhibited enhanced AR mechanical characteristics, a large AR, and biocompatibility [137]. The quaternary CTAB cationic surfactant is typically used for creating tubular surface structures [129].

5.2.5 Cluster-shaped nanocarrier

Clustered nanocarriers are particularly suitable for targeting the liver and spleen due to their rapid uptake by macrophages of the reticuloendothelial system. Drugs can also enter cells of interest using nanoclusters. Nanoclusters of boron nitride (BN), for instance, exhibit exceptional chemical stability [138]. Magnetic nanoclusters (MNCs) of Fe3O4 and gold nanoclusters offer potential for therapeutic delivery applications, despite the fact that cluster-shaped nanocarriers have been the subject of numerous studies [139]. According to the frequency dependence of LSPR on the dimension, shape, and dielectric atmosphere of GNPs, the particle's coupling becomes more significant as the GNP cluster alignment approaches, especially in surface-improved Raman scattering (SERS) studies, and the nanogap greatly enhances the near-field electromagnetic field [129].

5.2.6 Flower-like nanocarrier

Different flower morphologies are another attractive set of nanocarrier shapes for medication delivery and effectiveness [140]. Flower-like nanocarriers are particularly suitable for tumour targeting due to their large surface area, high drug adsorption capacity, and slow drug release. An organic–inorganic hybrid nanoflower (hNF) system was developed using glucose oxidase and copper ions trapped in amine-functionalized magnetic nanoparticles (MNP-GOx NFs) [141].

For the synthesis of Pd NP, the chitosan stabilizer serves as a multipurpose substance. various proportions of CS and vitamin C were used in the green biosynthesis of flower-shaped porous Pd NPs. By encapsulating the NPs through intensive interactions with Pd nuclei, CS played a critical role in developing Pd NPs with a floral morphology following the creation of Pd nuclei under the influence of vitamin C. The porous flower shape is the result of anisotropic directed development of Pd nuclei caused by the CS layer. Pd NPs get fewer as the concentration of CS rises. This principle states that the concentration and layer creation rate of the CS can be monitored to control the favorable size of Pd NPs [142]. Bacterial cell dispersion using H2O2 produced from GOx yielded the antibacterial property [143]. Compared to other morphologies, spherical Pd NPs having a plasmonic effect are more susceptible to changes in refractive index [144].

5.2.7 Spindle-like nanocarrier

Spindle-like nanocarriers are particularly suitable for lung targeting due to reduced phagocytosis and prolonged blood circulation. β-cyclodextrin-based supramolecular self-assemblies (β-CD) are a subunit of nanocarriers used to treat malignancy. A supramolecular self-assembly system was constructed using a curcumin anticancer medication as the guest and a β-cyclodextrin trimer (β-CD3) as the host. The β-CD3 ratio increased, producing a range of supramolecular self-organized carrier shapes, including as multi-compartment vesicles, spindle-shaped complicated micelles, and complex spherical micelles. Thus, complicated micelles with a spindle shape showed enhanced apoptosis and cellular uptake. The least effective micelles were the spherical complicated ones [145].

5.2.8 Needle-like nanocarrier

Needle-like nanocarriers are particularly suitable for targeting the brain, central nervous system, skin, and bone due to their enhanced membrane penetration and blood–brain barrier crossing potential.

The pressure-filter-template method can be used to create needle-shaped gallium nano swimmers covered with leukocyte membranes [146]. According to a different study, needle-shaped hydroxyapatite nanoparticles have a greater ability to permeate compared to other morphologies, entering the cell membrane. The needle-shaped NPs measured 246 nm in length and 43 nm in breadth, while the spherical NPs had an average size of 54 nm. At an optimal concentration of 100 mg L^{-1}, needle-shaped hydroxyapatite nanoparticles can effectively reduce cell growth and reproduction by over 73% [129].

5.2.9 Cubic-shaped nanocarrier

Cubic nanocarriers are particularly suitable for liver targeting due to strong cellular internalisation and shape-dependent uptake. For the following reasons, the cooperative inclusion of amphiphilic lipids in the production of cubic-shaped nanocarriers has garnered a lot of attention:

First, Under certain physiological circumstances, these types of lipids self-assemble into properly formed biomimetic NPs, like cubosomes with the previously indicated superiorities.

Second, the medications and active species are well protected from degradation by these nanocarriers, which results in an effective regulated release.

Third, a variety of structural configurations, including micellar cubic phase (I1), hexagonal phase (H1), lamellar phase (Lα), and bicontinuous cubic phase (Q1), are made possible by the slow increase in temperature and amphiphile concentration [147,148].

5.2.10 Disc-shaped nanocarrier

Disc-shaped nanocarriers are particularly suitable for targeting the heart, lung, gastrointestinal tract, lymphatic system, and eye due to their excellent margination and strong vascular wall interaction.

External stimuli and laborious deformation processes from spherical morphologies are the primary preparation techniques for disc-shaped nanocarriers. The disc's shape is determined by the diameter-to-thickness proportion of the particle [149]. To examine their cell absorption, hydrogels called nanodisc and nanorod were created. According to the results, disc-shaped hydrogels were more cellularly absorbed than nanorods [150]. Compared to their spherical counterparts, Higher cell uptake was seen in disc-shaped MSNs with a large surface area due to porosity [150,151].

5.3 Surface charge and surface chemistry

Generally, the cell membrane consists of a negatively charged phospholipid bilayer [152]. If NPs are positively charged, they have a high affinity with the negatively charged cell membrane and are more easily bound to the cell membrane via electrostatic attraction. In contrast, negatively charged or neutrally charged NPs typically exhibit a low cellular uptake efficiency due to their low affinity with the cell membrane [108]. NPs will first make contact with the cell membrane and then start the endocytosis process, tuning the surface property of NPs is the most common strategy to achieve a high cellular uptake efficiency. NPs may have different surface properties, such as different charges, different hydrophobicities, different chemical groups and different functional groups. All these properties could influence the cellular uptake performance [153].

For example, the cellular uptake of positively charged gold NPs modified with poly (acrylamide hydrochloride) by SK-BR-3 breast cancer cells is found to be 5–10 times higher than that of neutrally charged gold NPs modified with poly (vinyl alcohol) (PVA) and negatively charged gold NPs modified with citric acid. Besides the affinity, the surface charge of NPs also affects the cellular uptake pathways, with positively charged NPs favoring clathrin-mediated endocytosis and negatively charged NPs being taken up more easily via caveolae-mediated endocytosis [154,155].

The surface hydrophobicity of NPs also plays a crucial role in the cellular uptake [156]. Hydrophilic NPs are more likely to diffuse across the cell membrane or enter the cell via pinocytosis, while hydrophobic NPs tend to penetrate the cell membrane through the hydrophobic interaction with the cell membrane [157]. Generally, surface functionality of NPs plays a pivotal role in their cellular uptake across different cell types. To implement NPs with a tumor-targeting function and reduce the toxicity of NPs in vivo, functional groups are often decorated on the NP surface to regulate the interaction between NPs and targeting tumor cells [158]. Targeting tumor cells often show unique or overexpressed receptors on the cell membrane. Targeting the unique or overexpressed receptors, such as peptides, proteins, and antibodies, via functional groups provide the tumor-targeting function [159].

For example, PLA/shellac NPs functionalized with FA-polyethylene glycol (PEG)-COOH on the shellac bulb surface show a higher cellular uptake than unfunctionalized NPs [160]. PEG on the shellac

bulb surface increases the hydrophilicity of NPs and reduces the nonspecific interactions of NPs with cells, thus prolonging the blood circulation time of NPs [161].

Various surface modification approaches applied to nanocarriers have been demonstrated to improve drug delivery efficiency by increasing biocompatibility, prolonging systemic circulation, minimizing immune recognition, and facilitating targeted and stimuli-responsive drug release.

5.3.1 Bioinert surfaces

Bioinert surface modification of nanocarriers is a widely employed strategy in drug delivery systems to minimise undesirable interactions with biological components. A bioinert surface does not actively interact with cells, proteins, or immune elements, thereby reducing nonspecific adsorption and improving systemic stability. Such surface characteristics are particularly important for prolonging circulation time and enhancing therapeutic efficacy [162]. Zwitterionic, polyethylene glycol (PEG), poloxamer/poloxamine, and polyglycerol (PG) surfaces are the primary bases for bioinertness [163].

Many viruses can pass through cellular membranes and the mucus gel layer, among other biological barriers, thanks to their zwitterionic surfaces. Some viruses, including human papillomavirus, Norwalk, and Hepatitis B, have an equivalent diffusion coefficient in saline and mucus [164]. By adding both cationic and anionic charges to their surface, NCs can be made to resemble viruses and have bioinert qualities. While most other surfaces connect with the surrounding water molecules through H-bonds, zwitterionic surfaces bind water through Coulomb forces like ion-dipole interactions [163].

Surfaces made of polyethylene glycol (PEG) are widely employed to give the GI tract bioinert qualities. On the outermost layer of the nanocarrier, PEG chains can create a thick, hydrated brush that protects them from contact with mucus and GI fluids. PEG typically has a limited capacity to establish hydrogen bonds with GI substances [165].

Pluronic F-127 and other polxamers are polyoxyethylene–polyoxypropylene block copolymers. Because they contain polyoxypropylene blocks, they also have hydrophobic domains, unlike PEGs [166]. This characteristic allows them to be integrated into hydrophobic nanocarriers such liposomes, PLGA nanoparticles, SEDDS, NLCs, and SLNs. The mucus diffusing capabilities of PLGA nanoparticles and liposomes were markedly enhanced by the surface decorating with Pluronic F-127 [167].

Compared to PEGs, polyglycerols (PGs) have more hydroxyl functionalities, which give them a stronger hydrophilic nature and better hydration. Additionally, linear and branched PG chains are synthesized [168].

5.3.2 Adhesive surfaces

It may also be advantageous to develop sticky surfaces rather than bioinert ones. The adherence of nanocarriers to the mucus gel layer surrounding GI epithelia is of special interest for medication administration [169]. Mucoinert NCs are made to transport NCs in close contact with the absorbing membrane, thus they can communicate with epithelial cells, resulting in enhanced medication uptake, whereas mucoadhesive NCs are meant to remain in the mucus gel layer for a long time, releasing the drug in an emphasized manner close to the absorption membrane. Chain interactions between mucoadhesive polymers and mucins, as well as non-covalent interactions between the surface of NCs and the mucus gel layer, such as H-bonding, or the creation of covalent bonds, such as disulfide bonds, among thiolated polymers and cysteine-rich subdomains of mucus glycoproteins, can all contribute to mucoadhesive properties. Polymeric excipients can be employed as coating materials or directly to create NCs. The formation of NCs occurs when anionic mucoadhesive polymers, such as polyacrylic

acid, hyaluronic acid, alginate, or carboxymethyl cellulose (CMC), gel in situ with cationic polymers or multivalent cations, such as Ca 2+, Mg 2+, or Fe 3+. Still, the mucoadhesive features of these polymers are quenched, and due to their zwitterionic character, a mucoinert rather than a mucoadhesive surface is produced. Since NCs already stick to the mucus gel layer's surface, which is most quickly removed during the mucus turnover process, having excessively high mucoadhesive qualities is probably not beneficial. The more deeply NCs pierce the mucus gel layer, the better their mucoadhesive qualities should be. The more deeply thiolated NCs pierce the mucus gel layer, for instance, the more reactive they become [163].

5.3.3 Absorption-enhancing surfaces

Through a variety of interactions with epithelial cells, NCs can improve medication absorption [170]. They have the ability to endocytose into cells, merge with cellular membranes, and open tight junctions [171]. Clathrin-mediated, caveolin-mediated, clathrin- and caveolin-independent endocytosis, phagocytosis, and micropinocytosis are examples of endocytotic processes. Intracellular vesicles, such as endosomes, phagosomes, or macropinosomes, absorb endocytosed NCs, preventing them from entering the cytoplasm directly [170].

It appears that NCs' anionic surface charge causes tight junctions (TJs) to open. Compared to the identical nanoparticles with a positive surface charge, carboxymethyl chitosan/chitosan particles with an anion-based charge on their surface caused a greater breakdown of TJs and stronger paracellular permeability [172]. Moreover, it has been demonstrated that silica nanoparticles with an anionic surface charge can attach to epithelial cells and momentarily and reversibly open TJs [173]. In contrast to cationic surface charge, a study by Yu et al. found that anionic SLNs greater acceptance via the lymphatic channel [174].

It has also been demonstrated that several cationic polymers, including polyethyleneimine, trimethylated chitosan, and chitosan, can open TJs [175]. NCs can inherit the TJs opening characteristic found in cationic polymers [176]. Lin et al. further assessed the significance of NC size for diffusion enhancement via TJs opening, finding no improved paracellular transportation for larger carriers [177]. This suggests that polycations can increase the permeability of tiny drug molecules by opening TJs, while no improvement in drug absorption is shown for larger medicines [178].

The usage of NCs coated with cell-penetrating peptides (CPPs) is another strategy to boost medication absorption. Usually consisting of 5–30 amino acids, CPPs can help transport different biomolecules and NCs into cells so they can function at their intended location [179]. Based on their physicochemical characteristics, CPPs display a variety of absorption processes. These can be separated into energy-dependent uptake processes such as micropinocytosis and clatrin- and caveolin-mediated endocytosis, as well as energy-independent ones like membrane lysis [180]. Electrostatic interactions or covalent connections between the CPPs and NCs can be used to establish interconnection. Covalent bonds offer greater stability and binding region selectivity, whereas electrostatic interactions make it easy to immobilize CPP on the surface of NCs without requiring chemical changes to the CPP [181].

NCs with thiol groups on their outermost layer have the ability to cause endocytosis and open TJs [182]. The interaction between the thiol groups on the outermost layer of NCs and the thiol groups of membrane-bound proteins and enzymes is the mechanism that opens TJs. Clausen et al. shown that glutathione-mediated protein tyrosine phosphatase can be inhibited by thiolated polymers. TJs open as a result of this inhibition, which prevents the dephosphorylation of occludin's extracellular loops

[183]. Additionally, Zhang et al. showed thiol-dependent interactions with insulin-like growth factor and epidermal growth factor, which alter claudin-4 and cause TJ opening [184].

5.3.4 Charge converting surfaces

Intestinal alkaline phosphatase (ALP), an enzyme associated to the brush border membrane, is the primary source of charge-converting NCs. It cleaves and liberate phosphate from the NCs' surface [185]. Since nearly only positive charges are left on the surface, NCs lose their zwitterionic nature and/or change their charge to neutral or positive as a result of the removal among these anionic phosphate substructures. By coacervating a chitosan-phosphotyrosine conjugate with carboxymethylcellulose and a polyethylenimine-6-phosphogluconic acid conjugation, the first charge-converting NCs for oral drug delivery were created [186]. Such flip-flop devices can prevent unintended charge interactions, such as an ion pairing between cationic and anionic surfactants. Surface charges were shielded by PEG surfaces [187]. NCs without PEG are beneficial. As an alternative, PEG brushes can be covered with phosphate groups. N,N′bis (polyoxyethylene) oleylamine bisphosphate (POAP) and polyoxyethylene (9) nonylphenol monophosphate ester are examples of phosphorylated lipids with PEG spacer that exhibited a more noticeable charge conversion on the surface of lipid NCs than others [188].

5.4 Drug loading capacity and encapsulation efficiency

Simply, the amount of drug that a nanocarrier can carry is called its drug loading capacity. The percentage of Drug Loading Capacity (DLC %), simply referred to as the percentage of the drug that can be loaded within the total weight of the drug-loaded nanocarrier formulation [189].

$$\text{Drug Loading Capacity } (\%) = \times 100$$

Drug loading efficiency (DLE) refers to the percentage of the initially added drug that is successfully incorporated into the nanocarrier system.

$$\text{Drug Loading Efficiency } (\%) = \times 100$$

Therefore, the goal in nanocarrier design is to enhance the drug loading capacity during the preparation of the nanocarrier [189].

Current encapsulation technologies continue to face significant challenges related to safety, long-term stability, scalability, and clinical translation. As a result, our goal is to objectively examine the main encapsulation techniques, paying particular attention to their modes of action, therapeutic advantages, drawbacks, and prospects for further advancement.

5.4.1 Microencapsulation of drugs

Microencapsulated systems' typical particle size, which usually falls between 1 and 1000 μm, is a key factor influencing formulation behavior. The latter directly affects the drug's bioavailability, release profile, and encapsulation surface area. In fact, the pharmaceutical sector has made considerable use of microencapsulation [190]. This method uses a microcapsule made of biocompatible polymers to contain nanoscale drug particles. However, encapsulated drugs have limitations when it comes to organ-specific drug delivery. Excellent reproducibility of microencapsulated medications is still a challenge [191].

5.4.2 Nanoencapsulation of drugs

The nanoencapsulation of medications or other tiny molecule in nanocarriers is a very promising approach for the advancement of nanomedicine. By effectively packing drug molecules inside nanocarriers, innovative drug encapsulation techniques reduce the systemic toxicity associated with drugs [192]. The creation of drug-loaded particles with diameters ranging from 1 to 1000 nm is the method of drug nanoencapsulation. NPs are solid, submicron-sized drug carriers that can or can not be biodegradable [193]. Polymeric micelles, liposomes, dendrimers, lipid solid NPs, magnetic NPs, mesoporous NPs, gold NPs, carbon nanotubes, quantum dots, and others are examples of the evolving nanocarrier materials for drug delivery [194].

5.4.3 Polymeric encapsulation of drugs

Given their varied characteristics and colloidal particle networks with sizes ranging from 10 to 1000 nm, polymeric nanoparticles (NPs) appear to be a viable drug delivery vehicle [195]. To reduce their cytotoxicity and improve their water solubility, half-life, and bioavailability, drugs can be incorporated in polymeric systems. Additionally, the polymeric materials utilized to create NPs for drug administration must be biocompatible, biodegradable, non-toxic, and non-immunogenic. Poly (lactic-co-glycolic acids) (PLGA), chitosan, gelatin, poly-caprolactone (PCL), poly-alkyl-cyanoacrylates, and PEG are only a few of the numerous polymers that have been employed [196].

5.4.4 Molecular encapsulation of drugs

Since molecular encapsulation is naturally versatile and does not necessarily necessitate time-consuming synthetic processes, it is a preferred supramolecular strategy [197]. The confinement of a foreign molecule inside the cavity of a molecular host is known as molecular encapsulation. This method entails encasing compounds in molecular cages, like cyclodextrins, to improve the dissolution and long-term stability of medications. Calixarenes, carcerands, cucurbiturils, cryptoands, and cryptophanes are important developments in the chemical composition of molecular hosts and molecular encapsulation [198].

6. Characterization parameters and analytical techniques

6.1 Morphology, and surface characterization

Particle size, shape, and dispersity are the three most crucial aspects of nanocarriers [199]. The biodistribution and removal of nanocarriers are influenced by the size and form of the particles [200]. These biological processes are significantly impacted by a number of non-spherical shapes, such as discs, ellipsoids, cylinders, hemispheres, cubes, cones, and other complicated shapes [201]. Analytical methods including electron microscopy, zeta potential analysis, and dynamic light scattering (DLS) can be used to characterize some of these properties.

6.1.1 Dynamic light scattering (DLS)

Using light scattering properties and Brownian motion, dynamic light scattering (DLS) calculates the particle diameter. However, because their mobility may be too low, big particles might not be detected by the DLS approach [202]. A particle sizer with particle sizing software is used to determine the average particle diameter [203]. For statistical analysis, the acquired the dimension of particles and

size distribution data are useful. The use of DLS may be constrained by some issues, nevertheless, such as sample concentration, sedimentation of big particles, and the unsatisfactory outcomes of polydisperse and multimodal samples. By including a partitioning procedure to get fractions of varying particle sizes before analysis by the DLS method, these restrictions can be addressed. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are commonly used to validate the results of this technique [204].

6.1.2 Scanning electron microscope (SEM)

SEM makes it possible to see the topography of nanocarriers directly. Usually, a sputter coating method is used to coat samples with a metal that has a great deal of electrical conductivity such gold, after they have been dried and placed on a sample holder. A concentrated electron beam is utilized to scan the sample's surface, and the secondary electrons that are released from it are measured [205]. SEM-scanned nanocarriers is supposed to be able to tolerate the vacuum, coating material, and electron beam without significantly changing [206]. With the introduction of modified SEM techniques, sample drying is no longer required. For instance, hydrated samples can be analyzed with wet SEM without the need for fixation, coating, or drying [207]. Another modified method that necessitates sample freezing is cryo-SEM [208].

6.1.3 Transmission electron microscope (TEM)

TEM offers information on the shape of nanocarriers whereby electrons are traveled with. However, because samples should ideally be ultra-thin for proper electron transmission, TEM sample preparation is rather difficult and time-consuming [209]. The nanocarrier sample is fixed using either plastic embedding or a negative stain substance like phosphotungstic acid or uranyl acetate after being put onto support films or grids. An alternative method, called cryo-TEM, involves exposing the sample to liquid nitrogen after it has been placed in vitreous ice [210].

6.1.4 Zeta potential

The zeta (ζ) potential, which represents the surface charge, influences the aggregation tendencies of the nanocarrier units, suggests potential electrostatic interactions between them, and aids in the selection of suitable coating materials. It can be ascertained by passing a voltage through the sample and using laser Doppler velocimetry to record the nanocarriers' motion. All things considered, zeta potential measurement can be quite sensitive to pH and ionic strength. Usually, dilution of the sample is necessary prior to measurement. The dependability of the data interpretation may be impacted by mixtures of inversely charged nanocarriers [202].

6.2 Chemical and structural characterization

Chemical and structural characterisation is essential to confirm drug incorporation, drug–carrier interactions, crystallinity or amorphous nature, structural integrity of the nanocarrier, and overall stability of the formulation.

6.2.1 FTIR (fourier transform infrared spectroscopy)

The study employs FTIR spectroscopy to assess drug compatibility, utilizing spectral analysis to identify potential interactions and chemical changes, facilitating a comprehensive characterization of

drug interactions and their implications. Non-destructive, rapid, and offers qualitative and quantitative analysis of drug interactions. Provides information about chemical bonds and functional groups. Limited to surface analysis, may not detect subtle interactions, and cannot determine the degree of compatibility [211].

6.2.2 Raman spectroscopy

In Raman spectroscopy studies, drug compatibility is characterised by analysing molecular vibrational modes to assess potential drug–carrier interactions, providing valuable insights into the stability and compatibility of drugs in various formulations. It detects changes in molecular structure, chemical bonding, and functional groups, aiding in compatibility assessment. Raman spectroscopy offers valuable information about molecular fingerprint regions and structural modifications occurring during drug interactions. However, it does not provide direct thermal information, may be affected by fluorescence interference, and requires careful sample preparation and spectral interpretation [212].

6.2.3 NMR (nuclear magnetic resonance spectroscopy)

In NMR spectroscopy, drug compatibility is evaluated by analysing chemical shifts and molecular environments to detect drug–carrier interactions and structural changes. It provides detailed information on molecular structure, chemical bonding, and possible conjugation or complex formation, aiding compatibility assessment. However, it requires relatively high sample purity and concentration, is time-consuming, and may not detect very weak interactions in complex formulations [213].

6.2.4 DSC (differential scanning calorimetry)

In DSC study, Drug Compatibility is characterized through DSC, which measures thermal transitions to assess potential interactions, providing valuable insights into the stability and compatibility of drugs in various formulations. It also detects phase transitions, melting points, and heat capacity changes, aiding in compatibility assessment. Offers valuable information about energy changes in drug interactions. It does not provide structural and chemical information, may not detect subtle interactions, and requires careful sample preparation [211].

6.2.5 TGA (thermogravimetric analysis)

The study employs TGA to assess drug compatibility, analysing the weight loss and thermal behaviour of drug formulations to determine potential interactions and stability, offering a comprehensive characterization of drug compatibility. It also determines thermal stability and decomposition temperature, and offers insights into mass changes. Valuable for identifying degradation and compatibility issues. It provides limited chemical information, is not effective for amorphous materials, and cannot distinguish between multiple components [211].

6.3 Functional and performance characterization

In vivo studies evaluating pharmacokinetics (absorption, distribution, metabolism, and excretion), biodistribution, therapeutic efficacy, and toxicity through histopathological analysis to confirm the real biological performance and therapeutic effectiveness of the formulation [214]. It could be deceptive to forecast nanocarriers' in vivo performance just from their physicochemical characteristics and in vitro evaluation. This is due to biological factors including metabolic enzymes and efflux transporters that

have an impact on medication bioavailability. Determining the ingestion bioavailability of nanocarriers is essential since it indicates how effective they are pharmacologically. Analyzing medication plasma concentrations following oral administration is one way to evaluate it. The most costly and predictive experimental animals utilized in bioavailability testing are non-human primates [215].

7. Challenges and limitations in characterization

Characterisation of drug delivery systems, particularly nanocarriers, presents several challenges and limitations despite advances in analytical techniques. A major issue is the poor correlation between in-vitro and in-vivo results, as formulations that perform well under controlled laboratory conditions often behave differently in complex biological environments. Instrumental limitations further complicate analysis; for example, dynamic light scattering may overestimate particle size due to aggregation, electron microscopy requires dried samples that may not reflect native conditions, and zeta potential measurements are highly dependent on medium composition and pH. In addition, variations in preparation methods and analytical protocols create reproducibility and standardisation challenges across studies [216].

8. Future perspectives

Nanomedicine has advanced significantly over the past few decades, enabling the development of nanoparticulate drug carriers with precise control over size, shape, surface properties, drug loading, and release profiles using both organic and inorganic platforms. However, clinical translation remains limited, with only a few early products such as liposomes and micelles reaching the market. A major challenge lies in the limited predictive value of in-vitro and preclinical studies for clinical outcomes. Currently, the key limitation is not the ability to design nanoparticles, but the ability to accurately evaluate their biological behaviour. Therefore, it is essential to critically evaluate the limitations of current characterisation methodologies and develop advanced strategies that can more reliably predict clinical outcomes at early stages of nanomedicine development. Such approaches may include improved physiologically relevant cell models, advanced labelling and detection techniques, sophisticated analytical technologies, predictive mathematical modelling, and animal models that better replicate key features of human diseases [217].

9. Conclusion

We can conclude that the field of stimuli-responsive nanocarriers represents a transformative shift in medicine, evolving from the macroscopic delivery systems of ancient civilizations to the sophisticated, “smart” nano systems of the modern era. By exploiting the unique physicochemical properties of materials at the nanoscale, such as high surface-area-to-volume ratios and controllable particle sizes, researchers have developed various types of nanocarriers, including SLN, NLC, liposomes, dendrimers, and carbon nanotubes, each tailored to bypass biological barriers such as the blood-brain barrier. However, significant challenges remain. Although inorganic nanoparticles offer excellent stability and cellular uptake, their non-biodegradability raises long-term safety concerns. While polymeric systems are often biocompatible, they may suffer from limited drug-loading capacity or

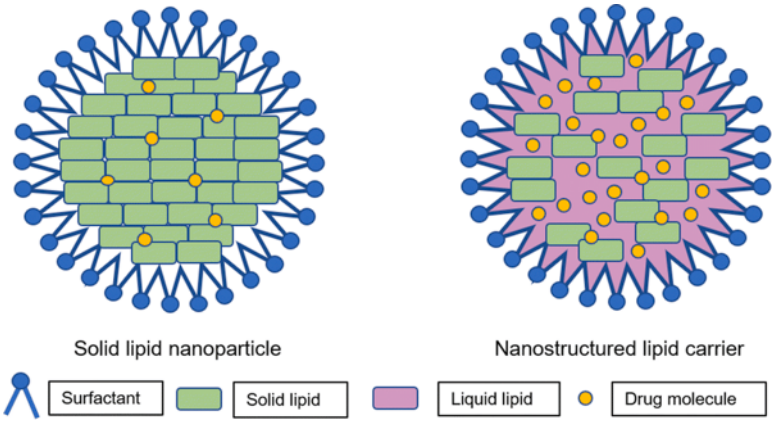


FIGURE 2.1

Schematic illustrations of a rigid solid lipid nanoparticle with low drug load versus a flexible nanostructured lipid carrier with high drug load. Ref. [218], copyright @ Elsevier, 2020.

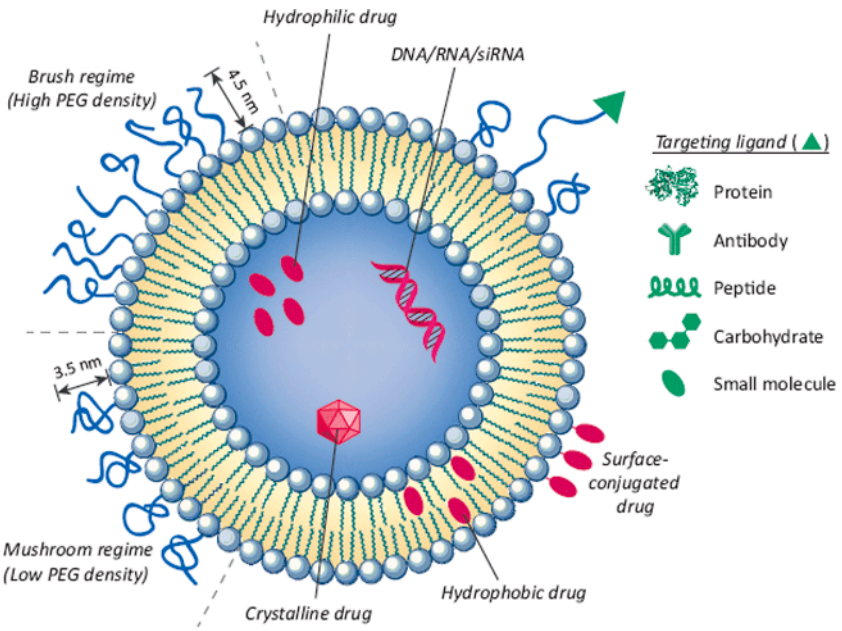


FIGURE 2.2

Schematic illustrations of liposomal drug delivery system. Ref. [219], under CC by license.

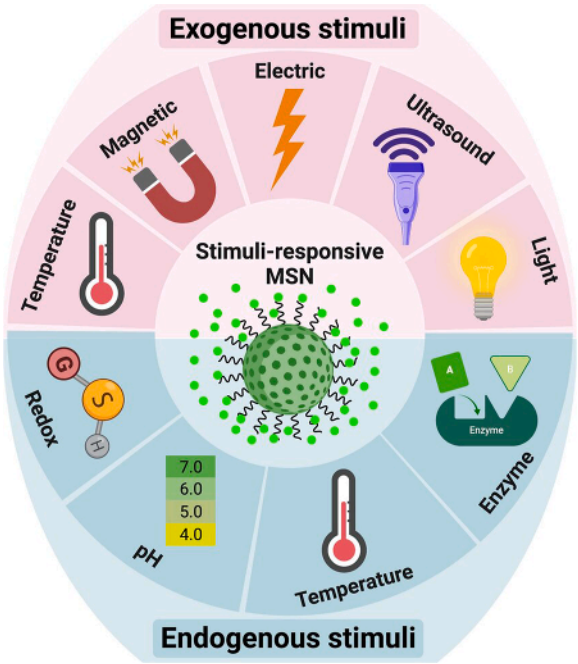


FIGURE 2.3

A general representation of the classification of stimuli-responsive drug delivery systems, reused from Ref. [220], under CC by licence.

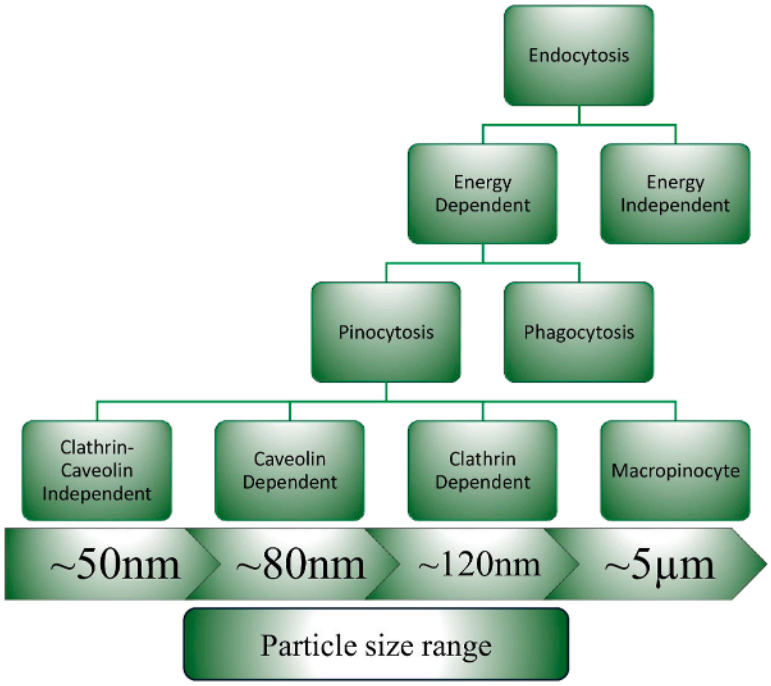
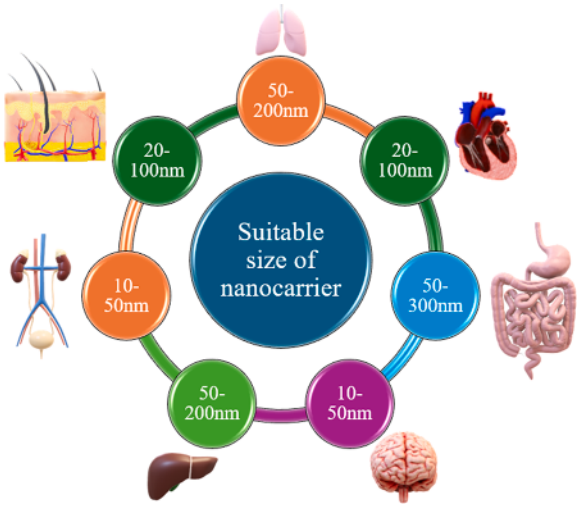


FIGURE 2.4

Different endocytotic routes allow for cellular uptake and ingestion of particles and nanocarriers of varying sizes.

FIGURE 2.5

A representation of the size range of nanocarriers suitable for drug delivery to different organs.



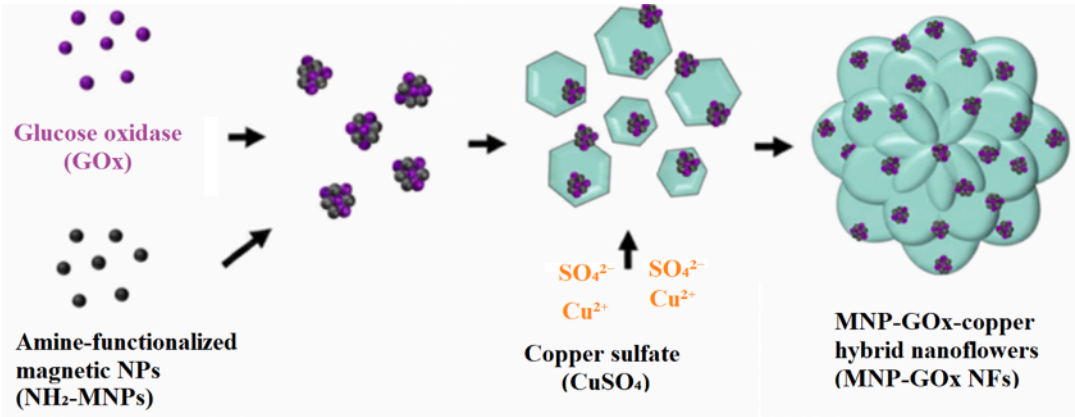


FIGURE 2.6

An illustration the mechanism of drug delivery using flower-shaped nanocarriers, reused with permission from Ref. [221], copyright @ Elsevier, 2020.

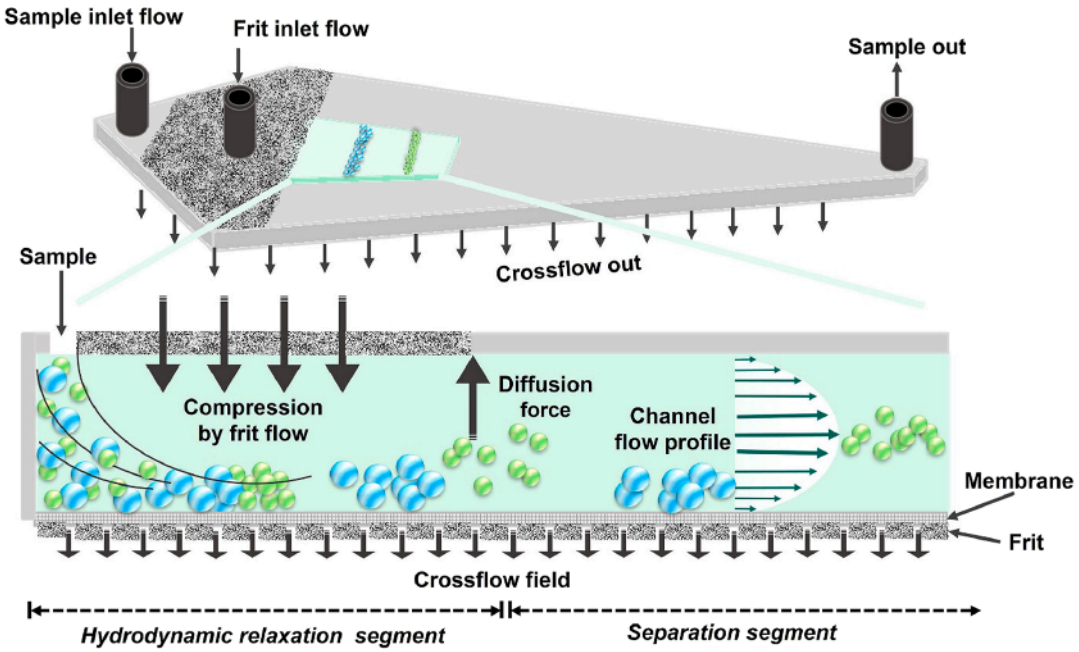


FIGURE 2.7

An asymmetric flow field flow fractionation channel with a frit-inlet (FI-AF4) is shown schematically. The components of the sample are propelled towards the accumulation wall by the frit-inlet flow, which permits their hydrodynamic relaxation without halting their axial movement reused from Ref. [222], under CC by license.

complex degradation profiles. Furthermore, the transition from successful preclinical "in-vitro" models to human clinical outcomes is hampered by the limited predictive value of current characterization methods. As we look to the future, we must focus on developing more reliable, physiologically relevant evaluation strategies than simply designing complex nanoparticles. By integrating advanced labeling, predictive mathematical modeling, and well-cultured animal models that better mimic human disease, we can bridge the gap between laboratory innovation and clinical reality. Ultimately, the goal is to improve these stimulus-responsive systems so that they become reliable, safe, and standardized treatments, helping to make personalized nanomedicine a practical reality (Figs. 2.1–2.7).

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PART

II

External-responsive nanocarriers for biomedical application

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CHAPTER

Ultrasound-responsive nanocarriers for biomedical application

3

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1. Introduction

The intrinsic benefits of NPs over other imaging modalities—such as their broad flexibility and availability, noninvasiveness, nonradioactivity, real-time monitoring, and affordability make them ideal in clinical imaging [1]. US molecular imaging may be performed using US contrast imaging using US contrast agents [2]. The most commonly employed US contrast agents are microbubbles, which have great scattering and high compression, making them seem brilliant on US images [3]. Conventional microbubbles, on the other hand, are usually too big to leave the vascular system [4]. Additionally, traditional microbubbles have poor stability [5]. With the advent of interdisciplinary research, nanomedicine has recently been investigated and used (NPs) have become widely used in clinical medicine because of their physicochemical characteristics at the nanoscale and distinctive structure [6,7].

First, the nanoscale NP size is limited to 100–200 nm, thus allowing for effective increases in intratumoral NP penetration and significantly augmenting bioavailability, achieving the extravascular US imaging. Furthermore, when compared to traditional microbubbles, the ratio of the surface area to the volume is noticeably higher [8–10]. As a result, the contact area and time of drugs and the intestinal canal can be enhanced with a better dissolution rate and solubility [11,12].Additionally, the use of NPs can enhance the drug effectiveness by removing the co-solvent during the formulation and minimize the toxicity of the emulsifying solubilizer [13]. Second, because of NPs' special structure, which makes them appropriate for tumors US imaging, better blood circulation durations as well as the substantial concentration in the tumor could be achieved [14].

Third, NPs can be used to target tumors in two different ways: passively and actively. In particular, in tumor tissue, they can passively accumulate through the improved permeability plus retention effect (Fig. 3.1), which, due to the unusually leaky vasculature found in tumor tissue, has been employed extensively in the treatment of cancer [15,16].Additionally, ligands can be included to enable active-targeting paths of NPs, which enable the NPs to avoid normal tissues and target cancer spots

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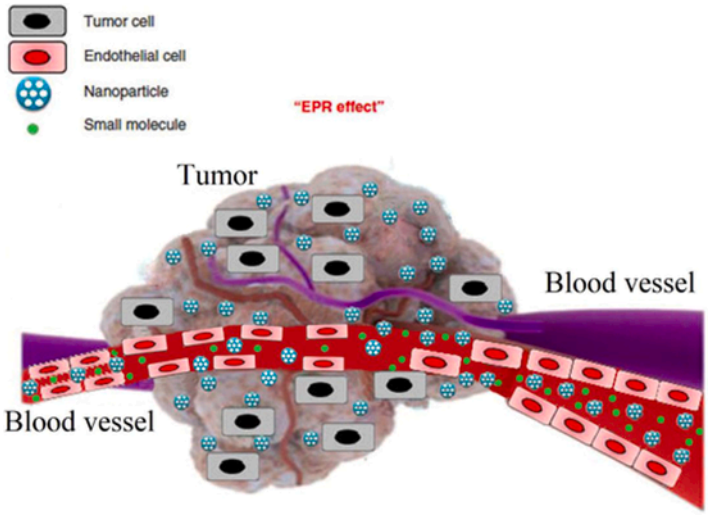


FIGURE 3.1

Illustration of enhanced permeability & retention effect.

specifically. Low-molecular-weight compounds such as aptamers, vitamins, and saccharides, as well as proteins, peptides, and antibodies, are examples of common ligands.

Fourth, when the NPs accumulate, the cancerous tissue is sonicated with US, which has been proposed to cause NP cavitation and drug release. It is also possible to use cavitation of the Nanoparticles to break down biological barriers [17]. Nanoscale agents may be utilized for extravascular ultrasonic imaging and therapeutic interventions through the introduction of various multimodal compounds [2]. The US imaging could be employed to guide the US treatment procedure and to monitor the effectiveness of cancer therapy and other severe diseases. Further, US also possesses suitable physicochemical properties for therapeutic use, including sonodynamic therapy [18,19], high-intensity focused US (HIFU) [20,21], US-activated drug release as well as US assisted gene transfection [22,23]. In the clinic, NP-based US imaging and treatment techniques have therefore been frequently employed (Fig. 3.2).

2. Ultrasound-based drug delivery systems

Targeted medication administration via ultrasound is a promising alternative to effectively treat medical conditions. By enhancing cellular membrane permeability through two methods, US makes it possible to transport medications across the membrane. The first involves the formation of microbubbles around cells, while the second involves sonoporation, which causes cavitation to create holes in membrane of the cell. This process improves the transport of genes in the target tissue, thrombolytic drugs into blood clots, and anticancer drugs into tumors [24]. A rise in temperature brought on by regulated and localized ultrasound (FUS) is known as ultrasonic hyperthermia, and it improves the uptake of anticancer medications [25].

FUS in combination with drug delivery systems facilitates improved delivery of chemotherapeutic drug [25]. The FUS generates acoustic-energy concentrated at a specific point. Acoustic energy

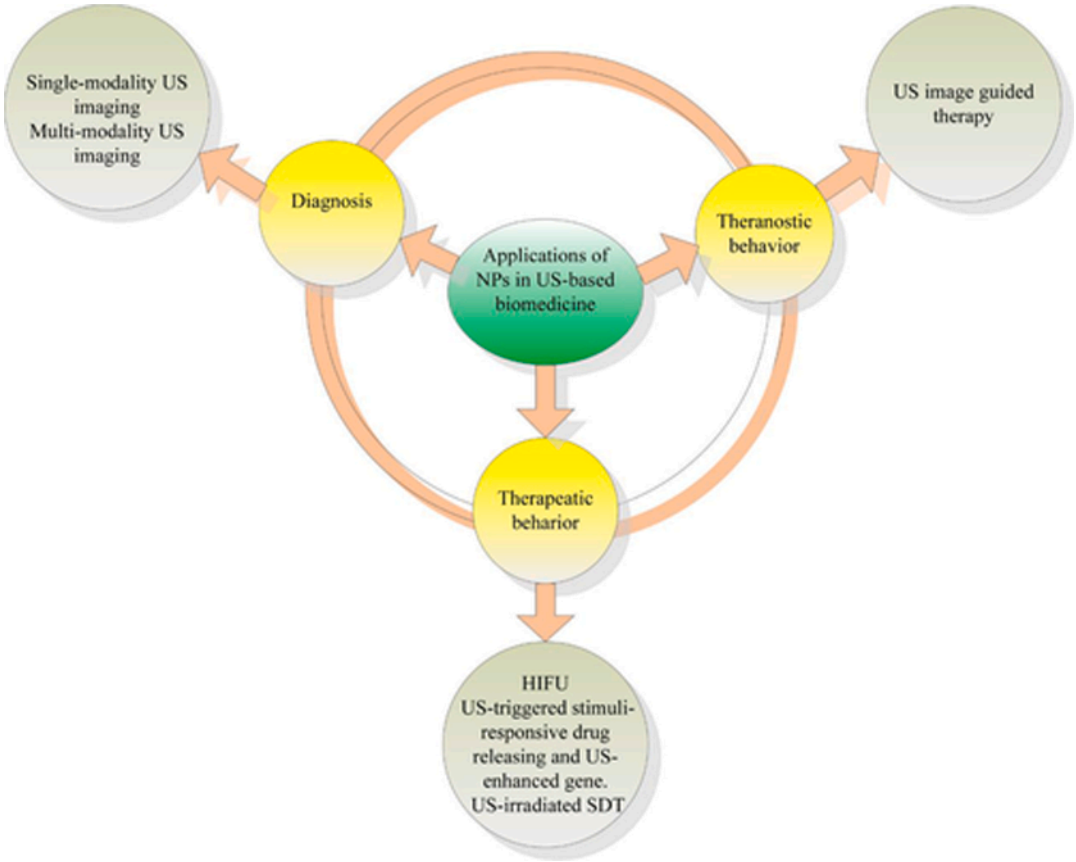


FIGURE 3.2

Applications of the nanoparticles (NPs) in the ultrasonography (US)-based biomedicine.

deposition in a localized focal area (1–10 mm) leads to different therapeutic effects including bone healing, thermal-tissue coagulation, lithotripsy, kidney stone modifications of the neural activities, and histotripsy. Traditionally, drugs can induce a broad range of toxicities and side effects following exposure to multiple normal cells. Some carriers such as liposomes, microspheres, nano particles, etc., are helpful in gene delivery, drug and macromolecular delivery for the brain tumor and neurological disorders treatment [24].

Diagnostic imaging is performed using low-energy US waves (0.1–100 mW/cm^2), while high energy US (100–10,000 W/cm^2) is researched for non-invasive treatments. US-based therapy can be utilized to set up a targeted and controlled release of actives. Certain applications of the technology have already been cleared for use commercially and are in use in medical treatment worldwide. Targeted delivery using ultrasound-based technology has great potential to create multiple applications, which are in an early research stage.

3. Physics of ultrasound-based drug delivery system

The US consist of compression longitudinal waves with a frequency higher than the range of audible human hearing (greater than 20 kHz). The sound waves travel in the medium by producing local oscillatory particle motion. Particle displacement from imposed ultrasound frequency, as well as sound pressure at a point, leads to a modification of the density of such a local medium. This variation in density causes physical injury to the tissues and cells [26].

4. Factors affecting ultrasound-mediated drug delivery

4.1 Frequency

Based on the tissue type and disease treatment procedure, ultrasonic waves penetrate at frequencies between kilohertz and megahertz. Unlike low frequency, with poor resolution and better tissue penetration (e.g., TDD (transdermal drug delivery), involving an enhancement of skin-permeabilization, needs ultrasonic waves of 55 kHz), high frequency possesses excellent resolution but insufficient tissue penetration. For the treatment of cancer using hyperthermia 1–3 MHz frequency, dissolution of intravascular thrombus requires a frequency of 2.2 MHz. The therapeutic ultrasound frequency is less than the frequency for the diagnostic use. As per [26]; a decreased ultrasonic frequency results in minimal attenuation with deeper penetration in tissues and optimal therapeutic effect. The frequency of the ultrasound must be in the vicinity of the microbubbles' resonance frequency to achieve microbubble cavitation. The ultrasound frequency is based on the microbubbles employed. Microbubbles implode due to inertial cavitation caused by increased acoustic pressures [27]. On average, we can say that low frequency (MHz) and elevated acoustic pressures (Mpa) preferentially increase inertial cavitation intensity.

4.2 Intensity

High-intensity ultrasound can potentially alter tissue and cause hyperthermia. The intensities that produce an increase in temperature of 1 °C or less have been cleared for use by the US-FDA. The therapeutic range of ultrasound is 0.3–3 W/cm^3. But lowering the temporal average-intensity (duty cycle 3-ultrasound intensity) by lowering the pulse length (i.e., pulse cycles/ultrasound frequency) can also be used to reduce greater intensity [26].

4.3 Treatment duration

Based on stable or inertial cavitation as well as sonoporation, the ultrasound is used to regulate the treatment time and avoid overheating. The nature of carriers used, their number, where the tissues are located to be treated, and the intensity of ultrasound used to treat influence how long an ultrasound treatment takes to administer a medicine. Medication administration can be enhanced by factors like inertial cavitation, high acoustic pressure, multiple or sustained microbubble injections, and longer treatment duration; however, high pressures will also induce undesirable tissue damage.

A lower pressure generates more heat because it necessitates longer treatment times. For each application, the treatment time regarding the frequency employed throughout the therapy must be optimized [26].

4.4 Ultrasound-mediated delivery implications

Because of the absorption of sound waves, medication delivery via ultrasound-mediated two side effects are microbubble-assisted cavitation and the increase in temperature effect, which is also known as hyperthermia.

4.5 Hyperthermia

The first effect of ultrasonography is hyperthermia, which is due to sound waves being absorbed. Bone tissues have greater temperature improvements compared to muscle tissues due to their high ultrasonic absorption coefficient [28]. FUS and high-intensity focused ultrasound (HIFU) are two methods for the treatment of hyperthermia.

Focused ultrasound (FUS): it is the most effective treatment for producing hyperthermia due to the ability to precisely focus and deposit energy. This allows for the non-invasive targeted therapy for the deep-seated tissue through focused ultrasound and the resulting localized heating [29,30].

High intensity focused ultrasound (HIFU)*:* It is most commonly applied to thermal ablation of the tumors or any diseased tissue. Through the use of interstitial thermocouples, ultrasound imaging or magnetic resonance imaging, to monitor the rise in temperature in real time, we can accurately control the treatment. Local hyperthermia has well-defined, transient effects that can lead to FUS, which is a valuable adjunct to drug therapy. When applied to treat hyperthermia, FUS can penetrate deeply into the body, resulting in different therapeutic effects, like tumor reduction and induction of an immune response against a few cancers [30].

4.6 Cavitation

The creation of vapor-or gas-filled cavities, or bubbles, in a material subjected to oscillating pressure is known as acoustic cavitation. This phenomenon results in the oscillation of bubbles. Bubbles are created when the pressure reduces below the liquid's vapor pressure or when gases are dissolved in the liquid. Inertial cavitation and sustained cavitation are the two main forms of cavitation [31].

When bubbles quickly generate and collapse, inertial cavitation produces shock waves with pressures greater than 10,000 atm and temperatures up to 5000K at the location of cavitation. Sonophoresis or sonoporation may be employed to enhance drug delivery since the size of the bubbles and the sudden disruption of cavitation bubbles can cause cell and tissue damage [32]. Histotripsy is mechanically destroying the diseased tissues with shock waves (Fig. 10.2). Accurate targeting and monitoring are facilitated by the high precision of this method, including least harm to normal cells and tissues, and the ease with which the bubbles are induced or generated during the cavitation can be visualized using ultrasound imaging [27].

Bubbles oscillate in stable cavitation, which also produces velocities that alter fluid velocities and apply shear pressure on the surrounding tissues (Fig. 10.2). Gene and medication transfer into cells

involves this kind of cavitation [32]. Cavitation causes significant biological, chemical, and physical changes in the tissues.

Although, the shock waves generated during the cavitation are short-lived, tissues can easily be disrupted by the large spatiotemporal pressure gradients they induce. When the core of the bubble collapses, its temperature increases by more than 1000K. This leads to a chemical alteration of the medium and produces highly reactive compounds, including free radicals, that induce a chemical transformation referred to as Sonochemistry [27].

The cavitation speeds up the generation of bubbles and lowers the bubbling surface tension of bubbles within an aqueous medium that is saturated with surfactant- and phospholipid-like surface-active molecules. Bubbles generated in solutions containing surfactants are not as very stable and destroy at relatively minute diameters compared to those generated under conditions without surfactants. Smaller cavitating bubbles in surfactant-rich media are formed by the breakdown of the bubble fusion rate due to surfactants in the water or gas interface [31].

4.7 Sonophoresis

Sonoporation is a short and energetic process that causes microbubbles to expand, contract, and collapse quickly as well as create a temporary enhancement in cell membrane porosity. The impenetrable agents are physically able to invade the cells owing to the higher permeability. An improvement in medication penetration into the cytoplasm of the cell ensues from the increased cell porosity. Sonophoresis with TDD applies low-frequency waves, e.g., 20–100 kHz. Protein and peptide distribution transdermal is also feasible in vitro; γ insulin (6 kDa), erythropoietin (48 kDa), and interferon (17 kDa) have been reported. US causes holes in the cell membrane to allow drugs and other medicinal chemicals such as DNA, genes, peptides, and proteins to permeate. Even though sonophoresis has been done under various ultrasonic conditions, the most appropriate ones for use in therapy are those with 1–3 MHz frequencies and 0–2 W/cm^2 intensities [33].

5. Different kinds of drug carriers

5.1 Liposomes

Liposomal nanocarriers are the highly biocompatible for targeted therapeutics. By crossing the barriers to cell entry, these NPs enhance the biodistribution and bioavailability of the compound at the target site [26]. Structurally, liposomes are bilayer phospholipids, self-assembled i.e., when dry phospholipids are rehydrated in water above the temperature of the lipid transition. The range of liposome sizes is 20 nm to submicron.

Liposomes are special in that they are capable of carrying both hydrophobic and hydrophilic molecules. Whereas hydrophobic molecules are injected into the lipophilic bilayer of the liposomes, hydrophilic molecules are assembled in the center of the liposomes. Liposomes are often used as carriers for the delivery of proteins, DNA, enzymes, chemotherapeutic agents, etc. due to their unique feature [34].

There are four different kinds of liposome-assisted delivery systems, i.e., conventional liposomes, sterically stabilized liposomes, ligand-targeted liposomes, and therapeutic liposomes. These types are categorized according to the chemical that is attached to them (Fig. 10.3). Typical liposomes

containing neutral, cationic, or anionic phospholipids and cholesterol have a lipid bilayer structure, which increases the drug's therapeutic index. By pharmacokinetic modification, cholesterol reduces the toxicity of the drug, stabilizes the liposome, and reduces its water permeability. To counteract the poor stability of this type, which also impairs circulation time, sterically stable ones are formed by incorporating polyethylene glycol (PEG) in the case of hydrophilic drugs.

This boosts the circulation period and minimizes the possibility of immune cell opsonization. The delivery of ligand-targeted liposomes enables site-specific targeting of the drugs. It is easy to target ligands like monoclonal peptides/proteins, antibodies, and carbohydrates to the diseased location. Liposomes are blended with some compounds which are functionally active to form ligand-targeted liposomes, which deliver the therapeutic agent and track the state of the treatment or disease [34].

5.2 Micelles

Micelles consist of amphiphilic block copolymers with polymeric core-shell structures. Upon achieving a threshold concentration of the polymer in the solution that is called the critical-micellar concentration (CMC) and the presence of a threshold temperature referred to as the critical-micellar temperature (CMT), such self-assembly structures are formed [35]. The polymeric micelles are generally in the range of 10–100 nm. Since it is complicated and difficult to get liposomes smaller than 50 nm, micelles have a number of advantages over other nanocarriers such as liposomes. The structure of the copolymers employed for synthesis can be changed to modify the size and shape of the produced micelles [36]. Due to their single layer structure, micelles' hydrophobic core allows for the trapping of poorly soluble medications within them. Additionally, this improves the medications' solubility, bioavailability, and circulation time [37]. Three primary methods are typically used to produce drug-loaded micelle structures:

1. Solvent evaporation technique: This process involves dissolving the copolymers and drug in a volatile solvent, which causes the solvent to evaporate and form a thin layer of self-assembled micelles.
2. Direct dissolution: This involves the drug and amphiphilic copolymer self-assembling in the solvent; however, the primary disadvantage of this approach is the minimal drug entrapment in the particles.
3. Dialysis method for micelle synthesis: The drug and the polymer in an organic phase solvent and then dialyzed against water in a dialysis bag. Micelles' high drug loading capacity makes them useful for targeted drug delivery; opsonization of these particles with peptide or sugar can also lead to receptor-mediated, tailored gene or drug administration. Currently, Intelligent vehicles are being employed to provide controlled release, are developed using pH and temperature-sensitive copolymers [36]. Micelles are created by intermolecular contact between block polymers and fall into one of the three kinds listed below.

5.3 Self-assembled micelles

Self-assembled micelles are nanoscale aggregates that spontaneously form when amphiphilic polymers are added to an aqueous solution at CMC, as well as at a temperature higher than CMT. The

hydrophobic and hydrophilic lipid molecules completely determine the micelle's form (Fig. 10.4). PEG and Poly (ethylene oxide) (PEO) are the most widely used hydrophilic blocks.

They both contain the same repeating monomer, CH2–CH2–O, but their terminal ends may differ depending entirely on the production. For instance, hydroxyl group HO-(CH2–CH2–O)n-H, and methoxy group CH3O (CH2–CH2–O)n-H etc. The PEO/PEG block has a range of 1–15 kDa. The main factor determining the selection of hydrophobic units, such as biodegradable poly (esters) and poly (L-amino acids), is drug compatibility with the core [38].

5.4 Unimolecular micelles

Single polymeric units covalently joined by amphiphilic chains make up unimolecular micelles; depending on the composition of the polymer subunits used to make the micelles, a dendritic or multiarm star (Fig. 10.4) structure may be formed. Because of their highly branched structure, dendrimers are typically utilized for the manufacture of unimolecular micelles. Multiple drug loading is restricted by micelles made of hydrophilic polymers. However, the drug loading capacity is increased by altering the cores of micelles with hydrophobic polymer units followed by PEG attachment.

There are two ways to generate star-type uni-molecular micelles: the arm-first method, which involves creating monofunctional linear macromolecular units and connecting them at the core, or the core-first method, which uses a chain initiator to grow the polymeric chain from the center core to the axis.

5.5 Cross-linked micelles

Multimolecular micelle formations known as cross-linked micelles are strengthened by crosslinking between the polymer chains. Because of their crosslinked micelles, these nanoscale molecules of micelles are resistant to shear stresses, dilution, and other changes in the environment. These micelles prefer to stay small because of the shape of their core shell [38].

5.6 Microbubbles

They are tiny, 1–8 μm gas bubbles that are encased in proteins, peptides, polymers, medications, etc. According to Ref. [39]; the microbubbles are employed as a contrast agent for ultrasound-based drug administration and ultrasound imaging. These microbubbles are divided into two categories based on their presence: external microbubbles and endogenous microbubbles. The tissue naturally contains endogenous microbubbles, and sonophoresis requires a strong ultrasonic acoustic pressure to create cavitation. In contrast to endogenous microbubbles, exogenous microbubbles are supplied externally and require far less pressure to overcome the cavitation threshold [40].

There are three methods for creating microbubbles that could be applied to sonoporation. The most often used method involves compressing the air to create tiny bubbles, which are then released through a nozzle system to dissolve the air into liquid. As a result of the supersaturated liquid's quick solution, tiny bubbles eventually form and merge to create larger ones. Using power ultrasound at a specific site of extreme rarefaction utilizing static ultrasonic waves is the second method of creating cavitation. Using a low-pressure air stream is the third method of creating microbubbles.

5.7 Microspheres

According to Table 3.1, microspheres are polymeric particles ranging from 1 to 1000 μm. Drugs could be encapsulated or trapped in these microspheres for use in drug delivery. Microspheres are divided into two types based on their polymeric makeup: natural and synthetic. Proteins like albumin and

Table 3.1 Ultrasound responsive nanocarriers related studies for treatment of breast cancer.

Nanocarriers	Platform used	Drug	Outcome	References
Carbon (C)	Perfluorocarbon-nanoemulsions	Doxorubicin	Excellent tumor regression, long-lasting display, strong ultrasound contrast	
Oxygen (O)	Nitrogen or oxygen recharged SE61 microbubbles	Radiation	Improved oxygenation levels in breast tumor, better survival rate of animals	
Oxygen (O)	Water-soluble vitaminE and Span 60	Radiation	Delay and slow tumor progression, improve survival rate of metastatic breast cancer murine models	
Micelles	Pluronic P123/F127	Curcumin	Improved circulating time as well as increased cellular uptake	[43]
Polymeric micelle	Polyethylene glycol (PEG) along with polylactic acid (PLA)	Doxorubicin	Higher apoptosis with fewer effect on the normal cells, more number of particles generated by ultrasound and greater surface area	[43]
Magnetic	Polylactic-co-glycolic acid (PLGA)	-	Better biocompatibility plus excellent imaging performance	[43]
Liposome	Perfluoromethyl cylopentan and Sonazoid	Doxorubicin	Acoustic cluster therapy produces improvement in efficacy of the therapeutic	[43]
Liposome	Saline and SonoVue	Docetaxel, epirubicin, cyclophosphamide	Ultrasound enabled to observe precisely how the tumor responds to the treatment	[43]
Liposome	Perfluoropentan	Doxorubicin	Well-controlled release of drug, adequate release of drug, better tumor penetration, and elevated antitumor efficacy	[43]

gelatin and carbohydrates like chitosan, agarose, starch, and alginate are examples of natural polymers. In contrast, synthetic polymers include both biodegradable (polyglycolic lactic acid/polylactic acid) and non-biodegradable (polymethyl methacrylate, epoxy polymers) [41,42]. There are four types of microspheres used in the drug delivery, and they are used for both prolonged and targeted drug release in a specific area. They protect pH-sensitive or unstable drugs, even before and after administration.

Bioadhesive microspheres: They have the muco-adhesive property, enabling the drug deposited on the surface of the polymer to adhere to the targeted region. This leads to better delivery of the medicinal products to the diseased site.

Magnetic microspheres: Made of magnetic particles, these microspheres may be utilized to deliver medications precisely where they are needed. These microspheres can be employed for drug delivery as well as diagnostics. An external magnetic source can be used to direct the medications coated or encapsulated in these particles to the sick location [44]. They are frequently used to treat tumor tissue with magnetic hyperthermia.

Floating microspheres: These are designed to deliver the medications that are contained within them into the stomach. For these drug-loaded microspheres to float on the surface and have a better drug release, their bulk density should be kept below that of the gastric liquid.

Radioactive microspheres: For therapeutic purposes, radioactive particles (10–30 μm) are directly injected into veins connected to the envisioned organ or tissue. Three distinct wave types are emitted by these radioactive particles: α, β, and γ emitters. Such microspheres can be prepared using a variety of methods, and the procedure varies according to the microspheres' size, the drug's cross-linking and release durations, and the route of administration.

6. Classifications of NPs

Based on their structural, NPs can be divided into three classes: inorganic NPs, organic NPs and hybrid NPs (Fig. 3.3). Both inorganic and organic NPs offer notable benefits as well as drawbacks for US imaging or treatment. Organic nanoparticles exhibit high acoustic responsibility, quick biodegradation, and strong biocompatibility. They are not very stable for long-term US imaging or treatment, though. Although inorganic nanoparticles often have strong stabilities and high biocompatibility, they degrade at comparatively slow rates. Moreover, the distinct physicochemical characteristics that cause US irradiation are displayed by inorganic NPs. Various types of US NPs are mentioned in this section [45].

6.1 Organic nanoparticles

They are carbon-based NPs and based on their chemical composition, can be divided into three categories: polymer NPs, lipid NPs, and lipid-polymer hybrid NPs.

6.1.1 Lipid NPs

The US-contrast materials that are commonly used in clinical settings are usually composed of bubbles encapsulated in lipid. Colloidal formations known as lipid nanoparticles (NPs) are composed of physiological or physiologically relevant lipids. Solid lipid NPs, nanoemulsions, nanostructured-lipid carriers, nanocapsules, and lipid–drug conjugates are examples of this type of NP. With a size range of 50–1000 nm, solid lipid nanoparticles are thought to be the most significant variety among them.

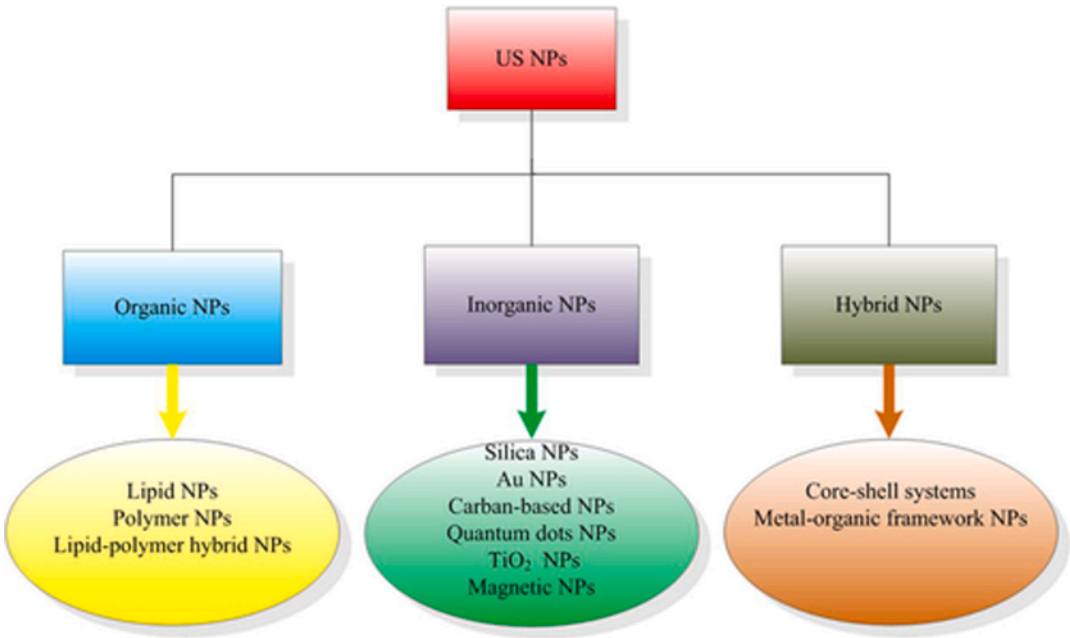


FIGURE 3.3

Classifications of nanoparticles (NPs).

These NPs are solid at room temperature and are nanospheres with lipid cores that are physiologically suitable [46]. Because of the lipids' versatility and biocompatibility, lipid nanoparticles (NPs) are also widely utilized for activities with a variety of goals and for the administration of numerous medications and genes [47]. By stopping drug leakage, lipid nanoparticles with disordered crystal structures can hold more medicines, exhibiting minimal toxicity in vivo, excellent stability, and biodegradation [47,48]. Lipid nanobubbles containing perfluoropropane gas, for instance, have developed demonstrating higher rates of gene transfection effectiveness without causing appreciable cytotoxicity when exposed to US irradiation in vitro.There have also been reports of improved gene transfection effectiveness and organ selectivity in vivo. When paired with US, the nanobubbles might survive longer than 24 h, allowing for effective gene transfection at room temperature [49,50].

6.1.2 Polymer nanoparticles

Natural, synthetic, soluble, and conducting polymers are among the polymers which make up polymer nanoparticles. Proteins, poly(lactic acid) (PLA), PEG, poly(glycolic acid) (PGA), poly(hydroxybutyrate), polycyanoacrylate, poly(D,L-glycolide) (PLG), polylactic acid (PLA), polylactide-co-glycolide (PLGA), and many other polymers can be used in biomedical disciplines.For instance, PEGylation, or coating the polymer nanoparticles with PEG, can both lengthen the time of NPs in the blood circulation and decrease the immunological effects [51,52]. In medical field, polymer NPs can be employed as carriers for anti-cancer medications and diagnostic agents. Excellent stability,

biocompatibility, and biodegradability, as well as excellent active substance encapsulation and transport efficiency to the target location, are among the unique benefits of using polymer NPs as the shell material for US-contrast agents [53,54]. Furthermore, by controlling the nature of polymer material, US-contrast compounds with a uniform size distribution and controlled size can be easily generated.

Certain polymer NPs can be synthesized to be more sensitive to environmental changes, such as US-irradiation, especially for controlled release delivery modes. For instance, polymer NPs coated with peptide shells were created for the anti-cancer medication's targeted delivery for breast cancer, as well as US/fluorescence imaging. On the surface of the breast cancer cells, Cathepsin D may accelerate peptide disintegration, which would release the medications contained inside the system [55,56].

6.1.3 Lipid-polymer hybrid nanoparticles

Combining lipids and polymers, lipid-polymer hybrid NPs offer the benefits of both lipid as well as polymer NPs. Drug administration, gene delivery, and diagnostic imaging are all successfully accomplished by lipid-polymer hybrid nanoparticles. Their unique Designs give them advantages for biological applications. The lipid-polymer hybrid NPs' solid polymer core offers a wide surface area and enhances stability and biodegradability. High biological hybrid nanoparticles are made from a broad range of biocompatible polymers and lipids [57,58].

Additionally, they can be used to deliver hydrophilic and hydrophobic medicines, both alone and in combination. For example, the hybrid NPs with stable PLGA NPs at the core and liposomes at the shell were created for US-enhanced drug delivery. After being exposed to high-frequency US radiation, the method involved the PLGA NPs creating vibrations and shattering the membranes, which allowed anti-cancer medications to pass through it. The use of high-frequency US, stable PLGA, and US-responsive liposomes produced encouraging outcomes [59,60].

6.2 Inorganic NPs

Nowadays, a wide variety of inorganic nanoparticles can be used in biological domains. Long-lasting US diagnosis or treatment is made possible by the comparatively stable inorganic framework. Additionally, the use of inorganic NPs can help overcome the drawbacks of low biodegradability and unknown biological side effects [61].

6.2.1 Silica/silicon NPs

There are several ways to synthesize silica in order to create NPs, such as solid silica NPs, porous silicon NPs, and mesoporous silica nanoparticles (MSNs). Silica condenses around biological micelles to create MSNs. Three separate domains, including the silica framework, the surface and the pores, can be individually functionalized are made possible by the special topology of MSNs. US imaging has made use of silica-based nanosystems. Additionally, due of their reactivity to external US irradiation, US-based treatment applications such as SDT, HIFU-based synergistic ablation, and US-triggered drug release have also been documented. These include MSNs and porous silicon nanoparticles, which exert theranostic capabilities [62,63]. For example, a longer blood circulation duration was achieved with the hydrophobic - hydrophilic US contrast chemicals based on the Janus porous silicon NPs.

6.2.2 Gold nanoparticles

The benefits of these NPs, including their facile production, high biocompatibility, and ease of modification to get over their low stability, make them widely used in many different disciplines. These NPs are capable of absorbing light for the optical detection of malignant cells with local cancer thermal therapy and drug delivery simultaneously. This ability has made Au NPs useful for medical and imaging therapeutic applications, such as ultrasound (US), computed tomography (CT), photothermal therapy (PTT), photoacoustics (PA), radiotherapy, and image-guided thermal therapy [64,65].

6.2.3 Carbon-based NPs

The high stability plus a high drug loading capacity are two benefits of carbon-based nanoparticles, which comprise of carbon and its allotropes. The carbon-nanotubes and fullerenes are two of the many varieties that have been created. Both single-walled carbon nanotubes (SWCNTs) as well as multi-walled carbon nanotubes (MWCNTs) are classified as carbon nanotubes. SWCNTs are made up of a single layer of the graphene sheets; on the other hand, the MWCNTs have several layers of graphene sheets [66,67].

PA imaging, Long-lasting US imaging, PTT, drug delivery, and the electrochemical biosensor systems are some of their biological uses. PEGylated MWCNTs, for instance, were created as US-imaging contrast compounds for prostate cancer after being modified with the prostate-specific membrane antigen (PSMA)-targeting aptamer, i.e., A10–3.2. Regarding the US-contrast agent's diameter, metabolism, and distribution, the findings were encouraging [68,69].

6.2.4 TiO_2 nanoparticles

Titanium dioxide (TiO2) NPs have been used in various industries, including glass, electronics, paints, and biomedical applications like biomedical imaging, gene or drug delivery, photodynamic therapy, and biological assays for malignant tumors, because of their photocatalytic activity, high stability, nontoxicity, and better compatibility. Specifically, TiO2 NPs have been described for SDT, which, when exposed to exogenous US, may produce ROS for cancer treatment. Additionally, it has been observed that TiO2 NPs and HIFU enhance cell toxicity and enhance the anti-cancerous effect of oral squamous cell carcinoma both ex-vivo and in-vivo. The findings demonstrated that using HIFU could improve sonodynamic activation [70,71].

6.2.5 Magnetic NPs

Materials that are primarily made of metals, such as iron, cobalt, nickel, or metal oxides, and are typically covered with either organic or inorganic substances, like PEG, organic polymers, or starch, are known as magnetic nanoparticles. Iron oxide-based magnetic nanoparticles (NPs) have been used for drug administration, heating cancer cells molecular detection, medical imaging contrast, and cell labeling. For MR T2 imaging, superparamagnetic iron oxide nanoparticles (SPIONs) have been specifically studied. Because of their strong negative contrast capabilities, SPIONs—particles larger than 50 nm—have garnered a lot of interest in prostate cancer imaging [72,73].

Magnetic nanoparticles have also been shown to work well for magnetic field-responsive ultrasound imaging but, their use in US-theranostics is still in its infancy. For instance, PFH-loaded hollow magnetite nanoparticles (NPs) were created that could convert PFH for US imaging and were sensitive to magnetic fields, producing heat [74].

6.3 Hybrid NPs

There are significant drawbacks to both inorganic and organic NPs. As a result, inorganic nanoparticles can be altered by adding organic components to give them low toxicity, high solubility, and excellent biocompatibility [75]. **Usually, both inorganic and organic components are included in these hybrid NPs. We primarily review two categories of hybrid US nanoparticles: MOF- NPs, core-shell NPs, and protein-inorganic NPs** [76].

6.3.1 Core-shell nanoparticles

The nanomaterial that coats the center of core-shell nanoparticles (NPs) can be a wide variety of materials. Increased therapeutic value can be achieved by using core-shell NPs because of their reduced cytotoxicity and enhanced biocompatibility, dispersibility, bridging molecule flexibility, and stability. For instance, a smart drug delivery system activated by US plus pH was constructed using a core-shell structure made up of polydopamine (PDA) and MSNs as the shell. Because PDA degrades, it was used in the system for responsive medication release and PTT under various stimuli. Both pH drop and the HIFU have been shown to activate the system's exceptional drug release behavior [77,78].

6.3.2 Metal-organic framework nanoparticles

Metal-organic framework NPs are made up of compounds that comprise metals joined by organic bridging-ligands. Metal-organic framework NPs can be used for numerous purposes, including drug delivery vectors, MRI, CT, optical imaging, and US, by utilizing their physicochemical characteristics, structural diversity, and flexibility of bridging ligands. Using ammonium bicarbonate, gold-nanorods, and liposomes conjugated with the folic acid, a highly multifunctional liposomal delivery system was created for dual-modality image-guided cancer therapy and US/CT imaging. By temporarily cavitating the CO2 bubbles, ammonium bicarbonate could produce a significant amount of CO2 in the delivery system and enable the effective release of anti-cancer medications [79,80]. Moreover, it was shown that the produced bubbles improved US imaging. The system's Au nanorods were employed for PTT as well as CT contrast imaging.

6.3.3 Biological effects of ultrasound

Ultrasound waves' impact on tissues or cells is important for treating breast cancer [30]. The effects of ultrasound on cells or tissues in therapeutic applications fall into two main types. The first is the thermal effect, in which a target tissue or cell's temperature rises when ultrasound is applied continuously to it. Depending on the intended therapeutic use, ultrasound can be used to generate either a high or low temperature. The second is mechanical, in which high-energy ultrasound has a mechanical effect on tissues or cells, such as making them vibrate. The four main methods of ultrasound-induced biological impacts (either mechanical or thermal) are acoustic streaming, cavitation, thermal, and bilayer-sonophore effects.

6.4 Thermal effect

The absorption of energy for ultrasonic waves is the main cause of the thermal impact of ultrasonography, which is an increase in the medium's temperature. The heat production rate by ultrasonic waves is inversely related to the targeted tissue's specific absorption coefficient and directly relates to the wave frequency and exposure duration. Therefore, the more the absorption coefficient of the

medium, the higher the temperature rises. Consequently, the thermal effect that the tissue experiences. The duration and amount of the temperature change are measured by the thermal dose that is applied to a cell or tissue. Ultrasound energy can cause thermal ablation, which is a brief, highly localized rise in temperature that kills tissue by denaturing proteins, or local hypothermia, which is a low level thermal rise that occurs over a few minutes or hours [81].

6.5 Cavitation effect

Cavitation is the process by which bubbles form in tissue microenvironment as a result of a change in the pressure brought on by the application of ultrasonic waves [82,83]. The creation, expansion, oscillation, and dissipation of cavities in the tissue environment stream as a result of pressure changes after the produced ultrasonic wave are all included in the cavitation effect caused by ultrasound [84]. In both external and endogenous gas bubbles, cavitation can occur. Exogenous gas vesicles are artificial microbubbles that are brought into cellular milieu from the outside, while endogenous gas microbubbles are cavities occurring naturally in the cytoplasm of cell [85]. Usually stabilized by phospholipids, a synthetic polymer, human serum albumin denatured, and an encapsulating surfactant, they are composed of spherical cavities filled with gas and/or saturated carbon. By creating pores on the membrane, the inner as well as outer microbubbles can make cell membranes more permeable. This is thought to be a cavitation brought on by sonoporation and results in acoustophoresis [86].

Upon reaching the cavitation thresholds, microbubbles may result in sonoporation. Microbubbles absorb ultrasonic energy and produce high-frequency oscillations when ultrasound is applied to them. The cell membrane structures are disrupted as a result of the formation of a liquid jet or a shock wave. Cells become more accessible to nanoparticles as a result of the oscillation plus expansion of microbubbles, which apply shear pressure to the cell membrane and improve its permeability [87]. As seen in Fig. 3.4, the two kinds of cavitation, including stable and inertial cavitation, depend on how bubbles collapse under ultrasonic treatment [88,89].

The non-linear, perennial, periodic bubble expansion and shrinkage is known as stable cavitation. In stable cavitation, the gas pockets in the liquid can linger for a long time and oscillate around an equilibrium radius. As a result, gas microbubbles may expand and contract over an extended length of time while being affected by ultrasound. The microbubble oscillates for a while until its gas component dissolves in the blood, at which point it is quickly expelled from the lungs through expiration. Conversely, inertial cavitation is typified by a dramatic bubble collapse, where the bubbles rapidly collapse when an ultrasonic pulse is applied [90]. Compared to steady cavitation, inertial cavitation causes greater pore diameters.

6.6 Acoustic streaming effect

High amplitude ultrasonic waves may cause unidirectional fluid current flow when they are applied to the medium due to momentum transfer from the ultrasonic wave to the medium; this phenomenon is called acoustic streaming [91]. The stream's velocity is inversely proportional to the speed of sound in the medium and the bulk viscosity. It is directly correlated with the medium's attenuation coefficient, ultrasonic intensity, and transducer surface area. The Ultrasound reflection and other distortions that take place during the wave propagation are the main causes of acoustic streaming [92]. The therapeutic potential of acoustic streaming has not been thoroughly investigated to far. Acoustic streaming

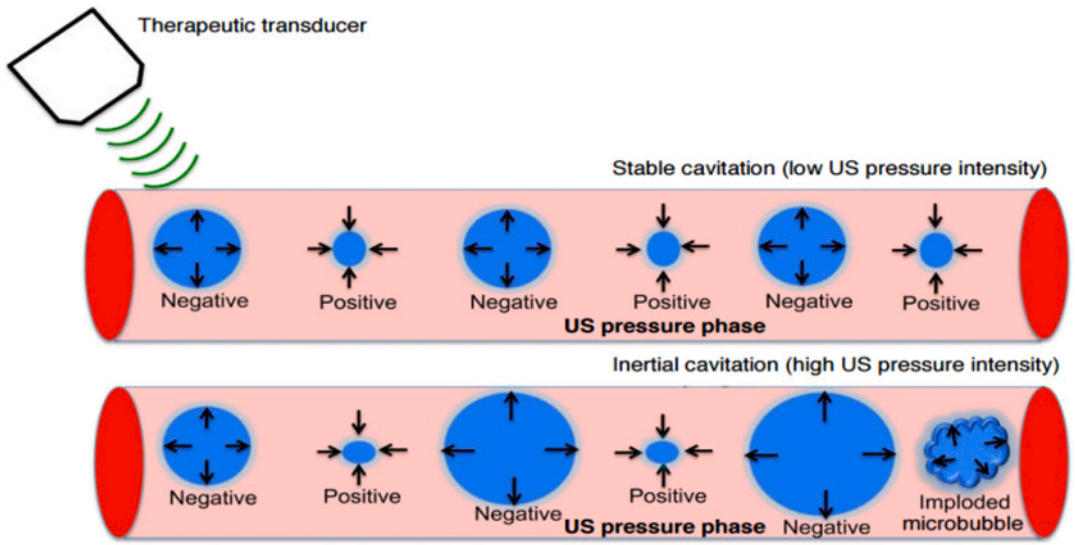


FIGURE 3.4

Stable and inertial cavitation.

Adapted from Chowdhury, et al. (2017). Ultrasound-guided drug delivery in cancer. Ultrasonography, 36(3), 171 with permission under the terms of the CC BY-NC 3.0 License, Copyright 2017.

generally occurs when a non-linear acoustic wave having a finite amplitude travels through a fluid. When exposed to sonic waves, the molecules are compelled to oscillate at the similar frequency. The acoustic wave's non-linearity causes wave propagation to produce a time-independent flow velocity in addition to normal oscillatory motion. As a result, the fluid flows in a certain direction that is determined by the characteristics of the applied ultrasonic wave, the system's structure, and its boundary conditions. Microstreaming is the term used to describe the tiny auditory streams in a fluid [93]. Cavitation microstreaming is the term used when connected with acoustic cavitation, which is a broad term for the actions of microbubbles [94].

6.7 Bilayer sonophore effect

Gas-filled bubbles as nanocarriers are injected in the blood as part of US-responsive treatment. The permeability of the bilayer membranes is momentarily increased as a result of the interaction between the bubbles and ultrasonic waves. The bubble in the bloodstream at the target area is activated by the ultrasonic wave once it reaches the region of interest. Within the capillaries, the bubble expands and contracts as a result of interactions between the ultrasonic waves and the acoustic energy it absorbs. The capillary walls expand and compress as a result of this process, which is known as the bilayer sonophore effect [95]. The membranes' tight connections are loosened as the protein complexes are disrupted mechanically. The therapeutic drug starts to leave the capillaries and enter the tumor tissue as the bilayer membrane's permeability rises. The sonophore effect only lasts for a minimum of one or 2 minutes. After the sonophore effect ends, the barrier is only permeable for a few hours [96]; which

allows paracellular transit and widens intracellular gaps. As endothelial cells move medications out of the artery lumen, transcellular transport may also rise. The therapeutic target of this medication can now be reached. Within minutes, bubbles leave the circulation when the process is finished.

The barrier completely regains its protective role within 24 hours or fewer following the sonophore effect, when the junction proteins re-associate and the endothelial cells return to their initial state. This impact reduces the region and duration of the increased tumor-blood permeability while allowing a range of medications to reach the target tumor for therapy.

6.8 Ultrasound responsive nano carrier treatment

These nanocarriers have been used in a few studies to enhance chemotherapy for the breast cancer. A few related researches are compiled in Table 3.2. Pluronic P123/F127 polymeric micelles containing Curcumin were created by Wu et al. [96] and were triggered by focused ultrasound to release the medication. By using ultrasound, they were able to boost absorption and prolong circulation. To enhance the therapeutic effects in breast cancer cell lines, Jablonowski et al. [97] combined doxorubicin co-encapsulation as ultrasound-responsive microbubbles with surface tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression. According to their findings, ultrasound protected healthy MCF-12A cells from being destroyed while reducing cancer cell survival the most.

Localized microbubbles were used by Eisenbrey et al. [126] to reduce hypoxia before breast cancer treatment. In their investigation, they created and administered intravenous surfactant-shelled oxygen microbubbles to mice with human breast cancer tumors in order to increase tumor oxygen levels locally when activated by non-invasive ultrasonography. In order to enhance patients receiving neoadjuvant chemotherapy for breast cancer, Soyemi et al. [127]; Rix et al. [128]; Wang et al. [129]; Dobruch-Sobczak et al. [130]; and Yang et al. [131] showed how ultrasonography can be used in conjunction with contrast agents. According to these investigations, an ultrasound-based method made it possible to precisely identify tumors that were responding and those that weren't.

For neoadjuvant breast cancer, Amioka et al., [132] suggested ultrasound-responsive chemotherapy and came to the conclusion that ultrasound-responsive techniques could be a novel way to establish treatment plans for breast cancer patients following neoadjuvant chemotherapy. For ultrasonic theranostics, Baghbani et al. [88] placed doxorubicin into alginate stabilized nanodroplets. They claimed that their suggested method demonstrated robust, long-lasting ultrasound contrast and had significantly improved anti-cancer effects under ultrasonography. The therapeutic effectiveness of liposomal doxorubicin in ultrasound-responsive nanocarriers was also investigated by Bush et al. [133]. They found that using acoustic cluster therapy increased treatment effectiveness.

The possibility that ultrasound-induced microbubble rupture improves breast cancer metastasis was examined by Delaney et al. [134]. They found that combining ultrasound ruptured microbubbles with radiation therapy slows the tumor growth as well as increases patient survival rates for metastatic breast cancer patients. Nie et al., [135] used a multimodal strategy for targeted imaging of breast cancer, where the cancer was exposed to a powerful ultrasound echo signal. When employing the multimodal strategy, they observed an improvement in performance. Nanodroplets with tunable qualities allow for effective site specific drug administration and demonstrate their effectiveness in cancer theragnostic, according to Sheng et al., [136]; who proposed ultrasound-guided breast therapy utilizing nanodroplets.

Table 3.2 Studies investigating the acoustically triggered drug-release from polymeric nanoemulsions and micelles.

Type of study	Encapsulated substance	US parameters	Effect	References
in vitro	Nile red/pyrene	1.1 MHz (0–150 W)	HIFU sonication and loaded agents are quickly released from the (PPG-[Cu]-PEG) micelles	[98]
In vivo using K12, DHD, and TRb cells	DOX	20 kHz (1.0 W/cm^2) or 476 kHz (23.61 W/cm^2)	The US improved DOX concentration by roughly 50% in the cancers. While a change in frequency is due to treatment.	[99]
In vivo using murine models, spontaneous breast adenocarcinoma	DOX	28 kHz (0.04 W/cm^2) & 3 MHz (2 W/cm^2)	Both frequencies used drug delivery as well as better uptake of the drug by the tumors.	[100]
In vitro study using MDR and A2780cells	DOX	1, 3 MHz and 20 kHz–3 MHz power densities (0.058, 6 and 0–0.2 W/cm^2)	Sonication improved the drug delivery from micelles and the intracellular uptake of the drug.	[101]
In vivo study using K12/DHD/TRb cells	DOX	20 and 70 kHz (0.048, 1, and 2 W/cm^2)	The US improves DOX release from micelles. The US may also assist drug/micelle uptake by the tumor.	[102]
In vitro study	Ruboxyl and DOX	20–90 kHz (0–3 W/cm^2)	Micelles disruption by cavitation, releasing their encapsulated molecules. Ruboxyl release was poorer than compared of DOX	[103]
In vitro study using HL-60 cells	DOX	80 kHz (3.6 W/cm^2)	Sonication resulted in reducing the $IC_{50\ of}$ DOX from 2.35 to 0.19 μg/mL.	[104]
In vitro study	DOX/DPH	70 kHz	DOX release increased with the increase in temperature from 25 °C (2%) to 37°C (4%). When sonication was halted, re-encapsulation of DOX occurred.	[105]
In vitro study using HL-60, MCF-7 and MDR cells	DOX	1.0-MHz (7.2 W/cm^2) 20-kHz (0.058 W/cm^2), 67-kHz (2.8 W/cm^2), and	DOX release was improved at densities of higher power densities	[106]

In vitro study with HL-60 cells	DOX	20 kHz (1.4, 14, & 33 mW/cm^2	Sonication caused better uptake of DOX by the tumors	[107]
In vivo study using A2780 cells	DOX	1 or 3 MHz (3.4 W/cm^2)	Sonication of the drug-loaded micelles inhibited tumor growth.	[108]
In vitro study	DOX	70 kHz (0.28 W/cm^2)	Release of the encapsulated drug by disassembling the micelles and transient cavitation disrupted	[109]
In vitro study involving HL-60 cells	DOX	70 kHz, (2.3, 1.3 W/cm^2)	US-mediated drug release from micelles caused apoptosis	[110]
In vitro study	Nile red	High intensity US	Cavitation disruption occurred at the HIFU focal point, causing the irreversible release of Nile red.	[111]
In vitro study using HL-60 cells	DOX	70 kHz, (1.3 W/cm^2)	DNA damage by US triggered DOX released from Pluronic P105 micelles	[112]
In vitro study with HL-60 cells	DOX	70 kHz (1.5 W/cm^2)	The US improved DOX toxicity by extravasation and permeabilization of micellar membranes.	[113]
In vitro study involving HL-60 cells	DOX	70 kHz, (1.2 W/cm^2)	The US application increases drug uptake through the cell membrane, not involving the acidic vesicles.	[114]
In vitro study	DOX	70 kHz (0.53–6 W/cm^2)	Maximum DOX release (14%) was measured at 5.4 W/cm^2 following a zero-order model.	[115,116]
In vitro study	DTT/pyrene	1.1 MHz (0–150 W)	Combining the effect of redox and HIFU improved drug release from copolymer micelles.	[117]
In vitro study	DOX	70 kHz (0.27 W/cm^2)	Micelles release the drug due to events created by cavitation.	[118]
In vivo study using MiaPaCa-2 and MB231 cells	PTX	1 MHz	Suppression of metastasis, tumor regression after US-mediated PTX-loaded PFCE nanoemulsion therapy	[119]

Continued

Table 3.2 Studies investigating the acoustically triggered drug-release from polymeric nanoemulsions and micelles.—cont'd

Type of study	Encapsulated substance	US parameters	Effect	References
In vivo involving MiaPaCa-2 cells	Green fluorescent PTX and PTX	1 MHz	Accumulation and extravasation in cancer sites occurred faster for micelles than for nanodroplets.	[120]
In vivo using MiaPaCa-2 cells	PTX	3 MHz (3.1 W)	Micelles having elastic cores with corresponding nanoemulsions gave better efficacy than ones with solid cores. Nanoemulsions showed lower cellular toxicity as compared to micelles.	[121]
In vivo using A2780, MiaPaCa-2 and MDA MB231 cells	PTX	1, 3 MHz	Without the use of US, PTX was tightly retained by the nanodroplets. While PTX was effectively released into the cancer cells when US was used,	[122]
		1 MHz (3.4 W/cm^2) 3 MHz (2 W/cm^2)	Droplet-to-bubble transition may be triggered by injection via fine-gauge needles along with LFUS in the presence of pre-existing microbubbles.	[123]
In vivo using MDA MB231 and A2780	DOX	1 MHz, 3 MHz	The US significantly improved the intracellular uptake of DOX in the presence of microbubbles compared to micelles.	[124,125]

To regulate cell growth, Song et al., [137] suggested ultrasound-responsive administration. They claimed that ultrasound and microbubbles had a beneficial effect on breast cancer and that their method might be applied to other cancer treatments.

7. Ultrasound and therapy

Both medical diagnosis and treatment have been transformed by ultrasound. Additionally, it has been used to promote and quicken the healing of soft tissue injuries, including bone fractures [138]. It is currently also utilized to stimulate bone formation. Diagnostic ultrasonography is inexpensive to operate and use, has good tissue penetration, and is generally safe [139]. As previously stated, the frequency and intensity of the acoustic waves, the duration of exposure, as well as the quantity of pulses, all influence the biological effects of ultrasound [140]. High-intensity-focused ultrasound (HIFU), has been used to treat cancer by directing high sonic energy toward a particular focal target. While not producing cytotoxic temperatures beyond the focal zone, this energy is absorbed by the cells at the treatment's center of gravity, resulting in high temperatures (over 80°C) [141,142]. This causes the targeted cancer cells to be completely destroyed to effectively ablate a whole tumor, HIFU ablation involves carefully positioning tiny lesions next to each other on the tumor tissues [143]. Although HIFU's thermal effect is the primary means of treating cancer, acoustic cavitation also produces nonthermal effects like microstreaming and radiation forces that destroy membranes and kill cells.

Skin burns of the second or third degree have been linked to problems from HIFU tumor treatment. Furthermore, although this has not been confirmed beyond a reasonable doubt, there have been worries that the tumor cells and the blood vessels that surround them may burst, leading to bloodstream metastases [144]. The immune response may be boosted by the secretion of inflammatory cytokines [145].

Sonodynamic therapy (SDT), a promising cancer treatment, heavily relies on ultrasound. SDT employs sonosensitizers, which are substances that promote the ROS generation, in conjunction with low-intensity ultrasound. In order to use this procedure, the cancer cells must absorb and retain the sonosensitizers.

The sonosensitizers are activated by US, resulting in the production of ROS and other harmful chemicals [146]. Sonosensitizers are activated from the ground stable state to the excited state via cavitation mechanisms. Large volumes of ROS, including singlet oxygen, oxygen ions, and peroxides, are produced as they settle back to their ground stable state, releasing energy that is then transferred to the surrounding oxygen. ROS can cause apoptosis and are extremely harmful to cells [147].

Since sonosensitizers are only bioactive after being exposed to US, which could be targeted at the tumor without damaging the surrounding tissues, they are believed to be safe. Additionally, their risk of systemic toxicity is extremely low [148].

Through the creation of microbubbles during the cavitation process, ultrasound contributes to improving cell membrane permeability (sonoporation), as was previously mentioned. Drugs and DNA can be transferred from extracellular to intracellular areas by sonoporation. Both in vivo and in vitro studies have shown that sonoporation improves the absorption of anticancer medications, such as adriamycin and bleomycin [149,150].

The application of ultrasound (0.5–4 MHz) has also been shown to improve gene absorption by cells [151]. As previously mentioned, ultrasound improves endocytosis or creates tiny holes in the membrane to increase the uptake of various substances [152].

7.1 Ultrasonically triggered nanoparticles for cancer treatment and drug delivery

Ultrasound radiation can be utilized as an external stimulation to cause medication release from NPs. Low-frequency ultrasonography deeply penetrates soft tissues, but other external stimuli, like light, do not permeate the body as well [152,153]. Safe and non-invasive accumulation of high amounts of energy into certain small volumes deep within the body while preserving the surrounding healthy tissues is made possible by the capacity to direct ultrasonic waves using probes applied to the skin [154].

Ultrasound's effective focus can reach volumes as small as only a few cubic millimeters [153]. The ultrasound may trigger nanocarriers in focal zone to discharge their payload, but it cannot trigger nanocarriers outside of this zone [155]. The mechanical effect of radiation forces or cavitation, as well as the thermal effect of the US, can cause ultrasonically triggered drug release from nanocarriers. These physical pressures cause structural destabilization in sonosensitive nanocarriers, which leads to drug release. Furthermore, these forces cause a brief rise in the permeability of artery, which facilitates the extravasation of medications or particles in tumor interstitial spaces and increases the cellular uptake of drug ultimately [26,156]. As previously stated, the disruption of the bubbles results in the generation of ROS, high temperatures, and pressures as well as inertial cavitation [139]. The ROS generated as a result of the ultrasonic and nanoparticles' combined effects result in oxidative stressors that lower cell viability by causing membrane disintegration, DNA fragmentation, and mitochondrial membrane damage [157,158].

7.1.1 Mesoporous silica nanoparticles triggered with US

Porous silica nanoparticles having pore sizes varying from 2–50 nm are known as mesoporous silica nanoparticles (MSNs). They have been thoroughly studied for their potential in the drug delivery and biological applications, along with their potential as sonosensitizers and cavitation nuclei [159]. Additionally, MSNs are biodegradable and biocompatible [160,161]. Their huge pore volumes provide them with enormous surface areas, which gives medicines a high loading efficiency. Horcajada ett al. [162], found that when pore widths were reduced from 3.6 to 2.5 nm, the delivery rate of ibuprofen enclosed in MCM 41-type mesoporous materials reduced. The release and loading efficiency of MSNs is determined by the drug interactions with walls of the pore and solubility [163].

By adjusting the time and the duration of pore closure or opening, different chemical groups can be functionalized into the pore walls to regulate the coating and release of the pharmaceuticals. When the MSNs get at the intended location, the cargo can be released, but the risk of an early drug release in the bloodstream is reduced [163].

Paris et al., [164], have employed ultrasound to cause Dox release from the MSNs. Temperature-sensitive copolymers (MEO2MA and THPMA) were used to graft MSNs in their investigation. These copolymers had the ability to open holes (the polymer had a coil-like shape during drug loading) while subsequently close pores (the polymer had a collapsed structure during blood circulation).

When the hydrophobic tetrahydropyranyl moieties are broken down by the ultrasonic waves' energy, the polymer's shape changes to a coil, allowing for the release of the medication. The same team recently examined ultrasound-mediated medication release from MSNs grafted with a PEG shell in a study [165] (107). The hydrophilic PEG shell separates from the MSNs that contain the medicine topotecan when ultrasound with high frequency is given. The exposed surface's positive charge causes the MSNs to internalize into osteosarcoma cells, releasing the medication within the cells.

In order to restrict the pores plus stop the release of the medicine that was encapsulated, Li et al., [166] manufactured MSNs loaded with the polymer sodium-alginate. The SA was cross-linked on the outer layer (SA-CaCl2) using carboxyl-calcium ($COO–-Ca2^{+}$). The coordination connections were broken, and the medication was liberated from the MSNs during the sonication with both low-frequency (20 kHz) as well as high-frequency (1.1 MHz) ultrasound. The team came to the conclusion that cavitation was the mechanism causing these coordination links to break and the ensuing medication release. Using folic acid (FA-β-CD) as ahighly targeting moiety, another group [167] has created multifunctional targeted β-cyclodextr capped MSNs to enhance absorption by cancer cells. Paclitaxel (PTX), a chemotherapeutic medication, was put into these MSNs.

Gas bubbles were kept in a hydrophobic interior channel to improve their sonosensitivity. Although the bloodstream release of these nanoparticles was modest, both in vivo **and** in vitro **drug release was greatly increased by triggering with low-intensity ultrasound (0.4–1 W/cm^2, 1 MHz). As previously mentioned, MSNs function as cavitation nuclei or sonosensitizers when stimulated by ultrasound. Remaining gas bubbles are present in the pores as a result of their rough surfaces. Hydrogen bubbles are also created as the silicon nanoparticles dissolve** [168]**. Low-intensity ultrasound and MSNs have a synergistic cytotoxic impact that has been shown to destroy cancer cells** in vitro [169,170]**.**

7.1.2 US triggered super magnetic iron oxide nanoparticles

Magnetite iron oxide (Fe3O4) or its oxidized counterpart maghemite (γ-Fe2O3) make up superparamagnetic iron oxide nanoparticles (SPIONs), having diameters ranging from 1 to 100 nm. SPIONs may be directed to a particular spot using externally applied magnets because they are made of iron oxide cores. The SPIONs are drawn to the appropriate position by a powerful magnetic field created by the external magnet placed over the targeted area. Because SPIONs are easy to produce and biodegradable, they are promising nanocarriers that can transport medications to the body [171].

The SPION surface can be grafted with various functional groups, such as carbodiimides and carboxyl groups. These functional groups can then be conjugated to chemotherapy medicines. However, it has been discovered that SPIONs release the drug shortly after being injected into the blood since pharmaceuticals are only loaded on their surface. At the targeted site, the antineoplastic drugs then fall short of their therapeutic levels. The metal cores are often coated with biocompatible polymers to prevent their early release. A number of polymers have been employed as coating materials in aqueous solutions, such as PEG, PLGA, and polyvinylpyrrolidone (PVP), polyethylene-co-vinylacetate [172].

By covalent bonds development, adsorption, or trapping on the particles, these coatings shield the magnetic core and enable drug binding [173]. For instance, compared to the noncoated SPIONs, the cross-linked polymer poly(ethylene glycol)-co-fumarate (PEGF) significantly decreased premature release (21%) when applied to the iron oxide core [174]. Targeting molecules, including RGD, folic acid, transferrin, proteins, and hyaluronic acid, can be added to the SPIONs' surfaces to enhance their

targeting abilities [174]. Numerous in vitro investigations have demonstrated that SPIONs have little to no cytotoxic impact on the cell cultures [175–177].

Other in vivo investigations, however, revealed conflicting toxicity patterns of SPIONs in a number of preclinical animal models, ranging from negative [178,179] to positive [180,181]. In general, the size, dosage, surface coating, and species of SPIONs all affect their toxicity [182]. Fard et al. [183] looked into how the viability of cancer cells was affected when Fe3O4 nanoparticles were subjected to ultrasound (1 MHz) at an intensity of 2 W/cm2. According to the study, sonication improved the amount of ROS in the cells, which led to apoptosis.

Additionally, the study found that high quantities of Fe3O4 nanoparticles (greater than 100 mg/mL) have a synergistic impact, acting as sonosensitizers and producing ROS. By causing the production of ROS, such as hydroxyl radicals, hydrogen peroxide, and superoxides, coating Fe3O4 NPs with sonosensitizers, such as titanium dioxide (TiO2), is a viable method for improving ultrasound-assisted stimulation [184]. Cytotoxicity of the DOX-loaded titanium dioxide encapsulated Fe3O4 NPs (Fe3O4–TiO2 NPs) in combination with ultrasonic (1 MHz, 1 W/cm2) was examined in an investigation by Shen et al. [185]. According to this investigation, these nanoparticles generated ROS after being exposed to ultrasound. Higher toxicities were observed when cancer cells were incubated with Fe3O4–TiO2-DOX and then sonicated, as opposed to when free DOX or Fe3O4–TiO2-DOX alone was used.

7.1.3 Gold nanoparticles triggered with US

The use of GNPs as nanocarriers for tumor drugs has exciting potential. GNPs have special physical (stability and size), optical (energy absorption), and chemical (reactive with thiols) properties that make them an extremely versatile platform for imaging, drug delivery, and surgical guidance [64,186,187]. According to research by Chithrani & Chan [188]; the receptor-mediated clathrin-dependent endocytosis pathway is the primary mechanism by which GNPs are taken in by cells. Additionally, the study found that, in comparison to bigger carriers, the nanocarriers' tiny diameter ($\leq$50 nm) results in faster uptake as well as improved concentrations of GNPs inside the cells.

It was discovered that GNPs interacted with heparin-binding glycoproteins, such as basic fibroblast growth factors mediating the angiogenesis and vascular permeability factors. Angiogenesis inhibition slows the growth of tumors. GNPs have been employed in the field of drug delivery, even though they have the potential to be therapeutic agents that kill tumor cells. Potential drug delivery vehicles are GNPs with from 10–100 nm, which can be coupled to chemotherapeutic medications [189]. Alkanethiol linkers and basic physical adsorption are used to accomplish the conjugation [75,189].

In order to decrease their absorption by the reticuloendothelial system (RES), GNPs can also be pegylated (decorated with PEG thiols) to increase their stability and solubility. Furthermore, GNPs can be engineered to conjugate with other ligands, including folic acid and transferrin (TF), to target malignancies [75,190]. (133,134) Once reaching the intended site, external (i.e., light, ultrasonic, laser) or internal (i.e., pH, redox state, matrix metalloproteinase, heat) triggers can be used to activate drug release from the GNPs [191].

Acoustic cavitation requires the existence of air bubbles, also known as bubble nuclei. Because of their rough surfaces, gold particles in liquid medium provide sites for nucleation to create bubbles when ultrasound is administered. This improves the cavitation processes by lowering the US pressure threshold required for cavitation occurrence. [192]. GNPs were shown to increase inertial cavitation when incorporated in to the culture media [193].

FA-PEG-GNP was created by Brazzale et al. [194] by using GNPs with the acid as a targeted moiety and PEG as a polymer covering. After these NPs were incubated with two different cancerous cell lines (HCT-116 and KB) and exposed to ultrasound (at 1.866 MHz, 8×10^{-6} and 8×10^{-5} J/cm^2 for 5 mintues), there was a significant decrease in both cell growth and proliferation as well as the production of ROS species. According to different investigations by Kosheleva et al. [195]; the lung cancer cell line (A549) was more sensitive to GNP in combination with 4 MHz HIFU than to GNPs alone.

Additionally, it was discovered that combining GNPs with US (1 and 3 MHz, 2 W/cm^2) and a strong light pulse greatly enhanced the therapeutic efficacy in animals with colon cancers [192]**. Ultrasound (1.1 MHz, 2 W/cm^2) applied to Au-PpIX, i.e., GNPs conjugated with sonosensitizer, resulted in better suppression of the colon cancer cell line (CT26) than when PpIX alone was used, according to an** in vivo **investigation by Shanei et al.** [196]**. This was explained by the fact that GNPs functioned as nucleation agents, which improved the tumor cells' absorption of Au-PpIX and caused more collapsing cavities.**

7.1.4 Carbon nanotubes triggered with US

According to numerous studies conducted over the last 20 years, carbon nanotubes (CNTs) can be used as gene and drug delivery systems because of special physical characteristics, which include their huge surface area, low mass density, and diameters of wide range (from a few to hundreds of nm), as well as their biocompatibility, non-immunogenicity, and improved drug carrying capacity [197]. A single-walled carbon nanotube (SWCNT) is a carbon allotrope made out of a single graphene sheet that has been coiled up [198]. Carbon nanotubes with many rolled sheets stacked in a concentric shape are known as multiwalled CNTs (MWCNTs) [197].

CNTs tend to agglomerate and dissolve poorly in aqueous conditions. The surfaces of CNTs can be altered chemically or physically, such as by hydrophobic, covalent, or noncovalent bonding, to get around these problems [198]. Their modification on the surface with polymers, acylation, carboxylation, PEGylation, amidation, esterification, and purification have all been extensively studied. They are also appropriate for targeted drug delivery since the surfaces can be attached to targeting molecules such as proteins, polysaccharides, folic acid, as well as antibodies [197,199,200].

CNTs have been studied for their potential in the treatment of cancer using both photodynamic and photothermal therapy. Since CNTs can convert near-infrared light into heat, they have been shown to play a part in photothermal therapy by encouraging hyperthermia, also known as thermal ablation, which improves tumor suppression. [201]. Apart from their capacity to load drugs, they can be designed to function as superior adjuvant contrast agents for imaging applications [198].

The MWCNTs' hyperechogenicity, for instance, encouraged their application as ultrasound contrast compounds. [202,203]. Functionalized MWCNTs show long-lasting ultrasonic contrast qualities, according to an investigation by Delogu et al. [204](147). In addition to breaking and shortening the individual CNTs, Pagani et al. [205] found that the cavitation created during sonication causes CNT bundles to exfoliate into individual units, which disperses the CNTs in liquids. Length of the CNTs decreased significantly as the sonication period increased, according to morphological and spectroscopic studies of CNTs subjected to sonication using a US exposure (power 260W, water 2.5L) [206].

Additionally, the topologies of CNTs were modified from solid to liquid when their length dropped as a result of the longer sonication period. [207]. According to a previous work by Ruan and Jacobi

[208] (151), MWCNTs' capacity to transfer heat rose nonlinearly as the sonication specific energy input increased. CNT drug delivery with ultrasound assistance is a potential method for treating cancer because of its capacity to effectively scatter CNTs and release their load in a regulated way. Furthermore, the potential of integrating MWCNTs with the HIFU ablation is demonstrated by the enhanced thermal conductivity of MWCNTs after sonication.

To fully understand the potential of US-linked CNTs in medication administration and to optimize the right sonication settings for the intended ultrasound-mediated effect, more research is undoubtedly required. However, MWCNTs' toxicity and biocompatibility are major concerns, and they were just listed as a nanomaterial of very high concern on the Substitute It Now (SIN) List [209].

7.1.5 Ultrasound-triggered polymeric micelles

Amphiphilic copolymers are used to create polymeric micelles, which are nanocarriers with a hydrophilic shell and a hydrophobic core. While polyethers, polyesters, or polyamino acids are utilized as the building blocks to make the hydrophobic core, PEG is frequently utilized to form the outer shell, which is hydrophilic [210]. (Polymeric micelles are effective drug delivery vehicles because they spontaneously self-assemble in water to make a stable and distinctive nanoscale structure with numerous biological and physicochemical benefits. [211]. The hydrophilic coating lengthens the blood's circulation time while preventing the immune cells from absorbing the polymeric micelles, which lowers the blood's ability to remove them.

Drugs that are insoluble in aqueous solutions could be readily encapsulated and transported to the tumor site via the hydrophobic core. [211]. Drugs can be coated on the polymeric micelles by chemical ionic bonding and conjugation, in addition to being physically trapped inside their cores. A variety of targeting moieties, such as folic acid, transferrin, growth factors, as well as antibodies, can also be used to create micelles. [212]. Drug release from micelles with spatial and temporal control can be triggered by intrinsic or external triggers once they reach the target location.

Ultrasound has been investigated in many studies as a means of initiating the controlled release of the active compounds from the polymeric micelles, primarily Pluronic micelles. [213,214]. Low-frequency ultrasound (20–90 kHz) causes reversible release of drug through inertial cavitation, while high-frequency ultrasound (1.1 MHz) can cause irreversible drug release from polymeric micelles through ester hydrolysis. [215]. According to Xuan et al. [216]; adding a tiny quantity of HIFU-labile 2-tetrahydropyranyl methacrylate (THPMA) comonomer units to the micelle core would increase its thermosensitivity and release more encapsulated medications when exposed to HIFU radiation.

By rupturing the formed polymeric micelles, cavitation causes the drugs' payload to be released from the core of the micelles. Polymeric micelles can successfully rebuild and re-encapsulate the pharmaceuticals inside their cores when ultrasound is stopped. Consequently, reversible drug release may result from the micelles' reversible disordering. [116,214]. According to a recent study by Wu et al. [217](161), P123/F127 mixed micelles coated with curcumin showed more absorption by cancer cells than free curcumin and circulated in the circulation for longer lengths of time. According to the study, when the copolymer chain was exposed to HIFU (3 W, 1.90 MHz) for 3 minutes, the Cu(II)–terpyridine dynamic link was cleaved, disrupting the structure of the micellar and causing the medication to be released.

Using five different kinds of poly(2-oxazoline) block copolymers to create the micelles, Salgarella et al. [215] discovered that sonication (20 W, 40 kHz, 10 minutes) increased the release of encapsulated dexamethasone from 6–105%. The amount of medication released was shown to be influenced by the type of copolymer employed. Table 3.2 summarizes some research related to ultrasound-mediated drug release from polymeric micelles.

7.1.6 Liposomes triggered with US

Liposomes are nanoscale transporters with lipid bilayer shells enclosing core aqueous compartments. Liposomes can effectively encapsulate hydrophilic pharmaceuticals in their cores and capture hydrophobic medications within the lipid bilayer [125]. To extravasate via the leaky vasculature of solid tumors, monodisperse liposomes with an optimal diameter (less than 200 nm) could be produced. Stealth liposomes, which are liposomes modified on the surface with polymers like PEG, have longer circulation durations and less absorption into the RES cells than liposomes that are not modified [218].

Accumulation of the drugs at the solid tumors can be greatly increased by drug encapsulation inside liposomes, with a 50–100 times increase in comparison to the normal free drug. [219,220]. However, this does not indicate an equally significant improvement in therapeutic effects because the drugs must first be freed from the carriers so that they can affect the surrounding area.

Since the FDA has already approved several of these, such as pegylated liposomal doxorubicin (Doxil), liposomal doxorubicin (Myocet), liposomal daunorubicin (DaunoXome) [221]; and liposomal doxorubicin (Lipodox), drug delivery of the anthracycline-containing liposomes has been extensively studied. These anthracycline-liposomes can transport high doses of anthracyclines to tumor cells selectively without damaging healthy areas like heart cells, which is useful for highly permeable vasculature tumors, such as Kaposi's sarcoma. This is because of their extended bloodstream circulation period and strong encapsulation and retention efficacy [222,223].

Furthermore, the liposomes' small size enables them to escape via the tumor's surrounding faulty vasculature and gather in great quantities in diseased tissues. [224]. The amount of free drugs at the cancer site will be below ideal since DOX stays in a crystalline state and is not available till it is encapsulated in liposomes. [225]. In multiple investigations, liposomes and ultrasound have been used together. Drug and gene absorption is improved by low-frequency ultrasound (LFUS), which increases the permeability of the membranes [226].

The LFUS administration also increases the permeability of the liposomes, which raises the rate of content release since the bilayer composition around the liposomes is similar to that of the cells. The LFUS (20 kHz) has been shown in numerous in vitro investigations to improve drug release from the liposomes. Combining ultrasound with the liposomal cytostatic drugs to treat tumors has been shown to have improved therapeutic advantages in in vivo trials [227,228] (200,201) [229,230]. The lipid composition of liposomes affects their sensitivity to acoustic waves. Because the membrane is irreversibly disrupted during sonication, DOPE-based (fluid) liposomes are extremely sonosensitive as well as efficiently release their payload, whereas HSPC-based (solid) liposomes have a lower sonosensitivity. In addition, after 6 minutes of sonication, DSPE-based liposomes emitted more DOX than DSPC-based liposomes (69% and 9%, respectively) [229]. After 6 minutes of sonication, liposomes made from DSPE-PEG2000/DOPE/DSPC/cholesterol (8:25:27 40 mol%) discharged 70% of their contents and had longer circulation durations [231].

The internal insertion of a gas can further improve the liposomes' sonosensitivity. Perfluorocarbon nanodroplets can be encapsulated in emulsion liposomes, or eLiposomes. [232]. The ultrasonic wave's

energy causes the gas to expand and evaporate, which ruptures the liposomes (drug release) and encourages sonoporation (drug absorption). At physiological temperatures, emulsion liposomes remain stable [233]. (204) eLiposomes have been destroyed by sonication for 15 min (1 W/cm2). [234]. After sonication, free DOX infused with eLiposomes dramatically reduced osteosarcoma cell proliferation, producing comparable effects to the free DOX at a substantially lower dosage (1/5 times the dose of free DOX) [235] (206).

When exposed to low temperature, thermoresponsive liposomes—which are quite sensitive to temperature increases—can be activated to unload their cargo. Despite reaching Phase III clinical trials, thermodox, temperature-sensitive liposomes (TSLs) containing DOX, were unable to achieve the main goal of a 33% increase in progression-free survival [236]. TSLs transition from a solid-like phase/structure to a liquid-crystalline phase/structure when the local temperature reaches their membrane solid to fluid transition temperature (Tm), at which point they release their payload [237]. This is a type of triggered release since cancer tissues are frequently warmer than healthy tissue.

For regulated medication release, there is a desire to use ultrasound with TSLs whose Tm is marginally greater than physiological temperatures. Medium frequency ultrasound often produces a slight increase in local heat (mild hyperthermia); by regulating the duty cycle, overheating can be prevented [238]. In contrast to TSL-DOX alone, Farr et al. [239] found that temperature-sensitive liposomes encapsulating DOX (TSL-DOX), which were induced by low hyperthermia created by controlled HIFU, enhanced DOX uptake by cancerous pancreatic cells of mice by a factor of 23. Patients with breast cancer were treated with TSL-DOX in conjunction with microwave and ultrasound triggering, which resulted in improved (48%) local response [240].

Recently, in a clinical experiment [241] (212), DOX was administered to patients with liver tumors using thermosensitive liposomes and targeted ultrasound. The study found that whereas HIFU-triggered TSL-DOX improved medication delivery to the cancer sites, the thermal ablation caused by HIFU did not have any negative consequences. A short summary of research on the acoustically triggered medication release from the liposomes is provided in Table 3.3 below.

7.1.7 Liposomes–microbubble conjugates triggered with US

Microbubbles of contrast agents are frequently utilized in diagnostic imaging. These improve blood-to-tissue contrast by enclosing a low-solubility gas in an outer shell made of albumin or a lipid monolayer. [89]. Both drug delivery and gene therapy may benefit from the use of microbubbles [84,266]. Perfluorocarbon (PFC) or SF6 gas is typically present in microbubbles, which have a diameter of 1–2 μm. Because the encapsulated gas's density differs greatly from that of the surrounding medium, the lipid-shelled microbubbles are highly echogenic and more pressure sensitive, created by the ultrasonic waves. This makes it simple to manipulate the microbubbles using the acoustic-radiation force [267].

When ultrasonic pressure (MI $>$ 0.3–0.6) is applied to microbubbles, mechanical stresses will be created as the microbubbles quickly grow, compress, or collapse. Via sonoporation, this oscillation or collapse produces a local microjet or microstreaming, which leads to membrane permeability. This will greatly improve the delivery of genes and drugs to the cells [267]. Hydrophobic medications can be administered via microbubbles inside the lipid monolayer that surrounds them for protection [268]. (240) However, the efficiency of employing microbubbles in medication delivery is generally limited by the limited area available for the pharmaceuticals within the gaseous core and the surrounding shell.

Table 3.3 Studies investigating the acoustically triggered drug release from liposomes.

Type of study	Encapsulated substance	US parameters	Effect	References
Both in vitro and in vivo TS/A	DOX	3 MH (3.3 W/cm^2)	Better therapeutic results due to the higher drug diffusion in the cancer following sonication.	[242]
Both in vitro and in vivo studies involving murine models of adenocarcinoma cancer	DOX	1.189 MHz (4 W/cm^2)	Combining HIFU with TSLs improved DOX delivery.	[243]
In vivo involving breast cancer murine models	DOX	1.54 MHz	Tumor elimination after the treatment with US-triggered liposomal copper-DOX, CuDOX-LTSLs.	[244]
In vitro study	Calcein	20 kHz (5 W/cm^2)	After the sonication, calcein unloading was significantly better from eLiposomes as compared to the conventional liposomes.	[245]
Phase-I trial	DOX	0.96 MHz	TSL combined with focused US hyperthermia is very safe and feasible	[241]
In vitro study involving EMT6 cells	DOX	15 kHz (20 W/cm^2, 10 W/cm^2, and 10 μW/cm^2)	Better DOX release from the temperature-sensitive liposomes activated with both non-thermal and thermal acoustic treatment.	[246]
In vivo study involving FaDu cells	DOX	1.2 MHz	Combining TSL and focused US enhanced drug delivery	[247]
In vitro study involving MDA-MB-231 and MCF-7	Calcein	20 kHz (6, 7, and 12 W/cm^2) and 40 kHz (1 W/cm^2)	Calcein uptake by the tumor was improved following sonication.	[248]
In vitro study involving melanoma cells	DOX	1 MHz (2 W/cm^2)	Following the US use, DOX liposomes having microbubbles were very effective in the tumor elimination.	[249]
i- vivo study involving (GBM) 8401	DOX	1 MHz (2.9 W/cm^2)	Sonication improved the drug delivery of the AP-1 Lipo-DOX, leading to the inhibition of the tumor growth in the brain.	[250]

Continued

Table 3.3 Studies investigating the acoustically triggered drug release from liposomes.—cont'd

Type of study	Encapsulated substance	US parameters	Effect	References
In vitro study	Calcein	1 MHz	The mechanical stresses caused by the focused US improved drug release from the TSLs.	[251]
In vivo study involving 9L gliosarcoma cells	DOX	1.7 MHz	Liposomal DOX's therapeutic impact in the brain was enhanced when paired with US-mediated BBB breakdown.	[248,252]
In vivo study involving HeLa cells	DOX	1 MHz	When paired with TSL liposomes, HIFU dramatically reduced tumor regression.	[253]
In vivo study involving DHD, K12, and TRb	DOX	20 kHz (1 W/cm^2)	Tumor growth was lessened when US, along with DOX liposomes, were mixed than when DOX was administered by micelles.	[254]
In vitro study	Calcein	20 kHz, 1 and 3 MHz with different power densities	At the low frequency (20 kHz) under investigation, calcein release was greater.	[255]
In vivo study involving prostate cancer cells	DOX	1 MHz and 300 kHz	The tumor absorption of DOX from sonosensitive liposomes increased with both frequencies.	[256]
In vivo study involving Vx2 tumor cells	DOX	MR-HIFU	After sonication, rabbits' Vx2 tumors received 3.4 times more DOX from LTSL than from LTSL alone.	[257]
In vitro study involving MDA-MB-231 & MCF-7	Calcein	20 kHz, 1.07 & 3.24 MHz	The cells' increased absorption of calcein was seen after exposure to LFUS.	[258]
In vivo study involving U87 MG	PTX	1.1 MHz	When PTX-liposomes and US were combined with microbubbles, the drug's effectiveness against glioblastoma was increased.	[185]
In vivo study involving rat gliosarcoma cells	DOX	690 kHz	The US improved liposomal DOX delivery from pegylated liposomes.	[259]

In vitro study involving the HeLa cell line	DOX	300 kHz	DOX absorption by cancer cells was improved by sonicating DOX liposomes and microbubbles.	[260]
In vitro study involving KB-V1 (DOX resistant) along with KB-3-1 (DOX sensitive) cell lines	DOX	20 kHz (1 W/cm^2)	The US released 78% of the DOX that was encapsulated in folate eLiposomes (feLD). The survival of both cell lines after feLD therapy was unaffected by US.	[261]
In vitro/in vivo study involving IGROV-1 cells	Topotecan	1.3 MHz	Better absorption of TSLs and topotecan as a result of the targeted US therapies.	[262]
In vivo study involving cell line LM8	DOX	1 and 2 MHz (0.5 and 2 W/cm^2)	When bubble liposomes were present, sonication greatly enhanced the therapeutic efficacy of DOX.	[235]
In vitro study involving the HeLa cell line	DOX	20 kHz (1 W/cm$_2$)	In contrast to liposomes without emulsions, eLiposomes showed a greater release of DOX after sonication.	[263]
In vitro study involving 293T cells	DOX	2 MHz (0.25–1 W/cm^2)	A US-enhanced drug release was seen when DOX liposomes and bubble liposomes were combined.	[264]
In vitro study	Calcein	20 kHz (6, 7, and 12 W/cm^2)	After being exposed to LFUS, HSA-PEG and Tf-PEG liposomes showed greater sonosensitivity than the control pegylated liposomes.	[265]

Microbubbles can be mixed with other therapeutically loaded nanocarriers to increase their load capacity. Drug-encapsulating liposomes, for instance, can be coupled to the microbubbles' surfaces. These liposome-microbubble conjugates exhibit potential as drug delivery carriers for solid tumors when combined with ultrasound-triggered drug release directed by ultrasound imaging. Radiation forces can be used to spatially position microbubbles at low intensities [269]. The microbubbles' oscillation grows with increasing ultrasonic intensity, which ultimately causes the microbubbles to completely collapse and be destroyed.

The permeability of cancer cells and the vasculature around them is increased by these physical impacts, which improves the absorption of medications that are released close to the target cells [154]. The conjugated liposome's structure within 40 μm is typically disrupted by the pressure generated by the collapsing microbubble [154].

Both covalent thiol-maleimide linkages and biotin–avidin linkers could be used to bind drug-coated liposomes to the surface of microbubbles [268,270]. Conventional ultrasound contrast imaging modes can be used to trace microbubbles once they have been introduced into the bloodstream. High-intensity therapeutic ultrasound starts the destruction of the microbubbles, the release of the medication, and the sonoporation of the tissues in the acoustic field after the liposome-microbubbles have accumulated at the cancer site [271,272]. A recent in vivo study by Zhang et al. [273] (244) found that iRGD-targeted PTX-loaded liposomes were bound to the surface of microbubbles using biotin–avidin linkages.

The produced iRGD-PTX liposome-microbubble complex demonstrated active US-triggered drug release while maintaining its ultrasound imaging capabilities. According to histological analysis, tumor xenografts had more apoptotic tissues and higher medication concentrations inside the cancer cells, which inhibited the growth of the tumor. In contrast to both the non-conjugated liposomes and non-sonicated tumors, another in vivo investigation by Yan et al. [274] revealed improved antitumor activity due to sonication of breast cancer cells after previous injection with PTX-loaded liposomes conjugated to microbubbles.

According to Yu et al. [275]; almost 70% of the DOX loaded inside liposomes coupled to polymer microbubbles was released by noninertial ultrasound exposure. According to the study, ultrasonic exposure enhanced the cancer cells' uptake of DOX and, at 48 hours, decreased their growth.

7.1.8 Nanocarriers used in drug delivery

Numerous nanocarriers with sizes ranging from around 10–1000 nm were developed in response to the urgent requirement to enhance biodistribution, pharmacokinetics, as well as therapeutic ability of anticancer medications. These nanocarriers have now received multiple clinical approvals. The in vivo biodistribution of these nanocarriers is significantly influenced by their size. Particles greater than 220 nm typically gather in the spleen, liver, and the bone marrow, but particles smaller than 5 nm are quickly removed from the blood [276](12,13). Most of the items utilized in the clinic are approximately 60 and 200 nm in size [277].

An essential feature is long circulation half-lives, and nanocarriers need to avoid being eliminated by biological processes like mononuclear phagocytosis. Additional crucial characteristics include: (1) safety and biocompatibility; (2) effective drug loading; (3) effective drug delivery to specific body regions; and (4) absence of immunogenicity [278]. Over the last 20 years, the nanocarrier field has been expanding steadily. Tumors and infections have been treated with a variety of nanoparticles that vary in size, composition, shape, and physical characteristics [279].

Nanocarriers can be made of inorganic materials like carbon nanotubes, gold nanoparticles, MSNPs, superparamagnetic iron oxide NPS, MOFs, and quantum dots, or they can be organic (like micelles, liposomes, and dendrimers) [191]. Table 3.4 lists the benefits as well as drawbacks of the various nanocarriers that are depicted in Fig. 3.2 and will be covered in more detail below. Liposomes and other lipidic nanoparticles have been the most effective therapeutic products to date. Fig. 3.2. Nanocarriers could be composed of inorganic and/or organic materials. This figure represents the kinds of organic and inorganic NPs discussed in this paper.

7.1.9 Passive targeting of the cancer cells by nanocarriers

Numerous uses for nanocarriers have been discovered in medication delivery, most notably in the management of inflammation, infection, and cancer. These nanocarriers' passive (or nonselective) targeting of tumors through the "improved permeability and retention" (EPR) effect is linked to their potential for treating cancer. This happens when tumors undergo angiogenesis, which produces new blood vessels to meet their high oxygen and nutritional needs. Tumor tissues have a disordered, leaky capillary endothelium that has poor lymphatic drainage and endothelial cell coverage [284]. (19) The tumor's abnormal molecular and functional dynamic behavior results from this. If the circulation half-life is long enough, the small size of NPs enables them to extravasate via leaky vasculature and collect at the tumor site (Fig. 3.3).

Grafting polymers like PEG chains to the particle surface is a popular method for extending their blood circulation times. This allows the particles to enter the circulation of the tumor and extravasate to the interstitial space at higher concentrations [285] (area with better angiogenic blood vessels). Some cancers have very variable vascular permeability, while others exhibit intrinsically poor drug accumulation due to the EPR effect [286]. PEG is very soluble in organic solvents and water due to its nonionic nature. Hepatic and splenic macrophages quickly remove conventional liposomes—ones which are not grafted with PEG or comparable polymers—from the bloodstream by the opsonization process, in which they interact with certain proteins (opsonins) in the bloodstream.

The delivery of PEGylated particles (also known as "stealth" liposomes) to the tumor site through the blood circulation (also known as "passive" targeting) does not, however, guarantee that the cancer cells will absorb the nanoparticles. Normal uptake mechanisms can absorb drugs released from the NPs in the tumor cells, for example, by diffusion through the NP membrane. Therefore, passive targeting mechanisms should be improved.

7.1.10 Ligand-mediated targeting of tumor cells by nanocarriers and passive-triggered release

The absorption of nanocarriers into cancer cells has been considerably increased through the application of tumor-targeting ligands. Targeting sites that attach to tumor-specific surface ligands are conjugated to the surface of the nanoparticle to achieve this. Ligand-targeted nanocarriers are made to attach to the specific or over-expressed receptors and cause the targeted nanocarriers to internalize into the tissues. Several cancer types are already being targeted by molecules like hormones, proteins, aptamers, peptides, antibodies, and oligonucleotides [14]. Until the carrier and its coated drugs are transported to the specific site, the targeted NPs are made to contain their drug molecules.

At that time, the most efficient method for increasing the drug concentration in the cancer cell is a stimulus-triggered burst release of the payload rather than a gradual release of the medications from the NPs via passive diffusion [154]. A nanocarrier with a low release rate can become unstable and

Table 3.4 Pros and cons of NPs used in drug delivery [280–283].

Nanocarrier	Pros	Cons
Micelles	Biodegradable, biocompatible, smaller size promotes extravasation, possibility of controlled drug release, surface functionalization, protects the drug from the environment	Low drug-loading capacity, used only for lipophilic drugs, difficulty upscaling, fast release
Liposomes	Biodegradable, low toxicity, biocompatible, small size promotes extravasation, amphiphilic, restricted release of drugs, surface functionalization, protects the drug from the environment	May induce an allergic response, are costly to produce, need special storage, phospholipids can undergo hydrolysis and oxidation
Nanoemulsions	Stable for a longer time & upon dilution, no sedimentation occurs during storage, possibilities of controlled drug release and drug targeting, carries both lipophilic and hydrophobic drugs, nontoxic	Stability influenced by environmental parameters, toxicity of some surfactants, high-cost chemicals,
Gold nanoparticles	Tuned biodistribution and pharmacokinetics, better surface area, better loading, ease of synthesis and conjugation of different bioactive agents, biocompatible, control of particle size	Not biodegradable, toxicity, costly, bigger scale production, nanoparticle aggregation, difficult to synthesize homogeneous formulations
Iron oxide nanoparticles	Uniformity in size & shape, antifungal and antibacterial properties, stability	Costly large-scale production, high toxicity, nanoparticle aggregation
Carbon nanotubes	Soluble in water, efficient loading, biocompatible, better surface area, ease of conjugation, and production of multiple bioactive agents, sustained release	Potential material toxicity, non-biodegradable
Mesoporous silica nanoparticles	large surface area, biodegradable, high stability, biocompatibility, surface reactivity, controllable, and better porosity, easy functionalization	Nanoparticle aggregation, very toxic

release drugs at a fast rate due to the triggering mechanism. The special properties of the tumor's microenvironment can be used to trigger this medication release.

Low tumor pH, high production of tumor enzymes, temperature variations between the healthy tissues and the tumor, and other factors are intrinsic to responsive targeted drug delivery. Additionally, nanocarriers can be made to react to the external stimuli including light, magnetic fields, ultrasonic, even [287] magnetic fields [255]. The three primary elements of targeted medication delivery using nanocarriers are depicted in Fig. 3.4, and Table 3.5 lists the pros and cons of these various triggering methods.

7.1.11 Active targeting of tumor cells by the external triggering of nanocarriers' drug release

It is possible to design nanocarriers reactive to electromagnetic fields like light [287] and magnetic fields, as well as "external stimuli" like ultrasonic [255] and more [290]. Although ultrasonography is the main topic of this review, electromagnetic field triggers are compiled here for completeness. Metallic NPs, such as gold nanoshells and nanorods, react with visible and NIR light to produce considerable heating. As the particles are lit, the medication is released due to changes in the surrounding polymer brought on by hyperthermia. Mesoporous silica covered with a polyurethane-amine coating that is responsive to heat was applied to gold nanorods in a recent study of release from such nanocarriers [291].

The polymer was used to encapsulate the porous silica after it had been loaded with doxorubicin (DOX). The DOX was released after being illuminated with 808 nm NIR light. In a different application, clusters of gold nanoparticles were attached to the surface of temperature-sensitive liposomes

Table 3.5 Pros and cons of different external stimuli used in drug delivery [238,288,289].

Stimulus	Pros	Cons
Magnetic field	Deep penetration, no ionizing radiation, AMF energy modulation, better opportunity for imaging	Limited to surface tumors, expensive equipment, accumulation can cause embolus formation and/or higher cytotoxicity
NIL and visible	High spatiotemporal precision, no radiation exposure side effects, easily tuned, minimally invasive, inexpensive,	Limited penetration in tissues (can be enhanced by NIR)
Ultrasound	Easily accessible, non-invasive, non-ionizing radiation, spatiotemporal control, better acceptability among the patient, low cost,	Hard to irradiate moving targets, depth limit (cm), difficulty of homogeneous use to large volumes, can cause temperature to rise
X-rays	High spatiotemporal precision, deep penetration possible, easily tuned	Ionizing radiation, costly equipment
Microwave radiation and RF	Easy production and control, non-invasive, no ionizing radiation	Penetration is limited, causing the temperature to rise

that contained DOX. In a mouse model, the hyperthermia caused the liposome to rupture as well as release the DOX when the liposome was illuminated (808 nm) [292]. To enable the hyperthermic release of medications from a variety of carriers, magnetic nanoparticles (MNPs) are locally heated using alternating magnetic fields (AMF) [293]. Although the electromagnetic fields' oscillation frequencies are usually innocuous to tissues, they can cause local heating when they pair strongly with magnetic particles. One of two general methods is used to provide controlled medication release.

The first is the breakage of bonds by a carrier at or close to the surface of a nanocarrier made of magnetic nanoparticles under extreme heat [294,295]. The second is heat-induced modifications to the medicine's permeability that are retained around the MNPs, such as thermally sensitive hydrogels that encircle superparamagnetic iron oxide nanoparticles (SPIONS) like poly-N-isopropylacrylamide (pNIPAAm) [296]. One benefit of these technologies is that AMFs can penetrate tissue deeply. The size and position of the electromagnetic coils, as well as the inability to precisely focus on the targeted tissue, are drawbacks. The targeted delivery of MNPs to the cancer location is one of the additional difficulties.

Scientists have been interested in the directed heating of particle-free cells since modest concentrations of iron oxide particles are not thought to be harmful. Increasingly precise direct heating of cells can be accomplished through the use of radiofrequency and microwave beams [297]. The coupling of such direct fields with thermally triggered drug delivery has long been anticipated; however, bond breakage is not possible due to the mild temperature increases (40–44 °C). These temperature excursions, however, are adequate to rupture thermally sensitive liposomes and activate nanocarriers with pNIPAAm reservoirs or barriers. These applications are still far from being used in therapeutic settings.

Ultrasound and direct electromagnetic heating compete without using MNPs to heat the tissues. As will be discussed in the following section, ultrasound offers greater spatial control but has certain restrictions on the area of heating that can be used for controlled medication administration.

7.1.12 Uses of ultrasound-mediated drug delivery systems

Worldwide, cancer is one of the main causes of morbidity and death. In addition to the availability of chemotherapeutic medicines, low absorption and adverse effects are significant issues in cancer treatment. By employing a targeted medication delivery strategy, side effects from cancer chemotherapy can be reduced. When a solid tumor is removed surgically, the cancer cells are managed using various techniques, such as chemotherapy, radiotherapy, immunotherapy, etc., based on the severity of the condition. On the other hand, chemotherapy is the sole available treatment option for metastatic cancer [298].

The main causes of chemotherapy failure include its toxic nature, large dose requirements, and nonspecific cell targeting [85]. Liposomes and site-specific microbubbles are examples of nanocarriers used in ultrasound-based medication delivery for tumors.

7.1.13 Liposomes as a drug delivery system for cancer

Numerous therapeutic applications have made use of liposomes as carriers for drug delivery. Both conjugated and encapsulated forms of the medication can be put into liposomes. Due to their generic mechanism of action and inability to target the right cells, chemotherapeutic drugs, in particular, suffer from substantial toxicity when used in cancer therapy.

Liposomes are therefore recognized to function as superior carriers, which can raise medication selectivity and decrease drug toxicity. The field of cancer therapy is also known for ligand-based drug delivery, which is very selective to the target cells [34]. Two methods can be used to deliver liposomes into cells:

1. Passively targeted delivery: The increased retention and permeability effect is how the medications are passively targeted in the tumor environment. The leaky vasculature in the cancer tissues is a characteristic of the EPR effect. Leaky vasculature can have pore sizes ranging from 100 to 780 nm, although typical vasculature porosity among epithelial cells can be between 5 and 10 nm. Due to diffusion, small molecular weight medications cannot be held in the tumor microenvironment; this issue is resolved by encapsulating them in liposomes. The phrase "passive delivery" refers to the drug's targeting method in tumors, which is entirely dependent on the pathophysiological characteristics of the tumor and normal cells. Medication administration driven by ultrasound enables noninvasive medication penetration deep within the tissue. Thermal energy produced by ultrasound causes the blood vessels' membranes to become more permeable. This enables the passage of drug carriers past the barriers, for instance, US could be used to raise the permeability of blood blood-brain barrier (BBB) in brain tumors, allowing the medicine to reach the specific malignant tissue [85].
2. Actively targeted delivery: The main goal is to lessen side effects. This involves targeting receptors such as folate receptors (FR), which are highly expressed on the surface of cancer cells, with a specific moiety such as a ligand, peptide, monoclonal antibody, or chemotherapeutic medication. Certain cancers of the kidney, breast, lung, colon, ovary, and brain have been shown to overexpress FR. Three forms of active delivery are used: tumor microenvironment targeting, intracellular delivery, and cell surface targeting [299].
   a. Cell surface targeting: Eosin, progesterone, and human epithelial-cell receptors (HER-2) are among the receptors on the cell surface that are necessary for the drug delivery mechanism to work in the case of breast cancer. Breast cancers are characterized by an overexpression of HER-2. Depending on the type of cancer cell line, liposomes are loaded with an antibody that targets any of these receptors. Although cell surface targeting is an excellent strategy for addressing breast cancer, medication delivery has proven difficult for triple-negative cell lines, which lack the aforementioned three receptors [85].
   b. Targeting the tumor microenvironment: This approach has more benefits than cell membrane targeting because it targets tumor vasculature, which is not specific to any one type of tumor. For example, it targets vascular endothelial growth factor (VEGF) as well as its receptors, which are known to promote angiogenesis plus targeted for antiangiogenic therapy [300].
   c. Intracellular delivery: The effectiveness of drugs relies on how they interact with various cellular components, such as the endosome, mitochondria, lysosome, etc. Some liposomes exhibit endocytic activity, are phagocytosed by endosomes, and are then released to the lysosome, which contains lipase. This causes liposomes to degrade and release drugs that are trapped or encapsulated in a particular form. Since the medication only targets cells, this lessens the off-target effect [301].
   d. Organelle-specific targeting: To decrease the side effects and extend the therapeutic window, medicines are delivered directly into the sick tissue. Additionally, it reduces the possibility of therapeutic cargo deterioration. These therapeutics are transported by liposomes into

lysosomes, which function as the cell's digestive organelles. The liposomes are digested to transport the cargo [302].

An excellent illustration of an organelle-specific targeting system is the nuclease that targets liposomes using nucleosome particles. This idea uses two-step targeting to deliver a short-range auger electron that is specifically designed to release radionuclides to the tumor cells' nuclear DNA [303]. The delivery of the radioisotope to DNA is facilitated by the PEGylation of the liposomes with a particular kind of DNA-intercalating chemical that was directed toward the cancer cells. It has been demonstrated that this method delivers the medication with the least amount of side effects [304].

7.1.14 Microbubbles in the cancer drug delivery

By creating permanent or temporary holes in the membrane of a blood vessel, microbubble-mediated ultrasonic drug delivery increases the extravascular distribution of medicinal chemicals to the intended organ.

There are two methods for creating cavitation: creating inertial cavitation, in which microbubbles expand and collapse at random, or creating stable cavitation, in which microbubbles oscillate steadily without dissipating in an acoustic field. Therapeutic substances can be delivered using sonoporation without compromising the physiological barriers or defensive mechanisms of the cells. Since the pores formed by sonoporation are transient and typically recover to their original shape in a matter of seconds, it has been determined to be a safe technique [154].

7.1.15 Alzheimer's disease

AD is an irreversible neurological illness that progresses over time and requires ongoing care. Neurofibrillary tangles, neurotransmitter deficiencies, β-amyloid plaque, and neuronal loss are all consequences of AD. Only medications that increase neurotransmission are available to treat AD. The BBB, which restricts the drug molecules' ability to enter the brain, presents a challenge in the delivery of such medications to the brain [305].

FUS by itself causes tissue injury and hemorrhage by enlarging the BBB's pores, although microbubble-mediated ultrasonic focusing can address this problem. Targeting the circulation with microbubbles lowers the acoustic power needed to open the blood-brain barrier, which is significantly less than the power needed to cause tissue damage. Microbubbles that oscillate continuously create temporary and safe holes in the BBB [306].

Using microbubbles-mediated US administration, there are primarily four ways to move medications or other therapeutic molecules across the BBB: Tight junctions in endothelial cells can be mechanically disrupted, molecules can be carried by the receptors on endothelial cells (a process called transcytosis), endothelial cells' cytoplasmic tight junctions can be opened, and endothelial lining damage can occur. Different imaging techniques are connected to US-mediated drug delivery systems, like MRIgFUS, allowing for controlled drug delivery based on the tumor area, as in Parkinson's disease, to increase controlled targeting and decrease off-target effects in AD and other brain disorders [307].

The medication only affects a tiny portion of the brain, like in the case of AD, and does not affect the other areas. By improving the effectiveness and efficiency of direct medications, this enables the targeting of a particular region and lowers the toxicity for other brain regions [305,308].

7.1.16 Transdermal drug delivery

The most noninvasive and painless method of drug administration is TDD, which allows the medication to be delivered topically to healthy normal skin from deep within the dermis and epidermis. The medication is absorbed by dermal microcirculation in the dermis. In TDD, the drug's pharmacokinetic profiles are consistent, reducing the likelihood of harmful side effects. By preventing presystemic metabolism, TDD increases the drug's bioavailability [309].

Since dendritic cells found in the epidermis as well as the dermis layer are essential for producing an immune response, TDD is the recommended immunization method. Numerous needle-free vaccines have been created as a non-invasive drug delivery strategy [310]. Sonophoresis, also referred to as phonophoresis, is the process of delivering drugs via the skin using ultrasonography. For passive drug delivery, molecules must have a molecular weight of little more than 500 Da to overcome the stratum corneum, the most crucial barrier in TDD that varies depending on the body's location. Because ultrasound increases the skin's porosity, it improves TDD.

It has been demonstrated that, in comparison to high frequency ultrasound (13 MHz), low frequency ultrasound (20 kHz) offers very effective TDD across aqueous channels in the stratum corneum [311]. An electric signal that has been amplified and transmitted to an ultrasonic horn produces ultrasound waves. Piezoelectric crystals in the transducer tip transform the electric impulses into mechanical waves as soon as they reach the ultrasonic horn, and these waves are then transmitted to the intended medium. Ultrasound waves' most important properties are their frequency and amplitude. Low-frequency sonophoresis (20–100 kHz) and high-frequency sonophoresis (0.7–16 MHz) are the two most commonly used frequencies for sonophoresis [31].

The effectiveness of sonophoresis is dependent on the ratio of the duration of US exposure, also known as the duty cycle of US, the distance between the horn and the skin, the length of therapy, and the coupling medium's composition. For the topical administration of lidocaine, an anesthetic medication, SonoPrep is a sonophoresis system that is now on the market and has received FDA approval [31].

It is also reported that sonophoresis increases the skin's permeability through cavitation or heat effects. A combination of sonophoresis and microneedle-based technologies was created to improve medication penetration efficiency and achieve a controlled-release system. Research is also being done on the use of transdermal insulin delivery in diabetic patients [310].

7.1.17 Pulmonary diseases

There are two different methods for delivering drugs to the lungs: topical treatment of the pulmonary tract, involving the application of the drug topically to the respiratory tract, and systemic delivery, which involves the drug being absorbed and carried by the blood circulation. A large amount of medication can be inhaled using ultrasonic nebulizers. This ultrasonic nebulizer creates an aerosol by vibrating a piezoelectric crystal that is porous at a high frequency (1.3 MHz) [312].

The two types of ultrasonic nebulizers are the big volume nebulizer, which is mostly used to administer a hypertonic saline solution for induction of sputum, as well as the small volume nebulizer, which is used to administer inhaled drugs [313]. Since the ultrasonic nebulizer cannot aerosolize heat-sensitive compounds, viscous solutions, or protein medications, it cannot be utilized to deliver suspensions or proteins [313].

7.1.18 Cardiovascular disease

In the cardiovascular disorders case, microbubble-mediated ultrasound therapy may be used to deliver medications or genes. By destroying the microbubble and releasing the drug at that specific location, using the microbubble as a delivery system increases the local concentration of the drug. It also increases the biological barriers' permeability, which is very helpful for delivering genes or DNA because it makes the transfection process easier [314]. The barriers found in the endothelium region make it extremely difficult to administer medications for the treatment of atherosclerosis, as well as other cardiovascular conditions, such as cardiomyopathy, congenital heart disease, and rheumatic heart disease [314].

Nanoparticle-assisted therapies, US-mediated drug delivery by gene therapy, plus stem cell repair of damaged valves and ischemic myome trium are among the treatments for these disorders [315]. Sonothrombolysis is the process of destroying vascular thrombosis, the cause of ischemic stroke, using ultrasonic waves. Although sonothrombolysis can also be caused by ultrasound alone, the addition of a microbubble intensifies the phenomenon because of cavitation. Low-energy US is intended to track the entry of microbubbles into the area of thrombosis as a result of cavitation, whereas high-energy US is used to produce sonothrombolysis [316].

Targeting endothelial epitopes for both therapeutic and diagnostic purposes. For instance, antibodies against p-selectin were developed for imaging ischemic memory, providing information about the presence of p-selectin epitopes in heart muscles following an ischemic stroke [316].

Microbubble destruction is also employed in myocardial contrast echocardiography. Unlike other liquids and vehicles, microbubbles can also be employed to carry oxygen. Gases like oxygen can be held in microbubbles more effectively. Because dodecafluropentane emulsion contains a lot more oxygen, it is employed as an ultrasonic contrast agent for oxygen distribution [314].

The effectiveness of the medication therapy is increased when ultrasound-triggered drug administration employing various drug carriers lengthens the period the drug circulates in the body and raises the amount of the medication in the targeted organ or tissue. It lowers the frequency of drug administration by controlling the drug's release in the tissue. Despite the potential that ultrasound-mediated medication delivery has demonstrated, there are still several barriers that stand in the way of clinical trials and clinical entry. Combining therapeutic and diagnostic molecules to create a theranostic molecule is a promising approach to disease targeting [317].

This will help with both disease treatment and tracking the disease's progression. Specific targeting may be aided by ligand-targeted delivery; for example, receptor-based targeting of chemotherapeutics lowers side effects in the instance of breast cancer. Another interesting method for theranostic applications is ultrasound-guided magnetic resonance imaging, in which magnetic nanoparticles can be put into an ultrasound contrast agent to produce contrast in magnetic resonance imaging. Therefore, despite its promise, ultrasound-triggered drug delivery systems must overcome clinical translation difficulties in order to be useful therapeutic tools [318].

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CHAPTER

Magnetic-responsive nanocarriers for biomedical application

4

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1. Introduction

Magnetism's association with medicine dates back to thousands of years ago. It is believed that Thales of Miletus, a Greek scientist, first applied magnetism to organisms around 600 BCE, leading to belief in their healing powers [1]. In the 14th century, Paracelsus hypothesized that magnetic material could draw diseases out from the body and in 1962, Freeman et al. suggested that magnetism would hold potential for medical diagnosis leading to clinical detection of cancerous tumors by MRI (Magnetic Resonance Imaging). Ugelstad, a chemical engineer in 1976 marked a significant milestone by synthesizing magnetic polymer particles by embedding iron oxide in a biocompatible polymeric matrix, allowing it to be safely manipulated by magnetic field, thus bridging the gap between magnetic nanoparticles and biomedical technology. But it was not until 1990s that research in pursuit of developing magnetic nanoparticles (MNPs) expanded, seeking to develop varied diagnostic and therapeutic approaches. The early use of this new concept involved guiding catheters for treatment of arrhythmias and orthodontic tooth movement. Since then, MNPs have been successfully employed as MRI contrast agents for imaging [2], in cell tracking, immunoassays for diagnostics [3], drug delivery [4] and cell separation [5]. Many MNPs and approaches are still being researched, while some have reached clinical trials [6].

Magnetic nanoparticles (MNPs) belong to the class of nanomaterials that embodies magnetic material like Iron (Fe), Cobalt (Co) and Nickel (Ni), possessing magnetic responsive feature and super para magnetism, in addition to the typical characteristics of a nanoparticle, i.e., 1–100 nm size, large surface area, ability to be surface functionalized, high drug loading capacity and controlled drug released. The magnetic core in MNPs is surrounded by a biocompatible shell imparting it high surface area, which can be surface functionalized allowing versatile biomedical applications and the drug/molecule to be delivered is either encapsulated inside the MNP, around the metallic core or ligated to its surface. MNP's physical properties like size and shape; chemical characteristics like surface chemistry and magnetic properties like magnetic remanence and coercivity influences their functionality. Thus, thorough characterizing post synthesis is essential to ensure safety hazards before administering

MNPs are fabricated in several forms including nanorods, nanowires, nanocubes using various physical methods like - wet chemistry or bottom-up techniques such as hydrothermal, sol-gel, thermal

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decomposition; chemical methods like coprecipitation, microemulsion and biological methods using extracted reducing agents from microbes [7]. Likewise, fine powders are produced by laser ablation and evaporation synthesis [8]. However, co-precipitation is the most common way to prepare MNP [9]. Iron oxide nanoparticles (IONs) such as Magnetite (Fe3O4), Maghemite (ϒFe3O4) and superparamagnetic iron oxide NPs (SPIONs) are the most widely employed MNPs due to their stability against oxidation, sensitivity, low-toxicity and non-immunogenicity.

The use of MNPs is attractive due to its bioavailability, enhanced deep tissue penetration, activation of cellular signaling pathways involving heat sensitive proteins, an approach called magnetogenetics [10], transporting drugs across biological barriers like blood brain barrier, localizing ligand conjugated MNPs to the target tissue, addressing off-target issue and enhancing therapeutic efficacy. Moreover, the paramagnetic characteristic causes the metal to be magnetized only when exposed to magnetic field and loses its magnetization upon withdrawal of external magnetic field, ensuring safe elimination of MNPs post desired activity in vivo, avoiding unwanted aggregation. This is crucial particularly for clinical utility due to reduced probability of aggregation when applied field is withdrawn [11]. Magnetic metal's magnetization property has currently led to significant applications in precision nanomedicine and other wide spectrum of biomedical applications. The magnetic moments of MNPs can be directed by high-frequency alternating magnetic fields, in a way that causes localized heating enabling hyperthermia for cancer treatment or generates voltage pulses that allows tracking nanoparticle's location invitro and in-vivo for diagnostic purposes (Magnetic Particle Imaging (MPI)). MNPs also allow loading of cells in scaffolds for tissue regeneration [12] and induce differentiation of target stem cells [13]. Adhering MNPs with antibodies targeting cancer associated markers can be used as biosensors. 'Lab on a chip', a term for external use of microfluidics which enables separating a cell using antibodies or protein markers [14]. With deeper studies and advancement, magnetic nanorobots have also been developed to perform minimally invasive surgeries, detoxification and eliminates biofilms in vivo [15]. Further, attempts have been made to utilize FDA-approved iron oxide NPs as a tool to diagnose and combat Covid-19 [16,17].

2. Structure of magnetic nanoparicles

The magnetic nanoparticles constitute magnetic core or inner matter embedded in the thin shell or outer covering, with a therapeutic layer around the magnetic core or ligated on the surface of shell. Thus, an MNP comprises of a magnetic core, surface coating/shell and functionalized groups onto the surface, each of which is of significant importance (Fig. 4.1).

2.1 Core

MNP comprises of a metallic core, allowing the nanoparticle to be manipulated by an external magnetic field. Core can be made of highly magnetic metals like Iron (Fe), Cobalt (Co) and nickel (Ni); magnetic oxides like iron oxides (magnetite—Fe3O4, and maghemite- ϒFe3O4); and bimetallic alloy combining two distinct metals.

2.2 Shell/coating of MNPs

It is a thin layer surrounding and stabilizing the magnetic core of nanoparticle. A variety of materials that are aqueous-soluble, stable, highly-bioavailable, biocompatible, non-toxic, degradable, non-

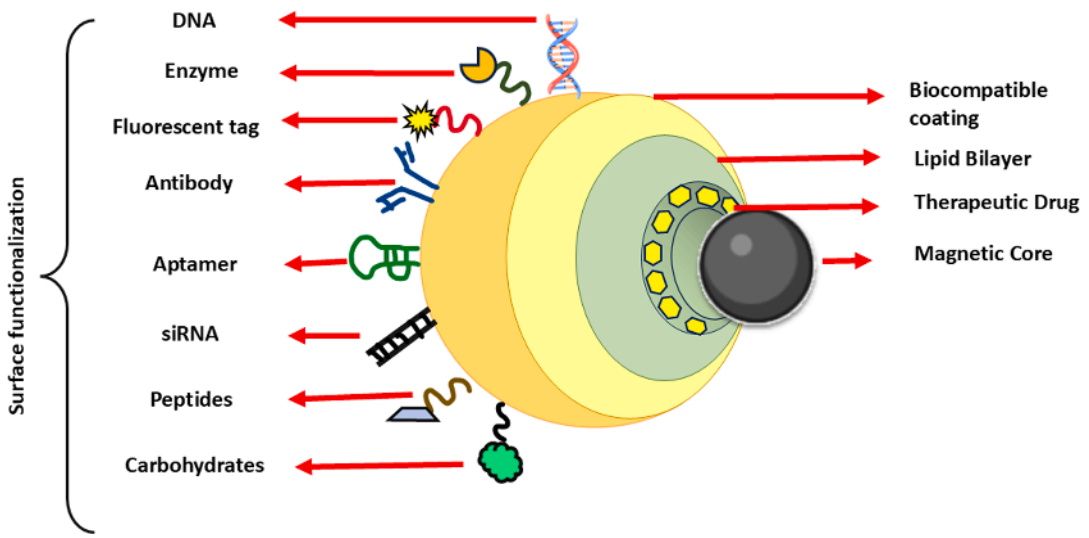


FIGURE 4.1

Illustrating structural components of a magnetic responsive nanoparticle.

immunogenic, and surface modifiable are used as shell/coating. Polymers, metal oxides, silica, small molecules and surfactants are the diverse array of material suitable for coating magnetic core. Coating of MNP with Polyethylene glycol (PEG), a Hydrophilic polymer makes the NPs more dispersible and prevents NPs aggregation by balancing the attractive and repulsive forces among them, which otherwise hinders NPs delivery to its site of action [18], causes thrombosis, induces Cytotoxicity if intracellular aggregates are formed and promotes cancer cell growth upon formation of extracellular aggregates [19]. Further, coating also allows us to deliver hydrophobic molecules and drugs/molecules with short half-life like Curcumin to its targeted location by employing proteins like Bovine Serum Albumin (BSA) as a shell [20]. pH sensitive coating material like Chitosan which is a cationic polyaminosaccharide, provides larger surface area and allows us to achieve higher cargo loading, controlled drug release and targeted therapies. To enhance the magnetic response, MNP can be coated with Dextran, a polysaccharide imparting superparamagnetic property to MNP [21,22]. Shell/coating is also necessary to functionalize the surface with specific molecules, in order to achieve desired adsorption, interaction and targeted delivery.

2.3 Functionalization of MNPs

It involves affixing various organic or inorganic moieties such as DNA, antibody, aptamers, saccharides, peptides, fluorescent tags, metals, metal oxides, metal sulphides, silica etc, covalently or non-covalently onto the surface of shell. This modifies its physical, chemical and biological properties making it suitable for versatile biomedical applications like targeted delivery of therapeutic cargo, diagnostics and tissue engineering. For example, functionalizing MNPs with Polyethylene glycol (PEG) conferred it stability, enhanced hydrophilicity and biocompatibility [23]. Moreover, PEGylated

nanocarriers exhibits increased antibacterial activity [24], decreased cellular toxicity of bare nanoparticles [25] and prevented immune clearance [26,27] and RES [28], thereby prolonging circulation time. Specificity can be achieved by immobilizing antibodies onto the shell, like MNP coated with anti-CD3 antibody exhibited stronger inhibition of T-cell mediated inflammation, when compared to MNP and antibody individually [29]. The ability of MNP in passing through the blood brain barrier for imaging and drug delivery has also been exploited by functionalizing the shell with folic acid [30], a tumor targeting ligand, fabricating contrast agents for MRI imaging; tagging FITC, a fluorescent probe for tracking cellular uptake and internalization [30]; and by conjugating lactoferrin (Lf), whose receptors are expressed on brain endothelial cells thereby promoting Lf-conjugated MNPs to efficiently deliver across the BBB [31]. Thus, functionalization has extended MNPs applications, holding immense potential in revolutionizing biomedical diagnostics and treatment.

3. Properties

MNPs besides possessing basic characteristics of nanoparticles i.e., nano size, stability, bioavailability, biocompatibility, ability to be surface functionalized, high entrapment efficiency, controlled drug release and targeted cargo delivery, also possess magnetic properties such as super para magnetism, saturation magnetisation, hysteretic magnetisation, slow relaxation time and blocking temperature.

3.1 Hysteretic magnetization

MNPs possess magnetic hysteresis, a phenomenon that occurs when MNPs are subjected to alternating magnetic field (AMF), causing magnetic material to retain its magnetization even after removal of the external magnetic field. It works by having the metal's atomic dipoles aligned with the external magnetic field, thereby increasing the metal's total magnetic flux which is dissipated in the form of heat. Hysteresis loss, occurs due to overcoming the hysteresis, resulting in energy being liberated as heat and relaxation loss, where either the entire particle rotates (in Brownian rotation) or magnetic moment within the particle rotates (in Neel rotation), are the mechanisms that dictate heat generation [32] allowing MNPs to achieve magnetic hyperthermia, a substitute for cancer treatment with no side effects. Hysteresis loss is dominant in Ferro/Ferri-magnetic NPs, while relaxation loss being the main mechanism in super papa magnetic NPs. The super para magnetism of MNPs causes them to be demagnetized after hysteresis when thermal fluctuations occur, which is very important when applied for biomedical application. Diameter, shape, crystalline anisotropy and degree of agglomeration, concentration and composition of MNPs are the factors affecting heating efficiency of Magnetic nanoparticles [33].

3.2 Ferromagnetism

It is a form of magnetism in which certain materials like Fe, Co and Ni display strong magnetism and exhibit hysteresis loop, where magnetization and demagnetization occur upon exposure to external magnetic field. Ferromagnetism arises under an external magnetic field, due to quantum mechanical effect, a phenomenon implying that spins of electrons and magnetic moments of nearby atoms are parallel leading to dipole alignment and net magnetization, even after removal of external magnetic field. This occurs particularly below curie temperature (Tc), which is specific to each ferromagnetic

material (Fe ~770°C, Co ~1131°C, Ni ~358°C). The alignment of spins is stable below Tc as the exchange interaction favoring parallel alignment is strong enough to overcome thermal motion, resulting in strong magnetism, but above Tc, the MNP loses its ferromagnetism and becomes Para magnetic as the thermal energy becomes dominant and fluctuates the spin orientation thereby disrupting the order of magnetic moments, causing the material to lose spontaneous magnetization and become magnetized only under magnetic field. Iron oxide nanoparticles are widely used among various types of ferromagnetic material, as they do not retain their magnetization when external magnetic field is removed. This property of Metallic core in MNP is exploited to improve various biomedical applications. For example, in enhancing imaging, the ferromagnetism of MNPs influence the local magnetic field, enhancing the quality of MRI imaging; Hyperthermia; targeted delivery by directing the MNPs to desired location and controlling the drug release by time variant magnetic fields

3.3 Super para magnetism (SPM)

It is a form of magnetism that occurs in small single domain ferro/ferrimagnetic NPs with diameter of less than 100 nm. Super para magnetism is the magnetic behavior in which magnetic material exhibits strong magnetization in the presence of magnetic field, but in the absence of which, electron spins become randomly disoriented causing drop in magnetic moment to zero [3]. Further, magnetization of NPs is considered as a single huge magnetic moment, the sum of magnetic moments of all atoms of the MNP. Super para magnetism is greatly dependent on diameter of MNP, type of magnetic metal, temperature and surface functionalization. SPM is a phenomenon where MNPs behave as paramagnets below its curie temperature. Blocking temperature (Tb), which is the temperature at which exchange interaction becomes zero, is one of the characteristics of super para magnetism. The metal displays hysteresis below its Tb while shows no hysteresis above Tb, which is the basis of super para magnetism. This hysteresis free, reversible magnetization and rapid magnetophoretic response makes this super para magnetic nanoparticles (SPMNPs) hold promise in varied biomedical applications like detection using immunoassays [3], gene delivery [4], MRI [2], cell tracking and mitigating neuron degeneration in CNS diseases [34].

4. Synthesis strategies

The synthesis of MNPs involves iron-based nanoparticles with various metals and a wide range of physical, chemical, and magnetic properties. These nanoparticles are produced through two groups of synthesis methods, categorized as bottom-up or top-down, falling into the physical, biological, or chemical techniques, both ultimately resulting in similar nanoparticles (Fig. 4.2).

4.1 Physical methods

The physical synthesis of magnetic nanoparticles mainly utilizes top-down approach but bottom-up method can also be used. Both approaches have their advantages and limitations. The choice between the two depends upon the specific requirement of the intended application of the MNPs.

4.1.1 Laser ablation

The laser ablation physical synthesis uses a high-energy laser to evaporate the solid target materials. In this method, the high-energy laser is focused on a specific target on the solid metal of which the

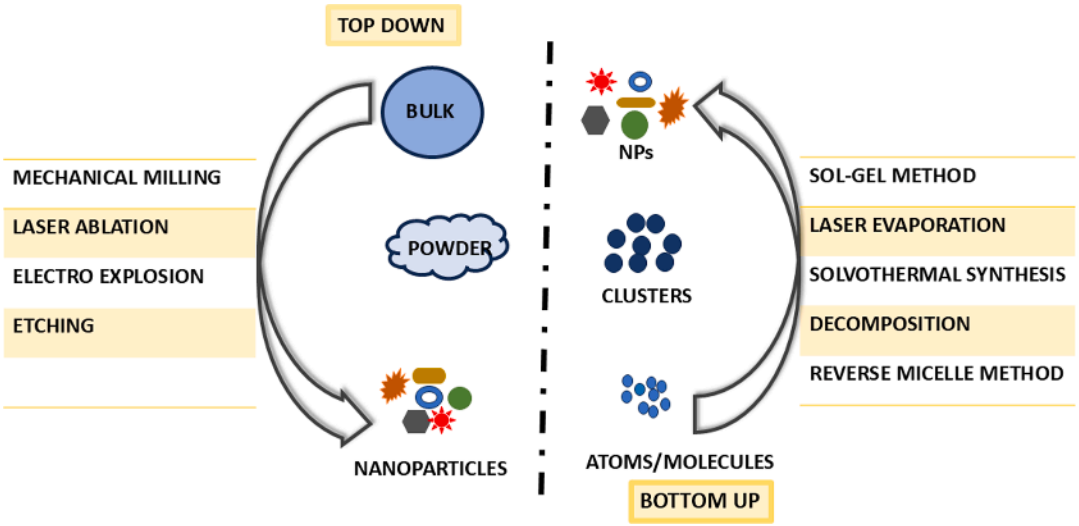


FIGURE 4.2

Representation of top-down and bottom-up approach for synthesis of MNPs.

nanoparticle is required, the laser vapourizes the material. The word "ablation" describes the process of removal of surface atoms and involves both single photon and multi-photon excitation. Laser ablation can produce high-purity nanoparticles because their purity of the particles largely depends purity of the target and the surrounding environment and eliminates the chances of contamination from the reactors. The major disadvantage of this method is controlling the size distribution, crystal structure, and agglomeration as nanoparticles are formed through random motion of molecules. Examples of nanoparticles developed by laser ablation are FeNi, CoPt, SiO2 TiOx [35].

4.1.2 Electric explosion of wire (EEW)

This Electric wire explosion is a less favored method of synthesis of nanoparticles, the procedure involves a thin metallic wire subjected to a short, intense electric current pulse. Initially, the wire melts and then disintegrates into evaporated atoms instantly, upon cooling they form spherical nanoparticles. Approximately 6 eV per atom of a current pulse is applied through the wire, which exceeds the specific heat required for evaporation of the respective metal. Several metals, including gold, silver, aluminum, iron, and nickel alloys, have been processed through this technique. The benefit of using this method is that it gives a perfectly spherical nanoparticle with high purity. The purity of nanoparticles completely depends on the purity of the metal wire used. The size of the nanoparticles can also be adjusted by changing factors simple factors like applied pulse energy, gas pressure, wire diameter and flow rate. The drawback of EEW method is that the fast-cooling of the nanoparticle preserves unstable oxides like Feo in the nanoparticles which affects the characteristics and application of the MNPs [36].

4.1.3 Mechanical milling

Mechanical milling is a cost-effective method for synthesis of nanoparticles from the bulk material. The process involves high-energy grinding tools. The process involves a high-energy mill having a

powder charge and milling medium, when the mill is rotated the balls impact the powder with kinetic energy to break them down [37]. There are a number of mills to carry out the process such as the shaker mills and planetary mills. Planetary mills consist of a rotating platform with many cylindrical containers attached to them. These mills involve both horizontal rotation around the container axis and rotation around the base center. The shaker mill includes high velocity oscillators which generates milling energy through oscillation of containers. The selection of the container material, powder to ball weight ratio, milling frequency and speed, and milling time all these factors are carefully regulated in the mechanical milling. The advantage of using the mechanical milling process is that it can be used to prepare a wide variety of MNPs in large amount, also the particle size can be controlled easily in this method. A major drawback of this method is the container may contaminate the nanoparticles altering their properties.

4.2 Chemical synthesis

4.2.1 Coprecipitation

The coprecipitation is a simple and most recognised technique for producing metal oxide and ferrate MNPs chemically. The technique is based on the simple principle of simultaneous precipitation of two or more metal ions in an inert alkaline medium at room temperature or higher. The coprecipitation synthesis have two main stages, nucleation and then the subsequent growth of the formed nuclei. The newly formed nanoparticles may form agglomeration, to prevent this, stabilizers like polyvinyl alcohol or trisodium citrate are used which increases the uniformity and narrows the variability in size of MNPs precipitated. The morphology, size, and dispersion of the precipitated particles can also be regulated by adjusting factors like pH, temperature and composition ratio to salt. For example, magnetite nanoparticles are synthesized by dissolving the nitrates, sulfates, or fe2+/fe3+ chlorides in an acidic aqueous solution. When a strong base like NH4OH or NaOH is added to this solution the mixture undergoes coprecipitation [38].

4.2.2 Thermal decomposition

In the thermal decomposition method, the metal precursor such as carbonyls, oleates, or acetylacetones are dissolved in the presence of high boiling point organic solvent such as benzyl ether or octadecane at temperature ranging between 150 and 300°C. The synthesis process consists of three steps, the process starts with constantly heating the solvent that have stabilizing agents, metalorganic precursor, and surfactant to start nucleation. Following the nucleation, the solution is brought to its boiling point of solvent to yield small nanocrystals. Finally, the solution is maintained at high temperature throughout the reflux phase before cooling down to room temperature. The complete process is performed in a closed system with inert environment. Hydrophobic ligands and dispersants are necessary to manage particle size and stabilize nanoparticles in organic solvent. The advantage of thermal decomposition over coprecipitation is that it produces nanoparticles with better structural properties and smaller size, however thermal decomposition requires high temperature which is an energy intensive process and only limited type of nanoparticles can be obtained through the process [39].

4.2.3 Microemulsion

Microemulsion techniques involves surfactant aided dispersion of two immiscible phase, water and oil. The surfactant molecule arranges themselves into a monolayer at the boundary of oil and water,

with their hydrophilic heads in water and the hydrophobic tail in oil. The surfactants such as dodecyl sulfate enables the water to stably disperse in the oil to create water-in-oil microemulsions, which are stable colloidal systems. The aqueous phase forms tiny nanodroplets which are encircled by surfactant molecules, attached with precursors for magnetic nanoparticles. The confinement within the stabilized micelles prevents the growth, nucleation and agglomeration of particles. To obtain a precipitate, second emulsion is added to the solution which causes micelles to collide, break, and fuse, thereby facilitating the growth of nanocrystals within them. Finally, the nanoparticles are isolated by incorporating a solvent like acetone. This method allows for synthesis of MNPs with size ranging from 1 to 100 nm. The microemulsion synthesis method gives a precise control over the particle size by regulating the microemulsion droplet size and reaction condition like pH and temperature and the microemulsion droplet ensures minimal agglomeration with comparable dispersion. The disadvantages of using microemulsion method is that the surfactants used for the method are costly also the MNPs produced may have remnant of these surfactant changing their properties [39,40].

5. Types of magnetic nanoparticles

Fig. 4.3 Based on their response toward the applied magnetic field the MNPs can be categorized as paramagnetic, ferromagnetic, and super-paramagnetic materials. Paramagnetic materials do not

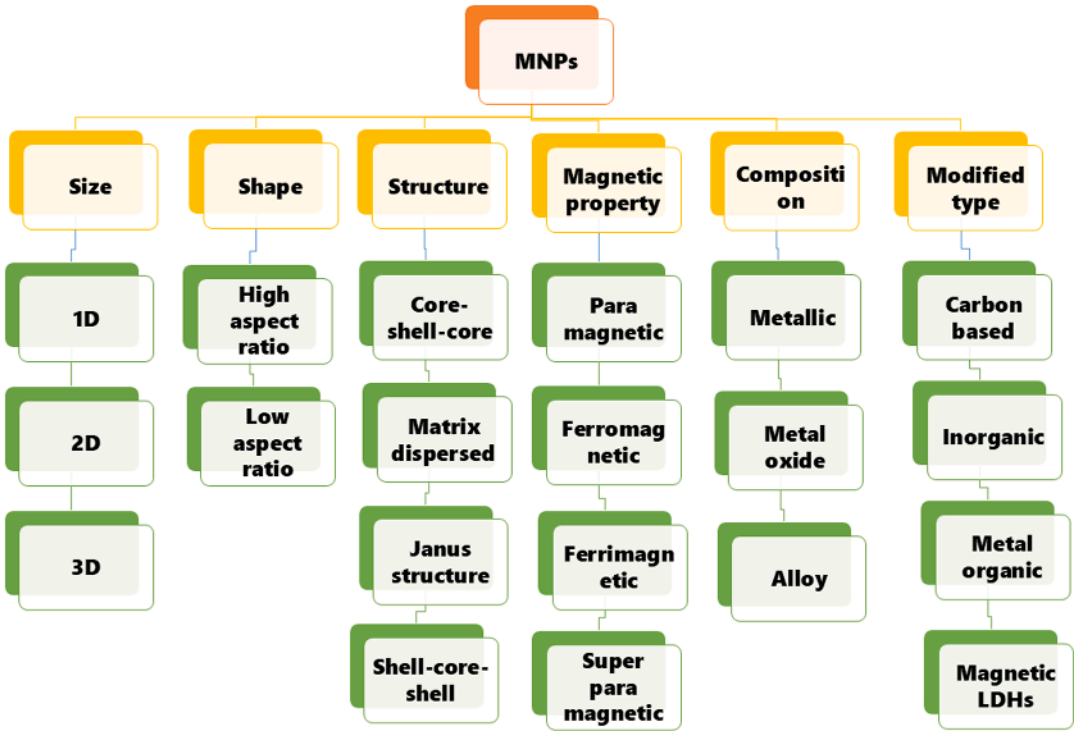


FIGURE 4.3

Classification of magnetic nanoparticles based on various aspects.

sustain their magnetic properties in the absence of a magnetic field, thus forming a weak magnetic moment e.g., Gadolinium chloride. Ferromagnetic materials remain magnetized even in the absence of an external magnetic field due to unpaired electrons, aligning their domains in the magnetic field direction, resulting in a significant magnetic moment e.g., FePt, NdFeB [41,42]. Superparamagnetic materials behave as a paramagnet in the presence of a magnetic field. Examples of superparamagnetic particles include magnetite (Fe_3O_4) and maghemite (Fe_2O_3) [43].

Based on their size dimension, nanoparticles can be classified into four types 0D, 1D, 2D, and 3D. Zero—dimensional nanoparticles have their all the axis (x, y, and z) within the nano range (1–100 nm). Quantum dots and fullerenes are examples of 0D nanoparticles. One-dimensional nanoparticles have one of the three-dimensional above the nano range and other two dimensions within the nano range. nanotubes, nano horns, nanofibers, nanorods, nanowires, and thin films, are examples of 1D nanoparticles. Two-dimensional nanoparticles have sheet-like shape with only one dimension within the nano range, while the other two dimensions is above the nano scale. Examples of 2D nanoparticles include nanosheets, fibers, ultra fine-grained over layers. Three-dimensional or bulk nanoparticles have all dimensions extending beyond the nano range but contain nanoscale structures in their bulk form [44,45].

The composition of MNPs is another way to categorize them as metallic, metal oxide, and alloy nanoparticles. Metallic nanoparticles are composed of single metal ions, they are less likely to dissolve and are very stable. They exhibit unique characteristics such as surface-enhanced Raman scattering, strong plasma absorption, enhanced Rayleigh scattering, and biological system imaging [46]. For example, gold nanoparticles when are coated with therapeutic agents, they can be used for DNA, antigen, and drug delivery [47]. The metal oxide nanoparticles are made up of a metal and one or more oxygen atoms, causing them to readily dissolve and release ions in the biological environment. They are less stable than the metal nanoparticles and may be cytotoxic [48]. Examples of metal MNPs that holds application in tissue and bone engineering includes TiO2, as it possess the ability to induce adhesion, healing, cell migration, and osseointegration [49]. ZnO is heavily utilized in food packaging, cosmetics, and sunscreens due to its biocompatible and nontoxic properties [50]. The alloy nanoparticles are made up of two or more metal ions combined, they are less stable and readily dissolve and release ions in a biological environment. Cu-Ag alloy nanoparticles are used over pure Ag or Cu nanoparticles due to their better antibacterial properties in *Escherichia coli* (DH5a) [51]. Alloy nanoparticles like Cu-Pt materials have a peroxidase-like activity that catalyzes H2O2, generating ROS and inhibiting bacterial growth [52].

6. Characterization techniques

In order to elucidate the physical, chemical and magnetic characteristics of MNPs and understand how inherent properties effect their function, it is essential to subject the synthesized nanoparticles to a wide range of advanced characterization methods which offers insights about their size, structure, composition and magnetic properties. It enables us to predict how MNPs behave when administered in vivo, helps assess the safety and health hazards and precisely enhance the development of nanoparticles to be able to make MNPs reach its maximum potential for biomedical purposes [7,53].

6.1 Characterization of physical properties

Physical properties like size and structure directly effects their functionality influencing magnetization, biocompatibility and efficiency of cellular uptake. As the size of MNP decreases, they undergo

shift from multi-domain to super para magnetism, displaying the desired property of negligible magnetic remanence and coercivity. Studies confirmed that larger MNPs exhibit greater cytotoxicity when compared to smaller MNPs because larger particles retain in tissues, undergo phagocytosis, trigger inflammatory response and induce oxidative damage, while smaller particles evade immune clearance [54]. Moreover, particle has to be small enough (<200 nm) to avoid being cleared by mononuclear phagocyte system (MPS) [55] and reticuloendothelial system (RES) [28], and large enough (>10 nm) to avoid renal filtration. Furtgermore, small size provides larger surface area allowing functionalization that enhances biocompatibility of MNP, suitable for drug delivery. However, the probability of aggregation by small MNPs is more than large sized particles. The techniques to characterize physical features of this class of nanoparticles are Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and magnetometry.

6.1.1 Transmission electron microsopy (TEM)

In TEM, high energy electron beam is imposed onto MNP sample, resulting high resolution images. This is the best technique that informs us the about the grain size, size distribution (Particle Distribution Index), morphology, magnetic core size, defects and crystallinity of magnetic nanoparticles. MNPs are prone to agglomeration, thus sample preparation is important to obtain high quality images. The sample is prepared by dispersing the MNPs in chemically inert solution that evaporates in few minutes at room temperature. Thus, limiting to characterization of dry sample. Particles in solution will have their size and behavior altered. Recently liquid-based imaging techniques have been developed to study inter-particle interactions and particle growth. TEM also allows elucidation of interaction of MNPs with cells. Other methods coupled to TEM offers additional information such as—TEM-electron energy loss spectra (HRTEM-EELS) offers compositional information and cryo-TEM sheds light on nucleation and growth of co-precipitation nanoparticles, structure of magneto liposome and cellular interaction.

6.1.2 Scanning electron microscopy (SEM)

SEM provides high resolution three-dimensional images, enabling the examination of surface, its modifications and functionalization. In addition to revealing surface morphology of individual MNPs, SEM also provides agglomeration state of MNPs, helping us understand their stability and behavior in different environments to apply them for suitable purposes. However, it is not SEM, but magnetometry that directly characterizes magnetic properties of particles.

6.1.3 Atomic force microscopy (AFM)

AFM provides surface topographical images of surface morphology as well as determines the magnetic behavior of MNPs including magnetic force, remanence, and coercivity.

6.1.4 Dynamic light scattering (DLS)

DSL is a powerful technique to unravel hydrodynamic size of MNPs in a liquid by examining the Brownian motion of particles. The patterns of light scattering can be measured to study the behavior of MNPs in colloidal solutions. One of the limitations of DLS is that it is more sensitive to larger particles than smaller particles, causing smaller ones to be left undetected. Moreover, mismatch in the results obtained from TEM and DLS upon size measurement have been observed which insists researchers to correctly interpret data as suggested by Filippov et al. [56].

6.2 Characterization of chemical properties

Chemical characterization involves determining composition and surface properties of MNPs. Surface is important as it engages in interaction, oxidation and heating. Chemical characterization helps ensure the stability, bioavailability, biocompatibility, precise targeting and controlled drug release of the therapeutic cargo of MNPs. Selection of right coating material is important for various reasons as already mentioned in sub-section 2.2.

6.2.1 UV-vis spectrophotometry

It is a commonly used technique based on Beer–Lambert law, which states that absorbance is directly proportional to concentration. It is used to examine the progress of MNP synthesis and quantify the sample.

6.2.2 Nuclear magnetic resonance (NMR)

NMR is a state-of-the-art technique to characterize surface chemistry. It provides insights into concentration of MNPs, composition and arrangement of ligands and surface coatings, and translation diffusion of particles. NMR relaxometry helps study size and dispersion. Although provides valuable information, it has some limitations like line broadening indicated by poor spectral resolution and lower sensitivity.

6.2.3 Fourier transform infra-red spectroscopy (FT-IR)

It is a versatile method that works by measuring the absorption of Infra-red Light by MNP sample, resulting in characteristic peaks depicting specific functional groups. Thus, allowing investigation on the chemical composition, ligands and reactive sites on surface, responsible for catalysis and surface interaction. FTIR is also used to evaluate MNP's agglomeration state

6.2.4 Small angle X-ray scattering (SAXS)

SAXS possess an extraordinary ability of assessing superstructure assemblies i.e., self-assembly of MNPs colloidal suspensions in various ways including- In-situ studies, time dependent and real time studies. It also informs about the crystal structure and lattice spacing.

6.3 Characterization of magnetic properties

6.3.1 Magnetometry

It is essential to characterize the magnetic properties like magnetic moment, magnetic responsiveness and coercivity of these NPs to determine and enhance their performance. Accordingly optimizing MNPs ensures efficient and precise action when applied to MRI, Hyperthermia and drug delivery. These properties are analyzed using Vibrating Sample Magnetometry (VSM) and Superconducting Quantum Interference Device (SQUID).

VSM is a simple, direct, but less sensitive technique which allows the evaluation of M-H loops and magnetic moment with respect to temperature, magnetic field and crystal orientation.

SQUID is a highly sensitive method used to assess super para magnetic material which is recognized for its inability to exhibit hysteresis loop. It enables determination of magnetic saturation, magnetic remanence and blocking temperature of superparamagnetic substances; and Curie and Neel temperature for ferro and anti-ferromagnetic material respectively.

6.3.2 X-ray diffraction (XRD)

XRD, also known as Wide-angle X-ray scattering (WAXS), is a non-destructive, non-contact technique allowing analysis of MNPs in desiccated powder form. XRD reveals information regarding crystallinity, lattice parameters and phase nature of MNPs, making this a crucial technique to evaluate magnetic properties of particles. Moreover, internal temperature of particles upon heating can also be measured.

7. Biomedical applications of MNPs

The biomedical application of magnetic nanoparticles can be broadly divided into three categories: Diagnostics, therapeutics and theranostics. some of the most efficient application of the MNPs are as follows.

Diagnostics—MRI, MPI and Immunoassays

Therapeutics -PDT, hyperthermia, immunotherapy, magnetofection (Fig. 4.4).

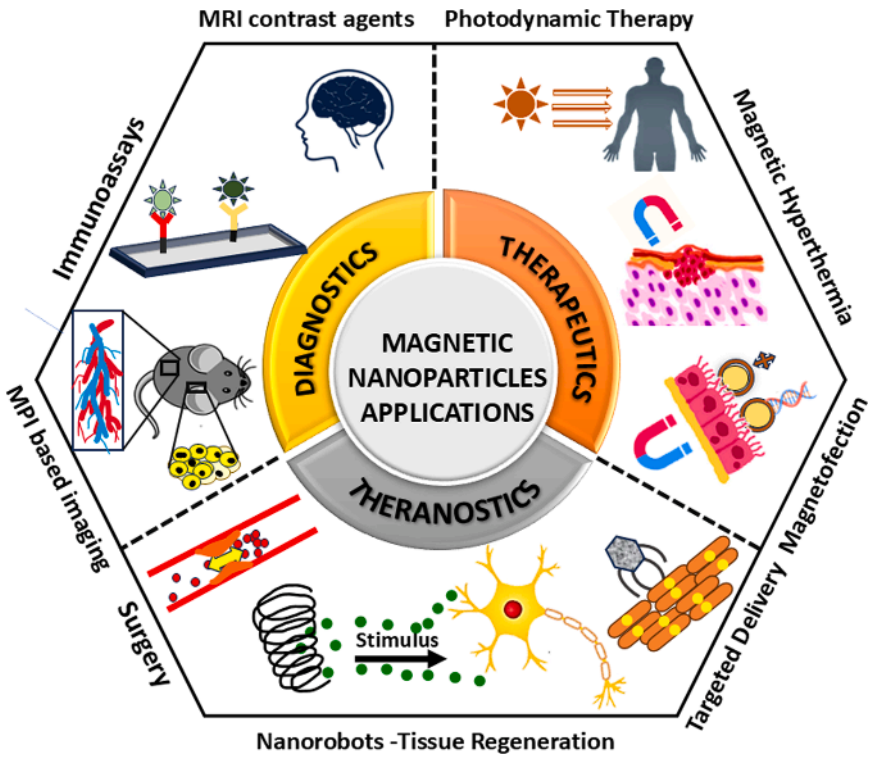


FIGURE 4.4

Schematic depicting diverse biomedical applications of MNPs.

7.1 Diagnostics

7.1.1 MRI

Magnetic nanoparticle, specifically superparamagnetic iron oxide nanoparticles (SPIONs) serve as advanced contrasting agents in magnetic resonance imaging (MRI). SPIONs compared to other paramagnetic agents can be modified in size and functionality to enhance the imaging of soft tissues. The MRI technique is based on the phenomenon called nuclear magnetic resonance (NMR) in which the behavior of atomic nuclei in a magnetic field allows for imaging based on the relaxation times (T1 and T1), which are affected by the presence of magnetic nanoparticles. SPIONs primarily induces outer sphere relaxation, leading to significant reductions in T2 relaxation time, while paramagnetic ions like Gd3+ affect T1 relaxation. Relaxivity measures the effectiveness of contrasting agents in enhancing image quality, with super-magnetic nanoparticles demonstrating high transverse relaxivity. Advances in MRI techniques and contrast agents aim to improve imaging capabilities, including the visualization of migrating cells loaded with magnetic nanoparticles. These nanoparticles can also be fluorescent- and radio-labeled, enabling the visualization through MRI and positron emission tomography (PET). Their application extends to monitoring stem cell differentiation and macrophage internalization. The use of Gd-complexes for molecular allows helps in the detection of the specific biomarkers associated with various diseases including cancer and cardiovascular conditions [57,58].

7.1.2 Immunoassays

MNPs have revolutionized immunoassays by acting as a versatile tool that enhances detection capabilities. They serve multiple roles by acting as carriers and labels simultaneously, hence significantly improving both selectivity and sensitivity in the detection process. MNPs work efficiently in concentrating the analytes from complex sample matrices and provide a good solid phase for immune complex formation. This dual functionality makes them particularly valuable in overcoming the limitations of traditional methods [59]. The effectiveness of MNPs is further enhanced through functionalization processes. Antibodies and oligonucleotide-modified MNPs demonstrate improved assay efficiency. This modification leads to notably faster reaction time compared to the conventional immunoassay methods, making them particularly valuable in time-sensitive diagnostic situations [60,61].

The integration of MNPs with microfluidic systems represent another significant advancement. In these systems, the MNPs serve as efficient carriers and labels, enabling complex reaction setups and facilitating reagent transport. This combination results in rapid analysis while maintaining high sensitivity levels [62].

7.2 Therapeutics

7.2.1 Magnetic hyperthermia therapy (MHT)

(Fig. 4.5):

Magnetic hyperthermia is a non-invasive method of treating tumors based on the principle that MNPs generate heat under the influence of an externally applied alternating magnetic field, while leaving the living tissue unaffected by the magnetic field. In MHT, the MNPs are induced to get heated up, while raising the temperature of the target tissue to 41–47^{0}C in which MNPs are accumulated, thereby resulting in apoptosis of target cells, usually tumor cells.

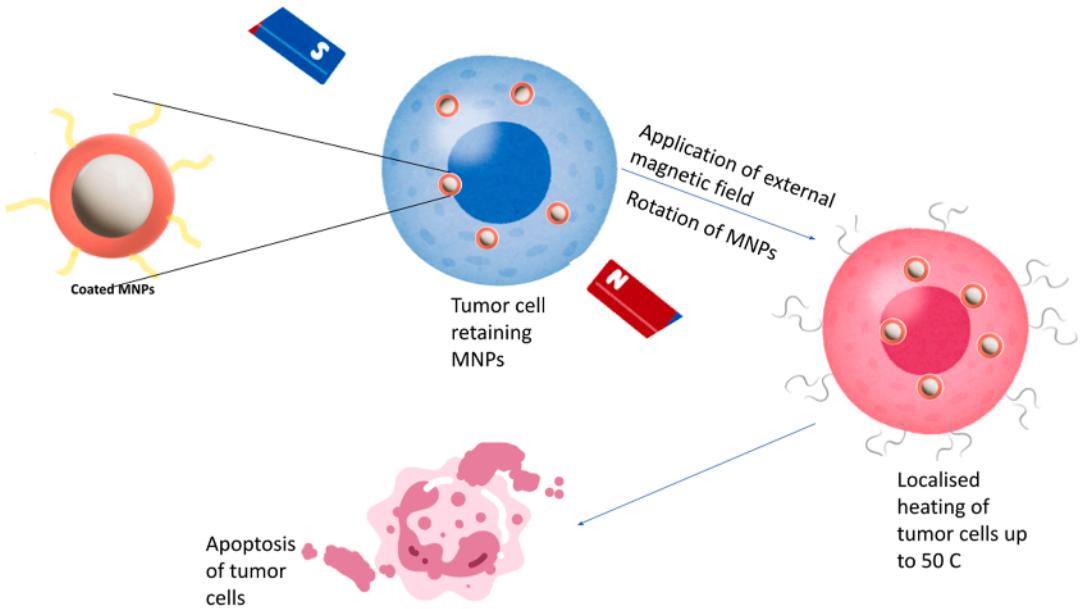


FIGURE 4.5

Representation of magnetic hyperthermia approach to destroy cancer cells.

In MHT, MNPs like Fe2O3 are administered inside the body by various routes such as directly injecting it into the tumor or in the systemic circulation depending on the type of tumor that needs to be dealt with. The MNPs get accumulated at the tumor site through active or passive targeting. Tumors being more sensitive to heat than normal cells, gets effected upon temperature rise induced by an external magnetic field. The rise in temperature is explained by Brownian and Ne'el relaxation.

Another important aspect of the MHT is the Specific absorption ratio (SAR), it is the measure of the transfer of magnetic energy to thermal energy. SAR is critical for selecting the MNPs. An MNP with high SAR value is injected in very low amount and the magnetic field exposure time is also very low, so the size, shape and surface of MNPs are modified accordingly to improve the SAR.

The advantage of MHT over chemotherapy is that it causes no toll on the healthy cells of the body and effects the specific targeted tumors.

7.2.2 Cancer immunotherapy

Cancer immunotherapy which utilizes the role of immune system in fighting cancer, is confronted with challenges like tumor microenvironment suppression and ineffective immune cell targeting. To overcome these obstacles, MNPs have been utilized and research proved that they hold potential in revolutionizing cancer immunotherapy. MNPs, in particular SPIONs serve as carriers to deliver therapeutics such as cytokines, immune checkpoint inhibitors and genetic constructs like CAR-T to cancer cells. Ongoing research is focused on advancing adoptive cell therapies (ACT), where MNPs are leveraged to modify immune cells, including NK cells and T cells by boosting their targeting efficiency, genetic reprogramming and longevity post reinfusion. Researchers fabricated a magnetic

polyplex composed of positively charged PEI-MNPs coated with folic acid and negatively charged siRNA against PD-L1 to enhance T-cell activation. Chiang et al. developed PD-L1 and anti-CD-3/-28 conjugated SPIONs to facilitate tumor specific delivery and reported that the strategy promoted localized expansion of tumor-infiltrating immune cells and reduced the systemic dissemination of antibodies thus improving therapeutic efficacy [63].

Natural killer (NK) cells are thought as the most promising agents for cancer immunotherapy, but due to their limitation of low cytotoxicity, employing MNPs to improve the anti-cancerous effect of NK cells can be beneficial. Nanoparticles like Fe2O3 coated with silicon dioxide are further modified by coating with cancer-cell membrane to make cell membrane-encapsulated nanoparticles (CMNPs), these biomimetic molecules can effectively present tumor-specific antigens enhance NK cell-mediated immunotherapy for cancer the treatment. The cancer-cell membrane retains tumor-associated antigen such as EGFR and MICA/MICB. The concentration of membrane protein used is lower than 25 μg, it activates the NK cells leading to increased secretion of perforin, INF- γ, and granzyme B. Peripheral blood is drawn from a healthy donor or cancer patient and NK cells are sorted from it afterward it is incubated with CMNP in vitro for activation, this increases the cytotoxicity of NK cells by four folds. The activated NK cells are put under the magnetic field which separates the CMNPs from cells. These cytotoxically enhanced NK cells are then injected back into the patient, once entering the circulatory system these cells mark the cancer cell for apoptosis. This method is very promising for the future of cancer treatment using MNPs as it does not involve any adverse side effects like other treatments of cancer [64].

7.2.3 Photodynamic therapy (PDT)

Photodynamic therapy is a non-invasive treatment approach for treating tumors and other diseases like cardiovascular or dermatological diseases. This therapy involves two key steps, first, administering a photosensitizing agent and then activating the drug by light of a specific wavelength. The activation of the drug promotes production of reactive oxygen species (ROS) including singlet oxygen that can damage the cells. The major limitations observed in the results of photodynamic therapy were the limited depth of light penetration, the delivery efficiency of the photosensitizer to the target cell, and the its potential toxicity. These challenges can be addressed by using magnetic nanoparticles, where the photosensitizers can be loaded onto the MNPs and by exposing the desired region of localization with external magnetic flux, the MNPs can be made to accumulate and perform the desired function. MNPs also contributes to PDT enhancement through their ability to generate hyperthermia and targeted drug release to specific target sites. This dual role allows for a combination therapy approach where the heat generation can work synergistically with the photodynamic effect to eliminate cancer cells more effectively. In a study, using cobalt magnetic nanoparticle conjugated with hematoporphyrin and folic acid (CoFe2O4-HPs-FAs) for PDT resulted in remarkable reduction of prostate cancer cells [65,66].

7.2.4 Magnetofection

Magnetofection is a technique that exploits magnetic nanoparticles to achieve delivery of genetic material such as, DNA, RNA, siRNA into the target cells. This method combines the properties of magnetic field with nanoparticles-based cargo delivery systems to improve gene therapy efficiency. This strategy involves compacting gene/carrier complexes with oppositely charged MNPs, i.e., negatively charged genetic material is bound to positively charged surface of MNPs coated with

cationic polymers, thus enables enhanced transfection under an applied magnetic field. These cationic magnetic gene carriers improve cellular uptake by overcoming the negative charge on cell membranes and serum proteins, with magnetic field facilitating this process. Research revealed that magnetofection accelerates the accumulation of MNPs/gene complexes on cell surface, achieving optimal transfection levels in a fraction of time compared to non-magnetic conditions. The internalization pathways for MNPs are similar to traditional methods, primarily involving clathrin and caveolin-mediated endocytosis. Additionally, MNPs aid in endosomal escape and nuclear import, enhancing gene stability and transfection rates. Magnetofection is capable of driving gene therapy where gene editing tools like CRISPR/cas9 or siRNA and miRNA can be delivered to treat the genetic cause of the disease; cancer immunotherapy where immunostimulatory genes can be transfected to amplify the immune response against tumors; and vaccination, thereby boosting clinical outcomes [67,68].

7.3 Theranostics

Theranostic application of MNPs represents the combination of diagnosis and therapy simultaneously in a unified system, thus making MNPs highly versatile in biomedical applications. This integrating of diagnostic and therapeutic capabilities, facilitates real time monitoring of disease progression and treatment efficacy. The dual functionality is highly advantageous in combating cancer, treating infection and management of neurodegenerative disorders. Scientists developed and revealed that 64Cu-labeled PEGylated melanin nanoparticles (64Cu-PEG-MNPs) is an excellent theranostic MNP that combines both diagnostic and therapeutic capabilities [69]. The system takes the benefit of melanin's natural ability to chelate radioactive copper ions, enabling PET imaging capabilities. The radioactive copper-64 isotopes, with its half-life of 12.7 h and β+ emissions, make it suitable for PET imaging [61]. Nanoparticles show excellent tumor targeting through the enhanced permeability and retention (EPR) effect, causing high tumor accumulation that can be visualized through PET/CT imaging [70]. The same MNPs are used for therapeutic purpose by utilizing the 64CU's β-electron emissions to deliver the targeted treatment to tumor cells. This method of delivery has significant advantages over conventional cancer treatments as it specifically targets the tumor cells minimizing damage to nearby healthy cells. The effectiveness of this therapeutic approach has been demonstrated through significant pathological changes in tumor tissues, with studies conforming minimal toxicity to normal cells [71,72].

8. Clinical trials

Any drug or nanoparticle need to undergo rigorous clinical trials before being approved by the FDA (Food and Drug Administrator) for clinical use. Table 4.1 below summarizes MNPs that are either in clinical trials and FDA approved [73,74].

9. Limitations of MNPs and their challenges for clinical application

(Fig. 4.6):

Although efforts to develop MNPs for biomedical applications has been commendable so far, with a few reaching clinical trials, many challenges still remain with these systems hindering and

Table 4.1 Current status of various magnetic nanoparticles in clinical trials.

Generic name	Brand name	Magnetic material type	Coating material	Size (nm)	Application	Clinical trial	Clinically approved
Ferumoxytol	Feraheme(US) Rienso(EU)	SPION	Carboxymethyl dextran	17–31	MRI contrast, iron deficiency treatment in chronic kidney disease, lymph node imaging		YES, but withdrawn from EU market
Fermoxtran-10	Combidex(US) Sinerem(EU)	SPION	Dextran	15–30	MRI contrast	YES	
Ferristene	Abdoscan	USPIO (ultra-SPION)	Polystyrene	300	MRI contrast		YES
Ferumoxide	Feridex(US) Endoren(UK)	ION	Dextran	50–100	MRI contrast, Cellular labeling and macrophage imaging		YES, but withdrawn from the market
N. A	NanoTherm	ION	Aminosilane	12	Cancer treatment (glioblastoma)	YES	
Ferumoxil	Lumirem GastroMARK	SPION	Siloxane	300	Oral GI imaging		YES, but withdrawn from the market
Feruglose	Clariscan		PEGylated starch	20	Blood pool agent	YES	
Ferucarbotran	Resovist (US, EU) Ciavist(France)	ION	Carboxydextran	80	MRI contrast, hepatocellular carcinoma, cell labeling		YES, Available in few countries

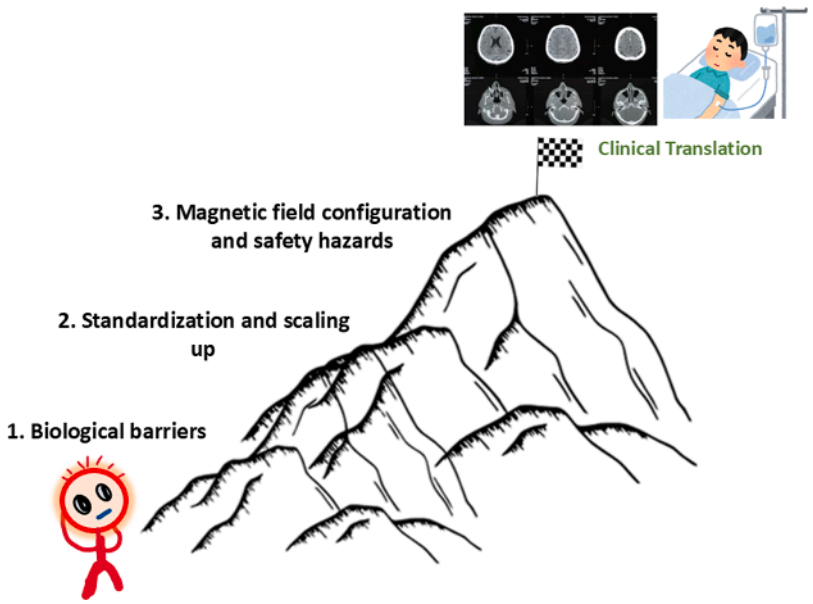


FIGURE 4.6

Illustrating the challenges in clinical translation of magnetic nanoparticles.

decelerating their implementation. The Primary reason being, limitations in scientific research which involves difficulty in studying toxicity of MNPs, as it depends on various factors, lack of development of apt procedures for synthesis and evaluation to ensure their desired behavior, as size and shape influences its efficacy. Aggregation of particles causing toxicity and uncontrolled heating of healthy tissues in hyperthermia are other concerns to be addressed. Requirement of strong magnetic fields at the desired target location raises questions on its possibility, as Thomson's magnetostatic theorem suggests that MNPs cannot stay stable just by using external magnetic fields. Thus, solenoids have been used but failed to work on deeper tissues. Likewise, ferromagnetic stent insertion into the tumor would work, but again defeats the major benefit of non-invasiveness of nanoparticle. Although the issue of chemical modification of surface of MNPs had tried to be addressed by biomimetics, scalable production of membranes remains a challenge. Moreover, Limited understanding of pharmacokinetics, and interaction mechanism of particles with biological components and tissues in vivo, different behaviors of MNPs in in vitro and in-vivo environment and uncertain data on biosafety are the unresolved issues slowing the progress in this area.

Further, clinical reasons for the same include, unacceptably high costs for clinical trials, flawed choice of dosage and treatment period and alteration in properties of MNPs while scaling up due to lack of standardization.

10. Future prospects

The future research needs to focus on standardization of synthesis, characterization and scaling up procedures that are a major hindrance for clinical translation of MNPs. One promising approach is by

integrating Artificial Intelligence and Machine learning that speeds up the process. Iron Oxide Nanoparticles shows promise due to its versatility but also comes with disadvantages of image distortions, short circulation time, and toxicity. Continuous efforts toward developing safer and more effective MRI contrast agents are the need of the hour. To translate MPI, a revolutionary non-invasive tool to track biological processes requires new data acquisition and image reconstruction algorithm models to improvize advanced tracers like Super ferromagnetic iron oxide nanoparticles (SFMIOs). To safely advance the employability of hyperthermia, working on developing and optimizing self-regulating magnetic hyperthermia NPs by top-down synthesis approach, tuning curie temperature and magnetic properties shall pave the way for successful clinical use in cancer treatment. Furthermore, researchers should also focus on optimizing magnetic field configurations, developing non-invasive neuromodulation strategies, ensuring long term safety of MNPs and address ethical concerns, to fully unlock the potential of magnetic nanoparticles for improved diagnostics and therapeutic outcomes. With continued research and rapid progress, the potential impact of MNPs on medicine over the next 4 decades is beyond imagination.

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CHAPTER

Light-sensitive nanocarriers for biomedical application

5

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1. Introduction

Nanotechnology has emerged as a transformative force in modern medicine, offering innovative solutions to longstanding challenges in disease diagnosis, treatment, and prevention. At the heart of this revolution are nanomaterials, which possess unique properties that make them particularly well-suited for addressing critical issues in drug delivery and tissue engineering [1]. One of the most significant advantages of nanomaterials is their high surface area-to-volume ratio, which enhances their reactivity and enables efficient loading of therapeutic agents. Additionally, nanomaterials can be engineered with tunable properties, allowing researchers to tailor their size, shape, surface chemistry, and functionality to meet specific biomedical needs. This adaptability is crucial for achieving targeted drug delivery, where nanocarriers are designed to selectively accumulate at disease sites, minimizing off-target effects and improving therapeutic outcomes [1,2]. Despite these advancements, conventional drug delivery systems meet several persistent challenges. Low bioavailability remains a major hurdle, as many drugs degrade before reaching their intended targets or fail to cross biological barriers such as the blood–brain barrier [3]. Systemic toxicity is another concern, as conventional therapies often distribute drugs indiscriminately throughout the body, affecting healthy tissues and causing adverse side effects. Furthermore, the lack of specificity in drug delivery systems can lead to suboptimal therapeutic efficacy, particularly in complex diseases such as cancer, where precision targeting is essential [4,5]. These limitations emphasize the critical need for advanced delivery platforms to overcome these barriers while ensuring safety, efficacy, and patient compliance.

Light-sensitive nanoparticles have arisen as a cutting-edge strategy in nanotechnology to address these critical limitations of traditional drug delivery systems. These photoresponsive nanocarriers enable precise, on-demand drug release, offering a solution to complex diseases such as cancer [6]. Light for photoresponsive nanocarriers is classified into three categories based on wavelength:

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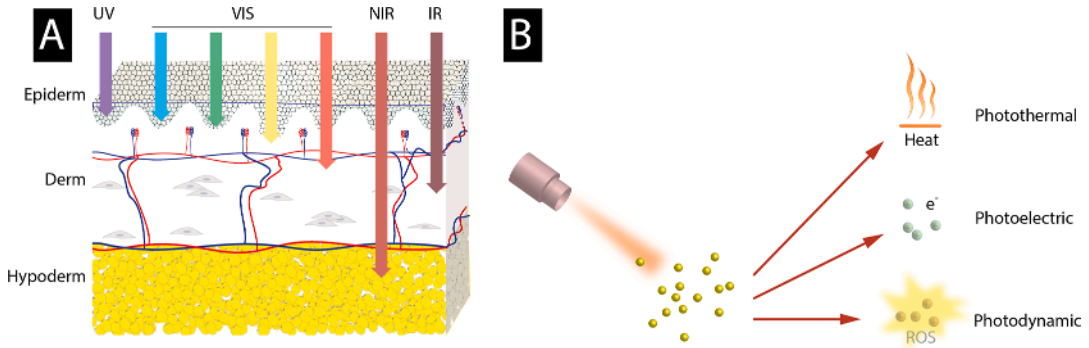


FIGURE 5.1

A schematic representation: A) The wavelengths of light determine how deeply they can penetrate the skin. B) Biomedical applications make use of nanomaterials' unique optical properties, which convert light into heat, electrical impulses, or chemical reactions.

ultraviolet (UV) (200–400 nm), visible (Vis) (400–700 nm), and near-infrared (NIR) (700–1000 nm) [7]. UV light is commonly used because it effectively triggers reactions such as cleavage, isomerization, or cross-linking. However, UV light has limitations such as poor penetration depth and high phototoxicity, making it less suitable for deeply located diseases Fig. 5.1 [8].

NIR and some Vis light have better tissue penetration due to minimal absorption and scattering by biological molecules Fig. 5.1 [9]. For instance, rat skin penetration depth varies with wavelength, with greater penetration for NIR light [10]. Despite this advantage, few compounds respond directly to NIR light because of its low energy [11]. Nanomaterials that convert NIR to UV or Vis light have been developed to overcome this. These materials enable remote-controlled drug release [12].

2. Types of light-sensitive nanocarriers and their biomedical applications

2.1 Polymer-based nanocarriers

2.1.1 UV/Vis light-based polymeric nanocarriers

Because it can cause structural changes in light-responsive polymers, UV light is generally considered the ideal light source for controlled drug delivery systems to trigger payload release. These photochemical reactions result in the disassembly of nanoparticles and enable precise control over payload release [13]. The structural changes in polymers induced by light can be grouped into three primary mechanisms: 1) photocleavage of light-sensitive units such as o-nitrobenzyl (oNB) and coumarin-based groups, 2) photoisomerization involving compounds such as azobenzene (AZO) and spiropyran (SP), and 3) photo-cross-linking or decross-linking processes, often utilizing molecules such as coumarin and cinnamoyl [14].

2.1.1.1 Photocleavable nanomaterials

Photocleavable polymers facilitate drug release through light-induced chemical bond rupture. They are categorized into four primary types according to the positioning of photolabile groups and

structural alterations: self-immolative polymers with photocleavable terminal groups, light-degradable polymers with photocleavable side chains, light-induced hydrophobic-to-hydrophilic transitioning polymers, and self-immolative polymers with multi photocleavable linkers [15,16]. Self-immolative polymers feature photocleavable protective groups at their ends, ensuring rapid breakdown into small molecules upon light exposure. For instance, polyurethanes with oNB or bromo-coumarin (Bhc) end groups have been used to create nanoparticles that degrade under UV light ($\lambda = 350$ nm), releasing encapsulated payloads such as Nile red through specific chemical processes [17–19]. Similarly, self-immolative dendrimers with oNB triggers enable head-to-tail depolymerization, releasing multiple drug molecules in a controlled manner [20]. Light-degradable polymers incorporate photocleavable species in their side chains, protecting nucleophiles such as alcohols, thiols, or amines. These groups split when exposed to light, which starts the polymer's breakdown and the payload's release. Olejniczak et al. developed nanoparticles with oNB-protected side chains that disintegrate under UV light, enabling the burst release of hydrophobic drugs such as Nile red or therapeutic agents such as nintedanib, demonstrating the potential for targeted ocular drug delivery [21].

In contrast, light-induced hydrophobic-to-hydrophilic transitioning polymers alter their solubility properties by removing photolabile groups, destabilizing nanoparticles, and facilitating drug release. For example, amphiphilic block copolymers with oNB-protected carboxyl groups have been used to create nanoscale polyelectrolyte complexes (PECs) that release up to 67% of encapsulated dyes upon UV irradiation [22–24].

Polymers with multiple photocleavable linkers have been synthesized to overcome limitations such as hydrophobic reaggregation after nanoparticle disassembly [25]. These systems incorporate numerous cleavable units along the main chain, ensuring complete degradation without secondary reactions such as intramolecular cyclization [15,26]. Without additional reactions, such as intramolecular cyclization or elimination, these polymers undergo degradation when exposed to UV rays. The degree of decomposition is proportional to the number of photocleavable groups present in the polymer chain. Functional monomers can be polycondensed to produce these polymers [26,27]. In this regard, a Passerini three-component reaction was utilized to prepare photocleavable poly(ester amide)s, which demonstrated rapid drug release upon UV exposure [28]. Additionally, Truong et al. developed photolabile hydrogels responsive to specific wavelengths, offering potential applications in selective cell release, tissue regeneration, and disease treatment [29].

2.1.1.2 Photoisomerizable nanomaterials

Photoisomerization, triggered by UV rays, is a key mechanism in designing light-responsive nanocarriers for controlled drug release. Commonly used molecules include AZO and SP derivatives, which undergo reversible structural changes [14]. AZO switches between its stable trans-isomer and metastable cis-isomer under UV light (300–400 nm), reversing the process by Vis light or heating. Similarly, SP can undergo a photochemical change from hydrophobic to hydrophilic when exposed to UV rays; this change can be reverted by Vis light or thermal stimuli [30,31]. These properties have been utilized to develop nanoscale polymer micelles with AZO side chains, enabling controlled drug release upon UV irradiation, which can be paused by switching to Vis light [32]. Additionally, dual-controlled systems using AZO-terminated polymers have demonstrated enhanced drug release under combined UV light and acidic conditions [33].

2.1.1.3 Photo-cross-linkable/decross-linkable nanomaterials

Reversible photoinduced cross-linking and decross-linking systems are utilized to stabilize drug-loaded nanoparticles and enable targeted drug delivery. Commonly employed molecules include coumarin and cinnamoyl, which undergo cross-linking under UV light and decross-linking with shorter wavelength light [34,35]. Coumarin derivatives cross-link under 350 nm UV light and decross-link under 254 nm UV light. Photo-cross-linking improves nanoparticle stability and drug retention. Coumarin- and cinnamoyl-functionalized polycarbonates can stabilize polymersomes with UV light [36].

UV- and pH-dual responsive iminocoumarin–chitosan nanoparticles showed higher stability and controlled drug release with photo-cross-linking [37]. Cross-linked micelles with coumarin and disulfide improve in vivo stability and tumor targeting and reduce toxic side effects [38]. Coumarin-anchored dendrimers self-assemble into nanostructures, efficiently load drugs and show improved DNA binding, gene transfection, and cellular uptake. UV light triggers the release of anticancer drugs and plasmid DNA, demonstrating effective cancer treatment [39].

2.1.2 NIR light-based polymeric nanocarriers

Most light-responsive nanoparticles for drug delivery rely on UV or short Vis light to trigger drug release. However, these wavelengths exhibit limited tissue penetration and can cause significant side effects, such as phototoxicity. It is crucial to develop systems that enable deeper tissue penetration and precise control to address these challenges while minimizing cellular damage [40]. A common approach in biomedicine involves using photosensitive nanoplatforms that convert NIR light into UV or Vis emissions, enabling controlled drug release within biological systems [41]. NIR light facilitates molecular transformations, allowing for efficient, light-controlled drug delivery with enhanced tissue penetration and reduced adverse effects, offering significant potential for advanced therapeutic applications [12].

2.1.2.1 Nanomaterials with two-photon absorption (TPA)

TPA, known for its ability to penetrate deep tissues, supplies a hopeful method for light-controlled drug delivery systems targeting diseases located deep within the body [42]. TPA can trigger drug release through several mechanisms, including chemical bond photolysis, energy transfer isomerization, and photoinduced hydrophobic-to-hydrophilic transitions [43]. In chemical bond photolysis, TPA has been used to cleave photocleavable groups such as coumarin or disulfide bonds [44]. For example, NIR-responsive block copolymer-coated hollow mesoporous silica nanoparticles (SiNPs) were developed. As coumarin units degraded under NIR light, they released over 50% of the anticancer drug doxorubicin (DOX) and demonstrated efficient tumor targeting through folic acid receptors. Similarly, mesoporous SiNPs with disulfide nanogates achieved two-photon-reductive cleavage, showing 70% cancer cell-killing efficiency in vitro [45].

Energy transfer isomerization has also been explored, particularly using AZO derivatives. While AZO typically responds to UV light, integrating it with two-photon fluorophores enabled efficient energy transfer for photoisomerization under NIR light. Such systems, such as nanostructures incorporating camptothecin (CPT), achieved controlled drug release upon NIR irradiation, leading to effective cancer cell killing [46]. Additionally, NIR-sensitive micelle systems combining hyperbranched conjugated polymers and AZO-functionalized poly(ethylene glycol) (PEG) demonstrated precise drug release visualized by confocal microscopy [47].

Photoinduced hydrophobic-to-hydrophilic transitions have also been achieved using groups such as 2-diazo-1,2-naphthoquinone (DNQ), which transforms into hydrophilic indene-carboxylic acid under NIR light [48]. DNQ-containing micelles loaded with DOX and functionalized with cyclic arginine-glycine aspartic acid (cRGD) peptides for cancer targeting showed enhanced therapeutic efficacy. Upon NIR irradiation, the micelles disassembled, releasing the drug and achieving superior inhibition of cancer cells compared with single treatments. These advancements highlight the versatility and potential of TPA-based systems for precise, spatiotemporal control in advanced drug delivery applications [49].

2.1.2.2 Upconversion nanoparticles (UCNPs)

UCNP systems involve the sequential absorption of multiple low-energy photons through intermediate states, resulting in the emission of a higher-energy photon. This process is more probable than TPA due to the involvement of real intermediate states [50]. Lanthanide-based systems are commonly used for UCNPs, where lanthanide ions serve as emissive centers [51]. These ions exhibit long-lived intermediate energy levels, providing stability and enhanced efficiency. Common sensitizers include Yb^{3+} or Nd^{3+}, while emitters such as Er^{3+}, Tm^{3+}, or Ho^{3+} are frequently employed. Other lanthanide ions, including Tb^{3+}, Eu^{3+}, Sm^{3+}, and Dy^{3+}, have also been developed as emitters through mechanisms such as energy migration or cooperative upconversion [52,53]. These systems can be integrated into micelles, hydrogels, or mesoporous structures for stimuli-responsive drug release, leveraging NIR-triggered mechanisms such as photolysis, photoisomerization, and redox reactions [54–56].

In UCNP-assisted photolysis, UCNPs are combined with photolytic materials such as oNB or coumarin derivatives to achieve smart drug delivery [57]. For instance, mesoporous silica-coated UCNPs equipped with light-responsive nanovalves have demonstrated precise control over drug release, enabling real-time monitoring and remarkable cytotoxicity in vitro [58]. Photoisomerization-based systems utilize photoswitchable compounds such as AZO [59] or SP [60] derivatives, which undergo reversible structural changes under NIR irradiation. For example, AZO-functionalized mesoporous silica systems have been developed to release anticancer drugs such as DOX through reversible trans-to-cis isomerization. This allows adjustable drug release by modulating NIR intensity and duration [61]. Similarly, nanocapsules incorporating AZO-functionalized polymers and UCNPs decompose upon NIR exposure, releasing encapsulated drugs due to photoinduced volume changes in the lipid bilayer [62].

UCNP-assisted reduction strategies have also been explored to minimize systemic toxicity while enhancing chemotherapy efficacy. Pt(IV) prodrugs, which are less toxic than their active Pt(II) counterparts, can be activated using NIR. UCNPs convert NIR light into UV emissions, activating Pt (IV) prodrugs grafted onto their surface [63]. A notable example involves PEGylated phospholipids coated with Pt(IV)-loaded UCNPs, which respond to the tumor's acidic microenvironment by converting it into cationic polymers. Upon NIR irradiation, the emitted UV light, combined with intracellular glutathione, reduces Pt(IV) to cytotoxic Pt(II), enhancing chemotherapy outcomes. This approach has shown prolonged blood circulation, improved tumor penetration, and superior tumor inhibition in both in vitro and in vivo studies [64].

2.2 Liposome-based nanocarriers

Researchers have focused much attention on liposomes because of their distinctive structure, biocompatibility, and capacity to encapsulate hydrophilic and hydrophobic compounds [65].

Liposomes have vesicular structures that contain a bilayer of phospholipids arranged in a sphere configuration with one or more buffer or aqueous spaces between them [66]. The hydration of the polar heads of dispersed phospholipids in an aqueous medium causes the formation of spherical structures known as Liposomes. Phospholipid bilayers organized in concentric spherical shells constitute the bulk of these vesicles (Fig. 5.2) [67]. Over the past few decades, liposomes have been widely used for drug delivery, diagnostics, and theranostics, with several liposomal formulations already endorsed for clinical application, such as Doxil (a liposomal formulation of doxorubicin) and Onivyde (a liposomal formulation of irinotecan) [68].

Functionalized liposomes encompass three main types: long-circulating PEGylated liposomes, ligand-targeted liposomes, and stimuli-sensitive liposomes (Fig. 5.2). Improved mechanisms that require a long circulatory half-life are needed to target drugs effectively. Liposomes with polymer coverings that inhibit plasma protein opsonization and are resistant to enzyme and immune system degradation are called stealth or long-circulating liposomes [69]. PEG is the most common polymer used to prevent liposome opsonization, and it is attached to liposomes by PEGylation. The global pharmaceutical market provides PEGs of various molecular weights, from pastes to waxy flakes. PEGs over 1000 are viscous liquids. PEGs on liposomal surfaces boost blood circulation, minimize reticuloendothelial system (RES) absorption, increase biodistribution and target accumulation, and stabilize the formulation [70]. Despite these advantages, PEG-functionalized liposomes may be immunogenic due to antibody-induced complement activation [71]. PEG-based delivery systems accelerate blood clearance because the spleen forms anti-PEG immunoglobulin M (IgM) antibodies after initial injection. After the second usage, the anti-PEG IgM binds to PEG groups on liposomes, activating the complement system and opsonizing them with C3 fragments, which increases their uptake by liver Kupffer cells and drug bioavailability [72].

Ligand-functionalized liposomes increase target cell drug storage, boosting therapeutic index and minimizing off-target effects. Active targeting moieties on liposomes aid ligand-receptor interactions

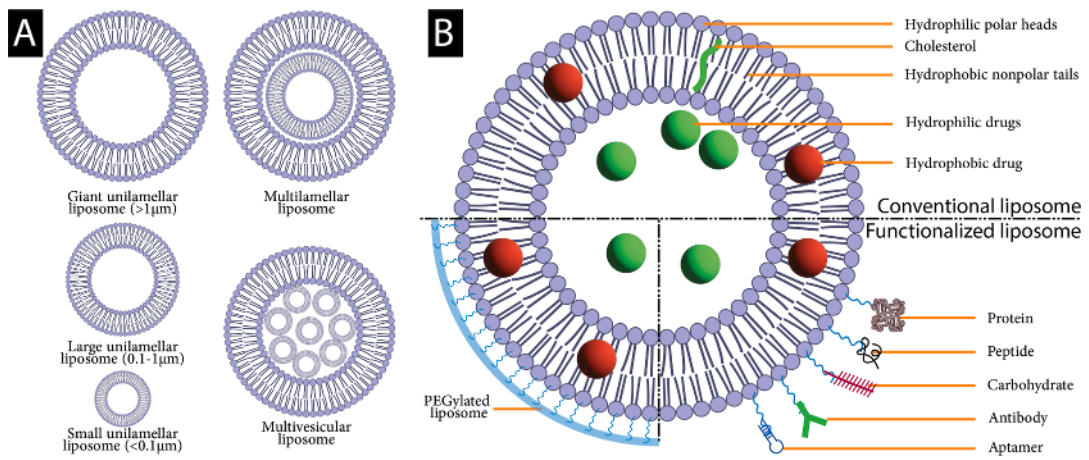


FIGURE 5.2

Liposome categorization based on size, content, and structural features; B: The differences between conventional and functionalized liposomes.

and target cell recognition [73]. Aptamers, polysaccharides, glycoproteins, monoclonal antibodies, fragments, peptides, proteins, and small compounds can be grafted onto liposomes (Fig. 5.2) [74]. Targeting moiety can be attached to the polymer distal end or lipid membrane. As drugs and photosensitizer targeting agents, aptamers are intensively researched [75]. Cellular internalization and liposomal drug accumulation require receptor-ligand interactions [72]. Monoclonal antibodies bind to cell antigens with great affinity, but antibody recognition and immunologic reactions limit their therapeutic application. Fab and scFv antibody fragments influence membrane antigen overexpression, making targets easier to reach than monoclonal antibodies [76]. Immunogenic, low-molecular-weight peptides penetrate tumors better than antibodies but have lower binding affinity. Synthetic peptides can be changed for physicochemical and biological features [77].

In recent years, integrating light-sensitive components into liposomes has opened up new possibilities for spatiotemporally controlled drug delivery and therapy [21]. Liposomes that are sensitive to light are engineered to release their cargo when exposed to light. This approach leverages the precision of light as an external trigger, enabling targeted and on-demand drug release with minimal off-target effects. The development of light-sensitive liposomes represents a convergence of nanotechnology, photochemistry, and biomedicine, offering innovative solutions for cancer therapy, antimicrobial therapy, gene delivery, and theranostics [72].

2.2.1 UV-triggered drug release mechanisms

2.2.1.1 Photoisomerization

Trans-cis light isomerization has achieved controlled drug release from photo-sensitive liposomes. In this regard, a liposomal curcumin formulation was developed incorporating 4-butylAZO-4-hexyloxy-trimethyl-ammoniumtrifluoro-acetate (BHA), which, when exposed to UV and visible light, trans-cis isomerizes in a way that can be reversed. The BHA-curcumin-liposomes (BHA-cur-lipo) were designed using thin-film hydration and SC-CO_2 techniques, achieving an encapsulation efficiency of approximately 88%. Upon UV irradiation, ~90% of curcumin was released within 6 h, with BHA demonstrating repeatable isomerization cycles. These findings underscore the potential of BHA isomerization as a precise mechanism for on-demand drug release [78]. In another study, Aryl-azo derivatives of thymol were synthesized and incorporated into liposomes composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine to create light-sensitive membranes. These hybrid systems were characterized using dynamic light scattering, atomic force microscopy, and Raman spectroscopy. The embedded compounds underwent reversible photoinduced isomerization upon exposure to UV and visible light, influencing membrane permeability and release behavior. Nonirradiated liposomes retained entrapped 5(6)-carboxyfluorescein (CF), while UV irradiation promoted CF release due to trans-to-cis isomerization. Cl-ion transport across the bilayer was enhanced by switching from trans to cis and restored by reverting to trans under blue light. The permeability could be precisely controlled by varying the wavelength and duration of light exposure [79].

2.2.1.2 Photocleavage

Encapsulating photosensitizers in nanocarrier systems, such as liposomes, address the significant issue of skin hypersensitivity reactions associated with their use. In this regard, an anticancer liposomal chemophototherapy (LCPT) was developed by incorporating a bilayer-loaded photosensitizer with the hydrophobic drug cabazitaxel (CTX) into porphyrin–phospholipid liposomes (CTX-PoP-Lip). The formulation included cholesterol and PEG-lipid to enhance stability and permeation using the hot

ethanol injection method. CTX-PoP-Lip exhibited excellent colloidal and photochemical stability over 3 months at 4 °C. Notably, when compared with chemotherapy or photodynamic therapy (PDT) alone, the tumor inhibition was considerably improved by combining CTX-PoP-Lip with the use of laser [80].

The novel light-triggered release and multifunctionality of lipid-porphyrin conjugates make them promising drug delivery vehicles. The combination of these substances results in the formation of porphysomes, which exhibit a marked reduction in fluorescence due to the dense packing of porphyrins. Upon NIR irradiation, porphysomes generate substantial heat, making them effective photothermal sensitizers. Cellular internalization through endocytosis disrupts their structure, resulting in increased fluorescence and confirming the successful targeting of the desired site [81,82]. Porphyrin rings in porphysomes offer chelating sites for metal atoms, making them promising candidates for multimodal imaging contrast agents. These structures combine photo-sensing capabilities with liposome-like characteristics and Enhanced photophysical properties [83]. Through surface modification, porphysomes can be actively directed toward tumor cells using strategies similar to those employed for conventional liposomes [84]. For instance, liposome-like assemblies were developed using phospholipid–porphyrin conjugates linked to pheophorbide-a (Pheo-a) or pyropheophorbide-a (Pyro-a) with varying alkyl chain lengths [85]. These structures, prepared through thin-film hydration and extrusion, included cholesterol, a phospholipid–porphyrin conjugate, and DSPE-PEG-2000. Pyro-a conjugates exhibited higher fluorescence quenching, while increased linker length enhanced stability due to deeper embedment of the porphyrin core. Based on these findings, Pyr3LPC and Ph3LPC liposomes were selected for further investigation. Similarly, another study [86] reported the design of photo-triggerable liposomes using a cholesterol-stabilized lipid–porphyrin conjugate, PhLSM. These stable liposomes encapsulated a hydrophilic probe and demonstrated efficient light-triggered cargo release, photothermal conversion, and photostability, underscoring their potential for controlled drug delivery.

2.2.1.3 Surface plasmon resonance absorption (SPR) (photothermal activation)

Photothermal methods employ metallic nanostructures, such as gold nanoparticles (AuNPs), to convert light into heat, thereby enhancing the permeability of liposomal membranes. These nanostructures exhibit unique optical properties, including SPR, which are influenced by size, shape, and environmental factors (Fig. 5.3) [87,88]. For example, bulk gold appears yellowish, while smaller particles display colors ranging from blue to red as their size decreases to approximately 3 nm. SPR arises from the collective oscillation of conduction electrons, enabling efficient light-to-heat conversion. Nanohybrids of AuNPs stabilized on thermosensitive liposomes demonstrated efficient NIR-triggered drug release while maintaining liposome stability [89]. Similarly, vincristine sulfate-conjugated gold nanoparticle-liposome hybrids with uniform 17 nm particles were developed, achieving enhanced antitumor efficacy. Encapsulation at a 6:100 M ratio produced 113.4 nm liposomes capable of UV-triggered drug release, significantly increasing cytotoxicity in HeLa cells. In vivo studies confirmed improved tumor inhibition with minimal toxicity, underscoring the potential of integrating the EPR effect with light-responsive mechanisms for targeted cancer therapy [90].

2.2.1.4 Photochemical hydrophobicity change (photochemical activation)

Amphiphilic block copolymers self-assemble into nanostructures such as polymeric micelles, which exhibit stability above the critical micelle concentration (CMC) but disintegrate below it, releasing

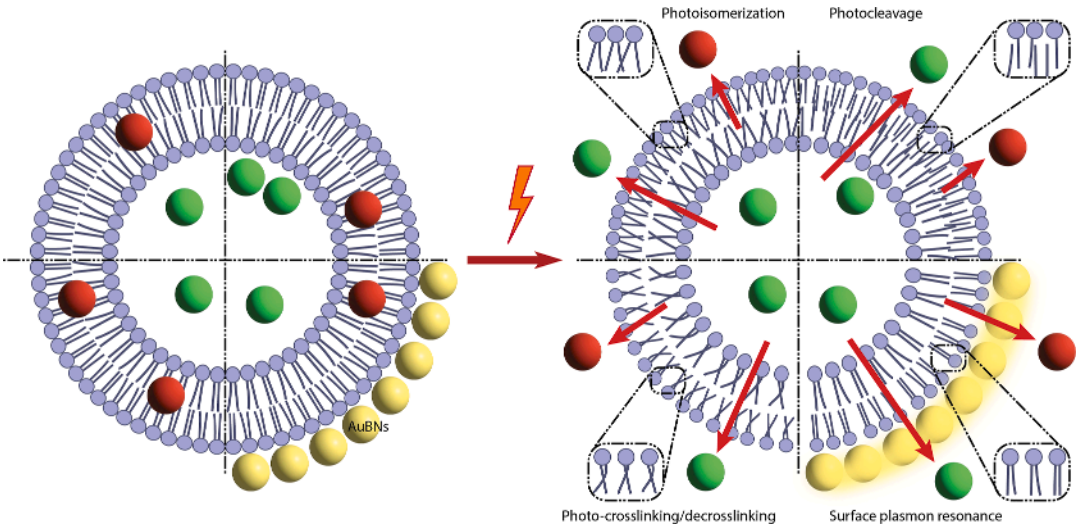


FIGURE 5.3

Light-triggered mechanisms used in triggering drug release from liposomes.

their payload [13]. Photochemical methods enhance stability by converting amphiphilic polymers into more hydrophilic forms, enabling controlled drug release [91]. Light-responsive chromophores such as AZO, SP, and diarylethene facilitate NIR-triggered transformations, offering deep tissue penetration with minimal damage. While micelles also function as Pickering emulsifiers in drug delivery, engineering light-responsive emulsions remains challenging. A nanocarrier system developed using β-cyclodextrin-grafted alginate (Ugi-Alg-CD) and AZO- PEG demonstrated reversible trans-cis isomerization under UV light, modulating the hydrophilic-hydrophobic balance for precise phase separation, showcasing the potential for advanced photo-responsive nanocarriers [92].

Polymersomes, biomimetic vesicles formed from amphiphilic block copolymers, exhibit superior chemical stability, resistance to oxidation, hydrolysis, and mechanical deformation during circulation or cellular processes compared with liposomes [93]. Their tunable composition, size, and surface chemistry enhance encapsulation efficiency (EE%) and loading capacity (LC%), while low membrane fluidity minimizes drug permeability [94]. Polymersomes disassemble upon light exposure, enabling controlled drug release. Studies on photo-sensitive polymersomes incorporating hydrophobic polymers, PEG, and photocleavable 2-nitrobenzyl compounds demonstrated structural integrity post-irradiation, with drug release driven by photo-induced permeability changes rather than structural breakdown, highlighting their potential for precise spatiotemporal drug delivery [95].

2.2.1.5 Photo-cross-linking–decross-linking

Photo-cross-linking induces drug release by polymerizing unsaturated bonds in the hydrophobic regions of lipid bilayers, leading to membrane disruption and increased permeability (Fig. 5.3) [96]. This approach was pioneered in liposomes using methacrylated PC derivatives, achieving enhanced stability, prolonged circulation, and improved tumor targeting. Additionally, photo-cross-linking has

been applied for sequence-specific DNA delivery into liposomes through structural transformations [97]. Reversible photo decross-linking, demonstrated using a microgel system with cross-linking at 365 nm and decross-linking at 254 nm, enabled controlled swelling behavior for doxorubicin release [98]. By monitoring the volume-phase transition temperature (VPTT), uniform distribution of the cross-linker CMA was achieved, underscoring the potential of hybrid liposome-in-gel systems for precise cancer therapy.

2.2.2 NIR-triggered drug release mechanisms

2.2.2.1 Photothermal effect

The photothermal effect involves converting light into heat using agents such as IR780 or gold nanorods (AuNRs) within liposomal nanocarriers, causing lipid bilayer disruption and controlled drug release [99]. For instance, liposomes encapsulating IR780 and plerixafor demonstrated heat-mediated disassembly and enhanced chemotherapy efficacy under NIR light. Similarly, thermosensitive liposomes incorporating AuNRs and Brij 58 enabled rapid, on-demand protein release, achieving 80.7% clot lysis in vitro after NIR irradiation, significantly outperforming free agents (36.3%) and nonirradiated liposomes (15.5%) [100] These advancements highlight the potential of NIR-responsive liposomes for precise, site-specific therapeutic delivery.

2.2.2.2 TPA systems

TPA enables chromophores to reach an excited condition by taking in two photons at the same time, facilitating specific photochemical reactions such as rearrangement or photocleavage. Chromophores such as DNQ, coumarin, and oNB can be activated by NIR light through TPA, inducing molecular changes that disrupt nanocarriers and release drugs [101]. For instance, NIR-responsive liposomes incorporating a 6-bromo-7-hydroxy-4-hydroxycoumarin (Bhc) unit demonstrated controlled release of hydrophilic molecules, with adjustable parameters for precise delivery. Despite its advantages, including deep tissue penetration and high spatial control, TPA's reliance on high-energy pulsed lasers restricts its application in vivo to small targeted areas.

2.2.2.3 UCNPs systems

The UCNP converts NIR light into UV light through sequential multiphoton absorption, enabling chromophore isomerization, such as the trans-to-cis transition of AZO [41,102]. Efficient energy transfer requires overlap between the UCNP emission band and the chromophore's absorption band [55]. For instance, UCNPs coated with an amphiphilic di-block copolymer featuring a UV-sensitive hydrophobic layer and a hydrophilic outer layer were developed to achieve NIR-triggered drug release [23]. Upon NIR irradiation at 908 nm, emitted UV light disrupted the hydrophilic-lipophilic balance, causing nanocarrier disassembly and drug release. Additionally, some NIR-responsive systems incorporate photosensitizers to generate ROS, enhancing chemotherapy effects [103,104].

2.3 Inorganic-based nanocarriers

Inorganic nanoparticles have emerged as a versatile and powerful platform for biomedical applications due to their unique optical, magnetic, electronic, and thermal properties. These nanoparticles, which include metals, metal oxides, semiconductors, and carbon-based materials, can be engineered to respond to external stimuli such as light, magnetic fields, or temperature. Among these, light-sensitive

inorganic nanoparticles have garnered significant attention for their ability to enable spatiotemporally controlled drug delivery, imaging, and therapy. By harnessing the interaction between light and matter, these nanocarriers offer precise control over the release of therapeutic agents, making them ideal for applications in cancer therapy, antimicrobial therapy, and theranostics.

2.3.1 Carbon-based nanoparticles (CNPs)

CNPs, including fullerenes, carbon nanotubes (CNTs), graphene, graphene oxide (GO), and nanodiamonds, have been extensively studied for their remarkable electrical, optical, mechanical, and biocompatible properties (Fig. 5.4) [105]. Surface-functionalized CNPs are hydrophilic, multifunctional, capable of simultaneously serving as PDT, photothermal therapy (PTT), and drug carriers [106]. For instance, CNTs, classified as single-walled (SWNTs) or multiwalled (MWNTs), exhibit strong light absorption, with MWNTs showing higher photothermal efficiency than SWNTs [107,108].

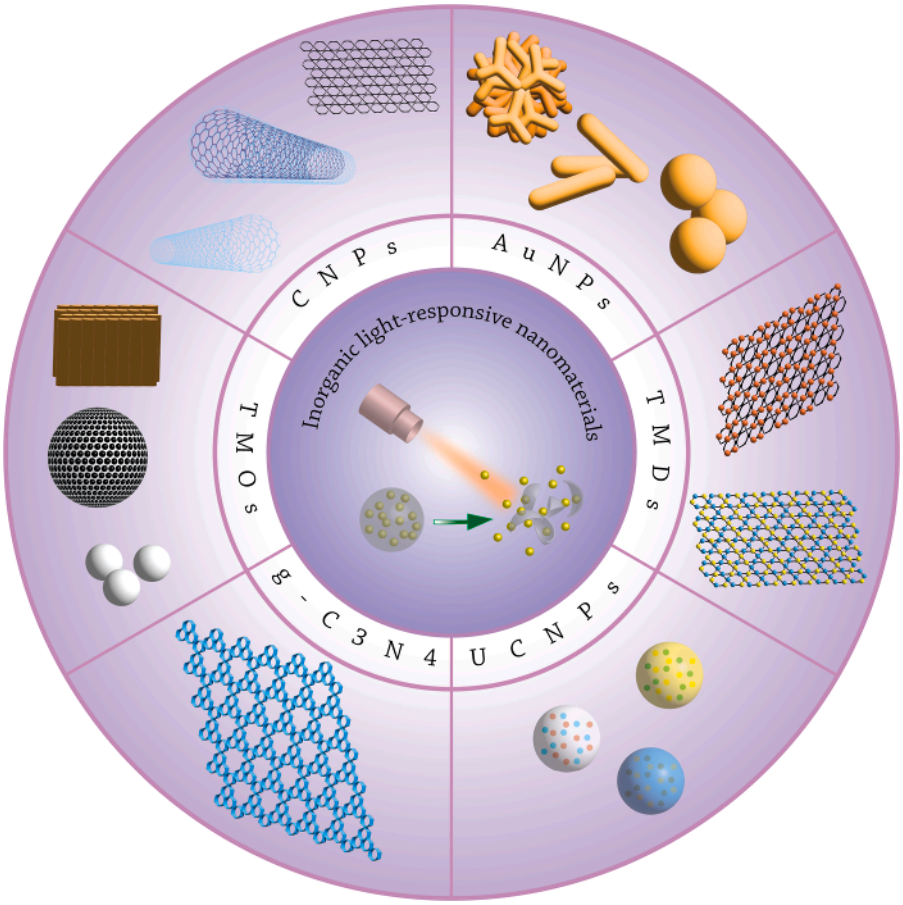


FIGURE 5.4

Inorganic light-responsive nanomaterials.

Semiconducting SWNTs, however, generate ROS effectively due to their suitable bandgap energy, making them ideal for PDT. Their hollow structure allows for high drug-loading capacity through π–π stacking, enabling multifunctional cancer therapy applications [109].

Graphene, a 2D material with sp^2 hybridization, demonstrates superior photothermal activity compared with CNTs and has been explored for cancer therapy and antiviral applications. For example, sulfonate-functionalized graphene mimics heparin sulfate moieties to capture and inactivate viruses through PTT [109]. Despite its potential, graphene's limited solubility poses challenges, making GO a more practical alternative due to its enhanced biocompatibility and water solubility. GO's photothermal properties and π–π interactions with drugs make it suitable for NIR-triggered drug delivery systems. However, its drug-loading capacity is limited by the chemical structure of the drug [110]. To address this, GO has been enhanced with the addition of thermosensitive polymers and siNPs to increase the drug-loading capacity and spatiotemporal precision. A system coated GO-silica nanocomposites with fatty alcohol and doxorubicin, achieving rapid drug release at 38°C under NIR irradiation, demonstrating clinical potential without toxic side products [111].

Hollow carbon nanospheres (HCSs) are another promising material, offering high drug-loading capacity, PTT capabilities, and ROS generation to disrupt cellular redox states. In order to conquer chemotherapy resistance, HCSs also contain sp^3 and sp^3 bonded carbons, which have great catalytic ability and can generate free radicals when exposed to near-infrared light [112]. For combined PDT and PTT, metal-organic frameworks (MOFs) integrated with mesoporous carbon nanospheres have been developed [113]. These systems exhibit strong NIR absorption for real-time monitoring and efficient singlet oxygen generation, comparable with common NIR dyes such as ICG [114].

Beyond cancer therapy, CNPs exhibit antibacterial properties, with GO showing notable cell membrane stress effects on bacteria under light exposure. Light-induced electron transfer from bacterial antioxidants to GO generates free radicals, enhancing antibacterial activity [115]. Dual light-responsive systems incorporating GO have been designed for wound healing, regulating temperature and pH to promote antibacterial activity or tissue regeneration. Additionally, GO-doped liquid-crystalline elastomers (LCEs) demonstrate improved photothermal effects and mechanical properties, enabling applications in 4D bioprinting and smart actuators. There are still unanswered questions regarding biocompatibility for in vivo applications and thermal injury [116].

Graphene also functions as a nanophotovoltaic device, converting light into electricity due to its zero bandgap and strong electron-electron interactions [117]. This property has been utilized for optical stimulation of cardiomyocytes, where light illumination triggers cell membrane depolarization, increasing action potential frequency [117]. Such studies highlight the versatility of CNPs in biomedical applications, though further research is needed to fully understand their light-induced activities in biological environments.

2.3.2 AuNPs

AuNPs, such as gold nanosphere, nanostars, and AuNRs, have been extensively studied in biomedical engineering due to their low toxicity, high conductivity, chemical stability, and ease of functionalization (Fig. 5.4) [118]. A key feature of AuNPs is their localized surface plasmon resonance (LSPR), which enhances light absorption and generates strong electromagnetic fields on their surfaces [119,120]. By tuning the shape and aspect ratio of AuNPs, LSPR bands can be extended into the NIR range, making them ideal for PTT and PDT. Among these, AuNRs are particularly promising due to their tunable NIR absorption and ability to perform PTT with high precision. However, the efficiency

of PTT is limited by the maximum permissible NIR exposure to avoid skin damage, necessitating strategies to reduce the required light intensity [121,122].

Adding peptides, DNA, or antibodies to the surface of AuNPs makes them more effective at targeting. For example, TAT peptides conjugated onto AuNRs enable intranuclear delivery, directly breaking DNA while minimizing damage to surrounding tissues [56]. Similarly, nucleus-targeting gold nanoclusters have been used for NIR-activated PDT, generating ROS with high reactivity and limited diffusion, leading to effective DNA damage [57]. Beyond cancer therapy, AuNPs have shown potential in treating neurodegenerative diseases such as Alzheimer's, where penetratin-conjugated gold AuNPs penetrate the blood–brain barrier to inhibit amyloid-beta fibril formation under NIR irradiation [58]. Anisotropic AuNPs, such as nanocrosses and multibranched structures, exhibit superior photothermal efficiency due to synergistic LSPR enhancement and have been used for antibacterial applications and deep-tissue PDT [59,61].

Recent advancements include integrating AuNPs with thermal-responsive polymers or hydrogels to fabricate innovative systems such as light-controlled actuators and cell capture platforms [123]. For instance, NIR-triggered gelatin phase transitions allow precise isolation of circulating tumor cells for personalized therapies [124]. Additionally, self-healing nanocomposite hydrogels using AuNPs as cross-linkers demonstrate dynamic mechanical property control through NIR laser exposure [125]. Despite being discovered decades ago, AuNPs remain attractive for light-controlled drug delivery, cancer therapy, and regenerative medicine because of their inertness, biocompatibility, and responsiveness to light [126]. These properties, combined with ongoing surface functionalization and composite design innovations, continue to expand their applications in regenerative medicine and beyond.

2.3.3 Graphite carbon nitride nanoparticles (g-C3N4)

g-C3N4, a Vis light-responsive nanoparticle made up of tri-s-triazine units, is synthesized through the polymerization of precursors such as cyanamide, melamine, or urea under varying conditions (Fig. 5.4) [127]. Its appealing electronic structure, characterized by a medium bandgap (2.7 eV), has enabled applications ranging from photocatalysis to photoelectronic materials. g-C3N4 has been engineered into various forms to enhance its photocatalytic activity, including nanosheets, hollow structures, and mesoporous frameworks [128]. For example, hollow g-C3N4 spheres have demonstrated enhanced ROS generation for PDT while serving as drug carriers through a photochemical internalization strategy. This approach overcomes endo/lysosomal sequestration by generating ROS to release drugs into the cytoplasm [129]. The photocatalytic efficiency of g-C3N4 can be further improved by embedding noble metal nanoparticles, such as silver, which act as electron acceptors to inhibit the recombination of photoexcited electron-hole pairs. Silver-doped g-C3N4 has shown superior antibacterial and biofilm-disrupting capabilities under Vis light [130]. Additionally, g-C3N4's large surface area and photoactivity make it suitable for photopolymerization applications, such as curing dental fillings or 3D bioprinting hydrogels using Vis light [131]. Different synthesis methods yield g-C3N4 nanosheets with varying surface properties, influencing their performance in photopolymerized hydrogels, where they act as initiators, reinforcers, and cross-linkers [132].

Beyond photopolymerization, g-C3N4 supports biomimetic photosynthetic reactions, such as NADH regeneration, by oxidizing electron donors such as triethanolamine (TEOA) and reducing NAD^+ to NADH [133]. Hybridizing g-C3N4 with self-assembled peptides enhances its photocurrent density, promoting efficient enzymatic reactions for synthesizing L-glutamate. Furthermore, g-C3N4

has been integrated into neural stimulation platforms, where it facilitates charge transfer across heterojunctions with GO, enabling spatiotemporal control of neurite outgrowth under Vis light [134]. Similarly, photoactivated g-C3N4 nanosheets combined with two-photon lasers have accelerated bone regeneration by modulating intracellular Ca^{2+} signaling to promote cellular proliferation and differentiation [135]. Given its low cost, chemical stability, and biocompatibility, g-C3N4 holds significant promise for advancing light-responsive biomaterials.

2.3.4 Transition metal oxide nanoparticles (TMOs)

TMOs have garnered significant attention for their semiconducting properties, including conductivity, adjustable bandgap, and light absorption, making them suitable for photocatalytic applications (Fig. 5.4) [136]. Photocatalysis in TMOs involves the excitation of electrons from the valence band to the conduction band upon light absorption, generating reactive species such as superoxide ions, hydrogen peroxide, and hydroxyl radicals [137]. Among TMOs, titanium dioxide (TiO_2) is widely studied due to its low cost, biocompatibility, and chemical stability [138]. TiO2 exists in various polymorphs, with anatase and rutile being the most efficient for photocatalytic applications. Its ability to generate reactive oxygen species under UV light has been utilized for drug delivery, surface coatings, and antibacterial wound dressings [139,140]. However, the wide bandgap of TiO2 (3.2–3.2 eV) limits its use to UV light, which has poor tissue penetration. To address this, modified forms such as hydrogenated black TiO_2 with enhanced NIR absorption have been developed for PTT and PDT [141].

Beyond TiO_2, other TMOs such as zinc oxide (ZnO) [142], tungsten oxide (WO_3) [143], and molybdenum oxide (MoO_3) [144] have shown potential in biomedical applications, particularly in antibacterial treatments and cancer therapy. For instance, oxygen-deficient TMOs such as WO_3-x and MoO3−x exhibit localized surface plasmon resonance (LSPR) in the NIR region, enabling their use in PTT and imaging [145]. These materials can be functionalized with biocompatible coatings such as PEG to improve tumor targeting and reduce toxicity [146]. Additionally, doping TMOs with metal ions or controlling their stoichiometry enhances their photocatalytic efficiency by creating oxygen vacancies and active sites, which improve charge transfer and radical generation [147]. TMOs also show promise in regenerative medicine and tissue engineering. For example, titania nanosheets have been used as photoinitiators for hydrogel cross-linking and as templates for micropatterning with high spatial resolution [148]. The unique wettability-switching properties of TiO_2 films under UV light have been explored for self-cleaning coatings and cell behavior modulation [90,91]. Despite their advantages, challenges remain regarding the toxicity, immune response, and long-term stability of TMOs in physiological environments [149]. Further research into their optical properties, structural modifications, and biocompatibility will expand their applications in phototherapy, drug delivery, and tissue regeneration.

2.3.5 Transition metal dichalcogenides nanoparticles (TMDs)

Because of their exceptional mechanical, electrical, and optical characteristics, 2D nanomaterials made of TMDs have recently gained attention as potential biological materials (Fig. 5.4) [150]. Unlike their bulk counterparts, exfoliated TMDs exhibit tunable bandgaps, typically within the range of 1.6–2.4 eV, making them suitable for Vis and NIR light absorption [151,152]. Among TMDs, molybdenum disulfide (MoS_2) has been extensively studied due to its negligible cytotoxicity, high NIR absorption, and potential for PTT and drug delivery [153]. MoS_2's surface can be functionalized with

biocompatible polymers such as PEG or biomolecules such as folic acid, enhancing its stability and targeting efficiency [154]. These modifications enable MoS_2 to serve as a versatile platform for combined chemo-photothermal cancer therapy, where drugs are released on demand through NIR irradiation [155,156].

Beyond cancer therapy, MoS_2 has shown promise in regenerative medicine and biosensing. For instance, its photothermal effects can modulate stem cell behavior, influencing gene expression related to wound healing and cellular migration [157]. Additionally, MoS_2-based materials exhibit peroxidase-like activity, enabling PDT by generating reactive oxygen species under NIR light [158]. Other TMDs, such as tungsten disulfide (WS_2) and molybdenum diselenide ($MoSe_2$), also demonstrate strong NIR absorption and photocatalytic capabilities, broadening their potential for deep-tissue therapeutic applications [159,160]. WS_2, in particular, stands out for its extended light absorption up to 910 nm and efficient charge carrier dynamics, which enhance its photocatalytic performance without inducing photothermal effects [161].

Despite their advantages, challenges remain in optimizing TMDs' exfoliation techniques and surface functionalization to ensure biocompatibility and physiological stability [162]. Furthermore, understanding the structure–property relationships and photoresponsive behaviors of TMDs is critical for advancing their use in light-guided drug delivery, PDT, and regenerative medicine.

2.3.6 UCNPs

UCNPs are unique nano-transducers enabling a wide range of phototherapy applications (Fig. 5.4) [163]. UCNPs typically consist of a host lattice doped with lanthanide ions, where ytterbium (Yb^{3+}) acts as a sensitizer and erbium (Er^{3+}) or thulium (Tm^{3+}) serves as an activator, with $NaYF_4$ being a common composition [134]. Factors such as particle size, dopant concentration, and surface coating influence their up-conversion efficiency, which has been extensively studied for biomedical applications [164]. UCNPs have shown great promise in deep tissue therapy and brain stimulation. For example, NIR-responsive UCNPs can enable transcranial optogenetic stimulation of deep brain neurons, overcoming the limitations of blue light penetration. By emitting blue light, these nanoparticles can activate light-gated ion channels such as channelrhodopsin-2 (ChR2), modulating neural activities and dopamine release in dopaminergic neurons.

Additionally, UCNPs can serve as multiwavelength light sources to control various neural processes, including inhibiting seizures and triggering memory recall [165]. In PDT, UCNPs address the challenge of limited light penetration by converting NIR light into UV or Vis emissions, activating photosensitizers for deep tumor treatment [166]. Dual-stimuli-responsive systems combining pH-sensitive polymers and NIR-responsive UCNPs enhance the selectivity of PDT by targeting the acidic tumor microenvironment. Similarly, UCNPs modified with virus-specific antibodies can selectively deliver photosensitizers to infected cells, offering a promising strategy for antiviral phototherapy [167].

UCNPs also function as on demand nanocarriers when coated with mesoporous silica, enabling controlled drug release triggered by NIR light. In tumor immunotherapy, the synergy between PDT-induced tumor antigen release and immune responses has been demonstrated using UCNPs loaded with photosensitizers [168]. Furthermore, UCNPs have been integrated into hydrogels containing UV-cleavable linkers for deep tissue imaging and controlled macromolecule delivery, minimizing damage to surrounding tissues [169]. Beyond cancer therapy, UCNPs have been explored in regenerative medicine. For instance, UCNPs conjugated with differentiation-inducing agents such as kartogenin promote chondrogenic differentiation of human mesenchymal stem cells (hMSCs), enhancing

neocartilage formation in vivo [170]. Additionally, UCNPs have been used in photochemical tissue bonding, where they replace optical fibers to deliver light for wound closure in deep tissues [171]. Recent advancements include NIR-induced 3D bioprinting, where UCNPs emit UV light to initiate photopolymerization, paving the way for high-resolution biofabrication [172].

3. Conclusion and future perspectives

Light-responsive nanomaterials, including polymers, inorganic materials, and liposomes, represent a rapidly advancing field with significant potential for controlled drug delivery and therapeutic applications. Polymers with photoresponsive properties have enabled precise spatiotemporal control over drug release through external light stimulation. However, challenges such as limited tissue penetration by UV and visible light have been a barrier to their clinical translation. The incorporation of strategies such as UCNPs and two-photon excitation has addressed these limitations, enabling the use of NIR light for deeper tissue targeting. Similarly, inorganic nanomaterials, such as TiO_2, MoS_2, and heterojunction semiconductors, offer enhanced stability, reduced photobleaching, and the ability to integrate multiple therapeutic modalities, including PDT and photothermal therapy PTT. These materials can be functionalized with biomolecules or natural polymers to improve tumor targeting and reduce off-target effects. Light-responsive liposomes further complement these systems by enabling targeted drug release. Recent advancements, such as photocleavable groups activated by long-wavelength light or efficient upconversion systems, have improved tissue penetration depth. Despite these innovations, several challenges remain. Nonspecific photoreactions in normal tissues must be minimized to avoid undesired toxicity, and real-time tracking of drug distribution is essential for monitoring treatment progress. Imaging-guided photoresponsive drug delivery systems that combine theranostics could address these issues. Furthermore, the safety and biodegradability of nanocomposites and photoresponsive compounds, such as AZO and oNB derivatives, require thorough investigation. In vitro and in vivo studies are needed to evaluate the toxicological properties, circulation, accumulation, and metabolism of these materials.

The complexity of biological systems demands multifunctional nanocomposites capable of integrating multiple therapeutic mechanisms, including direct photolysis, isomerization, and light-generated signals such as heat, ROS, or hypoxia. Recent advancements in heterojunction nano-semiconductors and carbon-based nanomaterials, such as SWNTs/TiO_2 composites, have improved photocatalytic efficiency and expanded light absorbance to include visible and NIR wavelengths. These innovations enhance energy utilization and support the development of next-generation photoresponsive materials for applications such as PDT, PTT, and genetic modulation. In regenerative medicine, light-responsive inorganic nanomaterials serve as energy transducers, converting photonic energy into heat or radicals to trigger stimuli-responsive biomaterials. However, while most systems currently rely on UV or visible light, which can cause cellular damage and limit tissue penetration, the development of red or NIR light-responsive hydrogels offers a promising solution to overcome these challenges, enabling the fabrication of larger, more complex 3D-printed tissues for advanced biomedical applications.

AI Disclosure

During the preparation of this work the author(s) used qwen in order to improve readability and language editing. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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CHAPTER

Electric field-response systems for biomedical application

6

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1. Introduction

Electric field-response systems represent biological mechanisms that detect and respond to internal and external electric fields, influencing cellular and molecular processes. At the molecular level, electric fields facilitate the transport of biomolecules through mechanisms like electrophoresis and electroosmosis, collectively termed iontophoresis [1]. At the cellular level, these fields interact with cellular components such as membrane proteins, ion channels, and the cytoskeleton, impacting activities like contraction, migration, and proliferation [2–4]. The study of bioelectricity began with Luigi Galvani (1737–1798), who demonstrated that animal muscles generate electrical impulses, coining the term "animal electricity." Alessandro Volta (1745–1827) contested this idea, attributing electricity to metallic interactions, leading to the invention of the voltaic pile, the first chemical battery [5]. Emil du Bois-Reymond (1818–1896) reconciled these perspectives by proving that nerve impulses arise from intrinsic electrical activity using sensitive equipment like the galvanometer, establishing the foundation of electrophysiology [6]. Julius Bernstein, a student of du Bois-Reymond, advanced this field by inventing the differential rheotome, enabling precise measurements of electrical currents, and introducing the membrane theory of electrical potentials [7]. Hermann von Helmholtz (1850-52) measured nerve impulse speeds, revealing that nerve signals travel at a finite speed rather than instantaneously [8]. Later, Hodgkin and Huxley formulated the Hodgkin-Huxley model, mathematically explaining nerve impulse propagation, earning the Nobel Prize in 1963 [9]. Katz's work on synaptic transmission and the contributions of Bert Sakmann and Erwin Neher in ion channel research further revolutionized neuroscience [10,11].

Recent innovations, such as optogenetics and neural prosthetics, have allowed for precise manipulation of neuronal activity and the development of brain-computer interfaces, respectively. These technologies have advanced neuroengineering, enabling therapeutic applications for neurological impairments [12–14]. Electric field-response systems have diverse biomedical applications,

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notably in diagnostics, regenerative medicine, and drug delivery. Tissue diagnostics leverage bioimpedance techniques to assess tissue resistance and capacitance, revealing physiological conditions like fluid distribution and edema. For example, electric fields measure breathing patterns for conditions like sleep apnea and tissue abnormalities like breast tumors, although current devices require further sensitivity improvements [15,16].

In wound healing, natural and externally applied electric fields guide cells like keratinocytes and macrophages in tissue repair. Studies have shown that electric currents between 250 and 500 microamperes stimulate cell migration and biochemical production, which is essential for healing. These principles extend to tissue engineering, where electric fields align and stimulate cardiac cells to beat or promote neurite growth for nerve repair [17–22]. Bone healing has also been enhanced through constant direct currents and piezoelectric scaffold materials [23,24]. Electroporation employs electric solid fields to temporarily increase cell membrane permeability, allowing the delivery of substances like drugs or genetic material into cells. This technique underpins applications such as Electrochemotherapy (ECT), which enhances drug delivery to cancer cells, and Electrogenetherapy (EGT), facilitating the introduction of therapeutic DNA or RNA for gene therapy. These methods promise to treat cancer and genetic disorders by improving therapeutic efficiency and targeting [25].

The chapter outlines the structural considerations of electric field-responsive devices and their biomedical applications, including diagnosis, drug delivery, and regenerative medicine. Despite promising advances, challenges remain, such as optimizing device sensitivity, addressing side effects, and scaling up applications. Future trends focus on improving therapy precision and expanding applications in tissue engineering and neuroprosthetics, offering transformative medical potential.

2. Basic principles of electric fields in biological systems

2.1 Understanding electric fields and their interaction with biological materials

Physics is intricately connected to natural cosmic occurrences involving electrically charged substances. An electrically charged object exhibits several significant phenomena, including the transfer of electric charge, electric force, and variations in electric potential energy [26]. Research has established the presence of electric fields surrounding any electrically charged item, which exert a repulsive force on like charges and an attracting force on opposite charges [27]. The detection of electric fields surrounding animals and within the environment has numerous possible ecological implications. Electroreception offers some of the more intricate and well-researched instances of the impact of electric fields. At the environmental level, other overlooked aspects of electric ecology exist that do not pertain to the biological detection of electric fields [27]. Research indicates that static electric fields (EF) occur naturally in the environment [28].

The biological system relies on inter- and intra-cellular connections for its growth, maintenance levels, and proliferation. This communication enables an individual cell to engage with adjacent cellular systems and its environment [29]. Electric and magnetic potentials are essential for the operation of living organisms, as these stimuli regulate and influence cellular processes, including proliferation, migration, differentiation, morphogenesis, and molecular synthesis, as shown in (Fig. 6.1) [30]. When a cell with an allowing transmembrane potential is subjected to an outside electrical field, the anode-facing side becomes hyperpolarized. In contrast, the cathode-facing side becomes depolarized, contingent upon the cell's size and shape [31]. The mitochondrial membrane

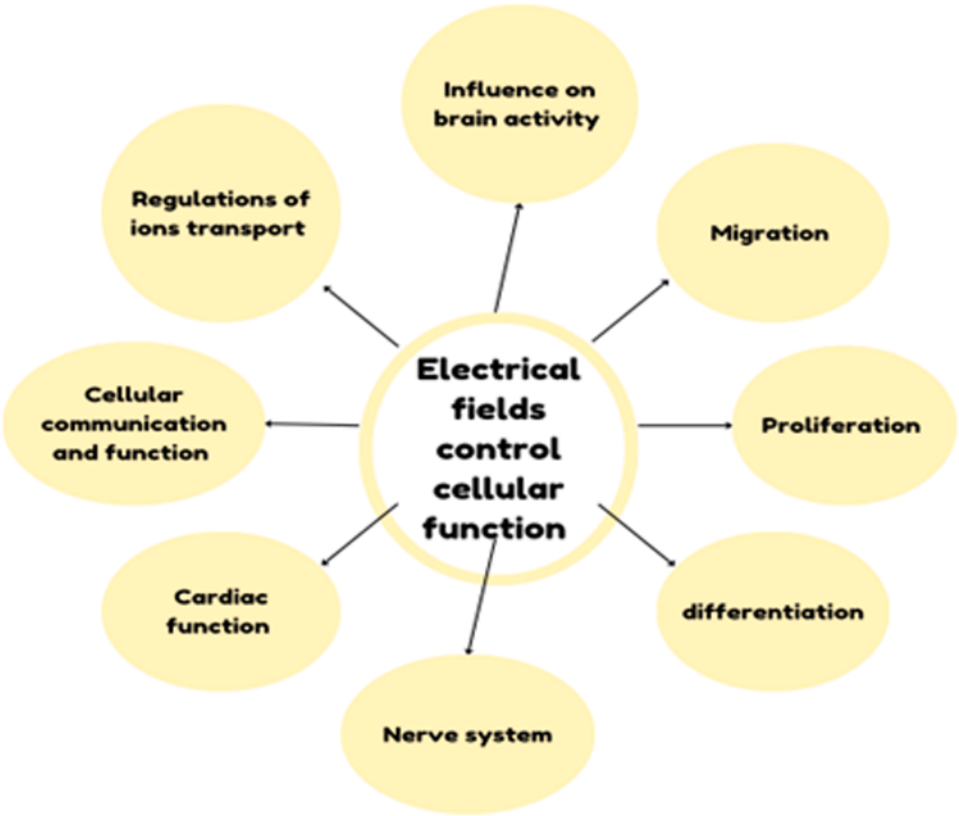


FIGURE 6.1

The diagram shows the effect of electrical fields on human biological processes.

potential ($\Delta\Psi m$) arises from redox changes linked to the Krebs cycle and functions as an intermediate storing energy form. These reactions provide an electrical potential due to separating the charges and the gradient of protons, collectively establishing the transmembrane potential of hydrogen ions [32].

2.2 Electric field effects on the cells and tissues

Electric fields (EFs) can modulate numerous cellular activities, including growth, adhesion, cytoskeletal reorganization, contractility, differentiation, proliferation, activation of intracellular pathways, protein secretion, and gene expression [33].A study conducted by Krassowska et al. Modeled a single cell to examine the impact of external direct electric fields on cell polarization and the physiological condition of the cell membrane [34]. The plasma membrane delineates the periphery of a live cell. It is a barrier and facilitates transit between the cell and its environment [35]. Research indicates that electric fields (EFs) facilitate the connection between nerve cells and the opening of signaling channels, enabling the transfer of chemicals from the extracellular matrix, as shown in (Fig. 6.2) [30].

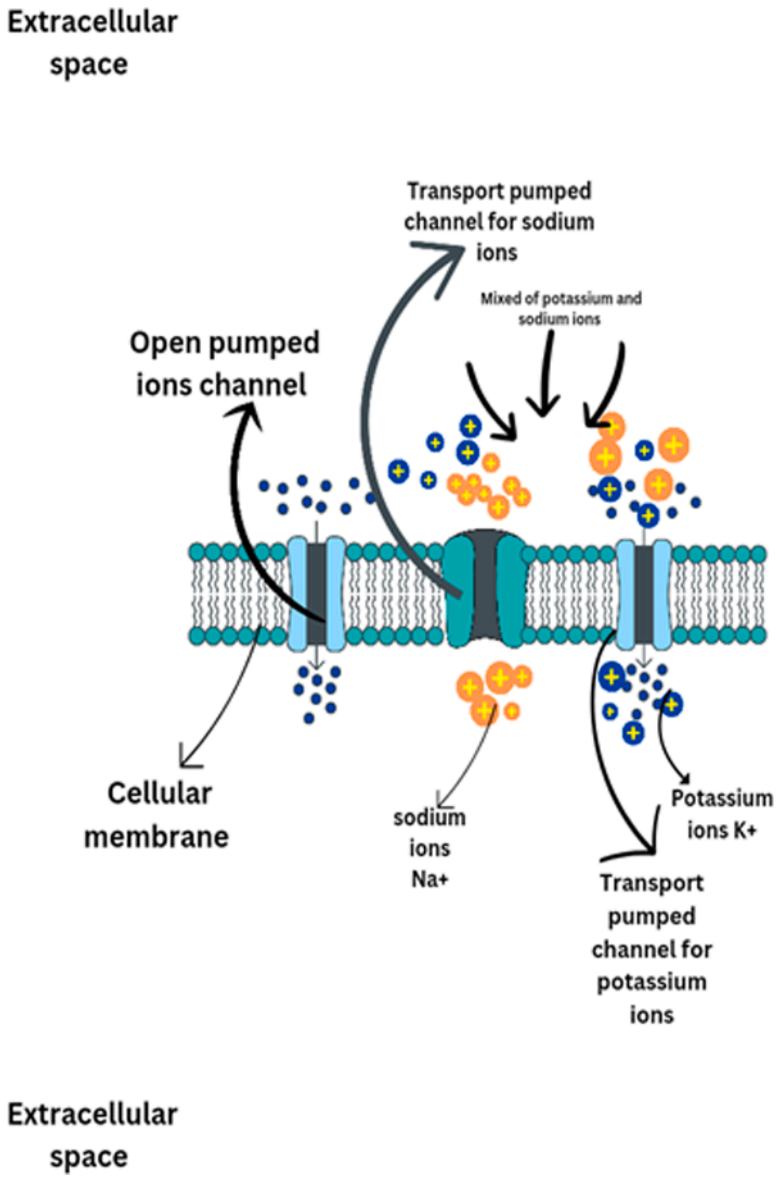


FIGURE 6.2

Ions are pumped across the cellular membrane through ion transport channels.

The anterior-posterior polarization of several cellular structures defines cell motility. We discovered that the plasma membrane is polarized in motile endothelial cells (EC) [36]. These movements produce varying electrostatic charges across the cell membrane [37]. The membrane potential arises from the disparity in electrical charge across the membrane, attributable to active electrogenic pumping (Na + -K+ pump) or passive ion diffusion as in (Fig. 6.2) [35].

The interaction of the cell with the electric field varies with increasing frequency. The electrochemical gradient in the membrane is preserved, but a progressively more significant electrochemical gradient emerges in the cytoplasm [38]. Ion channels are embedded in the cell or organelle membranes, facilitating the regulation of calcium, sodium, potassium, chloride, and other charged molecules within the cytoplasm and various cellular compartments [39]. While a few essential ions contribute to the resting potential, sodium (Na^+) and potassium (K^+) are predominant influences. The negative charges within the cell begin to apply a force to maintain the equilibrium of positively charged ions inside the cell [35].

2.3 Bioelectric phenomena in the human body

Research indicates that cellular behavior is modulated by various distinct stimuli that affect them in vivo. Additionally, bioelectricity pertains to intrinsic electrical signaling through ion channels and pumps at the plasma membrane; the extensive literature on external electromagnetic fields is omitted explicitly due to length limitations. Molecular bioelectricity: the influence of endogenous voltage potentials on cellular behavior and pattern regulation in vivo [40]. Electrical stimulation (ES) has been utilized in cell culture systems to augment neural stem cell (NSC) proliferation, neuronal differentiation, migration, and integration. Electrical stimulation manifests as electrical fields that induce directional cell migration, while electromagnetic fields promote neurogenesis and facilitate the differentiation of NSCs into functional neurons. [41]. Nervous systems are conventionally regarded as facilitating sensory perception and behavioral coordination at the organismal level [42]. Neurons convey information through chemical and electrical signals, with the transmission of action potentials along their axons being a crucial component of this communication [43]. As previously discussed, the neuron's membrane potential is formed by a disparity in the concentration of charged ions. Voltage-gated cation channels are the primary channels involved in the genesis and propagation of neural action potentials [44]. So synaptic connections are fundamentally responsible for presynaptic Ca_2^+ influx following action potential membrane depolarization and postsynaptic responses to neurotransmitter receptor activation [45]. The heart functions as a mechanical pump, essential for delivering blood to various organs. In humans, the heart contracts and relaxes approximately 60 times per minute, with its primary function initiated by a bioelectrical signal known as the action potential (AP) through excitation-contraction coupling [46]. Mechanical signals transmitted via the extracellular matrix (ECM) are essential for cellular function in all tissues. The interplay between electrical activity and intracellular calcium dynamics in cardiac tissues, known as excitation-contraction coupling (ECC), governs mechanical performance. Cardiac cells are responsive to electrical impulses that regulate their activity. They experience contraction and relaxation governed by electric fields [47].

3. Novel insights into advanced materials and architectures for next-generation electric field-response system

3.1 Advanced materials and technologies for systems reacting to electric fields

3.1.1 Conductive polymers

Polymers are essential in biomedical applications due to their diverse chemical properties, as shown in Table 6.1. Natural polymers can be modified top-down, while synthetic polymers are synthesized bottom-up or chemically adapted to meet specific needs. Their flexibility, strength, biocompatibility, and biodegradability vary depending on their chemical, physical, and mechanical structures, influencing their ability to support cell regeneration and differentiation [48]. Conductive polymers (CPs), capable of transmitting electricity, combine the benefits of metals and polymers, offering lightweight, ease of processing, and electrical responsiveness, which can trigger the release of encapsulated substances. Discovered in the 1970s by Shirakawa, Heeger, and MacDiarmid, doping polyacetylene with iodine significantly enhanced its conductivity [48,49]. CPs like Polypyrrole (PPy), Polyaniline (PAni), and Polythiophenes (PTh) are widely used in biomedical fields due to their electrical properties and biological compatibility. PPy is easy to produce and compatible with biological systems; PAni is cost-effective but lacks flexibility, while PEDOT, a PTh derivative, offers excellent conductivity and biocompatibility, ideal for tissue engineering, drug delivery, and biosensors [50].

3.1.2 Piezoelectric materials

Piezoelectric materials exhibit electrical polarization under mechanical stress, the direct piezoelectric effect. Ferroelectric materials, a subset of piezoelectrics, can change their polarization when exposed to an external electric field. This behavior is activated through a poling process, where a strong electric field aligns dipoles within the material [52]. Piezoelectricity involves a linear electromechanical coupling where mechanical stress deforms the material and generates electrical charges (direct effect) while applying an electric field induces mechanical deformation (inverse effect) [53]. Inorganic piezoelectricity arises from asymmetrical charge distribution in the crystal structure, often temperature-dependent [54]. Organic piezoelectricity results from reorientating permanent molecular dipoles under stress, producing net polarization [55]. These materials are applied in automotive, biomedical engineering, electronics, and military fields. For example, a lead-free ZnO piezoelectric

Table 6.1 Properties and conductivity of commonly used conjugated conductive polymers (CPs) for drug delivery and biomedical applications [51].

Polymer	Properties	Conductivity (S/cm)
Polythiophene	Exhibits excellent electrical conductivity, is easy to synthesize, and possesses favorable optical properties	$10–10^3$
Polyaniline	It offers a variety of structural forms, is environmentally stable, and has a low-cost	30–200
Poly(3,4-ethylene dioxythiophene)	A transparent conductor with environmental and electrochemical stability	0.4–400
Polypyrrole	Exhibits high electrical conductivity, straightforward preparation, and superficial surface modification	$10–7.5 \times 10^3$

device converts heart movements into electrical energy and a PVDF-based sensor—harvests biomechanical energy for real-time blood pressure monitoring, showing high sensitivity and linearity [53].

3.1.3 Nanomaterials

Nanomaterials (1–100 nm) possess unique properties, including a large surface area and specialized chemical attributes, making them ideal for biomedical applications. In biosensors, gold and silver nanoparticles improve detection accuracy, while in medical imaging, gold and magnetic nanoparticles enhance resolution, particularly in cancer diagnosis. For drug delivery, nanoparticles enable targeted, controlled release, boosting treatment effectiveness and minimizing side effects. They are also used in tissue engineering to promote regeneration and improve biocompatibility. Additionally, magnetic nanoparticles aid cancer therapies like hyperthermia and photodynamic treatment by generating localized heat or reactive oxygen species. In drug delivery systems (DDS), nanoparticles improve biocompatibility and controlled release through diffusion or biodegradation, with liposomes being a notable FDA-approved example despite challenges with hydrophilic drug solubility [56].

3.2 Structural design elements in electric field-responsive devices such as sensors, electrodes, and actuators

3.2.1 Sensors

Sensors detect physical, chemical, or biological stimuli and convert them into measurable signals. In biomedical applications, they are crucial for real-time monitoring and diagnostics by measuring biomarkers or physiological parameters. Fundamental sensor properties include sensitivity for detecting small changes, selectivity for targeting specific parameters, and accuracy for reliable measurements. Real-time connectivity allows continuous monitoring, while wearability, durability, and stability ensure user comfort and long-term function [57]. Advanced materials enhance sensor performance. Gold nanoparticles (AuNPs) and carbon nanotubes (CNTs) improve conductivity and surface area. At the same time, graphene and graphene oxide (GO) provide high conductivity, making them suitable for detecting compounds like Bisphenol A. Boron-doped diamond (BDD) adds chemical stability, and ionic liquids (ILs) improve electrode performance [58]. Conducting polymers like polyaniline (PANI) and polypyrrole (Ppy) increase sensor sensitivity, often in combination with molecularly imprinted polymers (MIPs) for selective detection of proteins and bacteria. These materials are ideal for wearable and implantable devices due to their biocompatibility [59]. Conductive polymer-based sensors are widely used in electrochemical devices, as in (Fig. 6.3) to monitor metabolites like glucose and cholesterol. In robotic surgery, tactile sensors provide feedback on tissue consistency, while thermal sensors detect temperature changes in diagnosing diseases like cancer and vascular disorders [60].

3.2.2 Electrodes in biomedicine

Electrodes deliver electrical currents to biological tissues for diagnostic and therapeutic purposes, such as transcutaneous electrical stimulation (TES). In transcranial direct current stimulation (tDCS), electrodes from materials like Ag/AgCl deliver low-level currents across the scalp to alter brain activity. The high-definition version (HD-tDCS) offers more precise stimulation and is often used in neurological treatments. Electrodes are also used in electrotherapy for rehabilitation and iontophoresis to enhance drug delivery by increasing skin permeability through electric currents [61].

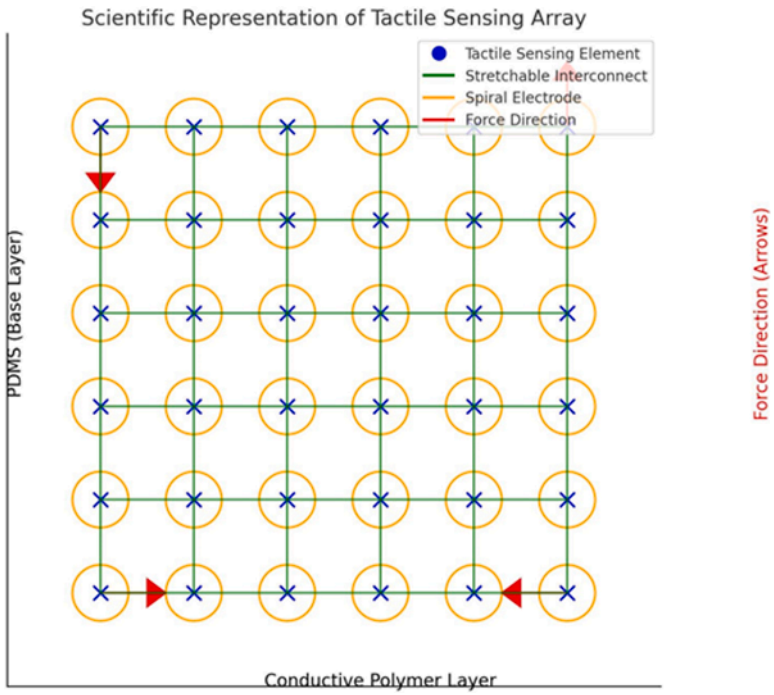


FIGURE 6.3

The diagram of the proposed tactile sensing array.(b) The proposed device while being stretched [60].

3.2.3 Actuators in biomedicine

Actuators are designed with materials like piezoelectric lead zirconate titanate (PZT), known for precise micropositioning in robotics and micropumps, though they require high voltage and careful management of nonlinearity. Shape memory alloys (SMA) are used in medical devices like stents and surgical tools because they can return to a preset shape when exposed to temperature changes, making them suitable for tasks requiring large force and displacement. Hydrogels and smart polymers, valued for their biocompatibility, are standard in drug delivery systems; piezoelectric actuators offer precision by using electric signals for controlled, small-scale movements, while pneumatic actuators with flexible polymer channels are ideal for soft robotics due to their ability to bend under pressure. MEMS-based actuators are critical in microsurgical tools and lab-on-a-chip devices, where precise fluid control is needed. Actuators are widely applied in surgical tools, drug delivery, cardiac implants, and assistive medical technologies [62].

3.3 Types of electric field response systems

3.3.1 Capacitive

Capacitive Power Transfer (CPT): Capacitive Power Transfer (CPT) is a wireless energy transmission technology that utilizes the electric field between two capacitor plates to transfer power without

physical contact. This method enables energy transmission through air or biological tissues, eliminating the need for wires. The energy transfer mechanism relies on the displacement current created by the electric field between the plates. The system typically consists of two metal plates, one placed on the skin and the other underneath it, forming a capacitor. The core principle of CPT is the displacement current, with energy transfer efficiency enhanced by increasing the electric field strength, enlarging the plate area, reducing the distance between the plates, and optimizing the operating frequency. Factors like circuit design, operating frequency, and matching between input and load resistance are critical in improving transfer efficiency.

Applications and Safety: CPT is widely utilized in implanted devices, as in (Fig. 6.4), such as pacemakers and cochlear implants, eliminating frequent battery replacements and enhancing patient convenience. Moreover, biological safety is a significant advantage of CPT. Its lower Specific Absorption Rate (SAR) ensures minimal health risks when used in biological tissues, making it a safer and highly effective choice for powering medical implants and other wireless energy applications. These features make CPT a transformative technology in medical and other wireless energy applications.

3.3.2 Hybrid systems

Hybrid Systems integrate multiple technologies or components to fulfill specific objectives, such as combining different communication methods or energy sources. These systems are precious in applications that demand high efficiency, as they enhance flexibility and reduce costs by blending various approaches. In medical implants, hybrid systems employ synchronized communication and power techniques to meet several essential requirements, such as Wireless Power Transfer, where RF (Radio Frequency) waves provide energy to the implanted device, eliminating the need for conventional batteries.

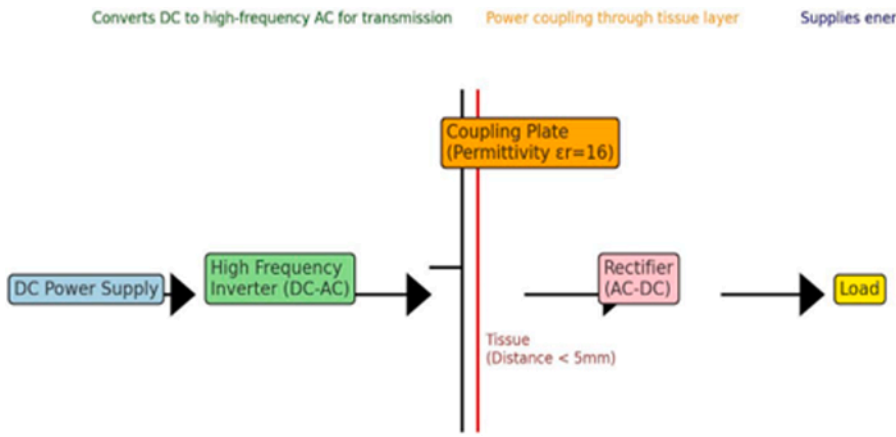


FIGURE 6.4

Dual coupling plate for implantable devices [63].

3.3.3 Backscatter communication

The implant transmits data by reflecting signals from an external reader, ensuring efficient communication with minimal energy consumption, as shown in (Fig. 6.5). A Galvanic Communication Link uses electrical impulses to facilitate communication between implants within the body, enabling data exchange and synchronization among devices. Applications of Hybrid Systems include Medical Implants, such as pacemakers and glucose monitors, which help track patient health and enhance medical care. Multi-implant Communication allows implants to connect and validate the collected data. Additionally, Wireless Capsule Endoscopy is used to diagnose gastrointestinal conditions by transmitting video data as the capsule travels through the body.

3.3.4 Inductive systems

Inductive Power Transmission (IPT) wirelessly transfers energy via magnetic fields, powering implantable medical devices (IMDs) like neural stimulators, cochlear implants, and retinal implants without wired connections. This reduces surgical battery replacement infection risks and enhances patient comfort. Key components include printed spiral coils (PSC) for efficient external power transfer, wire-wound coils (WWC) for internal efficiency, LC circuits for resonance tuning, and PMICs for stable voltage regulation. IPT also supports biomedical sensors for battery-free health monitoring, wearable devices like hearing aids, and telemetry systems, enabling data and power transmission for advanced patient care [65].

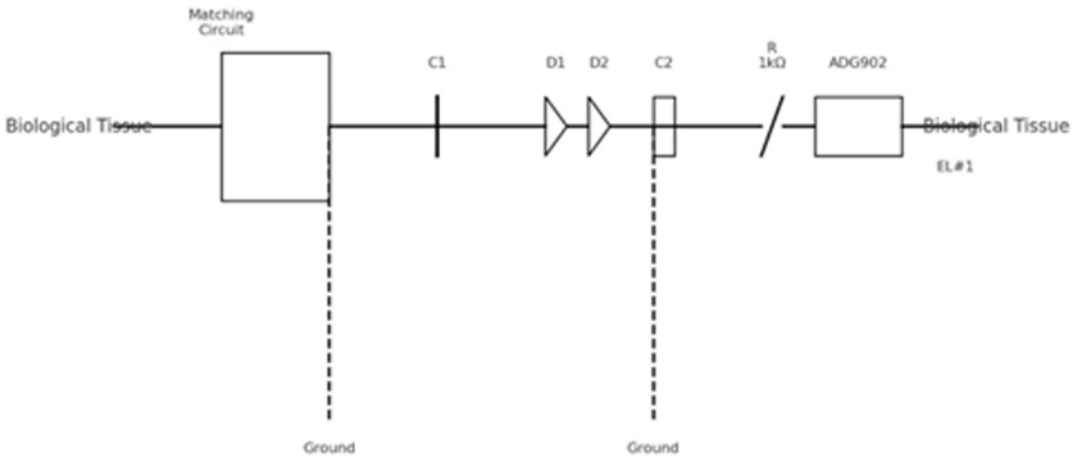


FIGURE 6.5

An electronic implant circuit designed for wireless power transfer, backscatter communication, and galvanic connectivity [64].

4. Applications for biomedical engineering

4.1 Diagnostic applications

4.1.1 Imaging techniques based on electric field response

4.1.1.1 Basic principles of electrical impedance tomography

Electrical Impedance Tomography (EIT) is a noninvasive medical imaging technique that reconstructs internal conductivity distributions by injecting a current or voltage pattern through electrodes placed on the skin and measuring the resulting voltages. The method relies on the tissue's electrical properties, with impedance varying based on frequency and tissue composition. At low frequencies, the current flows primarily through extracellular spaces, whereas at higher frequencies, it penetrates intracellular media, enabling differentiation of tissue types and physiological conditions. Building on these principles, EIT operates in two primary modalities: absolute EIT, which uses a single dataset to create an impedance map, and differential EIT, which compares datasets over time or frequency. The differential approach reduces modeling errors and allows for monitoring time-varying phenomena like lung ventilation while also identifying tissue differences based on electrical frequency. Additionally, current injection methods, such as adjacent, polar, and trigonometric techniques, provide varied imaging resolutions depending on electrode placement and impedance readings.

Moreover, EIT offers several advantages, including its noninvasive, radiation-free, and low-cost Nature, combined with high temporal resolution, making it highly suitable for real-time bedside monitoring, particularly in ICU settings. Emerging wearable EIT devices further expand their applications to home monitoring and early anomaly detection, providing a portable and efficient solution for chronic care management. Despite its lower spatial resolution compared to other imaging methods, the potential for wearable integration positions EIT as a transformative tool for enhancing patient monitoring and improving healthcare outcomes [66].

4.1.1.2 Applications of EIT

Pulmonary Monitoring with Electrical Impedance Tomography (EIT) is a highly effective, noninvasive tool for assessing respiratory mechanics and optimizing positive end-expiratory pressure (PEEP) in obese patients prone to respiratory complications. When combined with esophageal manometry during mechanical ventilation, it significantly reduces mortality in obese individuals with respiratory failure. In addition to this, EIT plays a crucial role in lung transplantation, where it evaluates organ viability and monitors lung function pre and postsurgery, with multi-frequency EIT and bioelectrical spectroscopy enhancing outcomes and patient monitoring.

Moreover, for pulmonary edema, EIT quantifies extravascular lung water (EVLW), using metrics like the lung-water ratio for bedside assessment and management. In pulmonary embolism (PE), EIT detects lung perfusion differences noninvasively, with proven efficacy in diagnosing high-risk cases using techniques like hypertonic saline boluses. Its portability, low cost, and radiation-free Nature make EIT valuable for a wide range of applications, from intensive care to outpatient settings [67].

4.1.1.3 Brain imaging and thermal monitoring in tumor treatment

Electrical Impedance Tomography (EIT) provides a noninvasive and real-time solution for brain imaging, enabling the monitoring of cerebral blood volume changes with high temporal resolution. Its applications include stroke detection and epilepsy monitoring, making it a valuable tool for

neurological diagnostics. Additionally, EIT plays a crucial role in thermal monitoring during tumor treatment, particularly in hyperthermia procedures like radio-frequency ablation. It ensures precise temperature monitoring, allowing accurate targeting of tumor tissues while protecting surrounding healthy structures, thereby enhancing the safety and effectiveness of treatment protocols [68].

Integrating AI with EIT and combining it with hybrid imaging techniques like EIT-MRI significantly enhances image accuracy and diagnostic capabilities. Deep learning models, such as CNNs and V-shaped dense denoising networks, improve the reconstruction of complex structures, preserve boundaries, and reduce noise. These advancements create detailed, high-quality images essential for precise diagnosis.

In addition to AI, hybrid imaging techniques, such as the combination of EIT with MRI, capitalize on the complementary strengths of each modality. While MRI provides high spatial resolution, EIT offers dynamic functional imaging, allowing real-time visualization of physiological processes and abnormalities. This synergy enhances diagnostic utility and expands the clinical applications of imaging, particularly in scenarios where traditional imaging methods may be insufficient. These innovations demonstrate the potential for integrated approaches to transform diagnostic imaging by combining the precision of AI with the strengths of multiple imaging modalities [67,68].

4.1.1.4 Magnetic resonance electrical impedance tomography

Magnetic resonance electrical impedance tomography MREIT integrates MRI technology to address the limitations of conventional Electrical Impedance Tomography (EIT), particularly its low data density and inability to achieve high-resolution conductivity reconstructions. By utilizing MRI to measure the magnetic flux density (Bz) induced by externally injected currents, MREIT achieves superior spatial resolution. This magnetic flux density reflects the conductivity distribution within the imaging object, enabling the reconstruction of internal conductivity images. The "harmonic Bz algorithm" development in 2001, which traces conductivity variations using the Laplacian of Bz, significantly improved conductivity image quality, making MREIT a reliable tool for advanced medical imaging.

In addition to the Advanced Applications of MREIT in Soft Tissue Imaging, MREIT has shown exceptional promise in various applications. Breast phantom and biological phantom imaging successfully distinguish different conductivity levels across tissue types, providing enhanced contrast that often surpasses conventional MRI in detecting soft tissue abnormalities. In postmortem animal imaging, such as canine brain studies, MREIT offers high-resolution conductivity maps, revealing structural differences between gray and white matter, highlighting its potential for postmortem analysis and in vivo applications. Furthermore, in ischemic region detection, MREIT effectively identifies areas of reduced blood flow, which is critical for conditions like strokes. Capturing conductivity changes in ischemic zones proves invaluable in brain research and diagnostics. These advancements position MREIT as a complementary and highly specialized imaging tool for enhancing soft tissue differentiation and clinical diagnostics [69,70].

4.1.2 Noninvasive monitoring systems: ECG, EEG, and EMG

EEG Signal Characteristics and Applications: EEG signals originate from ionic currents in the dendritic membranes of pyramidal neurons within cortical layers IV-V. These currents create electrical dipoles driven by excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials, requiring synchronization over ~10 cm^2 of cortical surface for influential recordings. Through volume conduction,

neuronal signals propagate through brain tissue and the skull to reach the scalp. The orientation of dipoles is crucial: radial dipoles (perpendicular to the cortical surface) generate localized potentials, while tangential dipoles (along the sulci walls) produce opposing charges. Critical components for signal generation include electrodes (silver/silver chloride or gold with conductive paste), amplifiers (enhancing weak neural signals while preventing feedback), and filters (high-pass filters removing low-frequency artifacts like movement and low-pass filters reducing high-frequency noise). EEG utilizes various montage types: bipolar (detecting phase reversals), referential (highlighting broader patterns), standard average (reducing bias via averaged signals), and Laplacian (focusing on localized activity). Clinically, EEG has diverse applications, such as diagnosing epilepsy (detecting interictal spikes, focal slowing, and seizures), assessing sleep disorders (analyzing patterns in sleep apnea and REM activity), tracking neurodegenerative diseases (cortical slowing in Alzheimer's or Parkinson's), monitoring anesthesia (guiding dosage and identifying surgical complications), and evaluating traumatic brain injuries (detecting regional slowing or suppression), as shown in Fig. 6.6.

Despite its benefits, EEG has limitations, including poor spatial resolution (difficulty detecting small or deep structures), susceptibility to noise and artifacts (e.g., muscle movements, environmental noise), and challenges in standardization (variations in electrode placement and montage selection). Additionally, interpretation complexity and older technological constraints hinder its widespread precision, though emerging digital tools address some of these issues at a higher cost [71].

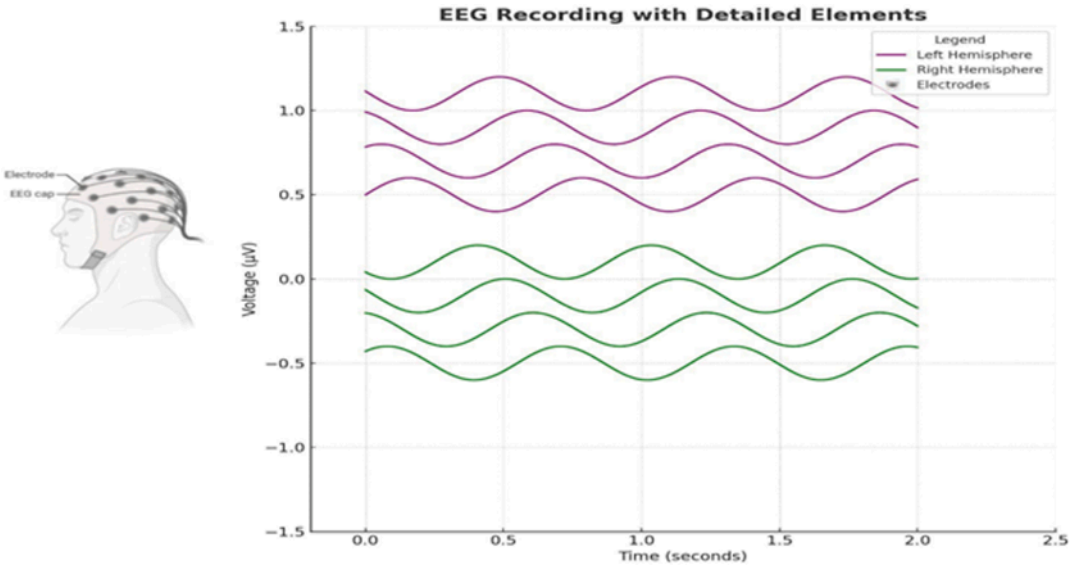


FIGURE 6.6

An EEG recording used for monitoring brain activity. The left section depicts the placement of the EEG cap with electrodes positioned on the scalp, while the right section illustrates voltage signals (measured in μV) recorded over time (in seconds) from the left hemisphere (*purple*) and right hemisphere (*green*). The observed irregular waveforms are indicative of potential epileptic activity, reflecting abnormal neural function.

Core Technologies for Space Neurology: Electroencephalogram (EEG) captures electrical activity from the cerebral cortex by measuring synaptic potentials, creating a map of brainwave patterns (delta, theta, alpha, beta) to analyze brain function. Its applications include detecting brain activity changes caused by microgravity and isolation, studying sleep disturbances and cognitive impairments during spaceflight, and monitoring real-time neurocognitive performance and decision-making. For example, the NEUROSPAT project on the ISS used EEG to assess visuospatial performance and revealed brainwave shifts during docking simulations under microgravity. Functional Near-Infrared Spectroscopy (fNIRS) measures hemoglobin concentration changes in the brain using near-infrared light, offering insights into cerebral activity, oxygenation, and blood flow. As a portable alternative to fMRI, it tracks attention, behavior, and workload sensitivity, especially under stress and sleep deprivation, making it ideal for long-term space missions. Its compact, energy-efficient design integrates well with EEG for a comprehensive view of brain function. Ultrasound uses high-frequency sound waves to measure intracranial pressure, cerebral blood flow, and vessel dimensions, tracking fluid redistribution caused by microgravity. It monitors changes associated with Spaceflight-Associated Neuro-Ocular Syndrome (SANS), blood pooling, and optic nerve sheath diameter, as demonstrated in the "Drain Brain" study on the ISS. Compared to larger imaging systems like MRI, CT, and PET, which are limited to pre/postflight assessments and diagnosing conditions like radiation-induced damage, technologies like EEG, fNIRS, and ultrasound excel in portability, efficiency, and real-time space applicability, making them indispensable for in-flight monitoring and research [72].

ECG Monitoring Systems: ECG monitoring systems are vital in diagnosing and managing cardiovascular diseases (CVDs), a leading cause of global mortality. These systems have evolved significantly with sensor technologies, IoT, mobile computing, and edge/cloud computing advancements. They are deployed in various settings, including hospital-based systems for ICU and nonICU environments, home-based monitoring for chronic conditions using wearable devices, ambulatory-based solutions for real-time daily activity tracking, and remote telemedicine through telemedicine for long-distance cardiac assessment. Modern features include real-time monitoring via wearable and noncontact devices and advanced data analytics like deep learning for enhanced diagnostic accuracy. Applications span across multiple areas: medical diagnostics, where they detect arrhythmias, atrial fibrillation, and myocardial infarction and provide predictive analytics in ICUs; telemedicine, which enables remote monitoring and reduces hospital visits; fitness and sports, offering wearable solutions for analyzing heart performance and recovery; driver and occupational safety, monitoring fatigue and stress in high-stress environments; and predictive health monitoring, where AI-powered systems predict diseases like arrhythmia or stroke and support personalized treatment. These advancements in ECG technology are revolutionizing cardiac care by improving precision, accessibility, and preventative health measures across various fields [67].

Types of Wearable ECG Devices: Wearable ECG devices come in various forms, including watches like the Apple Watch Series, Samsung Galaxy Watch, and Garmin Epix Pro, which offer single-lead ECG monitoring and additional functionalities such as heart rate tracking, as illustrated in Fig. 6.7, with some models receiving FDA approval. Bands/Bracelets, such as AliveCor KardiaBand and Fitbit Charge, focus on heart rate monitoring and arrhythmia detection with specific FDA-approved models. Patches, like the Zio Patch and Samsung S-Patch, provide adhesive-based continuous ECG monitoring, offering clinical-grade tracking suitable for prolonged use in patients with cardiovascular risks. Smart Textile Garments, including Hexoskin Smart Shirts and Omsignal System Garments, integrate multi-lead ECG monitoring into comfortable, noninvasive clothing for sports and daily activities.

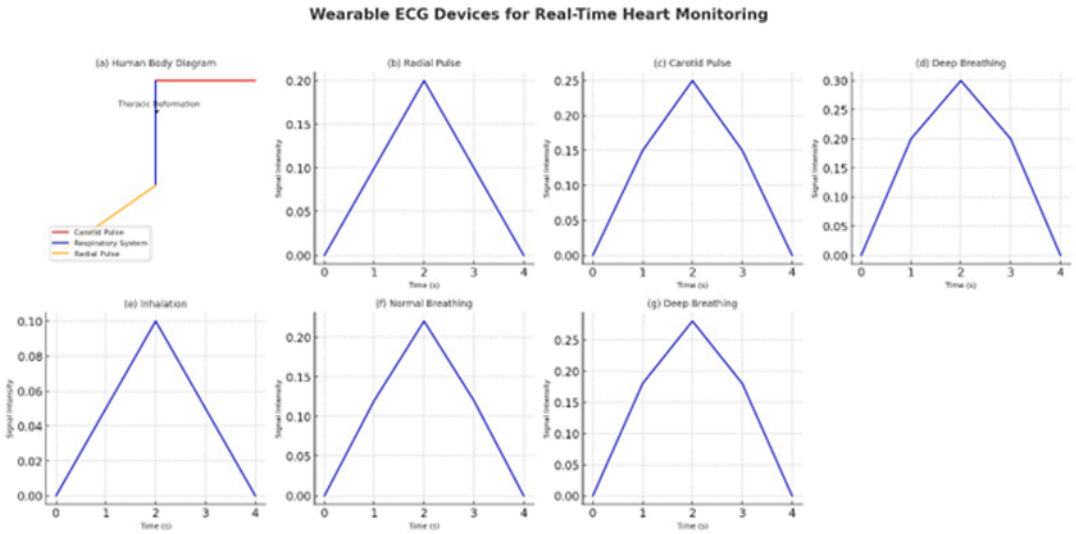


FIGURE 6.7

Wearable ECG devices used for real-time heart and respiratory monitoring. Panel (a) provides a schematic human body diagram showing the thoracic deformation and signal measurement locations, including the carotid pulse (red), respiratory system (*blue*), and radial pulse (*yellow*). Panels (b) and (c) represent the signal intensities over time for the radial and carotid pulses, respectively. Panel (d) highlights the increased signal intensity observed during deep breathing. Panels (e), (f), and (g) focus on respiratory signals during inhalation, regular breathing, and deep breathing, respectively, demonstrating the device's ability to monitor physiological changes in real-time.

Additionally, miscellaneous devices such as intelligent rings (e.g., Motiv Ring, Oura Ring), phone attachments, and ear-based monitors offer compact designs with basic ECG functionality. These devices have broad applications, including medical diagnostics, enabling real-time monitoring of arrhythmias, atrial fibrillation, and myocardial infarction, as well as long-term data collection for chronic conditions. In fitness and sports, they monitor athletic performance, recovery, and cardiac stress during activity, enhancing safety during training or competition. Telemedicine applications allow remote monitoring with real-time alerts for cardiac events, reducing hospital visits for chronic patients. For occupational health, they support high-stress professions like construction or deep-sea diving by managing stress-induced cardiovascular risks. Finally, home-based care offers continuous monitoring for elderly or disabled individuals, aiding in postoperative recovery or detecting sleep conditions such as sleep apnea. These advancements revolutionize healthcare, providing accessible, versatile solutions across medical, fitness, and daily care settings [73].

Electromyography (EMG) is a diagnostic procedure used to assess the health of muscles and the nerve cells controlling them by recording the electrical activity of skeletal muscles using electrodes. The key features include needle electrodes inserted directly into the muscle for localized monitoring and surface electrodes for less invasive and generalized monitoring. EMG aims to identify abnormalities in muscle function caused by nerve or muscle disorders and differentiate between myopathic

(muscle-related) and neuropathic (nerve-related) causes of muscle weakness. The procedure involves measuring electrical activity at rest and during voluntary muscle contraction, observing insertional activity, and identifying spontaneous potentials such as fibrillation and positive sharp waves. Applications of EMG include evaluating neuromuscular disorders like muscular dystrophy, amyotrophic lateral sclerosis (ALS), and peripheral neuropathy, detecting early stages of motor unit remodeling, providing intraoperative monitoring to assess nerve continuity during surgery, and determining the severity of nerve damage. Key clinical parameters measured include CMAP (Compound Muscle Action Potential) for motor nerve functionality, SNAP (Sensory Nerve Action Potential) for sensory nerve health, F-wave for proximal nerve conduction and root integrity, and Motor Unit Potentials (MUPs) for muscle innervation and motor neuron health. EMG is a critical tool for diagnosing and managing neuromuscular conditions, aiding surgical decisions, and tracking disease progression or recovery [74].

sEMG for Respiratory Monitoring: Surface Electromyography (sEMG) is a noninvasive technique for measuring the electrical activity of respiratory muscles. Unlike invasive methods, such as nasogastric catheters with electrodes, sEMG uses surface electrodes to monitor the diaphragm, parasternal intercostals, sternocleidomastoid, and scalene muscles. Its advantages include being noninvasive, patient-friendly, and capable of monitoring multiple respiratory muscles while providing insights into neural respiratory drive and muscle activation. However, it faces challenges, such as susceptibility to noise and artifacts like cardiac interference and crosstalk from adjacent muscles, necessitating high sampling frequencies (500–1000 Hz), advanced filtering techniques (e.g., gating, wavelet denoising), and precise electrode positioning. Applications in Respiratory Medicine span both research and clinical fields. In research, sEMG helps understand respiratory muscle dynamics by analyzing muscle activation during different states (e.g., rest, exercise, or mechanical loading), exploring mechanisms of breathlessness in COPD and asthma, and detecting early signs of respiratory muscle fatigue. In clinical settings, it is vital to monitor critical care, evaluate neural respiratory drive in ICU patients, predict weaning success from mechanical ventilation, and manage chronic respiratory diseases like COPD, asthma, and neuromuscular disorders. It also optimizes mechanical ventilation by detecting patient-ventilator asynchrony and assessing muscle contributions during spontaneous breathing trials.

Additionally, sEMG aids inspiratory muscle training (IMT) by tracking changes in muscle activation during training and evaluating outcomes. At the same time, in pediatrics, it monitors respiratory activity in children where voluntary tests are impractical, correlating data with clinical scores for conditions like asthma. This comprehensive utility positions sEMG as a critical tool for advancing respiratory research and patient care [75].

4.2 Therapeutic uses

4.2.1 Bioelectric mechanisms in wound healing

Electrical Stimulation (ES) is a technique to mimic the body's natural "current of injury," which is crucial for cellular activities in wound repair. These bioelectric currents drive essential processes such as cell migration, proliferation, and differentiation to aid wound closure and tissue regeneration. ES supports and accelerates healing by reestablishing these electric signals, particularly in chronic wounds with weak or absent natural bioelectric cues.

One of the primary mechanisms in this process is "galvanotaxis," which refers to the directional movement of cells under an electric field. This controlled migration allows cells to align and move

toward the wound site effectively. How galvanotaxis in ES overrides other environmental factors, directing a more organized and efficient cellular response toward the injury, thereby expediting wound healing.

The "current of injury" generated during wound healing creates an endogenous lateral electrical field. When the epithelium is disrupted, this current flows from the wound edges toward the center, promoting cellular activity to cover and close the wound. This is particularly important in chronic wounds that fail to heal due to a weakened bioelectric signal, as ES can be applied to mimic and restore this crucial aspect of healing. ES is found to be beneficial across various phases of wound healing. ES enhances vascular permeability in the inflammatory phase, allowing more immune cells to reach the wound. ES promotes fibroplasia, re-epithelialization, and angiogenesis during the proliferative phase, which is crucial for tissue regeneration. Lastly, ES helps form more muscular and more organized scar tissue in the remodeling phase. These effects demonstrate the potential of ES as a complementary approach to traditional wound care, especially for complex, nonhealing wounds [68].

4.2.2 Responses of stem cells to electric fields

Electric fields (EFs) stimulate stem cells, enhancing their regenerative capabilities for wound healing. EFs influence stem cell behavior by guiding migration and promoting differentiation, which is particularly useful for tissue repair. This response in stem cells is evident across various types, including neural progenitor cells, adipose-derived stromal cells, and mesenchymal stem cells (MSCs).

Neural Progenitor Cells (NPCs) are Electric fields that, when applied with specific pulse parameters, can stimulate NPCs to differentiate into various neural cells (e.g., neurons, astrocytes). This differentiation aids tissue repair and regeneration, potentially applicable to neural wound treatments.

Adipose-Derived Stromal Cells (ASCs) are EFs that guide ASCs to migrate toward wound sites, helping with skin regeneration. ASCs align perpendicularly to the electric field, enhancing their regenerative efficiency in wound healing applications.

Mesenchymal Stem Cells (MSCs), which play a significant role in wound healing, rapidly respond to EFs, migrating toward the cathode. EFs influence MSCs to release factors that attract other wound-healing cells, like macrophages and endothelial cells, facilitating coordinated tissue repair.

Additionally, the mechanisms by which stem cells interpret electric cues involve pathways like PI3K/AKT, which regulate migration and survival. Additionally, EF-stimulated stem cells exhibit polarized receptor distributions, optimizing their function in wound sites [76].

Role of electric fields (EFs) through electroactive biomaterials in bone regeneration:

The role of electroactive biomaterials in bone regeneration lies in their ability to replicate or amplify the natural bioelectric signals present in bone tissue, thereby promoting the healing and regeneration process. These materials, including piezoelectric and conductive polymers, help promote cellular activities essential for bone healing, such as osteogenesis, by providing a controlled bioelectric environment.

Piezoelectric Biomaterials: These materials generate electric charges in response to mechanical stress, similar to natural bone's piezoelectric properties. For example, materials like barium titanate (BT) and hydroxyapatite composites create electrical signals when subjected to pressure, aiding bone cell adhesion, proliferation, and differentiation. This mimics the natural electrical microenvironment of bone, supporting bone formation and repair.

Conductive Polymers are Polymers like polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT), which are widely used for their flexibility and conductivity. They support bone cell viability

and function by delivering stable electrical stimulation, thus enhancing implants' structural and functional integration within bone tissue.

Mechanisms of Action: These biomaterials work through pathways that activate ion channels, which lead to cellular behaviors like adhesion, alignment, and osteogenic differentiation. Electrostimulation also influences signaling pathways, such as the MAPK pathway, promoting the expression of growth factors necessary for bone regeneration [77].

4.2.3 Functional electrical stimulation (FES) and oscillating field stimulation (OFS)

Functional Electrical Stimulation (FES) is a technique that applies electrical currents to stimulate muscles in individuals with paralysis, particularly those with spinal cord injuries. This stimulation helps restore specific motor functions by generating muscle contractions lost due to nerve damage.

4.2.4 Mechanism of FES

Functional Electrical Stimulation (FES) uses low-level electrical currents applied through skin electrodes or implants near nerves to trigger action potentials, causing coordinated muscle contractions. Controlled by a microprocessor, these impulses mimic natural movement patterns, enabling functions such as standing, walking, hand grasping, and breathing for patients with spinal cord injuries (SCI). Innovations like the Freehand System allow tetraplegic patients to control hand movements, while other devices assist paraplegic patients in walking short distances. Additionally, the Oscillating Field Stimulation (OFS) technique promotes bidirectional axonal growth by alternating an electric field across injured spinal cord areas every 15 min, leveraging neurites' faster growth toward the cathode to achieve sustained axonal regeneration.

4.2.5 Applications of OFS

Has shown promise in animal studies. For example, in canine models with naturally occurring spinal cord injuries (SCI), OFS treatment led to notable neurological improvements, with some dogs regaining limited walking ability. So, this technique represents a significant advancement in SCI therapy, providing a foundation for combining electrical stimulation with other regenerative treatments to create a conducive environment for spinal cord regeneration [78].

4.2.6 Bioelectric applications in neuromodulation techniques

4.2.6.1 Transcranial magnetic stimulation

Mechanisms of TMS are a noninvasive technique that generates electric currents in targeted brain areas through magnetic induction, influencing neural activity. The primary action involves the depolarization of pyramidal neurons, which contributes to the activation of motor and nonmotor circuits. Different TMS methods include:

Repetitive TMS (rTMS): Delivers repeated pulses to stimulate or inhibit brain regions and is used for surface-level treatments. Deep TMS (dTMS): Employs H-coils for deeper brain stimulation, facilitating more profound brain structures.

Efficacy Across Disorders: TMS has shown promising outcomes in treating a variety of psychiatric and neurological disorders, including Major Depressive Disorder (MDD), obsessive-compulsive disorder (OCD), Schizophrenia, Parkinson's Disease (PD), and anxiety disorders [79].

4.2.6.2 Deep brain stimulation (DBS): Mechanisms and targeting approaches

DBS involves implanting electrodes in target brain areas to modulate neural networks, primarily used in Parkinson's disease (PD), essential tremor, and epilepsy. So, DBS affects brain connectivity, aiding the understanding of network responses and leading to more accurate programming for optimal therapeutic effects.

Advancements in DBS Technology: Newer DBS technologies incorporate MRI compatibility, rechargeable IPGs, segmented electrodes, and bidirectional DBS systems that allow real-time neural sensing. Such innovations enable clinicians to record neural activity and adapt stimulation parameters, improving treatment precision. Novel approaches such as interleaved stimulation, variable frequency stimulation, and closed-loop or adaptive DBS (DBS) are being tested to adjust therapy in response to patient needs, which minimizes side effects and extends device life [69].

4.2.6.3 The combining between (TMS), and (DBS)

Mechanisms of Combined TMS-DBS: Combining TMS and DBS enables simultaneous stimulation of cortical and subcortical brain regions, which enhances our understanding of the brain's connectivity and modulation pathways. TMS, being noninvasive, can influence cortical plasticity, while DBS targets deeper brain regions like the basal ganglia. This combination is primarily used to study and modulate excitability and plasticity in both cortical and subcortical structures. TMS-DBS pairing might enable cortical synaptic plasticity, potentially offering therapeutic effects by adjusting neurophysiological responses in a coordinated manner.

Clinical Applications in Movement Disorders: TMS-DBS combined application has shown promising results in treating movement disorders, particularly in conditions like Parkinson's disease and dystonia; in Parkinson's disease, TMS over the motor cortex can complement DBS in the subthalamic nucleus, potentially reducing motor symptoms by modulating the motor cortex's excitability and improving overall motor control, In dystonia, DBS targeting the globus pallidus internus (GPi) combined with TMS over motor areas may address abnormal cortical excitability, offering symptomatic relief through long-term changes in synaptic responses [80].

4.3 Targeted drug delivery systems Fig. 6.8

4.3.1 Electroosmosis for targeted drug delivery

Mechanisms of Electroosmosis: an innovative approach to enhance the deep penetration of drugs into biological tissues using an external electric field. This method leverages electroosmosis, a phenomenon in which fluid moves through tissue capillaries or porous membranes under an electric field without requiring direct contact with biological tissues or applying electric currents directly through them. A device designed for electroosmotic drug delivery consists of two electrodes placed above and below the treatment area. When an electric field is applied, the therapeutic liquid between the electrodes penetrates the tissue under the influence of the field lines directed through it. The device may include a heating element in one electrode, which warms the liquid and the target tissue, reducing viscosity and facilitating deeper penetration by expanding tissue pores. This device ensures the drug spreads evenly and efficiently into the affected tissue, making it ideal for cases where traditional methods fail to deliver adequately. This enhances drug delivery for more significant or dispersed areas, such as joint areas.

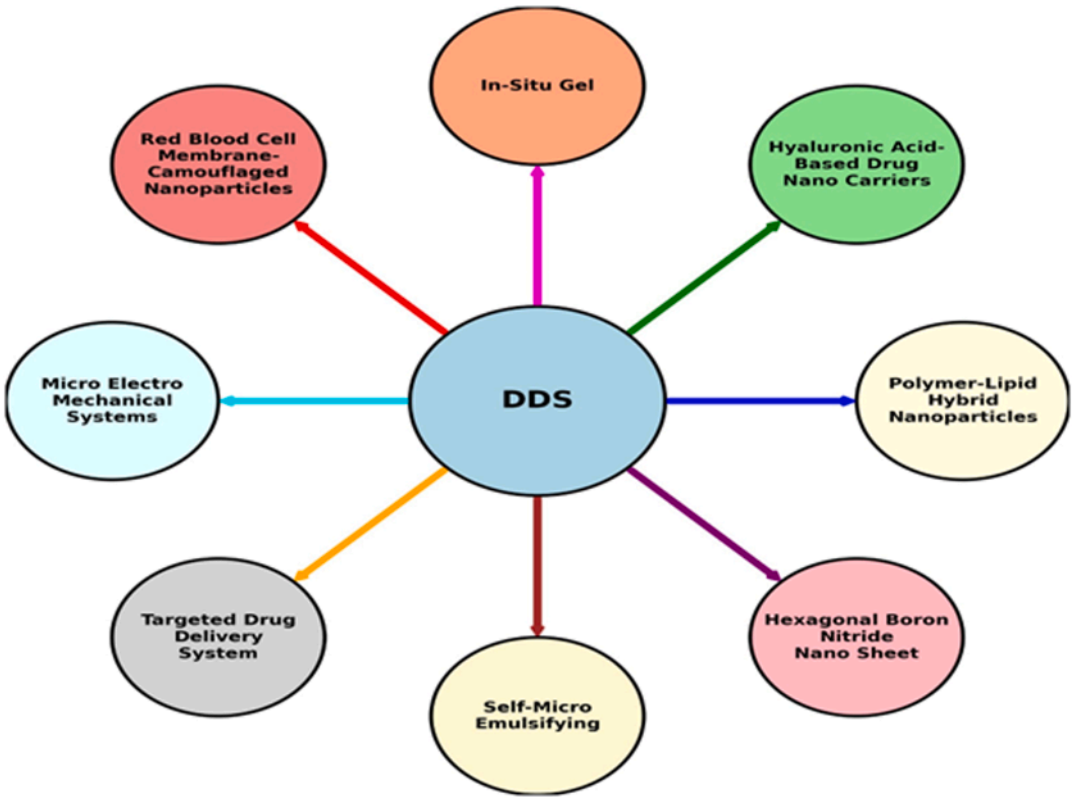


FIGURE 6.8

Drug delivery systems (DDS) integrate advanced technologies to enhance therapeutic precision and efficiency. Fundamental approaches include nanoparticles camouflaged with red blood cell membranes for biocompatibility, in-situ gels for sustained release, and hyaluronic acid-based carriers for targeted delivery. Other innovations, such as polymer-lipid hybrid nanoparticles and hexagonal boron nitride nano sheets, improve drug encapsulation and controlled release. Additionally, self-microemulsifying systems enhance the solubility of hydrophobic drugs, while targeted delivery systems and MEMS enable precise localization and efficient administration. Together, these advancements demonstrate the transformative potential of DDS in modern medicine.

Advantages Over Traditional Methods: Unlike conventional methods like electrophoresis and phonophoresis, electroosmosis avoids altering the drug's chemical composition, a risk associated with direct electric currents. Traditional methods also face limitations with drugs' depth of penetration (usually up to a few millimeters) and potential adverse effects on patients with cardiovascular issues. In contrast, electroosmotic delivery allows broader, deeper distribution of drugs without these restrictions, enabling treatment over larger, spaced areas, thus increasing therapeutic effectiveness [81].

4.3.2 Electric field-driven drug delivery

4.3.2.1 Electroporation for drug and gene delivery

Electroporation involves applying short, intense electric pulses to cells, temporarily permeating their membranes. This allows large molecules, such as DNA and drugs, to enter cells effectively. Initially used for in vitro gene transfer, EP has been instrumental in delivering DNA vaccines, anticancer drugs, and therapeutic genes directly into tissues like muscle, skin, and tumors in vivo. EP makes it possible for high expression levels to happen by increasing permeability. Clinical effectiveness is often reached without viral vectors, which can trigger immune responses. EP has shown potential in several diseases, particularly in oncology and infectious diseases, as it can enhance the immune response to DNA-based therapies, including melanoma, HIV infections, human papillomavirus (HPV), and hepatitis C [82].

4.3.2.2 Iontophoresis for transdermal drug delivery

Iontophoresis is a noninvasive technique that uses a low electric current to drive charged drug molecules across the skin, improving transdermal delivery. The stratum corneum, the skin's natural barrier, usually limits the delivery of high molecular weight drugs, proteins, and peptides. Iontophoresis creates an electrostatic repulsion that pushes ionic medications through the skin and into the bloodstream. This avoids first-pass metabolism, which can lower the drug's bioavailability when taken by mouth. Iontophoresis works in three ways: "electro-repulsion, electroosmosis, and increased permeability". Because of this, iontophoresis has much potential for treating localized pain and inflammation and giving localized anesthetic effects. It also helps deliver drugs throughout the body for long-term conditions requiring consistent dosing without injections or oral administration [83].

Iontophoresis-based transdermal systems surpass traditional methods by using electrical currents to bypass the stratum corneum, enhancing drug permeability and delivery efficiency. These systems provide controlled and sustained release, reducing variability in drug absorption and ensuring consistent therapeutic levels. Additionally, they minimize side effects by allowing precise dosing and enabling rapid cessation of delivery by turning off the current, offering a safer and more effective alternative to conventional transdermal systems [84].

5. Challenges and limitations of an electric field in biomedical applications

5.1 Evaluating the biological and safety impacts of exposure to electric fields

5.1.1 Safety and biological effects of static electric fields

Static electric fields (EFs), generated naturally and by HVDC lines, primarily interact with humans and animals through surface-level phenomena, such as hair movement caused by electrostatic forces. These interactions do not lead to more profound physiological effects. Studies on animals have noted changes in metabolism and immune function, but these are likely secondary responses to sensory stimulation rather than direct field effects.

Moreover, static EFs produced by HVDC lines do not penetrate deeply enough to affect internal organs and are not considered a significant health risk. There is no substantial evidence linking static EFs to cancer, and while microshocks from contact with conductive objects may cause brief discomfort, they are harmless. Overall, the effects of static EFs are limited to the skin and hair, with no evidence of profound biological impacts. Further research is needed to refine safety guidelines based

on human perception thresholds [85]. However, electromagnetic fields (EMFs) pose different biological and safety concerns. While EMFs have been explored for potential therapeutic applications in treating diseases like cancer and neurological disorders, their prolonged exposure raises safety questions. Research continues investigating safe exposure limits due to conflicting findings on health impacts. Mechanisms such as the radical-pair model and biochemical thermodynamic interactions explain how EMFs might affect biological processes. There is still no consensus on therapeutic EMF parameters, highlighting the need for more research to establish standardized safety guidelines and practical therapeutic uses [70].

5.2 Technical challenges in biomedical applications

Electric field-based technologies have shown remarkable potential in biomedical applications, including cancer therapy, tissue repair, and drug delivery systems. Despite these advancements, technical barriers must be overcome to enable full integration into current medical practices. Major obstacles involve system durability, the challenges of miniaturizing devices, and the compatibility of these technologies with existing medical infrastructures. This article will discuss these challenges and their implications for the future of biomedical technology.

5.2.1 System durability

The durability issue is significant in developing biomedical devices employing electric fields. Many such devices are designed for extended implantation or repeated applications, making them vulnerable to mechanical stress, environmental wear, and biological reactions over time. For instance, devices used for nerve stimulation or cardiac pacing must operate effectively in the human body's challenging environment, including exposure to fluids and immune system responses [71].

5.2.2 Miniaturization

Another major challenge is the miniaturization of biomedical devices. For many applications, these devices need to be small enough for minimally invasive procedures or to deliver therapies at a microscopic level, as seen in electroporation for drug targeting. However, as devices get smaller, they face issues like reduced battery capacity, weaker signals, and material limitations. Additionally, smaller devices are at a higher risk of mechanical failure or may not generate adequate electric fields for effective biological outcomes [72].

5.2.3 Integration with existing medical technologies

Integrating electric field-based devices with current medical technologies presents a significant challenge. Healthcare systems today utilize a wide array of sophisticated diagnostic and therapeutic tools, from imaging devices to robotic surgery platforms. To adopt new technologies, they must integrate seamlessly with these tools, enabling real-time analysis, treatment planning, and patient monitoring. However, data compatibility, signal interference, and inconsistent regulatory standards across manufacturers complicate the integration process [86].

5.3 Challenges in electromechanical system development (costs and regulatory barriers)

Highlights essential considerations related to the development costs and the regulatory landscape for approval in electromechanical system integration.

5.3.1 Economic hurdles

The economic challenges of integrating electromechanical systems are substantial, primarily due to the high initial investment and development costs. These systems often require specialized components, advanced materials, and state-of-the-art technologies, all contributing to increased production costs. Furthermore, the need for continuous innovation, such as developing new manufacturing techniques or utilizing costly components to ensure reliability, performance, and sustainability, can further drive-up expenses. Market competition also intensifies economic pressure. Companies must invest significantly in research and development (R&D) to foster efficient and innovative systems to remain competitive. The expenses associated with acquiring intellectual property rights, adhering to production standards, and managing supply chain complexities further challenge the financial feasibility of these systems [73,74].

6. Future application

6.1 Integration with AI and wearable technologies

Wearable health technology has significantly improved healthcare by offering cost-effective, convenient, and noninvasive patient monitoring through devices like fitness trackers and smartwatches [87,88]. These devices measure health parameters such as oxygen saturation, heart rate, blood pressure, and glucose levels, enabling pre-diagnosis and real-time therapy [75,89]. Their rise is driven by increasing chronic diseases and aging populations demanding personalized healthcare via real-time data analyzed by AI to aid clinical decisions [76,77]. AI enhances data accuracy, error correction, and power efficiency while resolving cross-sensitivity issues using pattern recognition algorithms [78,79].

Wearables promote preventive healthcare by notifying users of health issues early and encouraging healthier lifestyles with personalized recommendations [79,90]. Machine learning (ML) algorithms in wearables predict outcomes, detect patterns, and identify early disease indicators [80,81]. They have been applied to chronic diseases like cardiovascular conditions and diabetes by analyzing heart rate and glucose levels [82] and monitoring motor symptoms in multiple sclerosis and Parkinson's disease [83–85]. ML is also used in mental health tracking and includes systems like the Artificial Perception and Transmission (APT) nerve and AROMA for activity recognition using CNN and LSTM frameworks [91–93]. Deep learning algorithms analyze complex wearable data, aiding in detecting chronic conditions, cardiac abnormalities, sleep apnea, and epilepsy [87,88,94–96]. AI also enhances wearable accuracy by improving signal processing and noise reduction [97].

6.2 Speculative future applications

6.2.1 Quantum dock framework and wearable biosensing

An innovative tool, the Quantum Dock Framework, optimizes molecularly imprinted polymers (MIPs) for wearable biosensors. It improves selectivity, finds the best binding sites, and looks at different monomers to make them work better using Density Functional Theory (DFT) and molecular docking. This framework overcomes traditional limitations, making it ideal for wearable applications; the wearable gadget combines Quantum Dock-optimized MIPs with laser-engraved graphene (LEG) electrodes, enabling real-time monitoring of biomarkers like phenylalanine (Phe) in sweat without skin contact. Its autonomous design includes sweat induction, sampling, and sensing, achieving high sensitivity and calibration. This innovation marks the first wearable system for continuous phenylalanine monitoring, paving the way for personalized healthcare.

Its applications include continuously monitoring metabolic disorders, stress, and cardiovascular health. Phenylalanine detection plays a crucial role in managing conditions such as PKU; in addition to its strengths, It combines computational precision, flexible design, and scalability for diverse biomarkers, enabling accurate, real-world healthcare solutions; therefore, it aids in noninvasive, real-time health monitoring with minimal discomfort, thereby supporting early disease detection and management [98].

6.2.2 Electrical stimulation and self-powered drug delivery systems

Drug delivery systems (DDS) utilizing electrical stimulation offer precise control over drug release in both spatial and temporal dimensions. These systems enable applications such as pulsed drug delivery for intermittent release and continuous delivery to maintain consistent drug levels over extended periods. They integrate seamlessly with advanced technologies like sensors and microchips, supporting feedback mechanisms in precision medicine. Additionally, self-powered DDS eliminates the need for external power supplies by harvesting energy from the environment, making them ideal for wearable and implantable systems with long-term energy demands. Various energy sources include mechanical energy from piezoelectric (PENG) and triboelectric (TENG) nanogenerators, thermal energy from body heat, biochemical energy from biofuel cells, and environmental energy via solar and hydrovoltaic technologies. Biochemical energy from biofuel cells, along with ecological energy harnessed through solar and hydrovoltaic technologies, powers innovative applications such as implantable antiepileptic drug systems and wearable devices that enhance transdermal drug delivery efficiency by up to 50%. Additionally, Drug Delivery Systems (DDS) facilitate various administration routes.

6.2.2.1 Innovations in drug delivery systems

Transdermal systems use iontophoresis and electroporation for enhanced penetration, while injectable systems employ electrically responsive nanocarriers for localized delivery. Implantable systems rely on hydrogels and graphene-based devices for controlled, long-term release, and ingestible electronics like Intellicap integrate sensors and wireless systems for on-demand delivery. Moreover, gastric-resident devices enable continuous drug release for up to 36 days.

Additionally, Integrated Diagnosis and Treatment systems combine real-time diagnostics with therapy, creating a feedback loop for precision medicine. Wearable systems with biosensors track biomarkers, such as glucose, and administer drugs when needed, while microneedles sample

interstitial fluid for diagnostics and deliver medicines simultaneously. Gastric-resident devices further support the monitoring of internal conditions like pH and control drug release via Bluetooth, advancing minimally invasive, automated solutions [99].

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CHAPTER

Light-sensitive nanocarriers for *Helicobacter pylori* therapy

7

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Helicobacter pylori is considered a gastric pathogen associated with the development of several gastric diseases, including gastric cancer [1]. This bacterium is ubiquitous and is estimated to affect more than half of the world's population [2]. *Helicobacter pylori* infection, and in some countries, eradication is now recommended for all infected patients, regardless of symptoms [3]. Commonly prescribed therapies are based on a combination of broad-spectrum antibiotics and proton pump inhibitors [4]. However, over the years, their efficiency has declined dramatically, with eradication rates as low as 70%, well below the Maastricht Consensus Report definition of acceptable 90% [5]. This failure is related not only to the development of antibiotic resistance and low patient compliance with complex regimens but also to the stability and bioavailability of the drug in the gastric environment [6]. In addition, since these treatments are based on broad-spectrum antibiotics, they often alter the intestinal microbiota, triggering dysbiosis and further negatively affecting human health [7]. In summary, the World Health Organization (WHO) has suggested that *H. pylori* is one of the 12 most critical antibiotic-resistant bacteria that must be prioritized in the development of new antibiotic therapies [8]. Different antibiotic-free treatment strategies, namely, probiotics, antimicrobial peptides and plant compounds, have been explored [9–11]. Additionally, vaccines have been explored to prevent *Helicobacter pylori* infection [12]. However, although all these strategies are likely harmful to *H. pylori*, their effectiveness when translated into in vivo or clinical trials is low [13,14]. Similar to reports of antibiotics, these strategies also have low stability in harsh gastric environments (such as oxidation and proteolysis) [14].

Although antibiotic treatment has been the mainstream treatment for various bacterial infectious diseases for decades, the increasing emergence of bacterial resistance has brought great challenges to the application of antibiotics, and the number of approved antimicrobial therapies still ranges from 10% to 40%. fails in infected patients. In this context, therefore, the development of novel antimicrobial strategies is highly important.

Photodynamic therapy (PDT), a widely used cancer treatment, is a noninvasive treatment method that relies on the interaction between photosensitizers and light to induce local chemical damage [15,16]. It has been reported that reactive oxygen species (ROS), the final product of PDT, can damage biofilms and bacterial metabolism (such as proteins, nucleic acids, and lipids) but cannot induce drug

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resistance [17]. Antimicrobial photodynamic therapy (aPDT) has a broad antibacterial spectrum, shows great potential in the treatment of bacterial infections, especially multidrug resistance (MDR) [18], and has become an effective method for treating bacterial infections without causing drug resistance. Noninvasive and promising methods for generating reactive oxygen species (ROS) with photosensitizers (PSs) under light irradiation.

As one of the key components of aPDT, photosensitizers (PSs) can utilize light and oxygen to generate ROS, thereby determining the efficiency of antibacterial activity [19]. However, the current performance of PS, such as poor water insolubility and insufficient therapeutic penetration, is still unsatisfactory [20]. The development of a suitable PS and the achievement of sufficient therapeutic penetration are highly important for improving the antibacterial activity of aPDT [21].

However, problems such as insufficient therapeutic penetration, poor hydrophilicity, and poor biocompatibility of traditional PSs greatly limit the efficacy of aPDT. In recent years, studies have shown that nanomaterials have good photocatalytic activity, surface plasmon resonance, easy modification, and high drug loading capacity, which can improve the therapeutic effect of aPDT. In recent years, nanomaterials have attracted widespread attention in biomedical fields such as drug delivery, antitumor treatment, and antibacterial treatment [22–24]. Owing to their easy modification, high drug efficacy due to their loading efficiency and good photocatalytic activity, various nanomaterials used as carriers of PS or PSs are considered promising antibacterial agents for enhancing aPDT [25,26]. Specifically, functional nanomaterials such as gold nanomaterials and carbon dots have unique physical and chemical properties, such as surface plasmon resonance, making them a new generation of PS with a better ability to generate ROS [27,28]. The high loading efficiency of nonfunctional nanomaterials, such as liposomes, gives them the ability to carry PS, thereby improving the water solubility of PS and extending its working time [29]. In addition, aPDT based on nanomaterials can exert a variety of antibacterial effects through functional modification of the nanodelivery system [30]. In summary, the rapid development of nanomaterials has enhanced the efficacy of PDT and reduced side effects.

Targeted nanotherapeutics have become a powerful tool in the eradication of *Helicobacter pylori*. *Helicobacter pylori*-binding molecules have been developed for elimination by (i) blocking basic mechanisms of infection, such as adhesion to the gastric mucosa, or (ii) binding and killing *Helicobacter pylori* by releasing drugs within the bacterium or at the site of *Helicobacter pylori* infection. In this review, we aim to comprehensively understand the mechanism of nanomaterial-mediated aPDT and summarize representative nanomaterials used in aPDT, whether as PS or PS carriers. In addition, the combination of advanced nanomaterial-mediated aPDT with other therapies, including targeted therapies, gas therapies, and multidrug resistance (MDR) therapies, is reviewed. Additionally, issues and possible solutions for nanomaterial-based aPDT are discussed. Overall, this review may provide a theoretical basis and inspiration for the development of aPDT based on nanomaterials.

1. Nanomaterial-based aPDT and its mechanism of action

Photosensitive nanocarriers are nanoscale materials with light-responsive properties that enable precisely controlled drug release through illumination. These systems often contain photosensitizer molecules that can produce reactive oxygen species (ROS) or other chemical reactions after specific wavelengths of light are absorbed for therapeutic purposes. For phototherapy against *Helicobacter pylori* (*H. pylori*), photodynamic therapy (PDT) is the main method used. Photodynamic therapy is an

innovative treatment method that can not only avoid the possible side effects of antibiotics but also effectively address the challenges associated with drug-resistant strains.

Photodynamic therapy uses photosensitizers to trigger a reaction under specific wavelengths of light to produce cytotoxic substances, thereby killing bacteria. In the field of *Helicobacter pylori* treatment, PDT can accurately target bacteria while minimizing damage to surrounding normal tissues. Owing to its selectivity and high efficiency, this therapy provides a new strategy for the treatment of *H. pylori* infection.

In general, the antibacterial mechanism of nanomaterial-based aPDT is synergistic. First, excess ROS (such as singlet oxygen, superoxide anions, and hydroxyl radicals) induced by PSs play a key role in the antibacterial activity of PDT [31]. The ground-state PS (S0) absorbs photons and transforms into the excited singlet state (S1) under UV/NIR irradiation. This state undergoes intersystem crossing to form the excited triplet state PS (T1), which can interact with oxygen through two pathways. In Pathway 1, the excited PS generates ROS through electron exchange. In Pathway 2, triplet ground-state oxygen ($[3]O_2$) reacts directly with PS to generate excited singlet oxygen ($[1]O_2$). Eventually, the activated PS returns to the initial state and continues to generate ROS in a cycle [32]. Excess ROS can inhibit bacterial activity by damaging bacterial cell walls, cell membranes, proteins, and nucleic acids. Typically, oxidative damage caused by ROS leads to covalent modifications that destabilize and inactivate bacterial proteins, especially those containing cysteine (Cys) and methionine (Met) [33]. Therefore, in aPDT, bacterial adhesion can be inhibited by ROS-denatured adhesins, which are key proteins for bacterial adhesion [17].

In addition to the cytotoxicity of ROS, some nanomaterials also exhibit natural antibacterial activity due to their physical and chemical properties. For example, silver nanoparticles (AgNPs) can release highly toxic Ag+ in oxidative microenvironments because of the large surface area of metal nanocrystals. It has been reported that Ag+ can directly destroy bacterial cell walls, DNA and enzymes through chemical combination, inactivating bacterial activity [34]. In addition, two-dimensional layered graphene oxide nanosheets can damage bacterial cell walls through mechanical and chemical properties (such as sharp edge effects and membrane wrapping) as well as the ability to generate ROS and induce charge transfer [35]. Interestingly, aPDT also involves specific mechanisms against aerobic and anaerobic bacteria, depending on the type and depth of bacterial infection. For superficial infections caused by aerobic bacteria, PSs can consume oxygen in the bacterial microenvironment through Pathway 2, thereby limiting the growth of aerobic bacteria [36]. For deep infections caused by anaerobic bacteria, it is difficult for traditional PS to accumulate in deep tissues because of insufficient ultraviolet penetration [37]. Fortunately, nanomaterial-based PS can solve this problem by improving the local accumulation of PS and utilizing near-infrared light with deeper penetration [38]. Therefore, nanomaterial-based aPDT exhibits broad-spectrum and good antibacterial ability in most bacterial infections and even MDR infections.

The application of nanoparticles in antibacterial photodynamic therapy involves improving the penetration, stability and targeting of photosensitizers and enhancing the ability of photosensitizers to generate reactive oxygen species, thereby improving the therapeutic effect. The mechanism of drug release induced by photosensitive nanocarriers can be summarized as photochemical reactions, photothermal effects, and reactive oxygen species.

1.1 Photochemical reaction

The photosensitizer in the photosensitive nanocarrier undergoes a photochemical reaction under light, resulting in a reaction that can destroy the structure of the nanocarrier, resulting in the release of the

drug. Owing to their unique physical and chemical properties, nanomaterials such as metal–organic frameworks (MOFs) and inorganic nanomaterials have shown great potential in photodynamic antibacterial therapy. These nanomaterials can serve as photosensitizer carriers to improve the stability and targeting of photosensitizers. In a preliminary clinical study, photodynamic antibacterial chemotherapy was performed using methylene blue (MB) as a photosensitizer to improve diabetic lower limb ulcer infection. In this study, methylene blue was stimulated by light to produce reactive oxygen species, thereby killing bacteria, reducing the bacterial load on ulcer wounds, and improving infection [39].

1.2 Photothermal effects

Certain nanocarrier materials can convert light energy into heat energy under light, increasing the local temperature and triggering drug release. In this study, silver nanoparticles were also grown on two-dimensional MOF nanosheets through light induction, and two-dimensional Zr-Fc-MOF (MOF-Ag) nanosheets modified with silver nanoparticles (Ag NPs) were obtained and used in photothermal applications. Enhanced Ag+ release for antibacterial treatment. Under near-infrared (NIR) laser irradiation, MOF nanosheets can heat up in a short time, and an increase in temperature can accelerate the oxidation of Ag NPs into silver ions in solution, thereby achieving antibacterial effects. A new type of semiconductor polymer nanoantibacterial material (SP-PPh3 NPs) was developed on the basis of the synergistic effects of positive charge and photothermal effects. This material has photothermal conversion properties and is positively charged and can attach to bacteria. Through the synergistic effects of its photothermal effect and positive charge, it has antibacterial activity against both gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* [40].

1.3 Reactive oxygen species-mediated reactions

Photosensitizers generate ROS under light, and ROS can react with nanocarriers, leading to the release of drugs. Rare earth upconversion luminescent nanoparticles (UCNPs) can absorb near-infrared (NIR) light and emit ultraviolet or visible light. This property can be used to activate drug release. In some studies, by irradiating UCNPs with near-infrared light, the generated ultraviolet or visible light can excite photosensitizers to produce reactive oxygen species (ROS) and can also induce photosensitive "gate" structures (such as o-nitrobenzyl derivatives). breaks down, thereby releasing the drug [41].

Photosensitive nanocarriers can not only control the release of drugs but also enhance drug efficacy in the following ways: through surface modification of nanocarriers, the targeting of drugs to diseased tissues can be enhanced: nanocarriers can protect drugs from enzymatic hydrolysis in the body, prolong drug circulation time, and reduce damage to normal tissues, thereby reducing the systemic side effects of drugs. In addition, photosensitive nanocarriers can be used in combination with targeted therapy, gas therapy and multidrug resistance (MDR) therapy. The production of synergistic effects through photoactivation to improve therapeutic effects is further reviewed.

2. Photosensitive nanocarrier-based aPDT combined with other therapies

2.1 Bacteria-targeted PDT

Although aPDT offers great therapeutic potential in the antibacterial field, the lack of bacterial-specific targets may lead to off-target heat and ROS production, thereby damaging healthy cells and affecting

therapeutic efficacy. With the assistance of PS modified with nanomaterial-based cationic groups or corresponding bacterial ligands, aPDT has bright potential for advanced antibacterial therapy [42]. By reaching target bacteria and generating ROS locally, nanomaterial-based PSs greatly improve aPDT efficacy and reduce damage [43,44]. After exposure to body fluids, nanoparticles can absorb proteins such as albumin and form a negatively charged protein corona on the outside [45,46]. It is difficult for these nanomaterials to interact with bacterial cells because the cell walls contain large amounts of teichoic acid and lipopolysaccharide, and bacterial cells are negatively charged. Fortunately, cationic surface-modified nanomaterials are able to locally aggregate on bacterial biofilms through electrostatic attraction and generate ROS-induced cytotoxicity.

Another study proposed a polylysine-based photosensitizer (p3SLP) that targets *H. pylori* through specific interactions with the *H. pylori* surface. This photosensitizer can be administered orally to infected mice and works under irradiation with an endoscopic laser system, showing specific antibacterial effects against *Helicobacter pylori* without any side effects on normal tissues. Researchers have developed photosensitizers that specifically target *Helicobacter pylori*. For example, by combining 3′-sialyllactose (3SL) with polylysine (PLL) and a photosensitizer (such as pheophorbide-a, PheoA), a photosensitive nanocarrier (p3SLP) capable of specifically binding to *Helicobacter pylori* was formed. [47]. Recently, Dai Jianjun's team at China Pharmaceutical University and others designed and synthesized the protonated composite nanomaterial ZnO–Ag-mercaptoacetamide@chitosan (ZAN@CS). Research has proven that this nanomaterial has the following characteristics: it is not easily destroyed by gastric acid, can adhere to and penetrate the gastric mucosa, and prolong the residence time in the stomach; it can be targeted to the site of *Helicobacter pylori* infection through electrostatic adsorption; and it can clear mucosal pylori and its biofilm and reduce gastric inflammation while avoiding intestinal flora disturbance. ZAN@CS can penetrate the gastric mucosal barrier and be positively charged by protonation in an acidic environment, achieving active targeting and removal of *H. pylori* and its biofilm, leading to complete bacterial death. In addition, the nanomaterial has a good ability to reduce gastric inflammation, which is related to its ability to regulate immune responses. Unlike traditional triple therapy, ZAN@CS is negatively charged in the neutral environment of the intestine, is repelled by the intestinal flora and does not affect the abundance, function or species diversity of the intestinal microbiota. Therefore, this nanomedicine has good application prospects and provides an effective method for targeted eradication of *H. pylori* [48]. A research paper published by Professor Zhang Qianyu and Professor Xia Xuefeng in the pharmaceutical journal "Nanomedicine" noted that antibiotic-loaded nanocarriers are expected to overcome the limitations of *Helicobacter pylori* treatment, can penetrate mucosal barriers, and even have the ability to penetrate the extracellular polymeric layer to generate autogenous matrices and resolve persistent infectious biofilms [49].

2.2 Combined with gas therapy

Gas therapy, such as carbon monoxide (CO) therapy, nitric oxide (NO) therapy, and O_2 therapy, has received widespread attention in antibacterial treatment because of its small gas volume and good diffusion [50,51]. Typically, functional gases are released from precursor gas molecules upon stimulation and then exert therapeutic effects [52]. However, the uncontrollable local release of gas poses a threat to human safety and greatly limits its clinical application. Gas-generating nanoplatforms constitute a novel strategy to control the delivery of bioactive gases within a safe and effective concentration range [53]. In combination with aPDT, gas-generating nanoplatforms can exhibit stronger antibacterial capabilities.

NO is a physiological messenger that can achieve precise gas therapy. In recent years, it has been reported in the literature that it can inhibit tumor growth, inhibit bacterial adhesion, and kill attached bacteria. NO is an ideal antiinfection biological coating material [54][64]. NO can produce small-molecule hydrophobic free radicals, which can easily pass through cell membranes through free diffusion. NO can cause DNA damage after entering cells, and NO can easily be oxidized into nitroxyl free radicals, which can lead to lipid peroxidation and cell membrane disintegration. The therapeutic effect of NO depends on its concentration and location in the body, so a stable carrier is required for targeted transport while being able to control the release of NO. Carbon monoxide (CO), an essential biological signaling molecule, has a variety of antibacterial abilities, such as removing the bacterial ATP supply, inhibiting bacterial cell respiration, and activating host immune responses [55]. Nitric oxide also has inherent antimicrobial capabilities through multiple mechanisms, such as causing lipid peroxidation, DNA damage, and protein damage [56]. Combined with NO therapy, aPDT has enhanced antibacterial efficacy [57].

The hypoxic microenvironment in biofilms is a very important cause of antimicrobial resistance, which significantly limits the efficacy of aPDT [58]. To solve this problem, liquid perfluorohexane (PFH, an oxygen carrier), which was first used to treat tumor hypoxia, has also been used to treat bacterial hypoxia [59].

2.3 Multidrug resistance treatment

Although MDR infections have largely hindered the efficacy of conventional antibiotic treatments, nanomaterial-based aPDT offers an alternative approach to treat MDR bacterial infections for the following reasons. First, nanomaterials can enhance the performance of PS, thereby significantly improving the antibacterial effect of ROS without generating MDR bacteria. In particular, near-infrared-triggered aPDT with a nanoplatform provides safer, deeper and stronger therapeutic effects against MDR bacteria. Second, multifunctional modification and loading of nanomaterials/nanoparticles enables the specific design of antimicrobial agents that contribute to aPDT, achieving accurate and effective antimicrobial effects even at low drug concentrations, thereby mitigating any dose-related toxicity. Third, by combining synergistic therapies such as targeted bacteria and antibiotics, each other's antibacterial properties can be enhanced, thereby minimizing the ectopic effects of antibacterial treatments. This will help improve patient compliance with treatment and prevent the recurrence of infection. Finally, unlike other antimicrobial therapies that take time to take effect, PDT kills bacteria within seconds to minutes by causing oxidative stress. There are two basic approaches to improve the antimicrobial efficacy of nanomaterial-based aPDT against MDR infections. One strategy is to improve the performance of nanomaterials/PS, thereby increasing ROS production and causing greater damage to MDR bacteria. Some studies have proposed the use of an endoscopic laser system to activate oral p3SLP to achieve targeted treatment of *H. pylori*. This method has shown good therapeutic effects in mouse models without side effects on normal tissues [47]. In addition, nanozymes combine the advantages of natural enzymes and nanomaterials. They have intrinsic enzymatic activity, low cost, stable catalytic activity and multifunctionality and have become a research hotspot. In recent years, inspired by the catalytic activities of multiple protein cascades in biological systems, artificial multienzyme cascade technology has emerged. Through appropriate structural design, a single nanosystem can achieve multienzyme cascade activity and be used for the treatment of multiple diseases. Prussian blue, ferric ferrocyanide (PB), was the first coordination compound discovered in

humans. It is biosafe because of the high affinity between iron ions and cyano groups, has been approved by the FDA for the detoxification of thallium, and has great potential in biomedical applications. Moreover, research has shown that PB is an oxygen-functional nanoenzyme material that can use hydrogen peroxide in the microenvironment to catalyze oxygen production. Researchers have used new ferrocene-doped Prussian blue analogs (FPB NPs) as the core, modified Co3O4 NPs on their surfaces (FPB–Co NPs), and used chitosan with good biocompatibility and bioadhesion. With sugar as a coating, a nanozyme cascade oxygen production system (FPB–Co-Ch NPs) based on a core–shell structure was designed and prepared. Under acidic conditions, FPB-Co-Ch NPs can exert the cascade activity of four enzymes, effectively supply oxygen, and kill *Helicobacter pylori*; at the same time, they regulate the expression of inflammatory factors, alleviate the damage to gastric epithelial cells caused by oxidative stress, and reduce gastric epithelial cell damage. The inflammatory response can also upregulate the expression of mucosal repair proteins and restore damaged gastric mucosa. This platform integrates the ability to eradicate drug-resistant bacteria, regulate inflammation, repair functions and avoid intestinal flora imbalance and provides a new option for the treatment of drug-resistant *Helicobacter pylori* [60].

In one study, researchers synthesized zinc peroxide-based nanoparticles (ZnO_2–Ce6@lipo), zinc peroxide-based liposome nanoparticles loaded with the photosensitizer Ce6, and achieved gastric acid absorption of Zn2+, H_2O_2 and Ce6 responsive release. Under the action of blue light radiation, the material undergoes photooxidative cascade chemical reactions and photolysis reactions to form an ROS library with high antibacterial ability. Moreover, in a gastric acid environment, ZnO_2 is more efficient and has longer-lasting acid neutralizing ability than traditional proton pump inhibitors are. Under 460 nm blue light irradiation, ZnO_2–Ce6@lipo can produce a variety of ROS, including singlet oxygen, hydroxyl radicals and hydrogen peroxide. Blue light irradiation activate H_2O_2 to produce highly active ·OH; at the same time, the O_2 produced by the hydrolysis of H_2O_2 effectively overcomes the O_2-dependent limitations associated with Ce6-mediated photodynamic therapy (PDT). (3) Both in vitro and in vivo experiments confirmed that the photochemical reaction system has an eradication rate of >99.999% for standard strains and multidrug-resistant *H. pylori* strains. (4) Because ZnO_2–Ce6@lipo has superior gastric acid-responsive release and targeting properties in damaged gastric mucosa, the treatment process does not affect the intestinal flora. It is capable of acid-responsive release in the stomach to produce multifunctional reactive oxygen species (ROS) for the treatment of gastric infections. Research results show that this PDT system has significant bactericidal effects, with in vitro and in vivo experiments demonstrating an eradication rate of >99.999% against standard strains and multidrug-resistant *Helicobacter pylori* strains while minimizing damage to the intestinal tract. influences the microbiota and exhibits excellent biocompatibility [61].

Owing to the versatile modifiability of nanomaterials, multiple combination therapies, such as bacterial-targeted PDT and gas therapy, have been applied to nanomaterial-based aPDT. With precise bacterial targeting and excess air cell toxicity, nanomaterial-based aPDT exhibits excellent antibacterial activity against MDR infections in combination with multiple therapies.

2.4 PDT combined with PTT

Phototherapy is a laser-controlled treatment that exhibits antibacterial activity by converting light into reactive oxygen species (ROS) or heat energy, causing lipid/DNA peroxidation. Moreover, the photothermal therapy (PTT)/photodynamic therapy (PDT) effect can also lead to the ablation of bacterial

biofilms. Therefore, phototherapy is considered an attractive antibacterial strategy. In this context, researchers developed the near-infrared photosensitizer T780T. The T780T molecule has a highly efficient PTT/PDT synergistic effect. Moreover, organic photosensitizers have the advantages of fast metabolism and low biological toxicity. In addition, the team suggested that *H. pylori* and its biofilm components are negatively charged under physiological conditions. To enhance the antibacterial targeting of the photosensitizer, they used positively charged guanidine (Gu), which can target the photosensitizer more specifically. Langerhans-negative bacteria group was introduced into the T780T molecule to obtain the phototherapeutic agent T780T-Gu. Through integration with the Gu group, the hydrophilicity of T780T-Gu was improved compared with that of T780T. The positively charged T780T-Gu may penetrate the biofilm and connect with the negatively charged *Helicobacter pylori* on the surface through electrostatic interactions. Under near-infrared laser irradiation, T780T-Gu converts light energy into heat and ROS, leading to protein denaturation and cell membrane rupture. Studies have shown that this photosensitizer is able to effectively deliver phototherapy through structural defects, as revealed by RNA-seq analysis and a reduction in metabolic and defense activities. It exhibits synergistic photothermal therapy/photodynamic therapy effects against biofilms and multidrug-resistant clinical strains of *Helicobacter pylori* [62].

3. Summary and prospect

This article reviews the application of photosensitive nanocarriers in the treatment of *Helicobacter pylori* and explores the potential and mechanism of photodynamic therapy (PDT) as a novel antibacterial strategy. *Helicobacter pylori* is a pathogen associated with various stomach diseases, and traditional antibiotic treatment is limited in effectiveness due to drug resistance and side effects. PDT, as a noninvasive treatment method, generates reactive oxygen species (ROS) through photosensitizers under specific wavelength light irradiation to kill bacteria, providing a new approach to avoid the development of drug resistance.

The photosensitive nanocarrier improves the penetration, stability, and targeting of photosensitizers through photochemical reactions, photothermal effects, and reactive oxygen species mediated mechanisms, enhancing the ability to generate ROS and thus improving the therapeutic effect. These nanomaterials include gold nanomaterials, carbon dots, and liposomes, which not only improve the water solubility and working time of photosensitizers, but also exert various antibacterial abilities through functional modifications. The combination of nanomaterial-based aPDT with other therapies such as bacterial targeted PDT, gas therapy, and multidrug resistance therapy has demonstrated excellent antibacterial activity against multidrug-resistant (MDR) infections. For example, targeted modification of nanomaterials can reduce damage to healthy cells and improve therapeutic efficacy. The combination of gas therapy and aPDT enhances antibacterial ability by controlling the release of bioactive gases. In addition, in multi drug therapy, nanomaterials enhance the performance of PS, providing safer and deeper therapeutic effects.

Although aPDT based on nanomaterials has shown great potential, it still faces challenges such as biocompatibility, long-term stability, and potential toxicity of nanomaterials. Future research needs to address these issues and optimize the design of nanomaterials to achieve safer and more effective treatment of *Helicobacter pylori*. In addition, in-depth research on the mechanism of interaction between nanomaterials and *Helicobacter pylori*, as well as how to improve treatment efficacy and reduce side effects through nanotechnology, will be the focus of future research.

In summary, photosensitive nanocarriers have shown significant potential in the treatment of *Helicobacter pylori*, and their combination with multiple therapies provides new strategies for overcoming drug resistance. With the continuous advancement and innovation of nanotechnology, it is expected that aPDT based on nanomaterials will become an important direction for the treatment of *Helicobacter pylori* in the future.

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CHAPTER

Sonodynamic therapy nanocarriers for helicobacter pylori therapy

8

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1. Introduction

Helicobacter pylori (H. pylori) is an infectious agent that colonizes the mucous membrane of the human stomach, with over 50% of the global population being infected [1]. For instance, the prevalence of H. pylori infection among Chinese adults ranges from 40% to 50% [2]. H. pylori infection may cause a variety of complications, which includes chronic gastritis, peptic ulcer, gastric cancer, lymphoma and colon cancer, etc., seriously jeopardizing the physical and mental health of human beings [3–6]. It is important to note that H. pylori infection as a major risk factor for gastric cancer is the third most common cause of cancer-related mortality globally [7].

Presently, the standard clinical treatment for H. pylori infection is triple therapy (two antibiotics (amoxicillin and clarithromycin) and a proton pump inhibitor (PPI) and quadruple therapy (two antibiotics plus a PPI and a bismuth), which eradicates the H. pylori bacteria in 7 or 14 days [8,9]. However, Oral antibiotics can induce antibiotic resistance in H. pylori, increasing the recurrence rate after treatment [8,10–12]. Meanwhile, taking many types of drugs leads to poor patient compliance [13,14]. H. pylori eradication therapy with antibiotics results in patients prone to gut microbiota dysbiosis [15,16]. The problems caused by these treatments cannot be ignored and therefore new therapeutic strategies are essential to replace oral antibiotic therapy.

As nanotechnology continues to advance, it has shown great potential in the treatment of H. pylori infection. Nanostructured drug delivery systems, such as nanostructured lipid carriers, liposomes, nanoemulsions, metal nanoparticles, and polymer nanoparticles, can enhance drug bioavailability and enable targeted, controlled release of drugs [17,18]. For example, Muhammad Arif et al. [19] constructed novel pH-sensitive thiolated chitosan/PMLA nanoparticles for the delivery of amoxicillin. These nanoparticles exhibited pH-sensitive behavior, delaying amoxicillin release in gastric acid and efficiently targeting H. pylori, showing great potential for effective treatment. Additionally, Sun et al.

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[20] developed a comprehensive treatment platform consisting of liposomes loaded with a mixture of clarithromycin (CLA), bismuth (Bi), and ZnO nanoparticles (CLA-Bi-ZnO@Lipo). The findings demonstrated that CLA-Bi-ZnO2@Lipo was able to target inflammatory tissues and prolong the stomach residence time, and rapidly released the drug on exposure to the gastric acid environment, demonstrating potent antimicrobial ability against drug-resistant H. pylori.

Nanotechnology can improve the stability and efficacy of drugs, as well as reduce side effects of drugs and better overcome the problems of drug resistance, intestinal flora dysbiosis, and poor patient compliance faced by multiple antibiotics for H. pylori [15,20]. However, Application of nanotechnology to therapy for H. pylori is still at a research stage and there are still some challenges and problems. Current nanosystems primarily carry antibiotics, which still leads to bacterial drug resistance. Therefore, developing non-antibiotic approaches for the treatment of H. pylori infection is an attractive new direction. For the past few years, sonodynamic therapy (SDT) has shown enormous potential in disease treatment. For example, Li-Hua Yang's team [21] from the University of Science and Technology of China developed a nanoparticle-mediated SDT to treat H. pylori infection. The study promotes for SDT against H. pylori infection and is expected to be a safe and efficient replacement for traditional antibiotic therapy with significant potential for clinical application. The following sections will detail the development of SDT, the classification and development of sonosensitizers, and research on using SDT for H. pylori treatment.

2. Development of SDT

Ultrasound (US) is a type of sound wave with a frequency higher than the human hearing frequency (>20 kHz), which is widely used in the medicine because it penetrates deeply into the tissue and causes low damage to the nearby normal tissue [22,23]. Highly intense focused US can cause tumor thermal ablation and guided imaging for clinical diagnosis [24–26]. In addition to high intensity US, low intensity US exhibits promising nonthermal biological effects when combined with cavitation, increasing cell membrane permeability and facilitating delivery of drugs to deeper tissues under safe and controlled conditions [27,28]. Alternatively, a more prospective therapeutic application of low frequency US is to activate sonosensitizers to generate reactive oxygen species (ROS) to kill tumor cells. This new therapeutic approach, first proposed by Umemura in the 1990s, is called SDT [23,29].

2.1 Advantages and therapeutic mechanisms of SDT

SDT is a novel non-invasive therapeutic approach that combines low frequency and sonosensitizers to generate localized ROS to destroy pathogens or tumor cells [30–32]. It is characterized by good penetration of tissue, precise delivery and few adverse effects, and is mainly used to treat tumors that grow in the deeper parts of the body [33]. The main mechanisms that SDT involves are ROS generation, cavitation effect and thermal damage [31]. Among them, ROS production is the main mechanism of SDT, which induces apoptosis in tumor cells by destroying proteins in cancer cells, damaging DNA, and promoting lipid peroxidation [34]. Cavitation and thermal damage, which are physical effects produced by US, can lead to mechanical damage and death of cancer cells [35]. Similar to its antitumor effects, SDT also serves as an emerging antimicrobial technology that uses US energy to activate sonosensitizers that produce ROS with high cytotoxicity, thereby inducing bacterial death

[36]. Compared with traditional antibiotic therapy, sonodynamic antimicrobial treatment does not produce bacterial resistance and benefits from superior tissue penetration, good biocompatibility and US site specificity [37]. Therefore, SDT also has great advantages and perspectives in deep infections treatment.

2.2 Progress in SDT treatment research

SDT has garnered significant attention in recent years as a novel non-invasive approach with greater penetration depth (>8 cm), lesser adverse effects and no phototoxicity compared to phototherapy (PT) [21]. However, there are some drawbacks due to single SDT treatment, such as limitations of sonosensitizers: conventional organic sonosensitizer molecules used in SDT suffer from poor tissue accumulation, low bioavailability, low stability, and low ROS production, and can be easily cleared and excreted by this body, limiting its use in clinical practice [38]. Therapeutic efficacy is limited by oxygen (O_2) availability: SDT relies on the production of ROS by sonosensitizers activated by US, but SDT efficacy is hampered in microenvironments where there is a lack of O_2 (e.g., inside a bacterial biofilm or in a tumor microenvironment) [39].

To overcome these shortcomings, researchers are exploring using nanotechnology to enhance the effects of SDT by using nanoparticles as carriers for sonosensitizers and combining SDT with with other treatment approaches, including photothermal therapy (PTT), chemodynamic therapy (CDT), and immunotherapy, enhance the effects of SDT. Such as, in combined application with SDT and PTT, Yang et al. [40] succeeded in biosynthesising melanin with controlled particle size using gene edited bacteria with thermally induced genetic circuits. Biosynthesized 8 nm-sized melanin and dihydroporphyrin e6 (Ce6) were encapsulated in liposomes to obtain SDT/PTT bifunctional liposomes (named MC@Lip), which exhibited highly efficient singlet oxygen ([1] O_2) production and photothermal conversion upon US and Near Infrared (NIR) irradiation, and produced significant improved antitumor effects both in-vitro and in-vivo. In addition, Su et al. [41] proposed a fast photo-ultrasound therapy to create oxygen deficiency on titanium (Ti) implants by sulfur (S)-doping (Ti-S-TiO_{2-x}), which improves the efficiency of NIR photoabsorption and electron-hole separation, which results in implants with strong sonodynamic and photothermal capabilities, and in-vitro and in-vivo studies have demonstrated extremely efficient antimicrobial properties with exogenous stimulation. Regarding the combined application of SDT and CDT, Zhan et al. [42] designed a double cascade activatable nanoenhancer for synergistic immunotherapy of CDT and SDT in depth tumors by remodeling adenosine metabolism, and in US-irradiated tumor microenvironments, the NP MCA mediated both CDT and SDT in deep tumors to generate abundant ROS, and in-vitro and in-vivo studies have have shown promising therapeutic effects. In addition, Gao et al. [43] designed a Mn single-atom nanoenzymes functionalized 3D printed bioceramic (Mn/HSAE@BCP) scaffold that could enable CDT/SDT synergistic treatment to generate sufficient ROS, and demonstrated significant in-vitro and in-vivo antimicrobial efficacy. Studies of combined application with SDT and immunotherapy, Chen et al. [44] synthesized a well-stabilized titanium diselenide ($TiSe_2$) nanosheets, which acted as a reliable nano-sensitizer to induce high-level ROS-mediated SDT under hypoxic and normoxic conditions after exposure to US (USA). The combination of $TiSe_2$-mediated SDT with anti-PD-1 immune checkpoint inhibitor therapy can efficiently activate antitumor immune response and completely induce cell immunogenic death (ICD), thus inhibiting tumor growth and metastasis. In addition, Xu et al. designed a curcumin and platinum doped iron-based covalent organic framework

nano-adjuvant (CFCP) for efficient therapy of repetitive implant-associated infections (IRIs) by inducing systemic immune responses [45].

These research advances indicate that SDT, as an emerging tumor therapy, has a broad application prospect, especially when used in combination with other therapeutic means. These combinations can well make up for the deficiencies that exist in SDT therapy alone and amplify the therapeutic effect of SDT.

3. Classification and development of sonosensitizers

The choice of the sonosensitizer, one of the three elements of SDT, is crucial for therapeutic effects [46]. The three main categories of sonosensitizers that have been extensively studied are organic sonosensitizers (e.g., porphyrin derivatives and phthalocyanines, etc.), inorganic sonosensitizers (titanium oxides, etc.), and organic-inorganic hybrid sonosensitizers (metal-organic frameworks (MOFs), etc.) [47].

3.1 Organic sonosensitizers

Organic sonosensitizers mainly include porphyrin derivatives and phthalocyanines, which have a precisely defined chemical structures, excellent biodegradability and efficient production of ROS [48–50]. However, the stability of organic sonosensitizers needs to be further improved, while most organic sonosensitizers are also photosensitizers and their phototoxicity is problematic during tumor therapy [51,52]. To overcome these limitations, researchers have developed novel sonosensitizers. Such as, Chen et al. [53] designed a triaryl boron doped and benzothiophene-based compound that efficiently generates hydroxyl radicals under US, realizing high SDT efficiency and overcoming phototoxicity at therapeutic concentrations. Sun et al. [52] constructed an organic nanoparticle sonosensitiser, CCNU980 nanoparticles (NPs), which emits long wavelengths and targets mitochondria, with optical penetration into deep tissues, depth-activated ROS generation, high photostability, and low phototoxicity. Additionally, Chen et al. [54] developed a novel nano-sonosensitizer self-assembled from cell-cycled transferrin (Tf) and the organic sonosensitizer protoporphyrin IX, which has good biosafety, stability, and US-activated ROS production. These research advances suggest that the development of novel organic sonosensitizers is a key area of research in SDT, aiming to improve therapeutic efficacy while reducing side effects and promoting clinical application of SDT.

3.2 Inorganic sonosensitizers

Inorganic sonosensitizers, such as titanium dioxide and molybdenum disulfide, are widely studied for their higher stability and lower phototoxicity compared to organic sonosensitizers [55,56]. However, inorganic sonosensitizers have low sonosensitization efficiency as well as poor biodegradability, drawbacks that may limit their clinical application and therapeutic efficacy [55,57]. To overcome these shortcomings, researchers are exploring new design strategies, such as the development of sonosensitizers that are biodegradable and improve sonosensitization efficiency. For example, Cheng et al. [58] successfully prepared extremely fine nanorods of titanium monoxide (TiO_{1+x} NRs) with significantly enhanced sonosensitization and Fenton-like catalytic activity, and used them to enhance SDT. Cao et al. [59] prepared an enhanced piezoelectric sonosensitizer consisting of HNTM

(porphyrin-based hollow metal-organic framework), MoS_2 nanosheets, and red blood cell (RBC) membranes, and the US-mediated piezoelectric polarization of MoS_2 improved the HNTM transfer of charge at the HNTM-MoS_2 heterointerface, increased the ROS production, and improved the SDT antimicrobial efficiency. In addition, Jiang et al. [60] designed a piezoelectric sonosensitizer BTO-Pd-MnO_2-HA, where depositing palladium particles (Pd NPs) led to Schottky junction formation, facilitating electron-hole pair separation and improving ROS generation efficiency, thus enhancing SDT. In summary, inorganic sonosensitizers are of great value in SDT research and application, but require further research and optimization to achieve its wide application in clinical therapy.

3.3 Organic-inorganic hybrid sonosensitizers

Organic-inorganic hybrid sonosensitizers combining advantages from both organic and inorganic compounds, for example, metal-organic framework (MOF) materials, which are characterized by rapid ROS generation mechanism, porous structure, large specific surface area, and ease of modification, which can improve the efficiency and specificity of ROS generation [47,61,62]. These features make organic-inorganic hybridized sonosensitizers have great potential in SDT. Related studies have also shown that MOFs-based sonosensitizers can generate ROS through multiple mechanisms, including the catalytic effect of metal ions, the sonosensitivity of organic ligands, and linker-metal charge transfer [63–65]. Moreover, the versatility of MOFs allows them to be combined with other therapeutic agents (e.g., chemotherapeutic agents, immunomodulators) to realize combination applications of SDT with other treatment approaches, which effectively overcomes the shortcomings of SDT in the treatment process [66,67]. For example, Dong et al. [68] reported a MIL@Ag-PEG hetero structure for enhanced SDT as a US activated ROS generator., the MIL@Ag heterostructure consists of Ti-based MOFs (called MILs) on the surface of silver nanoparticles (Ag NPs) assembled in situ. Under US irradiation, doped Ag NPs are able to capture the electrons activated by the MIL to reduce the surrounding O_2 and generate super oxide radicals ($\bullet O^{2-}$), whereas activated holes oxidize the H_2O to generate hydroxyl radicals ($\bullet$OH), which showed a better management of both tumor and bacterial infections effect. Liu et al. [69] prepared a double layered MOF-derived hollow manganese silicate hollow nanoparticle (DHMS) obtained via growth of a layer of Mn^{2+} in situ at inner and outer $mSiO_2$ and ZIF-8 surfaces, which were completely etched, These nanoparticles exhibited high levels of ROS generation under US stimulation in multimodality imaging-guided SDT. In addition, Huang et al. [66] designed a sequential US/hypoxia-sensitive US chemotherapeutic nanopremedicine (CPT_2-Azo), which was then encapsulated in the mesopore of sonosensitizer-integrated MOF NPs to compensate for lack of therapeutic efficacy of SDT, by taking advantage of hypoxic microenvironment of tumor and the consumption of O_2 by SDT to achieve controllable release of the toxic chemotherapeutic drug (CPT). However, although organic-inorganic hybrid sonosensitizers have shown great potential in experimental studies, they still face several challenges before clinical application, including biosafety assessment, control of drug release kinetics, and long-term efficacy and metabolism studies [70,71]. Overall, organic inorganic hybrid sonosensitizers have promising applications in acoustic power therapy, but much more needs to be researched and developed to overcome the current challenges for their clinical applications.

In summary, the classification of sonosensitizers and their properties provide diverse choices for SDT, and different types of sonosensitizers have their own advantages and limitations, and future research on sonosensitizers may be focused on improving the stability, targeting, biocompatibility, and

combining with other therapeutic modalities of sonosensitizers. These research directions will help to overcome the limitations of existing sonosensitizers for safer and more effective disease treatment, enhancing the efficacy and clinical application potential of SDT.

4. SDT against H. pylori infection

Currently, in addition to the traditional antibiotic therapy, a series of non-antibiotic methods for treating H. pylori have been emerging from the research, such as hyperbaric oxygen or oxygen enriched environment therapy, probiotic therapy, photodynamic therapy, nano-materials, phage therapy, antimicrobial peptide therapy, and SDT, and so on [72–78]. These methods are effective in avoiding issues related to antibiotics use and drug-resistant strains of H. pylori, and several treatments have been demonstrated to have therapeutic efficacy against drug-resistant strains of H. pylori [79]. And SDT has non-invasive, good tissue penetration and other characteristics, which has great advantages for treating H. pylori infection [80]. Hence, the next section will summarize the research progress of Sonodynamic nanocarriers in fighting H. pylori infection.

4.1 Combined SDT with elimination of vacuolar cytotoxin A (VacA)

VacA is a key virulence factor secreted by H. pylori, which promotes H. pylori colonization and infection through a variety of actions on epithelial cells in the stomach [33,81]. Ideally, We hope that effective therapies for gastric H. pylori infection will be able to not only show robust antimicrobial activity against H. pylori, as well as preserve the integrity of the gut microbiota of patients and ensure biosecurity, but many previous studies have failed to specifically address the negative effects of VacA on the infection of gastric H. pylori [82]. Based on this, Yang et al. [82] developed an approach to non-antibiotic SDT by using lecithin bilayer-coated poly (lactic acid-hydroxyacetic acid) nanoparticles preloaded with Vitiporfin (small molecule sonosensitizer), Ver-PLGA@Lecithin. The results of the in vitro and in vivo studies showed that, without exposure to the US, Ver-PLGA@Lecithin first effectively removes VacA by protein corona, and then after 10 min of irradiation at 0.5 W/cm [2], Ver-PLGA@Lecithin was effective in killing H. pylori by producing ROS in simulated stomach juice. Using a female mouse model suffering from gastric H. pylori infection, Ver-PLGA@Lecithin-mediated SDT combined with US exposure of the supragastric skin (0.5 W/cm [2] for 10 min) was as efficient at reducing H. pylori infection as standard triple therapy. The study used a localized US exposure dose (0.5 W/cm [2], 10 min) within the allowable limits for US medical devices (i.e., output power $\leq$0.72 W/cm [2] and exposure time $\leq$15 min) [37,83]. Notably, compared to antibiotic therapies for H. pylori eradication, SDT does not affect the gastrointestinal microbiota [82,84]. Overall, SDT of Ver-PLGA@Lecithin provides a valuable reference for non-antibiotic approaches to treated H. pylori infections, and the three components used in the study (lecithin, PLGA, and vitexoporfin) have all been approved by the United States Food and Drug Administration (U.S. FDA) for clinical use, and have a high potential for clinical translation [85–87].

4.2 Combined SDT with chemodynamic therapy (CDT)

We know that hypoxia can greatly limit the therapeutic efficacy of SDT because oxygen is a key factor in producing [1]O_2 by SDT [88]. However, the site of bacterial infection is usually hypoxic, which

reduces the antimicrobial effect of SDT [39]. Hence, Strategies that promote ROS production or increase oxygen levels at infection sites are needed to enhance the efficiency of SDT in the treatment of bacterial infections [89,90]. CDT has received considerable attention as a promising treatment for bacterial infections and is generally based on the Fenton-like or Fenton reaction of hydrogen peroxide (H_2O_2) with metal ions (e.g., ferrous ions) to produce •OH [91,92]. Ferrous ions convert endogenous H_2O_2 at infection sites into high-reactivity -OH leading to bacterial death [93]. In addition, H_2O_2 can be converted to O_2 to overcome hypoxia in the tumor microenvironment using Fenton-like reactions [94]. Based on this, Yu et al. [78] designed a pH-sensitive ROS nanogenerator (Fe-HMME@DHA@MPN), which consists of an acid-sensitive metallopolyphenolic network (MPN) shell and a mesoporous metal-organic nanostructured core [Fe-HMME (Hematoporphyrin monomethyl ether, sonosensitizer)] encapsulated in dihydroartemisinin (DHA). In-vitro and in-vivo findings revealed that ROS nanogenerator under SDT was able to eliminate multi-drug resistant H. pylori and remove biofilm, demonstrating significant treatment effects in mouse model of H. pylori infection. Metal polyphenol network (MPN) shells in this material design dissociate at gastric acidic pH, triggering release of HMME and Fe-HMME from gastric acid by US to generate ROS to kill H. pylori [95]. Meanwhile, the dissociated tannic acid (TA) reduces Fe^{3+} to Fe^{2+}, which catalyzes formation of •OH for CDT from H_2O_2 located in microenvironments such as H. pylori infected biofilms [93,95]. At the site of infection where H_2O_2 is lacking, the DHA released by the nanogenerator can in turn provide hydrogen peroxide to eradicate H. pylori by generating •OH with Fe^{2+} via a Fenton-like reaction [96]. Moreover, O_2 production from peroxidase-catalyzed H_2O_2 mitigates the effects of hypoxia at the site of infection, which further enhances the effect of SDT [97]. Additionally, the excellent biocompatibility of the degradation products and in vivo safety results confirming minimal adverse effects to normal gut microbiota [78]. Hence, this Fe HMME@DHA@MPN nanogenerator, which amplifies ROS production under the action of US, could provide a promising alternative strategy to antibiotic therapy to treat H. pylori infections.

4.3 Combined SDT with gasotherapy

Methods to alleviate hypoxia to enhance SDT have been explored, such as in situ oxygen production or carrier-delivered O_2 [50]. However, the O_2 produced by these methods has limited effectiveness in the body [84]. Cavitation effect, as one of the main mechanisms of SDT, produces a mechanical effect under US that has antimicrobial properties and promotes nanoparticle penetration to disrupt bacterial biofilms [98,99]. On this basis, Fan et al. [84] developed a PtCu3-PDA@AIPH@Fucoidan (PPAF) sonodynamic nanocomposite consisting of a a platinum-copper (PtCu3) dopamine-modified inorganic sonosensitizer, an alkyl radical (R•) generating agent AIPH, and fucoidan, which could penetration through the layer of mucus, targeting the site of H. pylori infection, disrupting biofilm and exhibits significant antibacterial activity. The results of in vitro and in vivo studies showed that PPAF has strong sonodynamic activity and generates high levels of ROS and oxygen-independent R• for bactericidal purposes when US stimulated, and US-stimulated PPAF also showed significant antibacterial effects against H. pylori in a mouse model. Azide salt compound 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (AIPH) in this material serves as a free radical generator, which generates oxygen-independent R• and N_2 upon US stimulation [100,101]. R• induces free radical polymerization and oxidative stress reaction, leading to bacterial death, and N_2 enhances the US cavitation effect and promotes nanoparticle penetration, thus disrupting the bacterial biofilm and enhancing the bactericidal

effect [99,102,103]. $PtCu_3$ as a sonoensitizer can produce ROS sterilization under US [104]. Moreover, polydopamine (PDA)-coated $PtCu_3$ as a nanocarrier enhances the stability and increases the retention time for AIPH in vivo, and encapsulation of fucoidan prevents the leakage of AIPH and accurately targets to the surface of H. pylori, which reduces the damage to the normal cells and avoids dysbiosis of the intestinal microflora [84,105]. Hence, sonodynamic nanocomplex PPAF proposed by this work provides a promising therapeutic direction for non-antibiotic approach against H. pylori infection.

4.4 Combined SDT with photoacoustic (PA) imaging

PA imaging is a new medical imaging technology that blends the advantages of US imaging and optical imaging by offering strong optical contrast at high US resolution in deep tissues to aid in the evaluation and diagnosis of disease [106,107]. In addition, PA imaging, as a new non-invasive, radiation-free imaging modality, is being widely applied in biomedical research and is gradually moving toward clinical applications [108,109]. Based on the advantages of PA, Wang et al. [110] succeeded in developing a liposome coupled with a monoclonal antibody containing indocyanine green (ICG) (Hp Ab-LiP-ICG) for in vivo targeting of H. pylori infection by PA imaging-guided SDT. The results of in vitro and in vivo experiments showed that HpAb-LiP-ICG could specifically target H. pylori and produce ROS to induce the death of H. pylori under the action of US, and the in vivo PA imaging that showed a rapid accumulation of HpAb-LiP-ICG on the surface of H. pylori, and demonstrated that HpAb-LiP-ICG shows significant SDT antimicrobial properties in H. pylori-infected mice with no side effects on normal tissues. ICG in this material is loaded into liposomes, which overcomes drawbacks such as the instability of free ICG, and ICG acts as a sonosensitizer and PA signal to promote SDT against H. pylori [107,111,112]. Coupling of monoclonal antibodies against H. pylori to liposomes to achieve specific accumulation on the surface of H. pylori without side effects on normal cells [110]. Thus, HpAb-LiP-ICG is an ideally suited diagnostic agent for H. pylori targeted therapy and may have promise as a deep SDT antimicrobial application with greater clinical translational.

5. Conclusion and outlook

This paper reviews the application of nanocarrier-mediated SDT against H. pylori infection. We know that encapsulating the sonosensitizer in a nanocarrier can overcome problems such as poor stability of the sonosensitizer itself and enhance this treatment effect of SDT. These reports have highlighted that the combination of modern nanotechnology and SDT has demonstrated great advantages to treat H. pylori infection. Nanocarrier-mediated SDT overcomes problems of H. pylori resistance, patients' poor compliance, and disruption of balance of the intestinal microbial community associated with traditional antibiotic therapy. Not only can it achieve comparable results to standard triple therapy, but it also avoids the adverse affects on the gut microbiota. Therefore. Combination of nanotechnology and SDT provides a new and promising non-antibiotic therapeutic option to treat H. pylori infection, which is expected to become a new direction to treat H. pylori infection, particularly in combination with other treatment methods showing a more significant anti-H. pylori infection potential. Yet, little research has been done in this area, and no clinical trials have been conducted to confirm that SDT is safe and effective. In the future, more studies are needed to support the advantages of SDT

nanocarriers against H. pylori infection, to explore in depth the therapeutic frequency of SDT and the value of combining SDT with other methods of treatment, to confirm its safety and efficacy from clinical trials, and to promote the clinical application of nanocarrier-mediated SDT.

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PART

III

Internal stimuli-responsive nanocarriers for biomedical application

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CHAPTER

pH-responsive nanocarriers for biomedical application

9

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1. Introduction

It is well known that many materials at the nanoscale have unique properties compared to macroscale materials. However, besides the ability to express improved properties, nanomaterials can be modified at this dimensional scale to achieve specific physicochemical properties. Therefore, the search for new nanocarriers that allow improved drug loading, better stability, tissue-targeting capacity and blood circulation has been improved and frequently used, minimizing side effects compared to the administration of bioactive molecules alone. Some examples of nanomaterials, such as polymers conjugated with drugs, nanoparticles, dendrimers, micelles and liposomes, have been regarded for many years as promising avenues for biomedical applications, including drug delivery, bioimaging, diagnosis and therapy or both applications, named theranostics agents [1]. Nowadays, the applications of nanomaterials are still hampered by certain challenges, including an inadequate design for controlled drug release, nonspecific biodistribution, and off-target effects, which largely limit the therapeutic effectiveness [2,3].

For instance, to avoid side effects related to premature release, a stable loading of the drug into the nanocarrier is required, but it should be noted that if this increase in stability is very high, it could exhibit a poor drug release profile on the target sites. Thus, current designs have evolved, taking advantage of certain intrinsic properties of the human body or changes characteristic of the disease, such as enzyme levels, pH, levels of reduced glutathione and reactive oxygen species between nontarget and target sites for site-specific release of drugs. Another approach that has been explored is the use of environmentally sensitive nanocarriers able to control drug delivery when exposed to external stimuli such as temperature, electric field, magnetic field or ultrasound, producing physicochemical changes that favor the release of the bioactive agent on the target site [4].

Among environmental stimuli, pH gradients have been widely used to design novel, pH-responsive nanocarriers since different gradients exist among tissues and cellular compartments in the human body (Fig. 9.1) [5,6]. For instance, there is a wide range in pH values throughout the digestive tract,

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ranging from pH = 2 in the stomach to pH = 8 in the colon. Another example centered in cancer disease, a tumor tissue is 0.5–1 pH-units lower than the pH value in the surrounding normal tissue due to metabolic glycolysis and lactic acid production. Besides, at the cellular level, there are pH differences among cellular compartments such as lysosomes (pH = 4.5–5), endosomes (pH = 5.5–6), and the cytosol (pH = 7.4) [7]. On the other hand, the wounds themselves or the microorganisms that infect them can create acidic or alkaline environments depending on the biological environment, either through their own mechanisms or via the release of enzymes to promote their growth. With this approach, new nanocarriers can be designed to be responsive to defined pH values, can target a specific area in the body to release their encapsulated drugs with maximum therapeutic impact and minimum side effects [8–10]. This realization has made pH-responsive nanocarriers very interesting prospects to be used as drug delivery systems (DDSs).

2. Preparation methods

As mentioned above, depending on the organ, tissue or subcellular compartment—as well as their pathophysiological states—the diverse zones can be differentiated by their pH levels and gradients, and these differences can be exploited for site-directed design. Therefore, upon exposure to these pH changes, pH-responsive DDSs exhibit physicochemical changes in their structure and particularly in their surface characteristics. In this regard, some of the most widely used strategies focus on changes, including swelling, bond dissociation, or switching surface charge, favoring drug delivery to the target site rather than to the surrounding tissues. For these reasons, the following sections describe the various strategies currently used for the design of pH-responsive DDSs.

2.1 Linkers pH-responsive

The simplest design for controlled drug delivery based on pH is focused on the conjugation of a drug to a polymer using an acid pH-sensitive linkage (spacers) between the drug and the adequate polymer.

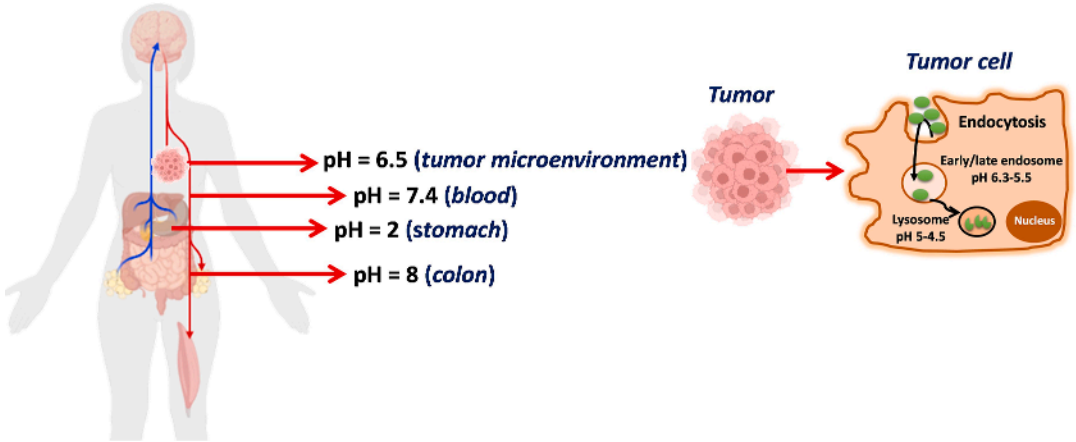


FIGURE 9.1

pH gradients in human tissues and pH changes observed in tumor cells.

This initial design responded to the need to improve the drug's physicochemical properties, such as solubility and stability. So, cleavage of acid-sensitive linkers at a specific pH range leads to drug release at the respective pH only. Based on this approach, various conjugates have been developed for targeted drug delivery, for example, to either relatively acidic extracellular fluids in a tumor, or after endocytosis, in the endosomes, or lysosomes in tumor cells. Table 9.1 shows the most frequently used labile linkers at acidic pH and their hydrolysis products [11].

2.2 Chemical groups that can be protonated or deprotonated

Protonation is one of the most widely used mechanisms for pH-induced drug release. The protonation and deprotonation of functional groups in a polymer at different pH values can trigger significant configuration changes, resulting in changes in its physical and structural properties, such as chain conformation, configuration, surface charge, stability, and solubility [12,13]. For example, a widely used classical strategy is the reversible ionization of carboxyl or amino groups to prepare pH-sensitive nanocarriers since these types of functional groups can undergo protonation and deprotonation in response to pH changes in their environment. In this way, these types of nanocarriers have the ability to swell, releasing the incorporated drug [14]. For this reason, in diverse designs is common to find the use of anionic polymers with carboxylic acid groups to prepare pH-sensitive nanocarriers, since when these systems are exposed to a neutral environment, the carboxylic acid groups are hydrophilic due to deprotonation, but when exposed to an acidic environment, they become hydrophobic due to protonation [15]. In this regard, an example of these polymers is polyacrylic acid (PAA), a pH-responsive material, which has strong hydrophobicity. However, it can self-interact to form precipitates under acidic conditions while it deprotonates and becomes hydrophilic at neutral or alkaline pH [16].

The change in the charge on a coating layer of a nano DDS when exposed to different pH values can result in either protection or deprotection of the same nano DDS. Nevertheless, if properly designed, after the coating layer is removed by a change in pH, the therapeutic cargo on the nano DDS interacts specifically with its target, e.g., a tumor cell, thus increasing the drug efficacy [17]. Polymers based on electrical charge can be divided into cationic, anionic, and zwitterionic polymers. Most cationic polymers contain ionized amino groups, anionic polymers have organic acids, and zwitterionic polymers bear both cationic and anionic groups on the same polymer chain [18]. The charge that a

Table 9.1 pH-sensitive chemical bonds for preparing pH-responsive DDSs.

Type	Structure	Metabolic products	Approximate pH of hydrolysis
Hydrazone			pH 5.0
Imine bond			pH 6.8
Amide bond			pH 4.5–6.0
Oxime			pH 4.8–5.0
Orthoester			pH 5.0–6.0
Borate ester			pH 5.0–6.5
Vinyl ethers			pH 4.5–5.0

nano DDS acquires when exposed to a biological system is crucial, not only for the targeted delivery of the drug but also for the interaction with other physiological components, in this sense, cationic nanoparticles are more easily absorbed by cells than anionic and neutral nanoparticles, but they can be eliminated during systemic circulation after binding to some serum component in the blood [19].

Zwitterionic polymers could maintain a negative charge in the physiological state (pH 7.4), thus reducing specific binding to blood serum components and preventing elimination by the reticuloendothelial system. Therefore, in the acidic microenvironment of tumor tissue, the negative charge of these polymers is transformed into a positive one, which improves their uptake by tumor cells [20]. An example of this is chitosan, which is protonated in a low pH solution because the pKa value of the amine groups in this polymer is 6.5 [21].

Carboxymethyl chitosan (CMCS) is an amphoteric polysaccharide because it has both an acidic group (-COOH) and a basic group (-NH_2) [22]. CMCS is pH-sensitive and negatively charged at physiological pH but positively charged under acidic conditions inside tumor cells. Therefore, 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) (chloride salt), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), and cholesterol were used to synthesize a cationic liposome (CL) for delivering sorafenib (Sf) and RNAi (Si). CMCS-SiSf-CL was obtained after modifying Si/Sf-charged CL (SiSf-CL) with CMCS. The electrostatic interaction allowed negatively charged CMCS to be coated onto the surface of a positively charged drug complex (SiSf-CL) at physiological pH. In the acidic microenvironment of tumor tissues, CMCS acquired a positive charge upon protonation and cleaved from the complex. The positively charged drugs in the complex interacted with the negatively charged tumor cell membrane, which enhanced the therapeutic effect of the drug on the nano DDS compared to its free counterpart [23].

As mentioned above, polymers containing pH-sensitive groups such as amine, sulfonic or imidazolium groups, which can protonate under acidic conditions, can be used for the design of a nano DDS, triggering charge reverse or hydrophilic/hydrophobic phase transition. This leads to changes in their physical and chemical properties or structural configurations to release the therapeutic load of these polymers [15]. In this way, polymeric compounds containing amino groups usually exhibit specific pH responsiveness, mainly due to the protonation/deprotonation effect of the amino group in different environments after changing the ionic state of these polymers [24]. In this regard, phosphorylcholine-b-(2-dimethylamino) ethyl methacrylate (PDMAEMA) has been widely used as an acidic pH-sensitive polymer to achieve controlled drug release. Another strategy used to optimize nano DDS is based on the protonation of tertiary amines under acidic conditions since this can induce changes from hydrophobicity to hydrophilicity, leading to the disassembly of nano DDSs to release the encapsulated drugs [25–27].

Thus, through surface modification of a nano DDS or covalent binding of specific functional ligands, it is possible to obtain nano DDS with the ability to deliver in a targeted manner through a drug release mediated by environmental pH changes, since after exposure of these nano DDSs to a different pH environment, protonation or deprotonation, charge reversal, or breaking of chemical bonds occurs, resulting in a structural conformation change, which could promote specific uptake of the release drug for example by tumor cells [28,29]. A variety of pH-sensitive nano DDSs have been developed, and some of them offer adequate controlled drug release [30]. Finally, it is worth mentioning that one of the design strategies for pH-responsive nano DSSs consists of facilitating hydrophobic-to-hydrophilic transformation at a low pH condition, leading to size swelling or changes in their phase behaviors [14].

3. Type of pH-responsive drug nanocarriers

3.1 Polymeric micelles

Polymeric micelles (PMs) are a thermodynamically stable colloidal solution that is formed from the self-assembly of graft copolymers or amphiphilic block copolymers with hydrophilic segments (shell) and hydrophobic segments (core) in water [15,31]. The hydrophobic core of micelles can load hydrophobic drugs with low solubility, while the hydrophilic shell can form a protective layer to shield drugs from exposure in an external medium, thereby prolonging drug circulation in the body [32,33]. PMs are promising as a nano DDS with an excellent ability of drug solubilization, high stability and a long circulation time [24,34].

However, PMs can release the encapsulated drug either slowly or uncontrollably. To resolve this issue, stimulus-responsive PMs have been developed, where pH is the most commonly used stimulus [35]. Generally, drugs are loaded into PMs via covalent bonds (*e.g.*, pH-sensitive chemical bonds) since they have lower drug leakage than those with noncovalent bonding (e.g., hydrophobic interaction) [36]. PMs with pH-sensitive chemical bonds can respond differently at various pH values, for example, in the tumor environment. Thus, pH-sensitive PMs could have slow or almost no drug release in a physiological environment, while they could rapidly decompose and release drugs under acidic conditions [37]. For this reason, pH-sensitive PMs are considered one of the most promising anticancer nano DDS due to their ability to respond to the tumor microenvironment, reducing side effects of encapsulated drugs, and improving drug bioavailability [38].

A wide variety of polymers can be used to make PMs, including amphiphilic di-block copolymers (*e.g.*, polystyrene and PEG), triblock copolymers (*e.g.*, poloxamers), graft (*e.g.*, G-chitosan), and ionic (*e.g.*, poly(ethylene glycol)-poly(ε-caprolactone)-g-polyethyleneimine) copolymers. The amphiphilic block copolymers making up PMs self-assembly above a given concentration are referred to as the critical micellar concentration (CMC). In diluted aqueous solutions, the polymers exist separately as unimers and act as surfactants to reduce interfacial tension. When the bulk solution saturation concentration exceeds the CMC, these unimers aggregate to form PMs [39]. This makes the CMC the most important parameter for controlling the thermodynamic stability of PMs [40]. Depending on the method of PM preparation and on the properties of the drug(s), these therapeutics can be encapsulated into PMs during their formation or incorporated postformation. Commonly used preparation methods for PMs include direct dissolution, dialysis, emulsion with solvent evaporation, and solution-casting followed by film hydration [41].

3.2 Liposomes

Liposomes are bubble like microscopic (0.01–5.0 μm) bilayered structures made of phospholipids. Liposomes have gained much attention and interest in targeted drug delivery during the last few years. Recently, new research and developments have been performed in the field of gene or drug delivery, for example, to preferentially destroy cancer cells. Liposomes can be easily formulated by hydrating phospholipids in an excessive aqueous medium. A very impressive characteristic of liposomes is that they can encapsulate both hydrophilic and hydrophobic drugs: hydrophilic drugs in the hydrophilic core, lipophilic drugs in the lipid bilayer, and amphiphilic drugs on the surface of the bilayer. They have the potential of targeting the drug at a specific site, thereby giving numerous advantages to liposomes for potentially safe and effective drug delivery [42].

Conventional liposomes have good biocompatibility, which is often accompanied by weak cell transfection ability [43,44]. However, PEG is a highly hydrophilic polymer that can be used for the modification of liposomes' surface since PEG can prevent recognition by the reticuloendothelial system, achieving extension of their blood circulation time [45–47]. Meanwhile, PEGylation can block cellular uptake and reduce intracellular drug release [48,49]. An effective strategy to address this issue is to endow liposomes with stimulus-sensitive properties. pH-sensitive liposomes have shown great potential in different areas of targeted drug delivery since pH-sensitive liposomes remain stable at physiological pH (pH 7.4) while they get destabilized under acidic conditions, and particularly these systems become fusogenic in nature [42]. These features destabilization results in the release of their aqueous contents. Furthermore, the idea of developing pH-sensitive liposomes was originally copied from certain enveloped viruses that take advantage of the acidification of the endosomal lumen to transmit diseases to cells. It is well known that some pathological tissues, such as tumors, inflamed and infected areas, possess a comparatively more acidic environment than normal tissues, and this feature can be exploited as a stimulus for drug release [50,51]. Furthermore, an important property is that liposomes can be sized for passive targeting or coupled to antibody fragments for active targeting.

3.3 Hydrogels

A hydrogel is a three-dimensional cross-linked polymer chain network with a large number of hydrophilic groups such as–NH_2, –OH, –COOH, and/or –SO_3H. It can absorb a great amount of water or biological liquids [52,53]. Hydrogels are formed by crosslinking polymer chains with ionic bonds, covalent bonds, hydrogen bonds and van der Waals interactions [54]. Hydrogels have important biocompatibility properties since their structures are similar to living tissue, minimizing the impact on surrounding tissue after self-implantation of hydrogels [55]. Optimizing these properties gives rise to nanogels, which are nanometer-sized cross-linked hydrogel particles, and have a large surface area, and their size and shape can be adjustable. To prepare them, polyelectrolyte polymers with acidic or basic functional groups can be used to form pH-sensitive nanogels after cross-linking. The functional groups in the backbone of the polymers used for the development of pH-sensitive gels have a capacity to donate or accept protons, responding to the pH of the environment to which they are exposed [56]. The electrostatic repulsive forces generated by the ionized pendant groups in the polymer network at appropriate pH and ionic strength are responsible for swelling or deswelling of the hydrogels. This pH-responsive swelling or deswelling of polymers regulates the drug release profile [42]. The pendant groups of the polymers of acidic (anionic) hydrogels remain unionized below the pKa of the used polymer and are ionized above this value. This leads to swelling of the hydrogel at pH above the polymer pKa due to excessive osmotic pressure coming from the presence of ions. In contrast, basic (cationic) hydrogels swell at lower pH (Fig. 9.2). Based on this approach, a number of trials have been done for targeted drug delivery in the acidic microenvironment of the endosomes/lysosomes or tumor tissues [57]. One of the most notable properties of hydrogels is that some structures can self-repair, which opens a great potential for diverse biomedical applications.

3.4 Dendrimers

A dendrimer is a type of hyperbranched polymer, which is characterized for bearing a tree like topology [58]. Dendrimer is a nano-scale spherical macromolecule that contains three parts from the

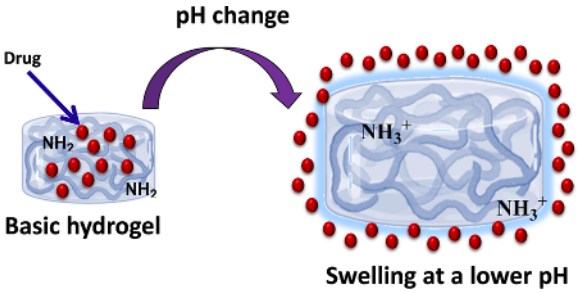


FIGURE 9.2

Illustration of the swelling phenomenon of a basic hydrogel.

inside out: (1) a central core (composed of two or more active groups), (2) branches that contain many structural repeats along the radial direction (named dendrons) and (3) functional groups in the periphery that modulate its macroscopic properties (Fig. 9.3) [58–60]. Dendrimers have attracted a lot of attention from researchers in recent years due to their excellent polydispersity, water solubility, and high surface functionality for drug delivery. In this regard, a dendritic configuration with a rigid structure shows a longer blood circulation time than a linear carrier. Dendrimers can be conjugated with drugs and target ligands simultaneously, and the dendritic structure is widely used due to its highly branched topology, a three-dimensional spherical shape, surface versatility, and well-defined composition [61]. Dendritic macromolecules can load drugs by attaching them to surface functional groups via covalent bonds or encapsulating them in the lumen via intermolecular interactions [62,63]. In this manner, pH-sensitive dendrimers can be designed and constructed by modifying the functional groups on their surface or by selecting a suitable linker between the dendrimer and the therapeutic payload (Fig. 9.3). So, the drug release is based upon quick hydrolysis of the linker in an acidic environment like the tumor tissue, while it remains stable in the systemic circulation. Using this design, the degradation of the linker leads to pH-responsive drug delivery.

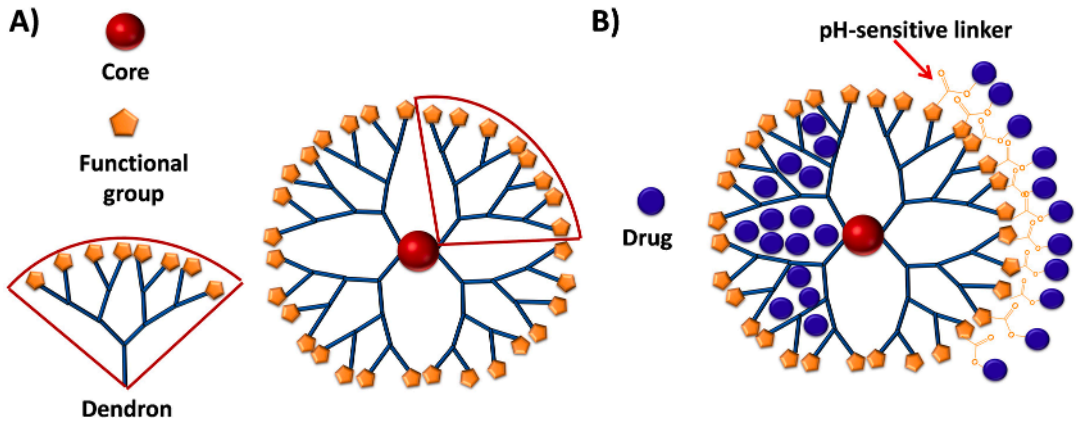


FIGURE 9.3

(A) Basic components of a dendrimer and (B) Illustration of strategies for the design of pH-responsive dendrimers.

3.5 Inorganic nanoparticles

Inorganic nanoparticles are considered to address issues associated with organic carriers, including unstable and uncontrollable drug release. Gold nanoparticles, magnetic nanoparticles, carbon nanotubes, silica nanoparticles and other inorganic carriers have been considered as substitutes for organic carriers [11]. Many inorganic materials, for example, calcium nanoparticles, metal oxides and graphene oxides, also have pH-sensitivity. Inorganic materials have their unique pH-sensitive mechanism. Calcium materials and metal oxides (iron oxides, manganese oxides and zinc oxides) are acid-soluble to deliver the drug. Other materials such as graphene oxides, gold nanoparticles, black phosphorous and mesoporous silica have particularly covalent and noncovalent interactions that can be changed by protonation, and changing of interactions triggered by pH is utilized for drug delivery. Moreover, there are different kinds of preparation methods in some inorganic materials, which are available for fabricating nanoparticles in various shapes (particle or layer) [12].

3.6 Organic-inorganic hybrid nanocarriers

While inorganic DDSs have demonstrated strong stability, controlled drug release and high drug loading capacity, their clearance in the human body can be very challenging [64]. Organic-inorganic hybrid nanoparticles have attracted attention because they may inherit advantages from both inorganic and organic nanoparticles. Metal-organic coordination materials (MOCMs) are an important class of organic-inorganic hybrid materials. This MOCM combines the physicochemical properties of metal and organic materials to achieve a variety of functions by tuning supramolecular structures in response to environmental stimuli [65]. In this regard, metal-organic frameworks (MOFs) are crystalline coordination-based materials, consisting of an infinite network of metal ions or metal ion clusters that are bridged by organic ligands through coordination bonds into porous two- or three-dimensional extended structures [11,66]. In general, several examples of organic–inorganic hybrid nanoparticles have been described with the ability to protect drugs, proteins and even nucleic acids from enzymatic degradation and improve cellular uptake of the nanosystem into a target cell.

3.7 Protein carriers

Albumin, lactoferrin (Lf), high-density lipoproteins and low-density lipoproteins can not only be used as a functional moiety of a nano DDS to improve the targetability of the DDS, but also act as a nano DDS to deliver drugs [67]. Albumin has great biocompatibility, biodegradability, stability, high solubility, abundant functional groups and many hydrophobic binding sites, and a myriad of ligands and drugs have been explored to bind to albumin. There are different types of sources for albumin. Currently, human serum albumin (HSA) and bovine serum albumin (BSA) are widely used as nano DDSs [68]. HSA is the most abundant circulating protein in the human body, and it has a half-life of ca. 19 days during systemic circulation. For this reason, albumin has been extensively employed for the formation of prodrugs and nanoparticles through covalent bonding or physical binding as described above [69].

Lf is an internal ferribinding protein in the human body, which shows a promising antitumor activity. Lf can be used as a target ligand of a nano DDS or act as a DDS. LF-based NPs have the capacity to penetrate the blood-brain barrier [67]. A protein cage, a nanoscale structure, has a hollow cavity after self-assembly of protein subunits [70]. Protein cages are made from protein molecules, and they

are readily biodegradable and have fewer adverse reactions [71]. Transferrin (Trf) can regulate the balance of iron ions in the human body, and it becomes a new DDS with targetability [72]. Ferritin is a protein for iron storage in cells, and it has a cage structure similar to the previously mentioned MOFs. Therefore, ferritin can be used as a DDS because a variety of drugs can be encapsulated in its hollow cavity [73]. Ferritin nanocages have been found to extend the blood circulation time of drugs. They act as a protective coating, reducing reactions of drugs within the cavity with other biological molecules. They also increase the biocompatibility of synthesized prodrugs. They have an intrinsic affinity for transferrin receptors (TFR-1), thus they can specifically target cancer cells with overexpressed TFR-1 on the cell membrane [74]. Reversibly covalent binding of proteins to small molecule drugs (e.g., pH-sensitive bonding) has emerged because the formed prodrug not only protects proteins from degradation and degeneration, but also prolongs their circulation time and increases the biocompatibility of small molecule drugs [75].

4. Applications

4.1 Tumor therapy

For many years, tumors have been the focus of lots of research. In this regard, due to excessive proliferation of cancer cells, intensive metabolism and inadequate oxygen uptake of tumor cells lead to increased extracellular acidity, resulting in a slightly lower pH value in the extracellular matrix (ECM) of tumor tissues than normal tissues [76,77]. Acidosis is one of the features of the tumor microenvironment, and the acidic microenvironment of tumors gradually becomes a key regulatory factor for tumor progression. This unique environment has been harnessed as a stimulus for smart DDSs to deliver drugs for the treatment and diagnosis of tumors [78,79]. Stable chemical bonds or linkers in DDSs at a physiological condition can be responsive in such a smart way that a weak acidic microenvironment of the tumor tissue can accelerate the release of drugs from DDSs [80]. The tumor microenvironment in the pH range of 7.2 to 6.5 can induce structural or physiochemical property changes of DDSs to facilitate endocytosis of them into cells, and at a pH level of 6.5 to 4.5 becomes another stimulus for promoting drug release from DDSs inside cells [81].

Some recent examples of applications in tumor therapy are described here. For instance, a dual-pH-sensitive micelle was constructed to deliver calcitriol (CTL) and paclitaxel (PTX). The shell of PTX/CTL-co-loaded dual-pH-sensitive micelle (PCDM) was composed of PEG and an anionic polymer, and the hydrophobic core contained *N, N*-diisopropylethylenediamine (DPA) groups. The surface charge was switched from negative to positive when the shell of PCDM was exposed to an acidic tumor environment. Therefore, the micelles entered tumor cells easily. The protonation of DPA groups inside acidic endo/lysosomes induced the dissociation of the micelles to release PTX and CTL [82].

On the other hand, highly active immunoliposomes have been reported by modification of the surface of pH-sensitive Taxane prodrugs with Ephrin A2 antibody to enhance liposome transport toward solid tumors [83]. Liposome surface modification with dendritic peptide fragments can achieve targeted delivery of drugs into mitochondria. Liposomes were modified with dendritic peptide fragments rich in an arginine structure to obtain a multi-functional prodrug. The dendritic peptide acted as a signal transduction agent to realize mitochondria-targeting delivery of the prodrug. A stearyl group was added as a hydrophobic segment to increase the lipophilicity of liposomes. In addition, 2,3-dimethylmaleic acid was loaded onto the surface of the prodrug to protect the cationic properties of

arginine. Therefore, the modified liposomes had the characteristics of pH sensitivity and charge reversal [84].

In this same way, pH-sensitive peptide hydrogels have been widely used as nano DDS. For example, a pH-sensitive octapeptide Phe-Glu-Phe-Glu-Phe-Arg-Phe-Lys (FER-8) hydrogel was prepared to load PTX for targeting tumors. The peptide hydrogel remained stable at a neutral pH of 7.4 and had a fast decomposition rate in an acidic environment because glutamate in FER-8 was ultrasensitive to acidic pH due to the presence of electrostatic repulsion between glutamic acid residues when the pH was far away from the pKa value of the peptide residues [85].

Other very versatile molecules are the polyamide-amine (PAMAM) dendrimers that have been widely used in drug delivery due to their simple preparation process and excellent physical and chemical properties [61]. In this manner, a nanocarrier was prepared by grafting PAMAM dendrimers onto persistent luminescence nanoparticles (PLNPs), and it was coupled with AS1411 on the carrier surface to target nucleolin overexpressed on the membrane of tumor cells. The DOX was loaded onto the carrier through an acylhydrazone bond, which is a pH-sensitive chemical bond. The release of DOX from the nanocarrier was confirmed to be pH-sensitive, and more than 60% of DOX was released at pH 5.0, whereas only 10% of the drug was released at pH 7.4 [61].

In recent years, owing to the nice compatibility of calcium phosphate (CaP), foreign substances doped with CaP have been popularly investigated to combine diverse properties. Fu et al. [86] have fabricated a biodegradable manganese-doped pH-sensitive CaP nanoparticle for traceable cascade antitumor theragnostic. The CaP systems consisted of glucose oxidase (GOx), DOX and Mn^{2+}, and since GOx realized starvation therapy through effectively eliminating intratumor glucose, Mn^{2+} caused chemodynamic therapy through toxic hydroxyl radicals that are generated by Fenton-like reaction, and DOX mediated chemotherapy. Besides, the enzyme activity remained stable for a long time because the CaP had served as a highly compatible and stable carrier for the GOx, thus the CaP had shown prominent application in enzyme delivery.

Zeolitic imidazolate framework (ZIF), UiO-66 and MIL-100 are the most used pH-sensitive MOF materials, which have permanent porosity and high thermal and hydrothermal stabilities [12]. In this regard, Zheng et al. [87] have synthesized DOX-encapsulated ZIF-8 nanoparticles through a one-pot method for pH-responsive drug delivery. The ZIF-8 nanoparticles exhibited excellent pH response; zero-release was observed at pH 6.5, whereas full-release was observed at pH 6.0. Wang et al. [88] have fabricated MIL-100-based nanoparticles for chemo/photothermal combinational cancer therapy. The nanoparticles were able to serve as a magnetic resonance imaging contrast and fluorescence optical imaging (FOI) agent to realize dual-model imaging guided chemo/photothermal therapy to treat cancer.

Finally, albumin was used as a drug carrier to load anticancer small-molecular drugs to extend their therapeutic window and reduce their side effects. Auristatins are potent anticancer agents, but their narrow therapeutic window has hampered their clinical application. To address this issue, an albumin-binding prodrug was developed. The prodrug of auristatin E was formed by connecting the drug with albumin-binding groups via acid-sensitive hydrazone bonds. After injection of the prodrug, the prodrug was bound to cysteine-34 (Cys34) of albumin, and the conjugated product stably circulated in the bloodstream. In an acidic environment of tumor cells, the hydrazone linker between the drug and albumin was ruptured to release the drug to exert its anticancer effect [89].

4.2 Tumor imagen

Unfortunately, cancer is often diagnosed after it enters the late stage; however, accurate and timely detection and diagnosis of cancer in the early stage have been actively pursued. Biomedical imaging can not only provide accurate pathological information, but also offer noninvasive detection; therefore, biomedical imaging methods via sensitive and specific molecular probes have attracted great attention [38]. Retention of the imaging probe at a pathogenic site is a prerequisite for long-term imaging and monitoring biological processes and therapeutic outcomes [90]. Nanoparticles have been used as imaging agents to detect biomarkers of tumor cells at disease sites, and they can act as contrast agents for magnetic resonance imaging (MRI) because they can improve the targeting ability to tumor cells and enhance the signal-to-noise ratio in comparison with low molecular weight contrast agents [91,92]. Acidity changes are seen at different stages of tumor progression, and imaging agents in response to a pH-varying environment can provide accurate information for localization and progression of tumors, thereby improving treatment outcomes [93].

Fluorescence sensing technology has the characteristics of high sensitivity, high resolution, simplicity, and noninvasiveness, and it can be used to monitor the progression of tumor *tissues* in vivo [94,95]. Nano DDSs can deliver drugs to the tumor site and offer fluorescence imaging at the tumor site, realizing the integration of tumor imaging and therapy [96].

MRI, which is a noninvasive imaging method, provides the advantages of no penetration depth limitation and a high spatial resolution. It has played an irreplaceable role in the diagnosis of many diseases [97,98]. Manganese oxide (MnO_2) nanoparticles (MONs) have negligible toxicity, and they can provide great T1-weighted contrast. pH-sensitive MnO_2@BSA nanoparticles were prepared. Under acidic conditions in tumor cells, MnO_2 in the MnO_2@BSA nanoparticles was dissolved to release Mn^{2+} to enhance the T1 signal of MRI. Therefore, the MnO_2@BSA nanoparticles could be used for in vivo dynamic imaging of an extracellular tumor pH microenvironment. In addition, MnO_2@BSA nanoparticles produced the strongest T1-weighted MR signal 45 min after injection, which may be the best imaging time point to monitor the treatment response [99].

Bioimaging-guided photothermal therapy (PTT) holds great promise as a strategy, but the delivery of the photosensitizer for precise cancer treatment remains a big challenge [100]. Visualization is achieved by loading a contrast agent into the DDS, and PTT is realized via loading a photosensitizer into the same DDS. Multi-modal imaging can diagnose cancer more accurately than a single imaging method. A multifunctional nano DDS was formed by coating manganese dioxide (MnO_2) onto the surface of Fe_3O_4 nanoparticles. A photosensitizer Ce6, and a drug celastrol (CSL) were loaded into the DDS via electrostatic interactions. Rapid decomposition of MnO_2 under acidic conditions in the tumor tissue resulted in the release of Mn^{2+}, Fe_3O_4, and loaded drugs. The nano DDS significantly enhanced the contrast of T1/T2 MRI and the sensitivity of fluorescence imaging, and it also increased drug accumulation in the tumor tissues for their anticancer effect [101].

4.3 Inflammation

The inflammation process is an immune response of the human body to infection or tissue damage [102]. The inflammatory microenvironment has unique characteristics, including a decreased pH value, an elevated body temperature, and special overexpressed enzymes [103,104]. A low pH value in the inflammatory microenvironment could be utilized to develop pH-sensitive nano DDSs for

delivering drugs to the inflammatory tissue, thereby improving the efficacy and reducing the toxicity and side effects of drugs [105,106].

One of the most recent examples of designs for the treatment of inflammation is the generation of octenyl succinic anhydride-modified pH-sensitive chitosan-octenyl succinic anhydride (OSA-CS), which was synthesized by direct acylation of octenyl succinic anhydride. OSA-CS self-assembled in an aqueous solution to form stable, charged nanoparticles, which were used to load curcumin (CUR) and quercetin. Therefore, in an acidic environment (pH 6.0), protonation of the amino group on chitosan resulted in a positive charge on the nanoparticles, leading to depolymerization of nanoparticles and structural disassembly for the release of the encapsulated drugs. The CCK-8 assay was used to examine the therapeutic effect of the drug-loaded nanoparticles on the proliferation of lipopolysaccharide (LPS)-induced macrophages. The drug-loaded nanoparticles significantly inhibited macrophage proliferation, suggesting that the nanoparticles could enhance the antiinflammatory and antioxidant effects of drugs, providing an attractive strategy for the treatment of inflammation [105].

Superoxide dismutase (SOD), an endogenous enzyme, can remove superoxide anions and reduce tissue damage caused by inflammatory reactions. However, it is challenging to deliver SOD to the inflammatory sites because it is unstable and easily cleared after it is administrated. Therefore, nano DDSs with high stability, targetability to inflammatory sites and long-term circulation are required to transport SOD. Hydrophilic modification of two polyketal compounds, poly (cyclohexane-1,4-dimethylacetone dimethyl ketal) (PCADK) and poly (cyclohexane 86.7%, 1,5-pentanediol 13.3%)-1,4-dimethylacetone dimethyl ketal) (PK3) were carried out by introducing PEG at different molecular weights. The prepared amphiphilic polymers were used to load SOD. The degradation rate of two copolymers at pH 4.5 was higher than that at pH 7.4, and MPEG2000-PCADK showed the best degradation rate and was selected as an acid-sensitive DDS in the body. MPEG2000-PCADK micelles demonstrated to target inflammatory tissues and exert a distinct antiinflammatory effect. After mPEG-PCADK micelles were injected into a rat model of adjuvant arthritis, the paw thickness was significantly reduced compared to the control (saline) group and the infiltration of ankle cells was decreased in the ankle joint. Therefore, the MPEG-PCADK copolymer could be an excellent DDS for treating acute inflammation and transporting unstable proteins [45].

As previously described, the construction of prodrugs is a promising strategy. A pH-sensitive acetone-based ketal-linked prodrug of dexamethasone (AKP-dexs, self-assembled nanoparticles) was designed. Surface modification of the nanoparticles with DSPE-mPEG2000 (1,2-distearoyl-sn-glycero-3-phosphoethanol-amine-poly(ethylene glycol)) was performed to improve their solubility and hydrolysis kinetics. In a rat model of collagen-induced arthritis, the nanoparticles exhibited a higher level of accumulation and a better antiinflammatory effect in inflamed joints. These nanoparticles lowered the side effects of the drug Dex phosphate [107].

Diabetes complications are the main causes for patient deaths, since diabetes can simultaneously cause various inflammatory injuries and long-term microinflammation [108]. Antiinflammatory agents and antioxidants can reduce inflammation and induce healing in the wound area [109]. It has been described that there is a lower pH and a higher amount of ROS in the inflammatory environment compared to normal tissue, so that both pH and ROS can serve as stimuli to design inflammatory disease-specific nano DDSs. For example, after reaction of the carboxyl group of 3-carboxy-phenyl boronic acid (BA) with the amino group of gelatin (Gel) to form an amide bond, 3-carboxy-BA was grafted to the main chain of Gel to form phenylboronic acid-modified gel (Gel-BA). It was mixed with

a polyvinyl alcohol solution containing rich o-diols to form a hydrogel. The hydrogel had a dynamic phenyl borate diol ester bond. Furthermore, silver nanoparticles with a glutathione protective coating layer were introduced into the hydrogel after loading vancomycin into the hydrogel. Finally, a nonsteroidal antiinflammatory drug, nimesulide (NIM), was loaded into the hydrogel through the hydrophobic effect of the polymer to form a mixed antibacterial and antiinflammatory system. Macrophages (RAW 264.7) were preactivated with LPS, and they were used to assess the antiinflammatory ability of the hydrogel DDS. Through western blotting, real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and immunofluorescence staining, the hydrogel DDS was demonstrated to significantly increase the expression level of IL-10 and decrease the level of TNF-α, displaying a strong antiinflammatory ability [110].

Another disease with a high global incidence and mortality rate is cardiovascular disease, and its pathogenesis is known to be closely related to vascular inflammation. For this reason, dual-stimuli responsive nanoparticles were developed, and they were shown to be sensitive to acidosis and oxidative stress at inflammatory sites. The nanoparticles contained ACD, which is sensitive at low pH and OCD, which is sensitive to a high level of ROS. The pH/ROS responses were modulated by adjusting the weight ratio of ACD and OCD. The nanoparticles were intravenously injected into an animal model of vascular inflammation in rats with carotid artery balloon injury. It was found that the nanoparticles could accumulate at the lesion site and inhibit neointimal hyperplasia in rats with a significantly enhanced efficacy in vivo [111].

Direct targeting of the colon could realize promising therapeutic outcomes for the treatment of inflammatory bowel disease (IBD). A nano DDS for the delivery of drugs for colorectal inflammation is different from that for vascular inflammation. Gastrointestinal administration of drugs should withstand the challenges of different pH levels in the gastrointestinal tract. Therefore, the nano DDS for colorectal inflammation should prevent drug release at the acidic pH of the stomach, and the drug could be released from the DDS into the colon at a high pH to prevent systemic adverse reactions and improve treatment effectiveness [112].

Dex can be used to treat mild to moderate inflammatory bowel diseases, but long-term glucocorticoid use of Dex is accompanied by systemic side effects. A colon-targeting DDS was prepared after coating Dex microcrystals with pH-sensitive layers via the layer-by-layer method. Based on this, a DDS was designed that increased the targeting effectiveness of the drug, and a significant structural change of the drug occurred only at pH 7.4 of the colon environment, reducing systemic side effects of the drug [113]. An oral chitosan/alginate hydrogel (CA-gel) was developed for the treatment of ulcerative colitis (UC). A pH-sensitive linear skeleton, PBAE, was synthesized by polymerization of hexane-1,6-Dioldiacrylate (HDD) and 4-Amino-1-butanol (AP) monomers. Since 4-(Hydroxymethyl) phenylboronic acid pinacol ester (PAPE) is responsive to the presence of ROS, a pH/ROS dual-sensitive/responsive nanocarrier, PBAE-SA-PAPE, was obtained by grafting PAPE onto the skeleton of PBAE. As chondroitin sulfate (CS) can bind to macrophages, PBAE-SA-PAPE was modified with CS to increase the targetability of NPs to macrophages. CUR was loaded onto the NPs, and a hydrogel formed from chitosan and alginate at a ratio of 3:7 was used to encapsulate the NPs, resulting in the final product, CS-CUR-NPs-Gel. Fluorescence imaging confirmed that CS-CUR-NPs-Gel had a great colonic targetability. Moreover, CS-CUR-NPs-Gel could reduce the overexpression of IL-6 and TNF-α, and decrease the levels of inflammatory markers including myeloperoxidase (MPO) and inducible nitricoxide synthase (iNOS). The antiinflammatory mechanism may be related to the signaling pathway of TLR4-MAPK/NF-κB [114].

4.4 Antibacterial infection

Bacterial infection can reduce the acidity of the infected site to a pH of 5.5, and such a low pH value could be due to the accumulation of by-products of bacterial sugar fermentation at the infected site, including lactic acid and acetic acid [115,116]. Taking advantage of this condition, antibacterial drugs could be encapsulated into a DDS to achieve targeted delivery to the infected cells across various biological barriers and improve their therapeutic efficacies [117,118]. When the bactericidal agent concentration is not maintained at a constant level, or the activity of the antimicrobial agent in the body is reduced, the bacteria acquire the ability to survive and also proliferate. When this happens, it is necessary to inject a high concentration of antibiotics directly into the site of infection, which may induce the emergence of antibiotic resistance [115,119]. pH-stimulus responses can be employed for DDSs to carry drugs for interfering with acid-producing bacteria. These pH-sensitive prodrugs could reduce the use of systemic antibiotics and treat local infections with less antibiotics. The preparation of pH-sensitive prodrugs has become a new antibacterial strategy [120,121].

Prolonged excessive acidification of a biofilm at the tooth and dentin interface leads to demineralization of the tooth, which in turn leads to the occurrence of caries. Caries induces significant pathological pH changes that can be monitored by pH-sensitive nanomaterials [122,123]. Periodontitis, an infectious disease induced by a microbial biofilm with a mixture of pathogenic microorganisms, is associated with progressive destruction of the dental supporting tissue. Albumin nanoparticles were loaded with antimicrobial agents specifically for microbial film. Zinc oxide was introduced to improve the stability of albumin nanoparticles, and these nanoparticles were then incorporated into a gel network. The nanoparticles were pH sensitive at the infection site and released the antimicrobial agents, thus significantly reducing cytotoxicity compared with free drugs [124]. Poly-L-Glycolic acid (PGA) was grafted onto the surface of MSNs to form pH-sensitive nanoparticles for loading chlorhexidine. SEM images confirmed that the nanoparticles had the ability to penetrate the gingival canal. The release of chlorhexidine in the system was found to be pH sensitive, and the released drug displayed a strong antibacterial effect, significantly reducing the metabolic activity of *S. mutans*. Therefore, the DDS has the potential to act as a binder for restorative dentistry [125].

To address the challenge of inducing infection during implantation, a pH-sensitive multilayer system was prepared on the surface of a magnesium alloy. Since the deprotonation of the amino group of chitosan occurs at a low pH, chitosan was used to coat ibuprofen (IBU) to form IBU@CS. IBU@CS and heparin were then assembled on the AZ31 alloy via the layer-by-layer method. The multilayer DDS system improved corrosion resistance of the magnesium alloy, and the release of ibuprofen in the biofilm was pH sensitive, which significantly improved the antibacterial activity of the multilayer system against *E. coli* and *S. aureus* compared to the AZ31 alloy alone [126]. Since pH-sensitive pharmaceutical agents can be used to control peri-implant infection, Dong et al. [127] developed a novel titanium implant. The implant was composed of titanium dioxide nanotubes, and silver nanoparticles were coated onto the surface of the nanotubes through acetal bonds. The nanotube was sensitive to a change in the pH value after bacterial infection. Within 30 days, the rate of drug release at pH 5.5 was about 2.5 times faster than at pH 7.4, and within 2 h, the antibacterial efficiency at pH 5.5 was much higher than at pH 7.4.

Another strategy is based on building an intelligent DDS that can simultaneously administer two antibacterial drugs. An example with this approach is based on a pH-sensitive hydrogel, where acid-sensitive imine bonds were formed after the reaction of sulfadiazine (SD) and tobramycin (TOB) in the

aldehyde group of glucan oxide. TOB and SD had different antibacterial mechanisms: TOB is a broad-spectrum aminoglycoside antibiotic for rapid bactericidal action, while SD is a commonly used sulfonamide for slow bactericidal action. Combinational use of both drugs in one single DDS significantly improved the bactericidal effect. The drug-loading hydrogel was applied against *S. aureus*, *E. coli* and *P. aeruginosain vitro* and on the back of mice after *S. aureus* and *P. aeruginosa* were subcutaneously injected into mice to induce an ulcer on the skin after their infection. In vivo and in vitro experiments confirmed that SD and TOB released from the hydrogel showed synergistic effects against these bacteria; therefore, the hydrogel could be used to deliver drugs for antibacterial infection [128].

Photodynamic therapy (PDT) is an effective method to treat drug-resistant bacterial infection, but it has a challenge of enriching photosensitizers in the infection site, especially in Gram-negative bacteria-dominant sites, because of its thick and dense outer membrane. Thus, PDT has not achieved satisfactory results in clinical treatment. pH-stimulus-responsive nano DDSs could be employed for PDT to improve its antimicrobial efficacy. A pH-responsive micellar prodrug was prepared via conjugation of Ce6 to PEG-b-poly(2-(hexamethyleneimino) ethyl methacrylate-co-aminoethyl methacrylate) (PEG-b-P(HMEMA-co-AEMA)). The micelle was subjected to charge conversion at different pH values. A negative charge was maintained in a normal tissue environment, and it turned positively charged in a bacterial infection microenvironment. Moreover, micelle significantly improved its antibacterial activity against methicillin-resistant *S. aureus* and *E. coli* under laser irradiation, so that it could have great potential in PDT for the treatment of persistent bacterial infections [129].

4.5 Neurological diseases

For many years, central nervous system disorders have lacked effective treatments because most therapeutic drugs cannot enter the brain due to the existence of the Blood-Brain Barrier (BBB) in the body [130]. To allow the permeation through the BBB, the use of nano DDS has improved the pharmacokinetics and biodistribution characteristics of a large number of drugs. They have also been used to diagnose and treat central nervous system diseases [131,132]. Very few therapeutic drugs are effective in treating Alzheimer's disease, a neurological disorder, due to poorness or lack of permeability of therapeutic agents through the BBB. Nano DDSs could be used for the transportation of therapeutic antibodies for Alzheimer's disease across the BBB. 3D6 antibody fragments (3D6-Fab) in the brain parenchyma are specifically effective against β-amyloid protein (Aβ) aggregation. The primary amino group of the Fab reacted with citraconic anhydride via an amidation reaction to yield negatively charged citraconylated-Fab. Cationic disulfide cross-linked poly(PEG)-poly(L-Lysine) block copolymers electrostatically interacted with anionic Fabs to form a dual pH/REDOX responsive polymeric nanomicelle. When citraconylated-Fab entered the endocytic (acidic) pH, the primary amino group of the Fab was exposed to charge reversal restoring the antigen-binding ability of the Fab. In addition, loss of ionic interactions between anions and cations due to charge reversal and the fracture of the disulfide bond led to the release of many biologically active antibodies, which could significantly inhibit the accumulation of Aβ in the brain and promote the clearance of Aβ from the brain parenchyma. Therefore, this DDS could be used to deliver functional antibodies or antibody fragments to the brain for the treatment of other neurological diseases [133].

In Stroke decreases in tissue perfusion, which in turn alter the metabolism and enhance concurrent accumulation of carbon dioxide. The pH of the ischemic area changed from 7.4 to 6.5 [134]. Since

ischemic stroke can induce acidosis in the ischemic area, pH-sensitive nano DDSs could be employed to carry drugs for the treatment of ischemic stroke. For example, cationic poly(ethylene glycol)-b-poly [4-(2,2,6,6-tetramylpiperidine-1-oxy) amino methyl styrene] (PEG-B-PMNT) and anionic PAA were used to form a polyionic complex, which self-assembled into nanoparticles. These nanoparticles were used to load tissue plasminogen activator (t-PA) to form t-PA@iRNP. In vitro thrombolysis activity experiments confirmed that t-PA@iRNP maintained its structure at pH 7.4, while significantly lysed at pH 6.2. Moreover, t-PA@iRNP extended the half-life of t-PA from 8.2 to 71.2 min in a physiologic environment. In a cerebral ischemia-reperfusion mouse model, injection of t-PA@iRNP led to a reduction in the infarct volume and improvement in neurological deficits. Therefore, t-PA@iRNP could be a promising candidate for treating ischemic stroke. In addition, t-PA@iRNP significantly improved the transformation of cerebral hemorrhage during t-PA treatment [135].

Urokinase (UK), a thrombolytic agent, was encapsulated in pH-sensitive PEG-conjugated nanogels to form PEG-UKs [136]. The PEG-UK nanogels increased the dissolution of clots. The introduction of nanogels also reduced the neurological deficits, protected the integrity of the BBB, and decreased apoptosis and neurotoxicity.

Nucleotides (NTs), such as adenosine triphosphate (ATP), have been previously reported that could generate bioenergy through phosphate group release. For this reason, an interesting design is focused on a pH-sensitive nanocomplex that was used to deliver ATP to treat an ischemic rat model. The Ca^{2+}/NT nanocomplex (NC) was prepared via the ionic complexation between Ca^{2+} and NT phosphates. Branched polyethyleneimine (bPEI1.8 kDa) was coated on the surface of the NC to serve as a protective layer by simple electrostatic interaction. The Ca^{2+}/NT/bPEI1.8 kDa NC was responsive to pH changes, and a lower pH led to a faster rate of ATP release. In a middle cerebral artery occlusion (MCAO)-induced postischemic rat model, an intranasal administration of the Ca^{2+}/NT/bPEI1.8 kDa NC was applied immediately after MCAO induction. The results suggested that the Ca^{2+}/NT/bPEI1.8 kDa NC reduced the infarct volume and neurological deficits significantly [137]. In a recent study, since breast cancer cells are demonstrated to be prone to crossing the BBB, membrane proteins of cancer cells could be coated onto the surface of nanoparticles to help nanoparticles cross the BBB. The 4T1 tumor cell membrane was isolated and coated onto pH-sensitive nanoparticles that were loaded with an antioxidant and antiinflammatory drug, succinobucol (SCB). Fluorescence labeling and MRI imaging confirmed that the nanoparticles targeted cerebral ischemic lesions and effectively helped, reducing the infarct volume of cerebral ischemic rats [138].

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CHAPTER

Hypoxia-responsive nanocarriers for biomedical application

10

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1. Introduction

Cancer has the potential to be an advancing illness that can metastasize to different areas of the body and result in severe harm. Despite significant efforts, cancer continues to plague the world due to the variety, complexity and diversity of cancer types [1–3]. Chemotherapy is the first choice for cancer treatment, alone or in combination with other treatments such as radiation therapy, photodynamic therapy and immunotherapy [4–6]. Nonetheless, despite their significance in treating cancer, these technologies encounter challenges like poor water solubility, quick elimination from the body, restricted availability in targeting tumors, absence of sedative prescription for tumors, drug resistance, and other significant obstacles [7]. The prompt advancement of chemotherapy methods relies on the creation of new drugs and delivery techniques that improve their effectiveness in targeting cancer with precise carriers. Scientists are actively working on different strategies to combat cancer and reduce the chances of the disease coming back [8]. Following this, there has been a sustained endeavor to develop a nanodrug delivery system (DDS) to combat cancer, believed to be both more efficient and safer compared to conventional cancer medications [9]. Unfortunately, despite significant work in the development of nanomedicines, many tumors still suffer from poor survival chances and are difficult to analyze [10]. Thus, the development of an effective and efficient sedative delivery framework (DDS) that will control the tumor microenvironment (TME) for cancer treatment is very important and crucial to overcome. Hypoxia is a prevalent characteristic of solid tumors, prompting tumor cells to adapt in ways that promote metastasis and resistance to cancer treatments. Various imaging techniques, such as MRI, FI, PAI, CT, and PET, are utilized for cancer diagnosis [11–13]. Nanomaterials serve as "theranostic" agents, integrating therapeutic and diagnostic functions to enhance treatment efficacy. These nanomaterials effectively target hypoxic regions within tumors, employing strategies that allow them to disassemble in low-oxygen environments or utilize surface modifications for precise delivery. Additionally, oxygen-releasing nanomaterials can enhance therapeutic outcomes in hypoxic conditions. Nanotheranostic agents target hypoxic tumors by generating oxygen for treatment. The development of nanomedicines, including various nanocarriers, aims to facilitate targeted drug delivery while minimizing toxicity [14]. Nanocarriers release drugs rapidly in response to stimuli to enhance their effectiveness. However, there is a notable gap in hypoxia-responsive nanocarriers specifically targeting hypoxic tumor regions, which could be valuable for early cancer detection and therapy

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selection. Promising advancements in cancer imaging and therapy are being explored through the use of amphiphilic copolymers or conjugates with hypoxia-responsive properties [15].

The purpose of this paper is to explore the complex role of hypoxia in cancer progression and treatment, focusing on the development and application of innovative hypoxia-responsive strategies. This study aims to provide a comprehensive overview of how low oxygen levels can be utilized to enhance drug delivery systems by examining the characteristics of hypoxia, the associated biomarkers, and the mechanisms through which hypoxia influences cancer advancement. Additionally, the paper will investigate the potential of nanoparticles sensitive to hypoxic conditions for targeted drug delivery, as well as the integration of various therapeutic modalities, including chemotherapy, photodynamic therapy (PDT), sonodynamic therapy, and photothermal therapy, to improve treatment outcomes.

2. Hypoxic characteristics

2.1 Physical and chemical properties

The occurrence of tumor hypoxia due to the extensive dissection of nearby blood vessels has been of interest ever since potential hypoxic zones were first discovered in tumors in 1955. These irregular tissues affect the tumor and lead to a decrease in the healing process. Brain tumors far from blood vessels have different physical and chemical properties compared to brain tumors in well-oxygenated conditions [15]. A deeper understanding of these properties will allow us to use key components to develop hypoxia-sensitive nanotherapeutic agents, thereby improving symptoms and outcomes in hypoxic tumors (Fig. 10.1) [16].

2.1.1 Distance from blood vessels

In several areas with inadequate oxygen caused by vessel inefficiency, various branches, types, and sizes are arranged within the blood vessels. Hypoxic regions in the body are known as hypoxic zones, and they harbor tumor cells known as hypoxic tumor cells [17]. When the blood vessels are farther from the brain, the oxygen level decreases(5 mmHg). The lack of blood vessels disrupts the supply of oxygen to the tumor environment and also prevents drugs from accumulating in this area. Consequently, this phenomenon impacts the achievement of therapeutic goals (radiation therapy and photodynamic therapy) [18–21].

2.1.2 Elevated oxidation-reduction potential (strong oxidizing ability)

Another important consequence of the hypoxic tumor area may be the high redox potential. In order to survive without sufficient oxygen and supplementation, hypoxic tumors produce reactive oxygen

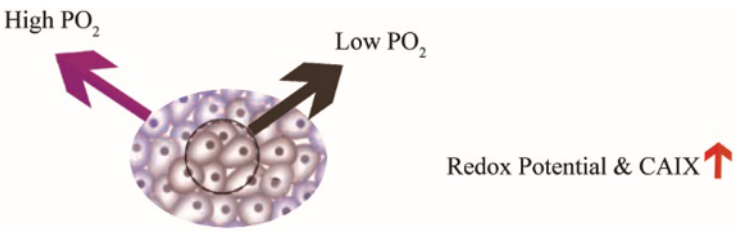


FIGURE 10.1

Schematic showing the characteristics of tumor tissue under low oxygen levels.

species (ROS), which act as free radicals and are important components of the electron transport chain (ETC) [22]. Increased ROS levels may be responsible for maintaining HIF-1 stability. To counteract unsafe ROS levels and maintain an altered redox state in the body, cells adjust their antioxidant activity to normoxic conditions. This includes supporting the activity of reductase functions such as NTR, azoreductase, and NQO1, as well as increasing the level of antioxidant atoms such as cysteine and glutathione [23–26]. The formation of hypoxia-induced chemical bonds involving reductase proteins like nitroimidazole and azo is influenced by key elements [27].

2.1.3 Acidic

When cancer cells experience a lack of blood flow, it can lead to reduced levels of glucose and oxygen in areas deficient in oxygen. This situation diminishes anaerobic glycolysis in the tumor cells, which in turn decreases the production of ATP and essential components required for cell growth [28]. Additionally, it results in the accumulation of high levels of carbon dioxide, lactic acid and protons as the tumor progresses. In order to anticipate acidosis, cancer cells control particle transport through cell membranes and the vital chemicals to expel acidic byproducts [29]. Due to a destitute blood supply, the end of metabolic waste such as lactic acid and carbonic acid is prevented, leading to a low pH of 6.2–7.2 in hypoxic tumor zones. As hypoxia compounds, tissue corrosiveness moreover rises [30]. Additionally, the move of the tumor microenvironment to an acidic pH level permits the breakdown of the extracellular lattice, empowering hypoxic tumor cells to emigrate and attack [31].

2.2 Biological features

2.2.1 HIF-1α

To survive in a challenging environment, hypoxic tumor cells modify specific intrinsic factors, such as carbonic anhydrase IX (CA IX), concentrations of HIF-1, and various other proteins. These alterations result in tumors developing resistance to chemotherapy, photodynamic therapy (PDT), and radiation therapy, causing significant local and clinical impacts in different tumor types.

Thus, gaining a deeper insight into the features of hypoxic tumors is expected to aid in the creation of biomarkers for nanotherapeutics [32]. HIF is possibly a DNA-binding protein containing HIF-1α and HIF-1β subunits that are crucial in regulating the response to oxygen levels. Semenza's 1999 study showed that HIF-1 is recruited to oxygen and affects the movement of HIF-1. Research in tumor tests has demonstrated that HIF-1 is elevated in various cancer types, such as colon, breast, and colorectal tumor [33]. Previous studies have shown that cobalt and oxygen can regulate HIF-1 degradation via hydroxylation of Pro-564. High levels of HIF-1 are associated with resistance to treatments such as PDT and RT due to tumor hypoxia, metastasis, invasion, and oxygen demand. Direct HIF-1 treatment is necessary to overcome the immune response caused by tumor hypoxia [34]. Semenza's group is of the opinion that breast cancer resistance could be overcome by blocking HIF-1 with either digoxin or acriflavine [35].

2.2.2 CA IX

CA IX, part of the carbonic anhydrase complex, is a transmembrane zinc metalloenzyme that regulates intracellular pH by converting carbon dioxide and water into bicarbonate and protons. This important factor allows tumor cells to thrive in tumors without blood vessels [36]. At the same time, monocarboxylate transporters release bicarbonate and protons from cells to lower tumor pH, contributing to

chemoresistance and metastasis. CA IX abundance is associated with hypoxia and HIF-1 regulation in various cancers [37]. Interestingly, CA IX is often found in healthy tissue of the intestinal tract, making it a specific marker for hypoxic tumor cells during the development of oncology theranostics. Utilizing the hypoxic characteristics of tumor cells has led to the development of ligand-targeted drugs, various small molecule inhibitors, and antibody-drug conjugates to enhance targeted therapy [38].

3. Hypoxia-responsive strategies

Although it is difficult to treat tumors in a deeply hypoxic environment, the characteristics of hypoxic cells offer the possibility of developing smart nanotherapeutic agents. Recently, many researchers have realized the negative effects of hypoxia on various cancer treatments, including chemotherapy, radiation, and photodynamic therapy [34]. Numerous strategies have been created to address these issues and leverage the traits of tumor hypoxia to enhance the chances of symptoms and current treatment [40]. The flexibility of nanomaterials allows image manipulation to be completed in a single step. Combining nanomaterials with hypoxia-inducing technology may help develop specific treatment plans targeting large tumors (Fig. 10.2). Another chapter describes how to create hypoxia-sensitive

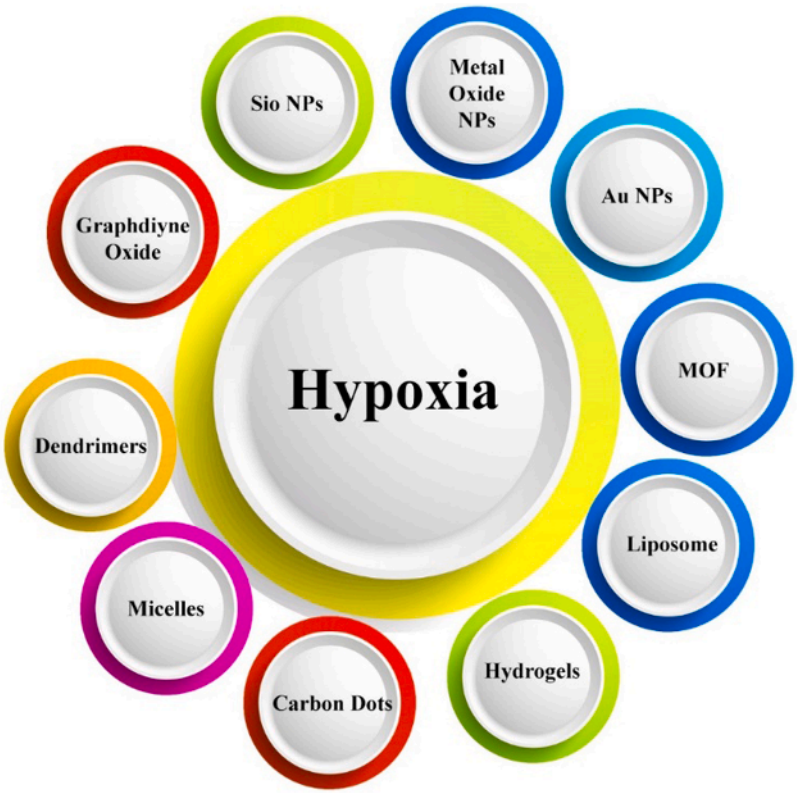


FIGURE 10.2

Demonstration of the principles for creating hypoxia-responsive nanotheranostic agents.

nanotherapeutic manipulators using three methods: specific chemicals activated by hypoxia, elevated oxygen levels activated by hypoxia, and hypoxia biomarkers [41–44].

3.1 Oxygen-sensitive chemical bonds

Recently, there have been important improvements in creating chemical bonds that are selective to hypoxia and can be activated in low-oxygen environments [45]. These bonds, which include N-oxide, quinone, azo, and nitro, undergo structural and characteristic changes in the absence of oxygen, resulting in increased hydrophobicity and enhanced electron affinity. The diverse responses of these chemical bonds to low oxygen levels provide nanomaterials with a variety of functions that improve their effectiveness in treatment and diagnosis [46–51]. In Table 10.1, the key points about nanotheranostic agents have been compiled.

3.1.1 Nitro

Unitroimidazoles in the 1970s enhanced radiosensitivity in hypoxic cells. Misonidazole became a marker for hypoxic tumors in 1981. The nitro group is converted to radicals by nicotinamide adenine dinucleotide phosphate (NTR) [49–51].

This particular pathway is less favorable in normoxic conditions, as molecular oxygen readily accepts electrons generated in the initial step, resulting in the formation of superoxide anions ($O2\bullet-$). This reduction mechanism enables these compounds to preferentially accumulate in hypoxic regions. Nitroimidazoles, nitrofurans, and nitrobenzenes are used to diagnose and treat hypoxic tumor cells [36].

The properties of the molecule can be altered by converting the nitro group into an amino group. This process results in Förster resonance energy transfer (FRET) [37].

Li et al. developed fluorescent probes under low-oxygen conditions, activating nitrofuran by releasing an electron-attracting group. Probe Cy7-1 showed quick response, sensitivity, and selectivity for NTR detection [38–40].

Theoretical evaluations demonstrated the critical role of hydrogen bond alignment in the development of hypoxia probes. Fluorescence imaging of A549 cells and a hypoxic mouse tumor model confirmed Cy7-1's strong response in low-oxygen environments. Nitroimidazole has held important biomedical roles since the 1970s [41].

Shi et al. demonstrated how the nitro group of nitroimidazole captures an electron under low oxygen conditions, resulting in the formation of a nitro radical anion. This anion then transforms into hydroxylamine, which interacts with thiol groups to create stable adducts [42]. In cells that contain thiol groups, proteins assist in retaining nitroimidazoles within hypoxic tumors. Hypoxic environments enhance the effectiveness of nitroimidazoles [43–45].

Additionally, incorporating a 2-nitroimidazole segment improved the uptake of macromolecule/gold gadolinium hybrid nanomaterials in hypoxic cells, leading to better CT/MR imaging and radiotherapy outcomes (Fig. 10.3) [46–48].

Tiny hybrid nanoparticles reach hypoxic regions due to their size, and the modification with 2-nitroimidazole aids in macrophage attachment, leading to enhanced cellular uptake [49].

Filippi et al. used metronidazole to modify dendrimers for the encapsulation of iron oxide nanoparticles. This method facilitates accurate tumor cell labeling and strengthens the signal. Moreover, replacing the nitro group with an amino group changes the hydrophobic characteristics [50].

Table 10.1 Nanotheranostic agents that activate chemical bonds specifically in low-oxygen environments.

Sample	Conclusion	References
AQ4N–64Cu-hCe6- liposome	Testing 95 nm on 4T1 cells.	[8]
c(RGDfK)- ppGO/VP-AQ4NsiHIF-1α	Tested a 91.3 nm reagent on HUVEC and PC-3 cells.	[12]
HAS-GOx-Fe3+-TA (HGTFT)	A 24.8 nm test in female mice demonstrated effective tumor growth inhibition using chemotherapy and chemodynamic therapy, along with imaging.	[17]
Lip/Ce6/TPZ NPs	An agent with a concentration of 162 $\pm$ 4 nm was tested on MCF-7 cells in mice. Optical imaging was used for the therapy.	[22]
TPZ/UCSs	Approximately 50 nm in size, tested on CT26 cells in mice.	[45]
Mn-APPMSF	Size is approximately 112.7 $\pm$ 8.3 nm, tested on HepG2 cells.	[11]
TENAB NPs	A 94 nm agent was evaluated on HeLa cells for chemotherapy.	[27]
YS-DMONs-AQ4N-GOx	An optical imaging technique that inhibits tumor growth.	[7]
UiO-66-H-P NMOFs	A 75 nm agent treats U87MG cells.	[12]
HA@AQ4N–Cu (II)-gossypol NPs	Male and female mice were tested with a chemotherapy dose of 88.7 nm.	[29]
HC/PN/DOX NPs	Research involved testing nanoparticles on mice for treatment.	[30]
PEG-Azo-PEI-DOPE	Tested 100 nm particles in cells and mice. Targeted chemotherapy for hypoxia and tumors	[32]
Pep/BDP-NO2@Lip	The 133 nm imaging agent detects myocardial hypoxia in mice.	[15]
Gd–Au DENPs-Nit	141.4 nm was tested on CNE-1 and NIH3T3 cells in mice, using CT, MRI, and optical imaging for radiotherapy.	[3]
DOX/CP-NI NPs	The 118 nm agent evaluated on HeLa cells in nude mice employs optical imaging for chemotherapy and photodynamic therapy, thereby reducing hypoxia.	[19]
NCs/DOX + Ce6 micelles	Testing nanoparticles (138.5 $\pm$ 3.6 nm) on mouse cells.	[37]
DOX@HMs	Tested a 55 nm agent on mice for chemoradiotherapy	[40]
DOX@NP	Tested on male BALB/c mice with HeLa cells, a concentration of 106.4 $\pm$ 2.8 nm inhibits tumor growth.	[42]
ALP-(MIs)n/DOX	The agent evaluated at 100 nm demonstrates efficacy for glioma therapy.	[28]
HRNP/siRNA	Optical imaging was used to assess chemotherapy in MCF-7.	[13]
mPEG-AzoPAsp-IM micelles	The agent was evaluated for photodynamic therapy on LLC cells.	[49]
DOX@AMOFs@ DRHC/CPPs	Around 200 nm nanoparticles were tested on MCF-7 cells in mice for chemotherapy.	[35]

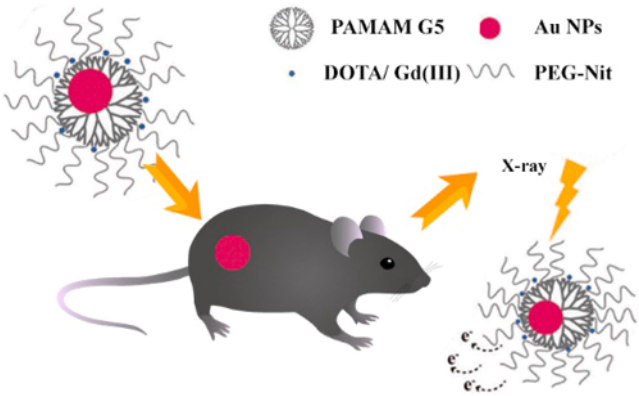


FIGURE 10.3

Demonstration of how Gd–Au DENPs-Nit can be utilized for both CT/MR imaging and enhanced radiation therapy of tumors.

This research highlights the important role of hypoxia-sensitive drug delivery using amphiphilic polymers. Gu et al. innovatively connected CP to 2-nitroimidazole (Fig. 10.4) [51].

Photodynamic therapy (PDT) utilizes a near-infrared (NIR) laser to transform three molecules of oxygen (3O2) into one molecule of singlet oxygen (1O2), generating hypoxic areas. These hypoxic regions enable the conversion of 2-nitroimidazole into 2-aminoimidazole, aided by NADPH and NTR enzymes [52–55].

Consequently, the DOX/CP-NI nanoparticles (NPs) disintegrate (as shown in Fig. 10.5b), resulting in a rapid release of doxorubicin (DOX) (illustrated in Fig. 10.5c). This strategic approach enhances the fluorescence imaging of tumors (depicted in Fig. 10.5e), while the combination of PDT and chemotherapy yields improved treatment outcomes (demonstrated in Fig. 10.5d) [56].

Zhao and his team [36] synthesized mPEG-P(Asp-NI) by attaching 2-nitroimidazole moieties to the side chain of methoxy poly(ethylene glycol)-co-poly(aspartic acid) [57–59].

The polymer, DOX, and Ce6 were combined to create a nanotheranostic agent. 2-nitroimidazole converts NADPH to NADP+ and depletes GSH, thereby enhancing the effects of PDT [60].

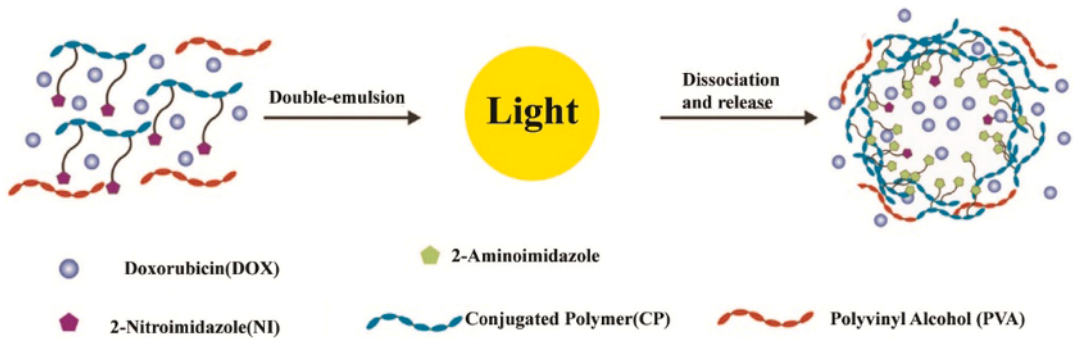


FIGURE 10.4

Creation and process of DOX/CP-NI nanoparticles in light-triggered, hypoxia-responsive drug delivery systems.

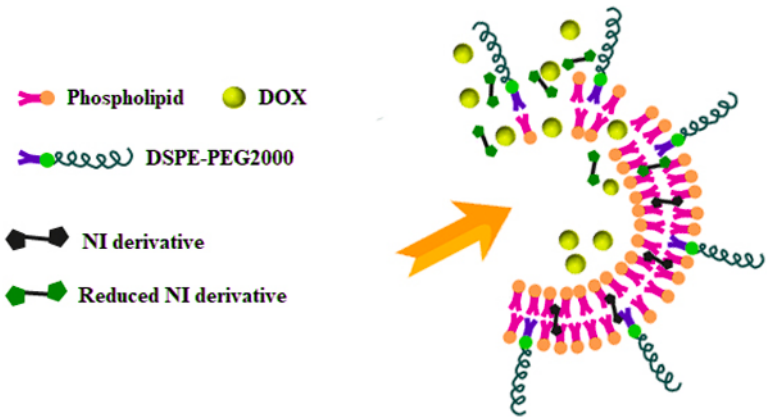


FIGURE 10.5

Demonstrating TaOx@PFC-PEG as a method to enhance radiotherapy using an oxygen carrier.

3.1.2 Azo

Azo compounds are used to detect low oxygen in hypoxic areas via azoreductases, converting them to amines [61].

A notable example is azobenzene, commonly used in the design of hypoxia-sensitive nanotheranostic agents. One such agent is PAPD, a nanocarrier developed by Torchilin's team in 2014 for targeted siRNA delivery to hypoxic tumor cells, enhancing cellular uptake [62].

Liu and his team created a nanocarrier that integrates photodynamic therapy (PDT) and chemotherapy by employing azo and cyclodextrin polymers [63].

In hypoxic tumor cells, the azo groups within the nanoparticles are broken down by azoreductase, which leads to the release of chemotherapeutic agents as oxygen is consumed during PDT.

Similarly, Yu and his colleagues introduced an innovative dual-target drug delivery strategy that tackles two challenges simultaneously with their new approach [64].

Ce6 was modified using an amphiphilic polymer featuring PCL and PEG via azobenzene linkage. This nanocarrier encapsulated DOX for a synergistic therapeutic approach. The system generated oxygen deprivation in tumor cells during photodynamic therapy (PDT), facilitating the release of nanoparticles for DOX delivery. It proved effective in hypoxic environments, demonstrating significant accumulation within tumors [65].

Yang and his team developed azoreductase-sensitive metal-organic frameworks (AMOFs) intended for nanotheranostic applications. These AMOFs release siRNA and chemotherapy agents in hypoxic tumor cells by reducing 4,4′-azobisbenzoinc acid via azoreductase, which in turn inhibits HIF-1α expression and improves the effectiveness of the drugs [66]. Meanwhile, Tan and collaborators added hypoxia-responsive azobenzene moieties to reduce off-target effects of aptamer and antibody therapies in healthy tissues, enabling effective recognition of tumor cells in low-oxygen conditions. Kim et al. improved drug delivery by utilizing mesoporous silica nanoparticles featuring customizable surfaces composed of 4-PA and β-CD. This innovation enhanced the precision of controlled drug release and increased selective cytotoxicity in in vitro studies [67–70].

3.1.3 N-oxide

N-oxide compounds are the most frequently employed class of prodrugs activated by low oxygen conditions. Two extensively researched drugs in this category are TPZ and AQ4N. The former is an N-oxide derivative known for its potent aroma, while the latter originates from aliphatic compounds as an N-oxide. Both drugs demonstrate heightened toxicity in hypoxic tumor cells compared to those in normoxic environments [1–3,71]. Table 10.1 outlines key findings related to the application of these two hypoxia-activated prodrugs [4].

TPZ induces cell death in cancer cells under low oxygen conditions by causing DNA damage through free radicals [5].

Unique active components present in tumor hypoxia can improve the effectiveness of TPZ treatment. The HGTFT nanoreactor modifies the tumor microenvironment by utilizing catalytic drug therapy (CDT) alongside the Fenton reaction. The absence of oxygen from glucose facilitates the conversion of the TPZ prodrug into an active form for chemotherapy. Glucose oxidase (GOx) aids in the rapid release of Fe3+, which generates hydroxyl radicals. Nanotheranostic agents exhibit anticancer properties [6–9].

Chen and team developed a method to increase tumor hypoxia using vascular disruption agents, leading to tumor shrinkage and prevention of spread. Li and team created a nanotheranostic platform combining TPZ and PDT for enhanced therapy [10].

In vitro and in vivo tests demonstrate effective treatment. TPZ/UCSs enhance the infiltration of T cells into tumors. AQ4N targets hypoxic cells to inhibit DNA processes [11–13].

Liu and his colleagues created liposome nanoparticles containing AQ4N and 64Cu-hCe6, triggering anticancer effects through laser-induced hypoxia. Yu's team developed organosilica nanoparticles for enhanced delivery of AQ4N and GOx, increasing hypoxia to induce apoptosis in tumor cells [14].

Wu's team created an AQ4N–Cu(II)-gossypol nanoplatform with hyaluronic acid (HA) for chemotherapy and tracking [15].

The nanoplatform appears to have no fluorescence when AQ4N is enclosed, but displays intense fluorescence when released in acidic environments [16]. These techniques improve the efficacy of treatment and imaging in both laboratory and live animal investigations. The new method provides significant potential for cancer treatment by allowing high drug capacity and effective drug distribution [17–19].

3.1.4 Quinone

Research is being conducted on the electronic and chemical properties of quinones and their derivatives, with an emphasis on creating treatments for cancer. The distinct redox capability of quinones enables the production of semiquinones or hydroquinones by undergoing reduction reactions [20]. Nishimoto and colleagues developed a 19F NMR detector for analyzing the decrease of indolequinones by NQO1 in oxygen-deprived cancer cells. During the research, a fresh signal emerges at −73.6 ppm on IQ-F when NADPH is present and in hypoxic environments. This IQ-F active probe exhibits potential in identifying hypoxic tumor cells using MR technology. A new drug was created by merging indolequinone and 5-fluorodeoxyuridine to specifically attack hypoxic cells without harming healthy cells [21–24]. Quinones provide a novel method for developing nanomaterials that can sense hypoxia, as shown by a polymer carrier releasing paclitaxel. Utilizing quinones and their derivatives

for creating hypoxia-responsive nanotheranostic agents has untapped potential, pointing toward areas for future research [25].

3.2 Increased oxygen levels are triggered by hypoxia

3.2.1 Hypoxia-triggered oxygen release

Studies concentrate on providing oxygen to cancerous tissues, utilizing hyperbaric oxygen therapy for more than 100 years to enhance delivery. Research is being conducted on the effects of reduced oxygen levels on the results of radiotherapy [26]. Hyperbaric oxygen (HBO) treatment offers advantages, but its effectiveness is restricted by complications such as barotrauma. Nanomaterials provide a more effective method for delivering oxygen to tumors suffering from severe hypoxia. Perfluorocarbon, recognized for its capability to absorb oxygen, is utilized in creating nanomaterials that transport oxygen. Liu and colleagues paired a radiosensitizer (TaOx) with PFC droplets and PEG-modified nanoparticles to improve delivery (Fig. 10.5) [27–31].

In live experiments, a technique was employed to increase oxygen levels in tumor cells, enhancing the efficacy of radiation therapy [72–74].

Hemoglobin, primarily located in red blood cells, is essential for transporting oxygen because it can carry oxygen efficiently [75].

Zhang and his colleagues developed a nanoplatform containing methylene blue, hemoglobin, polydopamine, and vesicles derived from red blood cells to enhance photodynamic therapy in low-oxygen environments. This approach effectively reduces hypoxia and improves the outcomes of PDT [76–79].

3.2.2 Oxygen generation is triggered by low oxygen levels (hypoxia)

A key strategy to improve the effectiveness of photodynamic therapy (PDT) and radiotherapy (RT) in tumor tissues involves generating oxygen within the hypoxic microenvironment. By leveraging the distinct properties of low-oxygen conditions, nanomaterials can facilitate the production of O_2 in tumor areas by catalyzing hydrogen peroxide (H_2O_2), driven by factors such as acidic pH and elevated redox potential [80–83]. This method can be divided into two categories: utilizing the naturally high concentrations of H_2O_2 present in oxygen-deficient tumor tissues, and employing transport molecules to produce H_2O_2 directly at the tumor site [84].

Metal nanoparticles are utilized in imaging, catalysis, and therapy due to their distinctive properties and diminutive size [85].

Metal nanoparticles inside oxygen-deprived tumors play a key role in converting hydrogen peroxide to oxygen, water, or radicals, particularly in acidic environments. Advancements in Mn-based nanoformulations and other nanomaterials improve Fenton-like reactions when combined with manganese-containing theranostic agents [86]. Different designs of manganese nanomaterials utilize its catalytic properties to target tumor hypoxia. Wu et al. synthesized A-MnO_2 by combining polymer and albumin, resulting in a 45% boost in tumor oxygenation and raising pH levels from 6.7 to 7.4 [87]. The combination of A-MnO_2 and radiation therapy decreased levels of HIF-1α and VEGF, and also increased DNA double-strand breaks, indicating potential in treating tumors. Hybrid MnO_2 nanoparticles enhanced the targeting of tumors as well [88].

Researchers have created a range of multifunctional nanoparticles, including MCOPP NE, HMCP, Ce6-MnO_2/CNTs, AMnO_2 nanosheets, Mn-CD assemblies, MOF nanosensitizers, and MnO_2@PtCo

nanoflowers, aimed at improving the effectiveness of photodynamic therapy (PDT) by producing oxygen from endogenous hydrogen peroxide (H_2O_2) [89]. These nanoparticles also function as imaging contrast agents, facilitating accurate monitoring throughout the treatment process. Furthermore, nanostructures like Fe-TPB nanorice have been engineered to catalyze the breakdown of H_2O_2 into oxygen and singlet oxygen, thereby addressing the issues associated with tumor hypoxia during PDT [90]. The synergistic interaction of these nanomaterials with PDT and other therapeutic modalities has exhibited encouraging outcomes in tumor inhibition and enhanced cancer treatment success [91].

Additionally, manganese ferrite nanoparticles have been integrated with mesoporous silica and Ce6 to formulate effective Fenton catalysts suitable for photodynamic therapy. These nanoparticles accumulate within tumors, thereby boosting treatment effectiveness while providing strong MRI signals for imaging purposes [92]. Catalase has been encapsulated within PLGA nanoparticles in conjunction with methylene blue (MB) and BHQ-3 to develop oxygen-releasing PDT agents specifically targeting tumor cells that express certain integrins. Hybrid nanoparticles that combine gold nanoparticles with doxorubicin have demonstrated therapeutic effectiveness by catalyzing the decomposition of hydrogen peroxide and subsequently releasing the drug in oxygen-deficient tumor environments [93].

Moreover, nanomaterials that can autonomously generate H_2O_2 have been developed to combat tumor hypoxia, showing promising results in impeding tumor progression. Another notable advancement is the POP-Gel hydrogel, which provides a continuous release of oxygen due to the incorporation of catalase, thereby contributing to effective phototherapy [94].

Hydrogel reduces hypoxia for 5 days, decreases levels of HIF-1α and VEGF, and inhibits tumor growth and metastasis [95].

An innovative approach by Zhang et al. introduced CaO_2@DOX@ZIF-67, which utilizes per oxygen compounds in acidic settings for a combination of chemotherapy and chemodynamic therapy. In acidic conditions, ZIF-67 disintegrates to release CaO_2, which reacts with water to generate H_2O_2 and oxygen [96].

The rise in oxygen levels helps to improve the effectiveness of doxorubicin (DOX) in tumor cells that are low in oxygen by reducing HIF-1α levels. Additionally, the cobalt ions that are released assist in transforming H_2O_2 into harmful hydroxyl radicals, which boosts the effectiveness of chemodynamic therapy [97].

4. Hypoxia-targeting biomarkers

CA IX, an essential hypoxia indicator found in abundance on tumor cells, controls acidic tumor conditions and is used in nanotheranostic tools. Sulfadiazine blocks CA IX and is utilized in nanomaterials that target hypoxia, such as NMOFs@BSA/SDs@MnO_2, to improve active targeting and MRI signals [98]. A hydrogel made from peptides containing a CA IX inhibitor improves the effectiveness of DOX treatment by destroying acidic vesicles and impeding endocytosis [99]. The combination of DOX with CA IX nanofibers enhances the effectiveness of cancer treatment both in laboratory studies and in living organisms. Kim and colleagues connect BODIPY with acetazolamide, increasing absorption by cancer cells. Zr-MOFs containing 1,4-benzenedicarboxylic acid bind to CA IX, releasing the inhibitor in acidic environments, leading to decreased HIF-1α levels and improving outcomes of radiation therapy [100]. Nanocomposites containing antiHIF-1α antibodies target cancer

cells that have high levels of HIF-1α for destruction. HIF-1α, a primary focus in hypoxic cancer cells, presents novel opportunities for hypoxia therapy. Novel strategies for addressing hypoxia in therapy can be developed using minor inhibitors targeting HIF-1α [101]. This new approach, which combines inhibitors with nanomaterials, has the potential to improve cancer treatment results and may transform photodynamic therapy [102].

5. Components triggered by hypoxia and their degradation processes

Hypoxia-responsive compounds such as azo linkers, nitrobenzyl alcohols, and nitroimidazoles are essential for the development of prodrugs, fluorescent indicators, and nanosystems due to their electron-accepting functional groups [103].

Nitrobenzyl alcohols have been utilized to develop therapies and fluorescent probes for hypoxic conditions, but their fluorescence diminishes before reaching hypoxic sites due to electron transfer. Azo linkages can respond to hypoxia by facilitating electron transfer [104].

Incorporating azo linkages into fluorophores and polymers creates imaging agents and nanocarriers responsive to hypoxic conditions. Fluorescent dyes with azo groups are initially quenched but can be restored in low-oxygen environments by azoreductases, regenerating fluorescence. Azoreductases can also disassemble micellar structures with azo-containing copolymers [105]. Phosphorescent materials using ruthenium, iridium, and copper show promise for hypoxia detection due to their sensitivity and rapid response to low-oxygen levels. Iridium complexes are particularly effective, with excellent phosphorescent properties and photostability suitable for ratiometric probing [106]. Combinations of copolymers with iridium-based compounds improve solubility and oxygen detection via enhanced phosphorescence, allowing for a clear distinction between hypoxic and normoxic environments [107].

6. The involvement of hypoxia in the advancement of cancer

Normal cells are tightly controlled, whereas cancer cells grow rapidly, creating an unpredictable tumor microenvironment in the body [108].

This setting plays a vital role in the advancement of cancer, infiltration, and the effectiveness of medications. The fast proliferation of cancer cells necessitates increased nutrients and oxygen, resulting in the formation of abnormal blood vessels and regions with low oxygen levels in the center of the tumor [109]. A high proportion of solid tumors experience hypoxia, as oxygen levels decrease considerably toward the center of the tumor. The absence of oxygen hinders both cell survival and metabolic functions. The presence of a dense extracellular matrix in pancreatic cancer causes unique difficulties for treatment, as hypoxia has a significant impact on this type of cancer. Hypoxia is present in cancer at every stage, including in small tumors, underscoring its significance in the advancement of tumors [110].

6.1 Effects of tumor hypoxia

The tumor microenvironment rapidly changes, forcing tumor cells to adapt to low oxygen levels by activating HIF-1 and undergoing genetic changes. This leads to biochemical alterations encouraging disease progression through ECM remodeling and epithelial-to-mesenchymal transition. Hypoxic

adaptation affects energy metabolism, endocytic receptor internalization, receptor recycling, and signaling pathways, inducing abnormal angiogenesis in primary tumors and fostering invasive tumor development and treatment resistance [111]. Acute hypoxia characterized by necrosis, indicates aggressive tumors with high oxygen depletion and increased production of free radicals and ROS, causing tissue damage, growth factor activation, and metastasis promotion. Additionally, hypoxia influences cancer recurrence by impacting cell processes like cell cycle regulation and resistance to apoptosis [112].

6.2 Resistance to drugs caused by a lack of oxygen

Hypoxia helps tumor cells survive treatment by promoting angiogenesis and metastasis. This leads to poorer clinical outcomes and increases the risk of cancer recurrence and mortality. Factors contributing to therapeutic resistance include difficulty delivering drugs to hypoxic tumors, low drug concentrations, and adaptive responses mediated by HIF-1α [113]. Hypoxia-related resistance involves reduced cellular senescence and gene activation, promoting tumor growth and survival. Overcoming hypoxia in tumors is crucial for improving treatment effectiveness by enhancing oxygen levels, activating prodrugs, and targeting molecular players like HIF-1α [114].

7. Nanoparticles sensitive to low oxygen levels for delivering drugs

Nanoparticles smaller than 50 nm have the ability to penetrate cells and engage with biomolecules, making them a promising option for cancer treatment [115].

However, clinical use is limited due to poor distribution and organ damage. Concentrating drugs in hypoxic tumors is difficult, despite promising lab results. Approved nanomedicines face obstacles in reaching the market [116]. Cancer heterogeneity highlights the need for targeted drug delivery responsive to low oxygen levels. The relationship between hypoxia, reoxygenation, and nanomedicine uptake challenges conventional design principles. Efforts are focused on targeting hypoxia and acidity in tumor microenvironments to release anticancer agents selectively in tumor cells while preserving healthy tissues [117].

Hypoxia impacts stimuli-responsive nanoparticles (NPs) formation and cancer therapy. Hypoxia-sensitive drugs and imaging advances drive research on hypoxia-sensitive nanocarriers for drug release in tumors [118]. Nanomedicines, typically intravenously administered, must efficiently target tumors from injection sites, regulated by tumor vasculature and oxygen levels. However, tumor reperfusion and physiological barriers hinder drug delivery to tumor cells [119]. Investigations focus on overcoming these challenges to safely reach hypoxic cancer cells. Current nanomedicines tackle the tumor microenvironment for improved chemotherapy, yet accessing anaerobic tumor regions remains difficult. Recent studies show hypoxia's influence on nanomedicine uptake and release in breast cancer cells, shedding light on potential advancements in drug delivery systems [120].

7.1 Bioreductive nanoparticles that are sensitive to hypoxia

Hypoxic and normoxic cells have distinct environments that can be leveraged for targeted drug delivery to tumors. Hypoxia-responsive nano drug delivery systems are created by attaching nitroaromatics and azo derivatives to amphiphilic copolymers, which self-assemble in low oxygen

conditions [121]. Enzymes like NTR and AzoR play a role in the rapid conversion of these groups to water-soluble amino groups under hypoxic conditions, leading to efficient drug release [122]. Self-assembled hypoxia-responsive polymer nanoparticles are thermodynamically stable and preferentially accumulate in tumor regions due to enhanced permeation and retention.

Thampi et al. conducted research on the creation of carboxymethyl dextran nanoparticles for delivering drugs to hypoxic tumors, utilizing a two-step chemical synthesis procedure. The amphiphilic conjugate formed hypoxia-responsive nanoparticles through self-assembly, demonstrating potential for targeted drug delivery [123].

Doxorubicin (DOX) was effectively encapsulated in stable nanoparticles measuring 176–192 nm, leading to selective and complete release in hypoxic conditions. This enhanced toxicity to SCC7 cells in just 12 hours compared to normoxic conditions [124]. Near-infrared imaging showed prolonged distribution in the body over 12 hours, with higher accumulation in tumor tissue than in vital organs. The DOX-NI-CMD nanoparticles targeted hypoxic tumors via the EPR effect, resulting in significant antitumor activity in mice. HR-CMD nanoparticles, introduced in 2017, incorporated a hypoxia-sensitive azo group for improved drug release under hypoxic conditions. This innovative approach showed promise for targeted drug delivery in cancer therapy [125].

The rapid and enhanced release of DOX from BHQ-CMD nanoparticles was observed under hypoxic conditions, leading to increased cytotoxicity compared to a slow release under normoxic conditions [126].

Systemic delivery in mice with tumors showed extended circulation and efficient targeting of tumors. Li et al. created HR-liposomal drug delivery systems by integrating NI derivatives into the lipid bilayer (Fig. 10.6) [127].

The NI derivative was used to synthesize spherical nanoparticles known as NI-LPs, incorporating various components. Doxorubicin was encapsulated within these liposomes, leading to rapid release triggered by hypoxia. In normoxic conditions, the liposomes remained stable with no significant changes in drug release [128].

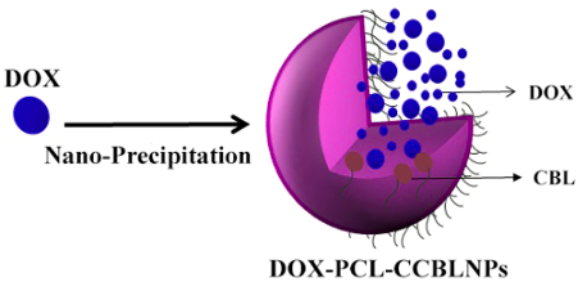
These DOX-NI-LPs demonstrated enhanced precision and treatment efficacy while minimizing overall toxicity when aimed at tumors with low oxygen levels [128].

A new amphiphilic copolymer vesicle system was developed for drug encapsulation, forming stable polymerosomes [128].

These polymerosomes have the ability to enclose hydrophilic substances in their core while also incorporating hydrophobic drugs within their bilayer. This design greatly improves the stability and release of the drugs.

FIGURE 10.6

A diagram showing how NI derivative-tagged liposomes are used for delivering DOX in response to hypoxia.



Gemcitabine and erlotinib were successfully encapsulated in the polymerosomes, targeting pancreatic cancer cells effectively [129].

The addition of PEG groups to the surface of polymerosomes enhanced their ability to target passively and increased their circulation time, resulting in improved drug release and reduced cell viability in hypoxic cancer cells [130].

Overall, this new polymerosome system shows promise for synergistic drug delivery and treatment of cancer.

Polymerosomes and mesoporous silica nanoparticles (MSNs) are utilized to target tumors for imaging and drug delivery. MSNs are effective due to their biocompatibility and ability to load drugs for controlled release [58]. DOX-loaded high-resolution MSNs with the NI-CD complex demonstrate potential for targeted drug delivery in cancer, responding to tumor microenvironments to achieve enhanced outcomes.

Lin et al. proposed a drug delivery method that uses tumor hypoxia as an internal stimulus and photoactivation as an external stimulus [131].

The system allows for controlled release of therapy using visible or NIR light excitation. A hypoxia-responsive phototrigger was developed by linking glycol chitosan and NI to act as both an electron acceptor and a hypoxic sensor. The phototrigger facilitated selective release of the anticancer drug etoposide [132]. The system formed stable nano-sized micelles that were internalized by cells and released the drug specifically in hypoxic tumor cells. This targeted delivery system was safe for healthy tissues, enabled deep tissue penetration, and achieved accurate drug release without UV light, showing promise for selective drug delivery to hypoxic tumor cells [90].

Glioblastoma is a deadly brain tumor treated with surgery, chemotherapy, and radiation therapy. Temozolomide (TMZ) crosses the blood-brain barrier to target glioblastoma cells, while metronidazole (MI) enhances radiation therapy by simulating oxygen effects. The BBB limits drug delivery, and intratumoral hypoxia reduces radiation efficacy. Zong et al. developed A2-P(MI)/TMZ nanoparticles for targeted glioma treatment, featuring a core with radiosensitizing MI and TMZ for synergistic effects with radiation [50]. These nanoparticles effectively crossed the BBB, infiltrated glioma tissue, and increased DNA damage in hypoxic tumor cells, leading to improved glioma targeting, inhibited progression, and extended survival in mice without side effects. The synergistic effects of chemotherapy and radiotherapy against glioma are enhanced by the combined use of TMZ and P(MI) [133].

7.2 Nanoparticles that respond to multiple stimuli

Stimuli-sensitive drug delivery systems can be classified into endogenous carriers using tumor microenvironment properties and exogenous vehicles relying on target site knowledge. Nanoplatforms for cancer therapy employ various stimuli like ROS, pH, and enzymes, or external factors such as NIR light, temperature, magnetic fields, and ultrasound. Exogenous systems are favored due to physiological diversity, with NIR activating nanoparticles for photothermia and tumor destruction. Ultrasound-induced hyperthermia can also trigger drug release, while AMF causes magnetic hyperthermia for targeted therapy [134]. To enhance specificity in cancer treatment, advanced multifunctional nanodrug systems have been designed using combination triggers for hypoxic tumors. These innovative approaches aim for synergistic therapeutic effects by activating additional triggers in a sequential manner for improved effectiveness [135].

8. Chemotherapy induced by hypoxia and photodynamic therapy

Photodynamic therapy (PDT) employs photosensitizers to produce reactive oxygen species, eliminating tumor cells and blood vessels. PDT induces a hypoxic environment. Qian et al. developed DOX-NI-CP NPs encapsulating doxorubicin with a ROS system and hypoxia-sensitive NI-CP coated with PVA for enhanced anticancer efficacy [136].

NI-CP, a polymer with CP and dithiophene-thienopyrazine, trapped DOX to create 118 nm nanoparticles with 20.8% efficiency. Light-induced ROS harmed membranes, leading to apoptosis and DNA damage, releasing DOX to treat tumors. Karthik's team used nanoparticles for CD44-mediated endocytosis in tumor cells, with light generating ROS for cell death and local oxygen deficiency for accurate nanoparticle release. Their polymeric system uses hypoxia and light for simultaneous delivery of DOX and chlorambucil, reducing harm to healthy tissues [137].

Karthik and team developed a hypoxia-sensitive, light-responsive polymeric drug delivery system for combining doxorubicin (DOX) and chlorambucil (CBL). The nanoparticles entered tumor cells via CD44-mediated endocytosis, and light irradiation generated reactive oxygen species, causing apoptosis [138]. Photodynamic therapy consumes oxygen, creating hypoxic conditions for more accurate drug delivery. This sequential approach successfully combined chemotherapy and PDT, minimizing damage to healthy tissues. The system is illustrated in Fig. 10.7 [139].

Amphiphilic polymer-drug conjugated nano drug delivery systems contain a photoresponsive CCBL ester core, with CBL masked by coumarin. The tail segment is PCL, synthesized through a two-step process. DOX is coencapsulated with CCBL ester via nanoprecipitation [140]. ROS generated during photo-irradiation hydrolyze the ester bond, releasing CBL and DOX. PCL unwinding aids in DOX release. The system allows hypoxia-triggered, sequential DOX and CBL release under H_2O_2 and light. The controlled release of both drugs shows enhanced anticancer effects compared to free CBL.

In general, this system offers a strategy for delivering drugs directly to oxygen-deprived cancer cells to improve cancer treatment [141].

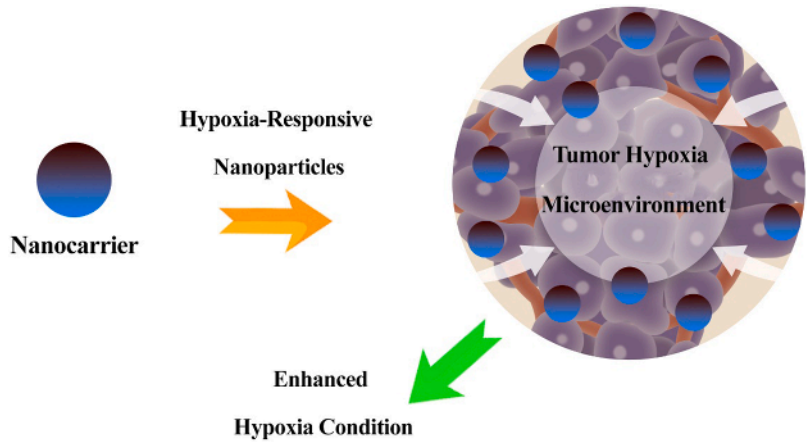


FIGURE 10.7

Synthesis of drug-loaded nanoparticles for light- and hypoxia-activated cancer therapy.

9. Nanoparticles for combined cancer treatments (photodynamic, sonodynamic and photothermal therapy)

W18O49 nanoparticles under near-infrared (NIR) light generate reactive oxygen species (ROS) and heat for enhanced photodynamic therapy (PDT) and photothermal therapy (PTT). However, challenges such as ROS depletion, oxidation, and rapid clearance can affect their effectiveness [142].

Tumor hypoxia further limits the efficacy of this therapy. To address this, MET was used to reduce tumor hypoxia by inhibiting oxygen consumption. Platelet membrane nanocarriers were then developed to coload MET and W18O49 NPs for improved delivery to tumor tissues and enhanced therapeutic effects. These biocompatible nanocarriers effectively targeted malignant cells, reducing tumor growth and increasing apoptosis through a combined PDT and PTT approach, while minimizing side effects on normal cells [143].

Zhang and colleagues developed DOX-Au-SiO2-MnO2 for treating hypoxic tumors through chemo-photothermal therapy with nanotechnology [80].

The SiO2 nanoparticles were synthesized using the Stöber method, followed by the formation of a gold nanoshell around them using reduction methods. Manganese dioxide nanosheets were then added to the shell to serve as a drug carrier and fluorescence quencher. The nanocomposite targeted tumor tissues with an aptamer modification and released doxorubicin in the presence of elevated intracellular glutathione in hypoxic conditions [144]. This approach inhibited tumor growth by overcoming hypoxia effects, utilizing chemophotothermal therapy, and various imaging modalities without toxicity to vital organs. Sonodynamic therapy was also considered for its effectiveness in treating deep tissues with minimal invasiveness [145].

Sonodynamic therapy (SDT) can trigger hypoxia, leading to p53 mutations that reduce treatment efficacy by promoting tumor resistance. Feng et al. developed a novel TPZ-SNT-HMT nanotheranostic platform using TPZ enclosed in HMT NPs with SNT modification to combat this issue. These nanoparticles localized to tumors and generated ROS under ultrasound, activating SNT to release NO and enhance SDT. High NO levels amplified the SDT effect, causing further damage to cancer cells [146]. The hypoxic environment from SDT activated TPZ, inducing DNA cleavage and specific cytotoxicity to hypoxic cells. This multi-functional nanoparticle system integrated SDT, hypoxia-activated therapy, and NO-sensitized therapy, boosting anticancer efficacy. The nanoparticles also served as ultrasound contrast agents, improving imaging without toxic effects in normal tissues. Initial trials on live subjects indicated that these nanoparticles could be suitable for medical applications [147].

10. Nano drugs

Hypoxia triggers the development of cancer stem cells (CSCs), which are crucial for cancer progression and spread. Zhang and his colleagues developed hypoxia-responsive drug-carrying nanoparticles to specifically attack and remove CSCs, cancer cells at different stages of development, and the blood vessel environment in oxygen-starved tumors [93].

These nanoparticles feature a unique azo linker that releases three drugs targeting different components of the tumor. The combination of hydrophobic combretastatin A-4 and hydrophilic irinotecan with a sedative cyclopamine can effectively inhibit the growth and migration of CSCs.

Nanoparticles efficiently stack, target hypoxic tumors, release drugs into cancerous tissues, are nontoxic to normal tissues, and inhibit cancer cell invasion, making them a promising option for cancer treatment [60].

11. Specialized nanocarriers

Nanomedicine, with its larger size compared to chemotherapy drugs, has limitations in spreading and penetrating tumors of 3–5 cells, but it is an effective delivery vehicle. Particles smaller than 10–20 nm can increase material in the body but may be quickly eliminated by the kidneys, reducing therapeutic effects [123–126]. To ensure tumor-specific transport, an ideal nanocarrier needs to be large in the body and convert to small particles in the tumor microenvironment. This shift eliminates the opportunity for extended circulation, causing a problem in tumors and increasing porosity during introduction. Nanoparticles taking advantage of oxygen content in the tumor microenvironment can deliver drugs to reverse tumor cell resistance by inhibiting hypoxia-induced cancer pathways. Polyamide amine dendrimers linked to PEG-2000 prepare a biocompatible delivery system that enhances drug penetration and inhibits tumor growth. This system also captures reactive oxygen species, potentially enhancing the effectiveness of chemotherapy [127,148,149].

Zebrafish tumors support the in vivo application of PAP for drug delivery in cancer treatment. Recyclable sedative nanocarriers are effective in drug delivery, but poor phagocytosis of nanoparticles hinders cell uptake. Wang et al. introduced a nanodrug framework with GA and Cy7 for cancer treatment. GA induces brain apoptosis and inhibits cancer progression, while Cy7 specifically targets cancer tissue. Surfactin, an amphiphilic lipopeptide, enhances nanopreparation stability [128].

GA-Cy7 units form controlled colloidal aggregates. In hypoxic conditions, Cy7 binds to cell membranes, enhancing drug uptake and offering potential benefits in cancer therapy.

In vitro studies on PC3 cells and xenograft mouse models revealed that GA-Cy7-surfactin NPs exhibited potent antiinflammatory properties in hypoxic tumor cells, affecting cell expansion, tumor growth, and angiogenesis. Compared to steady-state models, in vivo tests showed enhanced biodistribution and reabsorption of GA-Cy7-NP in tumors [129]. The use of a "mosaic-type" Cy7 interface provided a strategic model for delivering anticancer drugs to tumor cells by conjugation. Stem cells, particularly hADSCs, were explored as potential carriers due to their abundance, easy isolation, and autologous nature. Additionally, the study highlighted the potential of utilizing anaerobic bacteria in combination with nanoparticles for selective treatment of hypoxic tumors, offering promising advancements in cancer therapy [58,90,130–132].

Researchers developed center-shell-UCNR (CS-UCNR) nanoparticles for disease-enhanced bioimaging by coating UCNR and CS-UCNR with oleic acid etchant. Surface tuning was done to create hydrophilic ligand-free (LF) CS-UCNR [50,133–135].

The fluorescence of CS-UCNR improved with silica coating. Gold nanorods (Au NRs) increased the bacteria-Au NR loading capacity. Targeted therapy using pegylated Au NRs effectively removed tumors [136–138].

Anaerobic bacteria-assisted methods using nanomaterials have shown promise in cancer treatment by targeting specific antigens. Disease-induced hypoxia-specific methods are being explored for targeted cancer therapies. The research aims to find a cure for cancer through innovative approaches [139–141].

12. Limitations, future prospects and challenges

Nanocarriers encounter several limitations in their application for cancer therapy. A significant challenge is their difficulty in penetrating hypoxic tumor cores, which are located far from blood vessels and are characterized by increased efflux transporters [142,143]. Additionally, the short circulation half-life of fluorescence probes complicates real-time assessment of hypoxia levels, which is essential for selecting appropriate therapies. The oversimplification of tumor environments in preclinical models also restricts the translation of findings to actual patient scenarios. Looking ahead, there is considerable potential for advancements in the field of nanocarriers. Future research may focus on developing multifunctional drug delivery platforms that can effectively target and treat hypoxic tumors [80]. Innovations such as size-switchable nanomaterials could enhance penetration into hypoxic regions, thereby improving both imaging and therapeutic efficacy. Furthermore, exploring alternative delivery systems like liposomes and dendrimers, along with the development of self-reporter nanoplatforms for real-time feedback, could significantly enhance treatment outcomes [144]. Despite these promising prospects, several challenges remain. Drug resistance, tumor metastasis, and variations in cellular markers continue to limit the effectiveness of nanocarriers in active targeting and therapy. Additionally, the need for real-time assessment of hypoxia levels presents a challenge in selecting the most effective therapies. Ensuring the stability and biocompatibility of nanocarriers while evaluating their toxicity profiles is essential for clinical application. Overcoming these challenges will be crucial for the successful integration of nanocarriers into cancer treatment protocols [60,93,147].

13. Conclusion

Exploring low-oxygen environments within tumors presents an exciting opportunity to enhance cancer treatment and imaging through the development of advanced nanotheranostic agents. Scientists are developing intelligent nanomaterials that specifically target and treat tumors by utilizing the unique characteristics of hypoxic tissues, including their vascular distance, acidic pH levels, and elevated redox potential. These agents have the potential to improve treatment efficacy while minimizing side effects, as they are designed to release medications only in low-oxygen conditions. However, several challenges remain, including the necessity for real-time monitoring of hypoxia, improved penetration of nanomaterials into dense tumor structures, and the simplification of manufacturing processes for large-scale production. Additionally, safety concerns regarding the combination of organic and inorganic components in these agents must be addressed before they can be used in clinical settings. Future research should focus on developing biodegradable and biocompatible nanocarriers that can effectively deliver therapeutic agents to their intended sites, overcoming the limitations of traditional methods. By utilizing hypoxia as a natural trigger, these advanced nanomaterials have the potential to revolutionize cancer treatment, offering a more precise and effective approach to managing tumor diversity and improving patient outcomes. In summary, incorporating hypoxia-responsive techniques in nanomedicine holds significant promise for enhancing cancer therapy and imaging. Continued innovation in this field will be crucial for overcoming existing challenges and translating these promising technologies into viable clinical solutions.

CRediT authorship contribution statement

Dr. Mohammad Hossein Karami (First Corresponding Author): Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dr. Omid Moini Jazani (Second Corresponding Author):** Writing – review, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER

Azo-based compounds for hypoxia-responsive drug delivery

11

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1. Introduction

Cancer is one of the main causes of death worldwide, which is brought on by the uncontrolled growth and multiplication of abnormal tissues that develop into tumors. Through abnormal metabolic pathways and increased energy, these tumors develop into malignant cells. Malignant tumors spread to other areas of the body and actively attack tissues [1–3]. When cancer metastasizes, treatment options are limited. The world health organization (WHO) estimates that breast, lung, prostate, and colorectal cancers are among the most prevalent types of cancer [1]. Conventional treatments, such as surgery, radiotherapy (RT), and chemotherapy (CTx), focus on eliminating or killing cancer cells, but they often damage healthy tissues, leading to significant side effects [4,5]. Chemotherapy, despite being a common choice, often fails due to poor drug delivery and poor access to antineoplastic agents for tumors and requires high drug doses that can cause systemic toxicity [3]. In cancer treatment, all cancer cells must be destroyed, either directly or indirectly, to achieve complete recovery of the patient. Therefore, strategies for killing cancer cells without toxic effects on normal cells are needed [6]. Hence, drug delivery systems (DDS) are introduced to control the rate, time, and location of drug release in the body, thereby improving its effectiveness and safety [7]. Smart drug delivery systems (SDDS) have emerged as a promising and targeted therapeutic approach for cancer treatment [8]. These systems overcome the limitations of conventional therapies by targeting drugs to the cancer cells; thereby, they reduce toxicity and damage to healthy cells and improve treatment efficacy [8,9]. By minimizing drug entry into nontarget tissues, these systems direct optimal drug concentrations specifically to cancer sites. This approach while protecting other parts of the body, preserving the therapeutic benefits of the drug [10]. SDSS is an advanced method of drug-targeted (DT) delivery. This method emphasizes preserving the drug's properties, targeting the intended site only, and prolonging its interaction with cancer tissues in patient's body. Recent advancements in SDDS have led to the development of various drug-targeting strategies, including passive [11], active [12], dual [13] and physical targeting [14]. These systems use carriers such as liposomes, micelles, organic-inorganic hybrid smart nanoparticles, exosomes, and other nanomaterials for targeted drug delivery and controlled release [15,16]. Carriers designed for targeted drug delivery should be readily biodegradable and eliminated from the body without causing side effects [17]. The creation of nanocarriers has led to improved approaches for delivering drugs, particularly by focusing on specific targeting of

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cancer tissue and prolonged release mechanisms to improve safety and enhance cellular internalization [18]. Smart drug delivery systems can not only target specific sites on cancer cells but also respond to stimuli in the tumor microenvironment (TME), including pH [9], hypoxia, redox conditions, and enzyme activity, enhancing their efficacy [19]. One of these factors is hypoxia, characterized by low oxygen levels, frequently observed in fast-growing solid tumors, and it poses a major obstacle to cancer treatment [20]. In recent studies, various approaches have been investigated, including polymeric-based drug delivery systems, oxygen delivery systems combined with RT and CTx, and anaerobic bacteria for gene delivery. These techniques suggest that they could result in a better therapeutic approach and a lower risk of side effects in cancer treatment [21].

This chapter examines the potential of azo-based compounds as hypoxia-responsive agents in targeted drug delivery. Regarding the critical role that tumor hypoxia plays in drug resistance and treatment constraints, this study reviews the activation mechanisms, biomedical applications, and optimization strategies for azo-functionalized drug delivery systems. This strategy intends to promote precise and controlled drug release in hypoxic tumor microenvironments by overcoming the restrictions of current drug delivery systems, thereby improving therapeutic efficacy while minimizing systemic toxicity.

2. The importance of hypoxia in biological and drug delivery systems

2.1 Definition, characteristics and classification of hypoxia

Hypoxia is a condition in which the oxygen level in tissues is below physiological levels [22]. In other words, hypoxia occurs when an area of the body has insufficient oxygen to execute its normal functions, a condition that is a common feature among solid tumors and has a significant impact on cancer progression and treatment outcomes [23,24]. In healthy tissues, the partial pressure of oxygen (PO_2) generally ranges between 40–60 mmHg, while in tumor tissues, it lowers to as less as 10 mmHg. Due to abnormal metabolism and irregular vasculature in cancer tumors, the consumption of oxygen is higher in cancerous cells, which may be regarded as the main reason for this phenomenon [25,26]. The main characteristics of hypoxic tumors include enhanced tumor aggressiveness, reduced therapeutic effects, and poor prognosis of CTx, RT, photodynamic therapy (PDT), and sonodynamic therapy (SDT) [27]. According to classifications, there are three main types of cellular hypoxia in human tumors: chronic, acute, and cyclic hypoxia [25,28]. Thomlinson et al. [29] first outlined the description of chronic hypoxia in 1955. Chronic hypoxia, also known as diffusion-limited hypoxia, occurs due to overproliferation of cancer cells, which leads to increased cell density and an increase in the distance between some cells and the nearest blood vessel, thereby leaving insufficient oxygen for cells further away from the vessels. Chronic hypoxia is often associated with conditions such as lung disease, heart disease, and cancer [30–32]. In 1979, Brown [33] introduced a second form of hypoxia. Accordingly, acute hypoxia, a state of persistent oxygen deficiency for a short period [34], is caused by an aberrant shutdown of small blood vessels and irregular erythrocyte flow. This type of hypoxia is also caused by events such as asphyxiation, drowning, and ischemia [30,35]. Cyclic hypoxia is also caused by irregular blood flow and vascular remodeling in tumors [36]. In this case, the tumor microenvironment contains variable oxygen concentrations, leading to intermittent periods of hypoxia that can vary from minutes to days [37,38]. This type of hypoxia can greatly increase various cancer features and cause free radical formation, leading to tissue damage [30]. Following this, a more detailed examination of hypoxia and its tissue characteristics in various diseases will be presented.

2.2 Characteristics of hypoxic tissues in cancer, ischemia, and inflammation

As mentioned in the previous section, tissue hypoxia is caused by insufficient oxygen supply, which, in addition to compromising biological functions in the body, plays a role in many diseases, such as ischemic heart disorders, anemia, cancer, and even inflammation [39]. Hypoxia within the tumor leads to the overactivity of hypoxia-inducible factor (HIF) [40], which plays an essential role in vital aspects of cancer, such as metastasis [41], cancer cell resistance to RT and CTx [42,43], angiogenesis [44], and stem cell maintenance [45]. This transcription factor regulates a broad range of genes that tumor cells use for survival and resistance to therapy [39]. In other words, hypoxia-inducible factors (HIFs) play an important role in cellular responses to low oxygen levels (hypoxia) and the regulation of physiological and pathological processes [34]. These factors consist of two different subunits, α and β. The α segment, which is sensitive to oxygen, includes HIF-1α, HIF-2α and HIF-3α. Also, the β subunit contains HIF-1β, constitutively expressed, and forms a stable dimer with the HIF-α subunit stabilized in the nucleus [46,47]. Hypoxic conditions are evident in many pathological conditions, such as cancer, ischemia, and inflammatory diseases [34,48]. Based on studies of tumor development from initial formation to advanced metastasis, it is clear that tumor hypoxia is not only a hallmark of the tumor microenvironment but also plays a pivotal role in molecular and cellular responses to drive cancer progression [47]. Following the Warburg effect, cancer cells undergo metabolic changes under hypoxic conditions, often switching from oxidative phosphorylation to anaerobic glycolysis [49,50]. This process, stimulated by HIFs, helps cancer cells produce ATP more rapidly and supports tumor progression even under adverse conditions [51,52]. At the molecular level, hypoxia stabilizes HIF-1α and HIF-2α, which, by activating downstream genes, assist cancer cells in adapting to hypoxic stress [40,53]. The factors HIF-1α and HIF-2α have been reported in a large number of cancer types and are clinically associated with advanced stages of cancer [54]. On the other hand, hypoxic cancer cells typically do not show rapid cell division and are less sensitive to conventional chemotherapeutic drugs that target DNA replication [55]. Therefore, hypoxic microenvironments lead to limited therapeutic effects and increase tumor tissue resistance to radiotherapy [27]. For instance, hypoxia plays a significant role in tumorigenesis and progression in prostate cancer [56]. Prostate cancer is thought to be the second most common type of cancer, a prevalent disease among men [56,57]. Cancer metastasis reduces survival rates and causes neurological and motor impairment in the patient [31]. The accumulation of acidic metabolites or reactive oxygen species (ROS) is the main cause in the early stages of hypoxia in prostate cancer [58,59]. In this situation, the activation of the HIF signaling pathway, by accumulating in cells and affecting other components of the tumor microenvironment, activates multiple signaling pathways, such as PI3K/Akt/mTOR, to enhance tumor survival [60]. Additionally, HIFs, through epithelial-mesenchymal transition (EMT), cause further proliferation and spread of cancer cells and enhance androgen receptor (AR) resistance, ultimately contributing to the progression of prostate cancer [54,61]. Hence, hypoxia can act as a protective shield against various treatments, leading to genetic instability and increased tumor invasion in patients [62]. Cardiovascular diseases are another major cause of death worldwide, which can significantly damage organs, especially the brain and heart, by reducing blood flow [63–65]. The progression of this disease may be caused by sympathetic excitation disorder, oxidative stress, and hypoxia-induced lipid metabolism [66,67]. Ischemia is one of the main complications of this type of disease, which refers to the reduction or lack of blood flow to a specific body part [68]. Hypoxia occurs due to the restriction of blood flow to the brain in ischemia and, as a result, contributes to the reduction of oxygen in the neural tissue and energy

deficiency in the affected tissues [69,70]. Hypoxic-ischemic (HI) conditions lead to a lack of oxygen for cellular respiration and reduced levels of glucose and ATP, which causes neural cells to undergo anaerobic metabolism [65,71]. Reperfusion to restore oxygen can cause oxidative stress, thus leading to excessive glutamate stimulation. This process, in turn, increases intracellular calcium, intensifying damage and leading to the death of neuronal cells [72,73]. Inflammation is another aspect of the tumor microenvironment that plays a vital role in neoplastic progression [74]. Hypoxia and inflammation are interconnected at the molecular, cellular, and systemic levels [75]. In the clinical context, the interaction between hypoxia and inflammation may be associated with several metabolic diseases, cardiovascular conditions, and even chronic obstructive pulmonary disease (COPD) [76,77]. Factors such as increased cell metabolic demand, altered oxygen supply, trauma, and even atelectasis (airway plugging) can lead to tissue hypoxia [78,79]. Inflammation-induced hypoxia, in addition to altering the immune composition of tissues, can also promote the activation and survival of immune cells in a hypoxic environment [79]. The produced reactive oxygen species (ROS) are critical for the stability of HIFs. They can regulate genes related to inflammation and angiogenesis, which would otherwise damage tissues [80,81]. The interaction between the hypoxia-inducible factor and the transcription factor NF-κB, as a master regulator of inflammation, plays a vital role in modulating immune function and inflammatory signaling [82–84]. Accordingly, hypoxia can be both a consequence of inflammation and a factor that contributes to its development. Understanding these mechanisms would be essential for more effective treatment and reducing tissue damage.

2.3 The role of hypoxia in drug delivery and therapeutic challenges

Although hypoxia is implicated in cancer progression and treatment resistance [85,86], this low-oxygen environment plays a crucial role in tumor targeting and, especially, in targeted drug delivery [21]. According to researchers, the hypoxic environment has enabled the design of specific drug delivery systems [87]. Hypoxia-responsive drug delivery systems use low oxygen levels in tumors as a "trigger" for the selective and targeted release of therapeutic drugs in hypoxic tumor areas [21]. This process increases the efficacy of treatment and reduces chemotherapy-induced side effects and off-target toxicity [88]. As mentioned in the previous sections, many solid tumors show hypoxic regions due to insufficient blood supply and rapid cell proliferation. This hypoxic tumor environment may affect the distribution of drugs and their therapeutic effect [89]. Thus, the hypoxic environment in the tumor overactivates enzymes, including nitroreductase and nicotinamide adenine dinucleotide phosphate (NADPH), which can activate hypoxia-sensitive drugs [90,91]. Besides this, there is the possibility of developing hypoxia-activated prodrugs (HAPs) due to the hypoxic environment, such as quinones and nitroaromatics. These HAPs remain inactive until exposed to low-oxygen conditions and only release their cytotoxic effects, specifically on tumor cells at low oxygen levels, without harming healthy tissues [92,93]. Although prodrugs have a specific molecular structure, the therapeutic effect has not been very satisfactory due to inadequate drug concentration in tumors, toxicity at high drug doses, and short half-lives of small molecules in the circulatory system [90,94]. Scientists have developed hypoxia-responsive nanoparticles (HR-NPs) that selectively release drugs in low-oxygen environments and show improved efficacy [95]. These nanoparticles, which are sensitive to the tumor microenvironment, can prolong the circulation time of drugs and enable more effective tumor targeting [27,96]. Also, they are often modified with hypoxia-sensitive compounds, such as azo derivatives that enhance the drug cellular uptake into the tumor cells [97]. However, hypoxia-responsive

systems face significant challenges. Low-oxygen environments complicate the ability of drugs to penetrate tumors and can reduce the effectiveness of some drugs [20,98]. Poor blood flow in highly hypoxic areas can limit drug delivery to these areas [99]. In addition to these issues, hypoxia can promote metastasis and make treatment more difficult by stimulating further genetic changes in cancer cells and creating a more aggressive phenotype [100,101]. Moreover, since hypoxia levels can vary in different areas of a tumor, uniform drug activation is challenging and complicates treatment efficacy [97,102]. Overall, the use of hypoxia as a stimulus for targeted and controlled drug delivery could be a valuable tool in therapy. In the following sections, the types of hypoxia-responsive drug delivery systems will be reviewed in more detail.

2.4 Advantages of using azo-based compounds for targeted hypoxia therapies

Azo-based compounds are distinguished by the azo functional group (-N═N-) and exhibit considerable promise in a variety of biomedical fields owing to their unique structural and functional properties [103–105]. These compounds play an essential role in hypoxia-activated bio-reductive systems. Under hypoxic conditions, enzymes such as azoreductases are upregulated and facilitate the reduction and cleavage of the azo bond [106,107]. The reduction not only specifically activates the drug in hypoxic regions but also avoids off-target effects [108]. Such cleavage transforms azo groups to amine derivatives that enhances the hydrophilicity of drug molecules [87,109]. In other words, azo-based compounds can undergo reductive cleavage in response to low-oxygen conditions and be selectively broken down in the hypoxic environment of tumors, thereby facilitating targeted drug release [110,111].

For example, PEG-Azo-PEI-DOPE (PAPD) nanoparticles modified with azobenzene can shed their polyethylene glycol (PEG) layer under hypoxic conditions. In addition to increasing cellular uptake in cancer cells, this targeted release increases drug concentration within the tumor and reduces toxicity [97,112,113]. Azo-based compounds offer significant advantages for targeting hypoxic tumor environments. Because of their sensitivity to hypoxia, these compounds are activated exclusively in low-oxygen areas, allowing for precise targeting. This characteristic reduces side effects and enhances drug efficacy compared with conventional treatments. Furthermore, these compounds demonstrate theranostic potential, showing therapeutic actions such as simultaneous drug release and fluorescence imaging for diagnostics. This capability allows clinicians to monitor drug release in real-time [111,114,115]. For example, azo-iridium (III) complexes have been developed as phosphorescent probes for detecting hypoxia, allowing imaging of tumor regions with low-oxygen levels [116]. Azo compounds are also used in antiinflammatory, anticancer, and antibiotic treatments due to their ability to act as prodrugs that become activated under specific physiological conditions [104].

3. Properties and chemistry of azo compounds

3.1 Physicochemical properties of azo compounds and response to stimuli

Azo compounds are organic molecules that connect aromatic (aryl) and aliphatic (alkyl) groups [117–119]. Aromatic azo compounds are stable, with bright colors, and are often used as industrial dyes in the textile, food, and other industries. On the other hand, aliphatic compounds are mostly colorless and less stable. These compounds are commonly used as radical initiators [104,120]. Azo

compounds have attracted much attention because of their color-changing ability from conjugated systems that can undergo trans-cis isomerization upon light irradiation [106,121]. The specific structure of azo-bond compounds endows them with remarkable physicochemical properties in their response to various stimuli, making them highly valuable for developing targeted materials [122]. Photosensitivity is one of the stimulants that can cause an isomerization between cis and trans states when exposed to certain wavelengths of light. This process usually occurs under ultraviolet (UV) or visible light and causes significant changes in the dipole moment and molecular geometry of the compound. Under light or heat, this reversible isomerization makes azo compounds valuable for developing photosensitizing materials. This feature plays a vital role in phototherapies, molecular switches, and drug delivery systems [123–125]. As an example, according to the study by Yagai et al. [126], azobenzene-based liposomal systems utilize the photoisomerization of azobenzene to trigger the release of encapsulated drugs upon irradiation with UV light. Azo compounds can change their structure and absorption in response to pH, making them useful for pH-sensitive drug delivery systems, where drug release is triggered by changes in acidity or alkalinity [117,127–129]. In addition, some azo compounds in the body can be reduced by enzymes such as azoreductases. This process makes them suitable for targeted drug delivery to the colon, where they are degraded only in the anaerobic environment of the colon [109,130]. For example, drugs enclosed in azo polymer hydrogels are safeguarded against enzymatic degradation in the stomach and small intestine. This results in a potential therapeutic approach for localized disorders within the colon [131]. These properties can be tuned by adding various substituent groups. Electron-donating groups in the aromatic ring can influence the absorption spectrum and reactivity of azo compounds, optimizing them for specific applications [132]. Overall, azo compounds play an important role in various fields, especially in targeted therapies and imaging in medical sciences.

3.2 Synthesis of functional azo for drug delivery

Various methods are available for synthesizing functional azo compounds and derivatives, each with its own advantages and limitations. The most prominent methods include diazotization/azo coupling, the Mills reaction, electrochemical synthesis, solvent-free synthesis, and photocatalytic synthesis. Table 11.1 provides a detailed comparison of these techniques, highlighting their advantages and disadvantages.

Functional azo compounds for drug delivery are synthesized by incorporating drug molecules or responsive groups into the azo structure. These compounds are designed to remain stable and selectively degraded in target areas under physiological conditions. In other words, functional azo compounds, with their specific properties, can act as drug carriers and facilitate localized drug targeting [118,145]. Below are some examples of studies conducted on the synthesis of functionalized azos for drug delivery. For instance, azo linkages can be incorporated into metal-organic frameworks (MOFs) to act as scaffolds in drug delivery and catalysis. Azo-MOF compounds are synthesized by coordinating metal ions with azo-functional ligands, creating a porous structure with tunable properties [118,146]. Meng et al. [147] synthesized a zirconium-based MOF with an azobenzene moiety called UiO-68-azo. This compound can act as a cargo reservoir in water, with pores sealed by β-Cyclodextrin (β-CD), which attaches to azobenzene stalks on the surface of the MOF. This compound, with favorable biocompatibility and high stability in water, has been introduced as a promising platform for drug delivery and controlled drug release [147]. Fig. 11.1 shows a schematic of this process.

Table 11.1 Overview of functional azo synthesis techniques.

Method	Description	Advantages	Disadvantages	Ref.
Diazotization/Azo coupling	Conversion of an amine (aromatic or aliphatic) into a diazonium salt with nitrous acid (HNO_2), followed by reaction with a nucleophile to form an azo bond	High efficiency, wide substrate scope, diverse azo derivatives production with various colors, industrial use	Limited substrate range, temperature sensitivity, risk of explosion	[129,133–137]
Mills reaction	Reaction of nitroso compounds (–NO) with aniline derivatives in acidic conditions, producing azo compounds after dehydration	High selectivity, symmetric/asymmetric products, useful in dyes and materials science	By-products formation, limited substrate compatibility, reagent dependency	[118,138–141]
Electrochemical synthesis	Oxidation of aniline derivatives through electrodes in aqueous or organic electrolytic systems, producing azo compounds with minimal waste	Short reaction time, high efficiency, low chemical waste, precise control of the final product	Additional reagents requirement for stabilizing the reaction, electrode and substrate interface challenges, complexity for industrial scalability	[134,135,142]
Solvent-free synthesis	Grinding solid reactants with catalysts such as boron trifluoride (BF_3) or silica (SiO_2) without solvents, producing azo compounds	Ideal for green chemistry applications, environmentally friendly, cost-effective, nontoxic, diazonium stabilization	Solubility limitations, reactant compatibility issues	[143,144]
Photocatalytic synthesis	Use of light (UV or visible) and photocatalysts such as TiO_2 or Fe_2O_3 to synthesize azo compounds	Energy-efficient, minimal waste, sustainable, suitable for light-sensitive material	Limited substrate compatibility, requires specialized equipment, potentially longer reaction times	[134,139]

Other practical applications include integrating azobenzene derivatives into polymers and nanoscale materials such as dendrimers, micelles, and hydrogels, which create biodegradable matrices that provide sustained drug release [148,149]. For example, hydrogels based on methacryloyloxy azobenzene exhibit controlled swelling and targeted degradation and are used exclusively for the delivery

of drugs such as 5-fluorouracil, which selectively release the drug [150,151]. As illustrated in Fig. 11.1, Ling Chen et al. [152] synthesized Fe_3O_4-Azo-DOX nanoparticles system for targeted drug delivery. This combination aims to construct a stimulus-responsive system that releases the anticancer drug doxorubicin (DOX) upon exposure to near-infrared (NIR) light. The covalent bonding of DOX to Fe_3O_4-Azo builds up a stable drug delivery system and avoids premature drug release. Additionally, the Fe_3O_4-Azo nanoparticles have good biocompatibility in cancerous cells, causing fewer side effects within the body.

In addition to the mentioned aspects, different functionalities for drug delivery often require specific substituents or functional groups. For example, oxidative dehydrogenation is one of the approaches for synthesizing heteroaromatic azo compounds such as azopyrroles. Jiang et al. [153] demonstrated the possibility of generating azopyrrole derivatives through oxidative coupling and iodination of pyrazol-5-amine using Tert-Butyl Hydroperoxide (TBHP) as the oxidant and copper salt as the catalyst. The synthesis of azopyrroles, because of their stability and selective reactivity, is ideal for biological applications and could be crucial in the development of responsive drug delivery technologies.

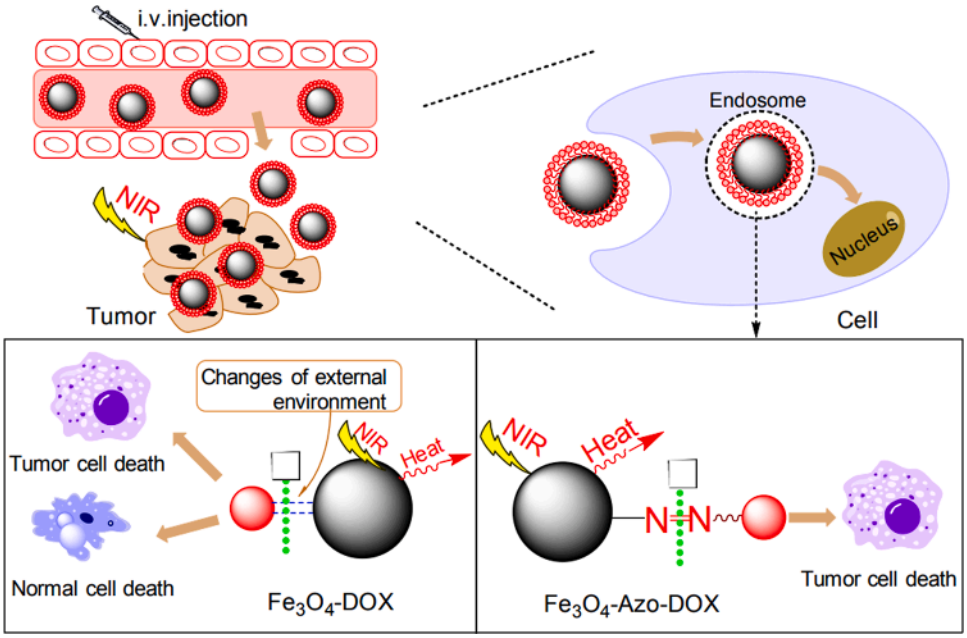


FIGURE 11.1

Schematic representation of drug release mechanisms and photothermal therapy applications of Fe_3O_4-DOX and Fe_3O_4-Azo-DOX nanoparticles under NIR irradiation.

Reproduced from L. Chen, L. Wu, F. Liu, X. Qi, Y. Ge, S. Shen, Azo-functionalized Fe3O4 nanoparticles: A near-infrared light triggered drug delivery system for combined therapy of cancer with low toxicity, J. Mater. Chem. B. *4 (21) (2016) 3660–3669.* https://doi.org/10.1039/C5TB02704G

3.3 Biocompatibility and cytotoxicity

The biocompatibility of azo compounds varies depending on the structure and the type of substituents. Aromatic azo compounds, such as azobenzene, are used in diverse industries due to characteristics such as reversible light sensitivity and the potential for degradable bonds [104,154]. A major concern in using these compounds is the production of carcinogenic aromatic amines, which can pose health risks [155]. Some nontoxic derivatives, such as sulfasalazine, have demonstrated clinical safety, allowing the design of azo-based prodrugs. In this case, these derivatives are essential to minimize toxicity and ensure therapeutic efficacy [124]. Also, aliphatic azo compounds can reduce risks compared with aromatic azo compounds, as they are less toxic if appropriately encapsulated [154]. In addition, the high molecular weight of azo-polymeric compounds prevents systemic absorption and reduces toxicity risks. Designs such as attaching bio-adhesive polymers further enhance localized action and reduce adverse effects [156,157]. Below are examples of azo-based compounds examined in terms of biocompatibility and toxicity. According to the research, azo-based PEGylated nanoparticles are nanoparticles attached to the PEG polymer to increase their biocompatibility and drug performance. These compounds are specifically designed to improve body distribution while assisting in more precise targeting in treatments. By adding azo groups to their structure, it is possible to provide greater control over drug release and their response to specific body conditions [86,158]. Based on the results of cytotoxicity tests, azo-based PAPD nanoparticles show no significant hemolysis or hepatotoxicity, even at therapeutic doses. This result indicates a favorable biocompatibility profile for these nanoparticles. The minimal toxicity, combined with the high ability of these nanoparticles to release hypoxia-sensitive drugs, emphasizes that azo-based nanoparticles are a promising option for cancer therapy [159,160]. Another study examined the biocompatibility and toxicity of AzP1, synthesized by coupling N, N′-dimethylaniline with SN-38 (irinotecan analogue). According to the results, AzP1 is stable across a wide pH range and showed no significant interaction with common biological analytes such as NADH coenzyme and hydrogen peroxide (H_2O_2). Moreover, this compound not only facilitates drug activation and release in hypoxic tumor environments but also induces cytotoxicity in cancer cells under hypoxic conditions, further demonstrating its potential for effective cancer therapy [111]. One approach to improve the biocompatibility of azo compounds is to use systems containing self-degradable azo linkages to control release. For example, self-degradable azo linkages conjugated to drugs such as doxorubicin are efficiently degraded in azoreductase, releasing the active drug specifically into the target cell [154,161]. In general, the new designs of azo-based compounds offer good biocompatibility performance. These compounds are activated explicitly in hypoxic environments and protect normoxic tissues from cytotoxic effects, thereby reducing damage to healthy cells.

4. Response to hypoxia by cleaving the azo bond

4.1 Response of azo compounds to hypoxia

As mentioned in section 2.4, azo compounds undergo a reductive cleavage in hypoxic environments, leading to significant changes in their photophysical properties [110,111]. The reduction of azo bonds in fluorescent probes occurs enzyme-mediated, where intracellular reductases such as cytochrome-P450 and NADPH-dependent reductases reduce the azo bond in a multistep process, effectively eliminating the N═N bond [98,111,162]. The azo bond, which is stable under normal oxygen conditions, is cleaved into two amines under cleavage, converting the compound from a stable and inactive

state to an active state, leading to a change in the chemical structure and behavior of the compound [109,111]. Cleavage of the azo bond recovers the fluorophore and generates a strong fluorescent signal that indicates hypoxic levels [163,164]. In other words, this mechanism provides an effective means to turn on fluorescence in hypoxic regions [165,166]. In one study, Piao et al. [164] introduced probes such as mono-azo rhodamine (MAR) and mono-azo Si-rhodamine (MASR) that can enable detection at different levels of hypoxia. MAR, which uses the fluorophore rhodamine, can detect hypoxia at various oxygen levels (<5%), making it sensitive to relatively mild hypoxia. In contrast, MASR makes it responsive only to more severe hypoxia conditions (<1%). These azo-based fluorescent probes provide a precise method for detecting hypoxia and utilize the sensitivity of the azo group to turn fluorescence on and off at low oxygen levels.

4.2 Kinetics and mechanism of hypoxia-based drug release

Hypoxia-responsive drug delivery systems exploit the specific features of the hypoxic environment of tumors, such as reduced oxygen levels, low pH, and high glutathione (GSH) concentrations, to achieve targeted and controlled drug release [85,86,167]. These systems are designed based on mechanisms that are activated under hypoxic conditions. Chemical linkers such as nitroimidazole, hydrazone, and disulfide bonds are among the most commonly used approaches [167–169]. For example, nitroimidazole undergoes reduction in hypoxic environments, converting into an amine that triggers drug release. Hydrazone bonds are also rapidly degraded at low pH conditions (~5–6) inside cells, such as lysosomes. On the other hand, disulfide bonds are broken in the presence of glutathione, which is highly concentrated in tumors, and cause drug release [170,171]. Drug release kinetics strongly depend on environmental conditions; low pH and high GSH concentrations are key factors that accelerate the release process. Enzymes such as oxidoreductases, which are more active under hypoxic conditions, enhance these processes [106,172,173]. Prodrugs such as Mitomycin C and Apaziquone are activated by chemical reduction in the hypoxic environment, becoming highly effective therapeutic agents [168,174]. For example, DOX nanoparticles conjugated with nitroimidazole and paclitaxel (PTX) linked through disulfide bond have shown high efficacy in reducing tumor growth and precise targeting in vitro models. This technology offers a promising future in targeted cancer therapy by regulating the timing of drug release and the possibility of combining it with imaging methods [167,175,176].

5. Types of hypoxia-responsive drug delivery systems

Hypoxia-responsive drug delivery systems are designed to target the microenvironment of hypoxic regions [177]. Various systems have been developed over the past years, including hypoxia-activated prodrugs, nanoparticle-based drug delivery systems, anaerobic bacteria for gene delivery, polymer-based carriers, and hypoxia-responsive oxygen carriers [21,177,178]. All these systems aim to increase accuracy and provide more effective treatments for hypoxic tumors while minimizing adverse effects on healthy tissues [177]. This section will describe the most important of these systems in more detail.

5.1 Hypoxia-activated prodrugs (HAPs)

Prodrugs are bioreducible compounds activated by enzymatic reduction under hypoxic conditions, allowing them to release cytotoxic agents specifically in hypoxic regions [92,179]. Tirapazamine

(TPZ) was identified as the first hypoxia-activated prodrug in 1986 [180,181]. TPZ is an aromatic N-oxide that, when reduced in low-oxygen environments to form oxidative radicals, can act as DNA alkylators or inhibitors of certain enzymes involved in DNA replication and repair [182]. However, studies have shown that this prodrug causes side effects such as nausea, vomiting, and muscle cramps [183,184]. Another example of a bioreductive drug is Banoxantrone (AQ4N) [181]. This prodrug was introduced in 1993 as an example of an inactive aliphatic N-oxide prodrug [181,185], which, in addition to its ability to release cytotoxic drugs under hypoxic conditions, can improve tumor specificity and minimize side effects [182]. The most important disadvantages of these prodrugs are their potential for activation in normal tissue conditions and their limited efficacy in deep hypoxic areas, indicating the need for further optimization [186].

5.2 Nanoparticle-based systems

Nanoparticles are being used as a new form of drug delivery systems [187]. Their nanoscale size increases cellular uptake and allows them to penetrate biological barriers effectively [178]. Common nanoparticle carriers include "liposomes, micelles, dendrimers, polymeric and metallic nanoparticles," each designed to release drugs in response to hypoxic conditions, improving drug delivery and oxygenation in hypoxic areas [86,188]. Among the advantages of nanoparticle-based drug delivery systems are precise targeting of drugs, controlled release, stability and increased absorption, and the ability to load multiple drugs [189,190]. These nanocarriers, in addition to being highly biocompatible, can carry both hydrophobic and hydrophilic drugs [191]. LipoMB/CaO_2 is an example of a lipid-based nanoparticle that uses calcium oxide (CaO_2) and methylene blue (MB) to generate oxygen and enhance PDT. When this compound comes into contact with water, CaO_2 reacts to release oxygen and reduce hypoxia, which further enhances the effect of MB. This material is known as a photosensitizer in PDT and could help improve the targeted treatment of hypoxia [192]. Trans-crocetin (L-TC) liposomes, another example of liposomal nanocarriers, have been developed to enhance oxygen delivery to tissues and help with hypoxia. This material has been tested in phase II clinical trials to improve radiotherapy outcomes [177,193]. However, using liposomal nanocarriers can be challenging due to stability issues, premature degradation, and difficulties maintaining drug concentration [177]. According to a study, polylactic-co-glycolic acid (PLGA)-based manganese dioxide (MnO_2) nanocarriers have been designed to address oxygen deficiency in the tumor microenvironment by scavenging tumor-generated H_2O_2. These nanocarriers enhance the cytotoxicity of natural killer (NK) cells by reducing hypoxia-induced immunosuppression while exhibiting good biocompatibility [194]. In a study, Thambi et al. [95] prepared hypoxia-responsive carboxymethyl dextran-based nanoparticles (CMD-NPs). The CMD polymer was chosen as a drug carrier due to its biocompatibility, biodegradability, hydrophilicity, and drug-protecting ability. These nanoparticles encapsulated hydrophobic drugs such as DOX and rapidly released the drug under hypoxic conditions due to biodegradation. Luo et al. [195] designed a micellar nanocarrier using biocompatible materials PEG and polyamino acids that can respond to hypoxic environment and high glutathione concentration in tumor. This nanocarrier, using nitroimidazole, is hypoxic-sensitive and penetrates deep into the tumor, releasing drugs such as PTX and gemcitabine-C18 (GEM-C18). According to the results of this study, in addition to enhancing the effectiveness of drug delivery, this system improves the treatment of pancreatic cancer by reducing hypoxia and destroying the tumor matrix and offers high safety. Despite challenges such as the complexity of the manufacturing process and the possibility of nanoparticles accumulating in

nontarget organs, nanoparticle-based drug delivery systems have opened up new perspectives in the treatment of complex diseases such as cancer. Advances in material engineering and surface modifications have played a crucial role in enhancing nanoparticle functionality, improving drug loading, and optimizing targeted release [177,190,196,197].

5.3 Polymer-based carriers

Today, polymers are recognized as one of the most widely used materials in the biomedical field [198]. Polymer carriers enhance drug delivery by improving pharmacokinetics, targeting specific tissues, and reducing systemic side effects [178]. These carriers are engineered to deliver drugs to the target site in a controlled manner and improve therapeutic outcomes. These systems utilize hypoxic-sensitive functional groups for the selective release of drugs into tumor environments [95]. Hypoxia-responsive carriers often incorporate 2-nitroimidazole derivatives into their structure, and under hypoxic conditions, the nitro group (NO_2^-) is converted to an amino group (NH_2^-), leading to drug release [199]. Biodegradable polymers such as PLGA, PLA, and PEG can be more precisely tuned for drug release and targeted delivery [200].

6. Applications of azo-based hypoxia-responsive drug delivery

As mentioned in the previous sections, hypoxia, a condition in which there is insufficient oxygen to meet metabolic demands, plays a key role in the progression of diseases such as cancer. Hypoxia can promote tumor metastasis, angiogenesis, and resistance to conventional therapies [27,41,44,201–203]. Meanwhile, hypoxia-responsive drug delivery systems can achieve targeted drug release by providing a targeted therapeutic approach and utilizing the unique properties of azo compounds (their reduction capability under hypoxic conditions) [111,204]. Targeting tumors, triggered drug release, diagnosis and imaging, cancer therapy, treatment of ischemic and respiratory diseases, targeting hypoxia in chronic inflammation, development of new drugs, enhancing therapeutic efficacy, and reducing side effects caused by drugs are among the applications of hypoxia-responsive systems based on azo compounds [104,115,205–207]. Due to their high sensitivity and tunable capabilities, azo-based materials offer an efficient mechanism for activating hypoxia-related responses. These materials are recognized as a promising class in the design of hypoxia-responsive nanocarriers and probes, opening new horizons in treating and diagnosing diseases [114,201]. Table 11.2 presents examples of hypoxia-responsive drug delivery systems based on azo compounds, along with their mechanism of action under hypoxic conditions and their applications. This table highlights the unique capabilities of azo compounds in the design of smart drug delivery systems and precisely examines the relationship between the mechanism of action, targeted applications, and findings from recent studies.

7. Analytical techniques for characterizing hypoxia-responsive azo compounds

Advanced analytical techniques are required for characterizing and identifying hypoxia-responsive azo compounds. Ultraviolet–visible (UV-Vis) spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy are three of the most commonly

Table 11.2 Investigation of some recent studies on hypoxia-responsive drug delivery systems based on azo compounds.

System/ Compound	Mechanism of action	Application	Results	Challenges	Ref.
PAO-L (PEG-AZO-Oleic acid liposome)	Cleavage of azo bonds under hypoxic conditions and removal of PEG from the liposome surface	Melanoma and breast cancer therapy	Higher cellular uptake in B16F10 and 4T1 cells, significant tumor volume reduction	Limited circulation stability, laser activation requirement	[208]
TPA-Azo (Triphenylamine-Azo)	Cleavage of azo bonds under hypoxic conditions enabling fluorescence activation and production of ROS for type I PDT	Imaging and therapy for hypoxic tumors with dual-organelle targeting	Enhanced tumor suppression in vivo, increased ROS production in hypoxic environments	Complex synthesis process, requirement for light activation	[209]
AzP1	Cleavage of azo bonds by NADPH-cytochrome P450 reductase under hypoxic conditions	Targeted cancer therapy and theranostics	Reduction in tumor volume in xenograft models, fluorescence activation dependent on oxygen levels in cancer cells (HeLa, MCF-7)	Limited depth of activation	[111]
PEG-C6-AZO-CA4 micelles	Reduction of azo bonds under hypoxic conditions	Chemotherapy for hypoxic tumors	Rapid release of DOX and CA4 in hypoxic conditions, enhanced cytotoxicity in hypoxic cancer cell	Requirement for optimized dosing	[210]
GlyAC4A (Glycosylated Azocalix[4] Arene)	Cleavage of azo bonds under hypoxic conditions by azoreductase enzymes	Ischemic stroke therapy	Increased drug delivery to hypoxic brain regions, significant reduction in inflammation	Limited systemic stability	[211]

Continued

Table 11.2 Investigation of some recent studies on hypoxia-responsive drug delivery systems based on azo compounds.—cont'd

System/ Compound	Mechanism of action	Application	Results	Challenges	Ref.
T80-HACB/ BCNU nanomicelle	Cleavage of azo bonds under hypoxic conditions combined with esterase-mediated micelle disassembly	Glioblastoma treatment	Increased drug penetration, dual-stimulus targeting, simultaneous release of BCNU and O^6-BG in hypoxic environment	Need for further optimization to enable clinical translation	[212]
Azo-BODIPY dyes (3-azo-conjugated BODIPY dyes)	Cleavage of azo bonds under hypoxic conditions enabling fluorescence activation and targeted drug release	Theranostics (dual imaging and therapy for cancer diagnosis and treatment)	Enhanced imaging contrast in hypoxic tumor regions, improved drug targeting efficiency	Requirement for dual functionality optimization	[213]
Amphiphilic azo polymers	Reduction of azo linkers in hypoxic environments	Cancer therapy (targeted delivery of chemotherapeutic agents)	Selective drug release in hypoxic cancer cells, minimal off-target effects	Dependency on external conditions for self-assembly	[86]
PAPD (PEG-Azo-PEI-DOPE) nanoparticles	Cleavage of azo bonds under hypoxic conditions resulting in PEG shedding	Codelivery of siRNA and DOX for hypoxia-targeted cancer therapy	Minimal systemic toxicity, improved cellular uptake under hypoxic conditions	Requirement for the optimization of PEG shedding kinetics and systemic stability	[97]
PEG-Azo-PS4	Cleavage of azo bonds under hypoxic conditions enabling PDT through singlet oxygen ($^1O_2$) generation	Cancer therapy (combined imaging and PDT)	Significant suppression of tumor growth in hypoxic conditions, effective ROS generation	Limited efficacy in deep tumor regions	[214]

Table 11.2 Investigation of some recent studies on hypoxia-responsive drug delivery systems based on azo compounds.—cont'd

System/ Compound	Mechanism of action	Application	Results	Challenges	Ref.
PEG-Azo-TPE	Cleavage of azo bonds under hypoxic conditions enabling fluorescence activation of TPE and enhanced imaging contrast in tumor microenvironments	Fluorescence imaging of hypoxic tumors	Enhanced fluorescence intensity in hypoxic conditions, negligible fluorescence in normoxic tissues	Limited to diagnostic applications	[215]
azoSeR	Reduction of azo bonds under mild hypoxic conditions (<5% O_2) enabling activation of $^1O_2$ generation for PDT	Hypoxia-specific PDT for targeted tumor treatment	Selective activation in A549 cells under hypoxia, minimal cytotoxicity in normoxic conditions	Ineffectiveness in anoxic environments	[110]
NI-Azo (Naphthalimide-Azobenzene supramolecular vesicles)	Reduction of azo bonds under hypoxic conditions by azoreductase enzymes, leading to disassembly of vesicles and targeted release of curcumin	Cancer therapy (targeted drug release using curcumin as the therapeutic agent)	Significant inhibition of tumor growth in preclinical models, 2.4-fold higher cytotoxicity in hypoxic cancer cells compared with normoxic conditions	Dependency on azoreductase levels	[216]

used technologies for this purpose. These techniques enable the identification of the chemical structures, morphological properties, and behavior of materials under specific conditions. The following sections explore the role of each of these methods in the analysis of azo compounds.

7.1 UV-Vis spectroscopy

UV-Vis spectroscopy is one of the key tools for studying structural changes in azo compounds under hypoxic conditions. This method is especially useful for detecting absorption bands related to $\pi \rightarrow \pi^*$

and $n \rightarrow \pi^*$ electronic transitions in the –N═N- functional group [217]. $\pi \rightarrow \pi^*$ transitions are typically detected in the UV region due to their higher energy need. They occur from bonding to antibonding orbitals, which corresponds to a greater energy difference. On the other hand, $n \rightarrow \pi^*$ transitions, which involve the movement of an electron from a nonbonding (n) electron-pair orbital to an antibonding (π^*) orbital, require less energy. These transitions may take place in the long-wavelength UV region or even extend into the visible spectrum, depending on the compound's molecular structure and ambient conditions. The exact position of these bands varies depending on the chemical structure of the compound, the substituents attached to the azo group, and the type of solvent, which allows UV-Vis to precisely analyze the chemical behavior of azo compounds [218]. Also, this technique is particularly helpful for monitoring the response behavior of azo compounds to hypoxia, the decrease or shift in absorption bands at specific wavelengths can indicate azo bond cleavage and the formation of reductive products such as amines [216,219]. For example, in a study conducted by Sarkar et al. [216], NI-Azo compound was investigated as a hypoxia-sensitive material. In this experiment, the UV-Vis spectrum of NI-Azo (10 μM) was recorded in the THF−water (1:9 v/v) solvent system. As illustrated in Fig. 11.2, this compound showed a distinct absorption peak at 458 nm that belonged to the azo group. With the addition of sodium dithionite ($Na_2S_2O_4$) as a reducing agent (at concentrations ranging from 0 to 1 mM), the intensity of this peak gradually decreased and nearly disappeared at 1 mM, indicating the complete cleavage of the azo bond. At the same time, an increase in absorption was observed in the range of 350–370 nm, which was attributed to the formation of reductive products such as amines. These findings well confirm the importance of UV-Vis in assessing the structural changes of azo compounds under reducing conditions.

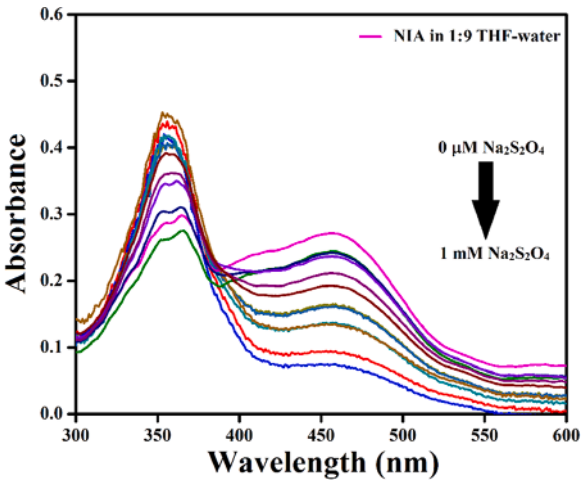
7.2 FT-IR spectroscopy

FT-IR spectroscopy is a powerful technique for identifying functional groups and analyzing the structural properties of compounds. This method provides valuable information about chemical groups such as the azo group by detecting specific vibrational frequencies of molecular bonds. The stretching

FIGURE 11.2

UV−visible spectra of NI-Azo (10 μM) in 1:9 (v/v) THF-water solvent mixtures in the presence of $Na_2S_2O_4$.

Reproduced from D. Sarkar, M. Chowdhury, P.K. Das, Naphthalimide-based azo-functionalized supramolecular vesicle in hypoxia-responsive drug delivery, Langmuir *38 (11) (2022) 3480–3492. https://doi.org/10.1021/acs.langmuir.1c03334*



vibrations associated with the azo bond usually appear in the range of 1500–1600 cm^1, whereas other groups such as hydroxyl (-OH) or carbonyl (-C═O) can be identified in other specific regions of the spectrum. The intensity and position of these bands may vary depending on the substituents and environmental conditions [217,220]. FT-IR is particularly useful for studying azo compounds under hypoxic conditions. Under these conditions, azo bond cleavage induces significant changes in the FT-IR spectra, resulting in the reduction or disappearance of the –N═N- stretching band and the addition of new bands corresponding to reductive products such as amines [118,216]. For instance, Mahmudi et al. [221] synthesized an azo compound (mPEG-Azo-Chitosan) to explore its responsiveness to hypoxic conditions. According to Fig. 11.3, the FT-IR spectrum of this compound showed that the azo group has a distinct absorption band at 1557 cm^{-1}, which is related to aromatic C═C bonds. Additionally, the presence of carboxylate groups was confirmed by a characteristic band at 1658 cm^{-1}. Other important bands included the stretching vibration of the C–O bond at 1110 cm^{-1}, the bending

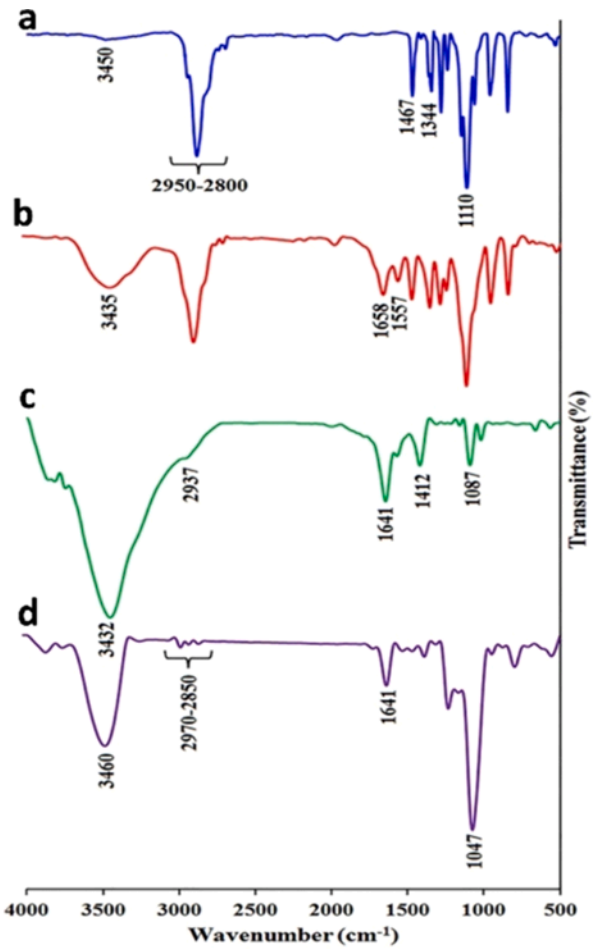


FIGURE 11.3

FT-IR spectra of (a) mPEG, (b) mPEG-Azo, (c) native Chitosan (CS), and (d) CS-g-Azo-mPEG, showing characteristic absorption bands of the azo group.

Reproduced from H. Mahmudi, M. Shahpouri, M. Amin Adili-Aghdam, M. Akbari, A. Salemi, S. Alimohammadvand, A. Barzegari, M.A. Mazloomi, M. Jaymand, R. Jahanban-Esfahlan, Self-activating chitosan-based nanoparticles for sphingosin-1 phosphate modulator delivery and selective tumor therapy, Int. J. Biol. Macromol. *272 (Pt 2) (2024) 132940.* https://doi.org/10.1016/j.ijbiomac.2024.132940

vibrations of C–H at 1344 and 1467 cm^{-1}, and the hydroxyl group vibration at 3450 cm^{-1}. Upon exposure to reductive conditions, the intensity of the –N═N- band decreased, while new bands appeared in the range of 3300–3500 cm^{-1}, which were attributed to the formation of amine groups. These changes indicate the successful cleavage of the azo bond and its conversion into new reductive products.

7.3 NMR spectroscopy

NMR spectroscopy is a powerful tool for analyzing the molecular structure of azo compounds. By examining the chemical shifts of hydrogen (^{1}H NMR) and carbon (^{13}C NMR) nuclei, this technique enables for the detailed study of structural changes including cis-trans isomerization, azo bond cleavage, and the effects of reductive conditions [147,216]. One of the main applications of NMR in azo compounds is the analysis of isomeric configuration. Cis and trans isomers exhibit different chemical shifts in the NMR spectra due to variations in their nuclear electronic environments. This feature helps researchers to obtain detailed information about the stability and structural transformations of azo compounds [220,222]. In addition, NMR is useful in understanding the behavior of azo compounds under hypoxic conditions. This method can detect chemical changes caused by oxygen depletion and reduction of the azo group by azoreductase or other reducing agents [216]. Based on the research of Zhao et al. [223], the azo compound Azo-M was investigated to evaluate its behavior under hypoxic conditions. As can be seen in Figs. 11.4 and 11.5, the ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of this compound confirmed the presence of azo groups and aromatic structures with signals observed at 8.077–8.057 ppm, 7.986–7.945 ppm, and 7.896–7.875 ppm. Similarly, the ^{13}C NMR (100 MHz, DMSO-d_6) spectra revealed signals at 176.48, 154.65, and 144.19 ppm, corresponding to carbonyl groups and aromatic rings. The NMR spectra changed noticeably once the compound was subjected to reductive conditions. The intensity of the signals related to the azo group decreased, while new peaks appeared in the 6.5–8.5 ppm range in the ^{1}H NMR, which are attributed to the formation of reductive products such as amines. The results suggest that NMR spectroscopy can be an effective method for studying the structural alterations of azo compounds in low-oxygen environments and understanding their reductive pathways.

In addition to the aforementioned analytical approaches, various other techniques are utilized to examine hypoxia-responsive azo compounds. Among these, scanning electron microscopy (SEM) is used to examine surface morphology and transmission electron microscopy (TEM) enables visualization of the internal structure and phase distribution of these compounds. Also, X-ray diffraction (XRD) is an effective tool for determining the crystalline phases and structural features of these substances. Furthermore, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are widely utilized to assess thermal stability and phase transitions in azo compounds. The combination of data obtained from these methods provides extensive insights into the physicochemical properties of azo compounds, which might be useful in the design and development of advanced drug delivery systems.

8. Future perspectives and challenges in azo-based hypoxia-responsive drug delivery

Hypoxia-responsive drug delivery systems based on azo compounds have emerged as one of the novel and efficient approach in the medical field, owing to their capability to target and release drugs

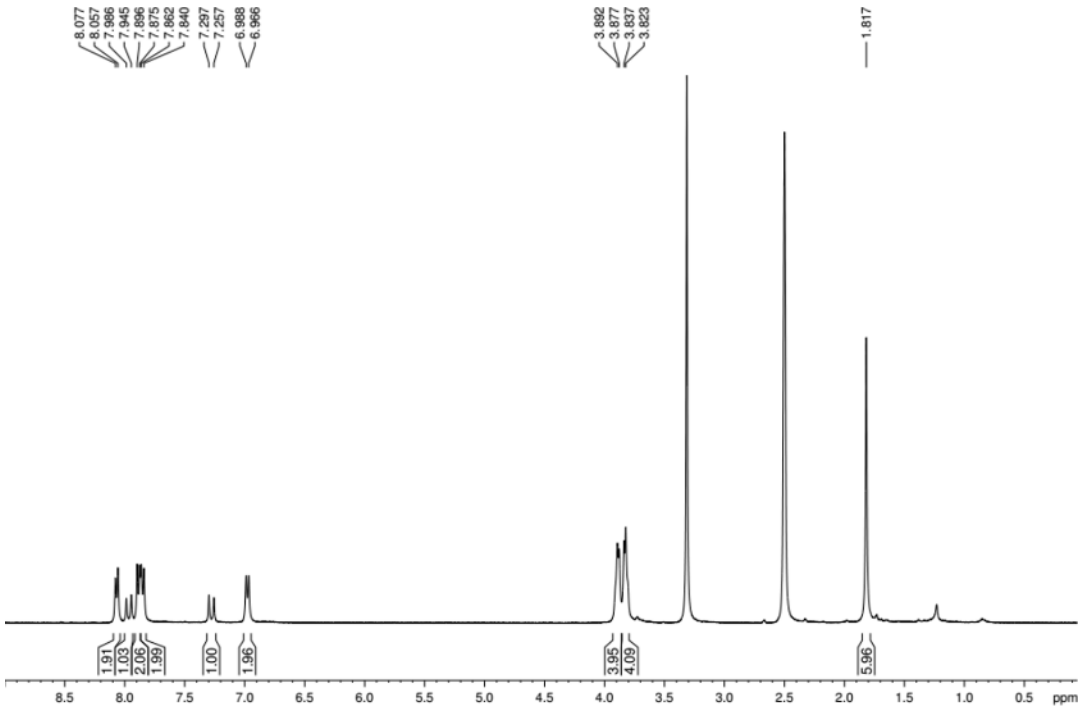


FIGURE 11.4

^{1}H-NMR of Azo-M in DMSO-d_6.

Reproduced from Y.Q. Zhao, S. Biswas, Q. Chen, M. Jia, Y. Zhou, S. Bhuniya, Direct readout hypoxia tumor suppression in vivo through NIR-theranostic activation, Appl. Bio Mater. *4 (7) (2021) 5686–5694. https://doi.org/10.1021/acsabm.1c00467*

specifically in hypoxic conditions [201,224]. These systems, through their innovative design and leveraging the unique characteristics of azo compounds, enable effective treatment while minimizing side effects [111,114]. However, despite significant progress, there are still challenges that need to be addressed for further development and improvement of this technology [154]. Recent advancements in combination technologies have enabled the development of advanced azo-based drug delivery systems. Studies have shown that modifying azo structure, especially by incorporating electron-withdrawing groups, enhances the efficacy of these compounds under hypoxic conditions. These chemical modifications facilitate the design of smart nanoparticles for targeted drug release [124,224,225]. Azo nanoparticles, combined with NIR sensors, have been able to improve imaging accuracy and monitoring of treatment progress while simultaneously enabling targeted therapy. These systems, with their ability to regulate drug release, play an effective role in enhancing the effectiveness of targeted therapies [201,206]. The use of hypoxia-sensitive hydrogels has also demonstrated that these materials can release drugs in a controlled and precise manner [148,226]. Furthermore, MOFs in combination with azo groups have exhibited unique capabilities in controlling drug release. These systems, in addition to reducing side effects have shown superior performance in complex tumor environments [147,227]. The integration of these technologies with hypoxia-responsive systems has

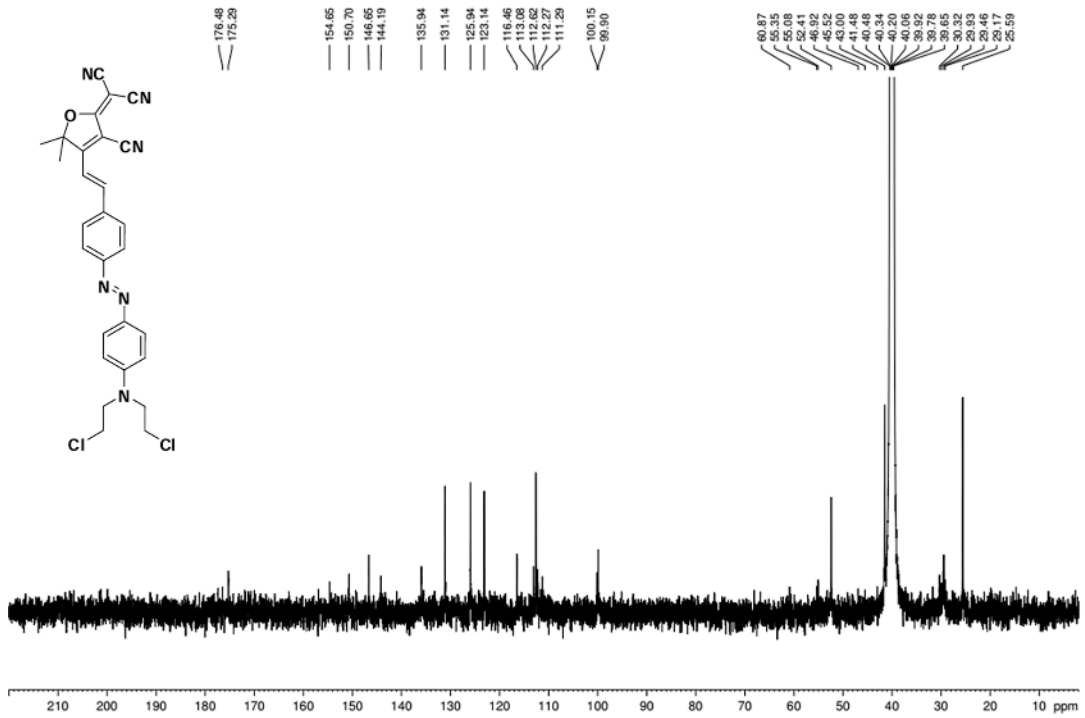


FIGURE 11.5

^{13}C-NMR of Azo-Min DMSO-d_6.

Reproduced from Y.Q. Zhao, S. Biswas, Q. Chen, M. Jia, Y. Zhou, S. Bhuniya, Direct readout hypoxia tumor suppression in vivo through NIR-theranostic activation, Appl. Bio Mater. *4 (7) (2021) 5686–5694. https://doi.org/10.1021/acsabm.1c00467*

paved the way for the development of personalized and precise treatments. These advancements, in addition to enhancing treatment efficiency, have also enabled the use of new tools for monitoring therapeutic outcomes. Despite these advances, there are several challenges to the widespread use of these technologies that need to be addressed and resolved. One of the fundamental challenges in using azo compounds is ensuring the stability and biocompatibility of these systems. Strategies such as the use of biocompatible materials such as PLGA and hyaluronic acid into the structure of nanoparticles have successfully reduced the toxicity of these systems and optimize drug uptake in target cells [158,206,228]. Moreover, the use of protective coatings and improved material designs to prevent unwanted drug release and to overcome the instability of azo compounds under some environmental conditions such as changes in pH or temperature, have significantly enhanced the stability of these systems. These achievements, along with the use of systems that activate exclusively under hypoxia conditions, have played an important role in improving drug delivery performance and reducing side effects [97,229]. On the other hand, regulatory and clinical challenges remain significant barriers to the widespread application of these systems. Azo-based drug delivery systems require rigorous research to demonstrate safety and comply with regulatory standards. These processes are often time-consuming and costly, which may delay the commercialization of these technologies [154,229]. Furthermore, the

performance of these systems is highly dependent on the oxygen levels in the target tissues, and fluctuating hypoxic conditions can reduce the effectiveness of the treatment. This highlights the need for designing systems that can perform optimally in variable hypoxic environments [230,231]. Another obstacle is the cost of production and development. The industrial-scale manufacturing of hypoxia-responsive azo-based systems requires advanced technologies and special raw materials, which increase the costs of research and production [154,227]. By addressing the existing challenges and focusing on further research, this technology has the potential to become one of the key tools in the targeted treatment of complex diseases [104].

9. Conclusion

This study investigated hypoxia-responsive drug delivery systems using azo compounds. Because of their unique structure and capabilities, these compounds allow for controlled and selective drug release in hypoxic conditions, enhancing the precision and efficacy of targeted therapies. Their mechanism of action is based on the biochemical reduction of azo bonds under hypoxic conditions, which results in drug activation at the site of the tumor while preserving healthy tissues. Compared with conventional methods, this technology offers reduced systemic toxicity, improved cellular targeting, enhanced drug efficacy, and the possibility for integration with imaging technologies and theranostic procedures. However, challenges involving the stability of these compounds in circulation, fluctuations in oxygen levels within tumors, synthesis complexity, and high production costs continue to impede their clinical application. For further advancements in this field, it is critical to optimize the structure of azo compounds in order to increase stability, minimize production costs, and improve drug release precision. Combining this technique with smart nanomaterials and hybrid medication delivery systems has the potential to improve its performance even more. Furthermore, performing extensive clinical studies is an important step toward determining the safety and efficacy of this approach and easing its commercialization. Overall, azo-based drug delivery systems are a promising strategy in targeted medicine, offering fewer side effects and superior therapeutic efficacy, ultimately contributing significantly to patient quality of life.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to enhance the readability and language clarity of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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CHAPTER

Redox-responsive nanocarriers for biomedical application

12

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1. Introduction

1.1 Approach and classification of stimuli-responsive drug delivery

This section will give a brief idea regarding certain widely preferred exogenous and endogenous stimuli-responsive techniques, followed by this, in the upcoming sections we'll have an in-depth look into the redox-responsive nanocarriers (Figs. 12.1 and 12.2).

1.2 Exogenous stimuli-responsive drug delivery

Amongst the extensively studied stimuli-responsive drug delivery techniques, thermos-responsive drug delivery has received a lot of attention in the field of cancer. The characteristics of no less than a single element of the NC often undergo a nonlinear, abrupt shift in response to temperature, which controls thermos-responsiveness. Following a change in the ambient temperature, this rapid reaction sets off the medication's release. To counterbalance quick blood-passage duration and runoff from the tumor, thermosensitive NCs ought to optimally hold their contents at body temperature (~37°C) and deliver the medication promptly inside a selectively warmed tumor (~40–42°C). Thermosensitive liposomes (TSLs) represent one of the most advanced thermoresponsive nanocarrier systems, with their use in numerous clinical studies showcasing their potential. Doxorubicin-loaded TSLs (marketed as ThermoDox by Celsion Corporation), when used alongside hyperthermia or radiofrequency ablation, are currently being evaluated in phase II clinical trials for breast cancer and colorectal liver metastases. Additionally, they have reached phase III clinical trials for hepatocellular carcinoma treatment [1].

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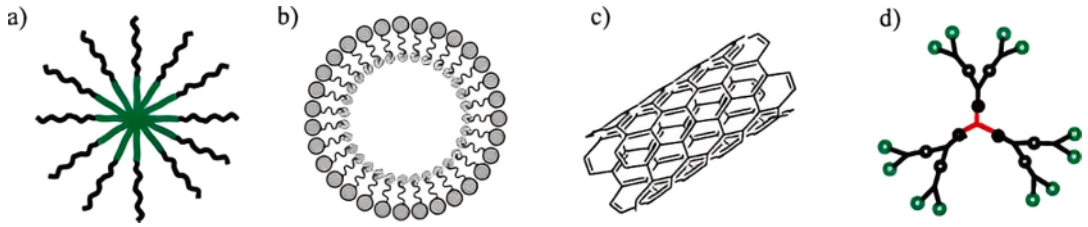


FIGURE 12.1

Various kinds of NCs used in DDSs especially for cancer therapy, (a) Polymeric micelles, (b) liposomes, (c) carbon nanotubes, and (d) dendrimers.

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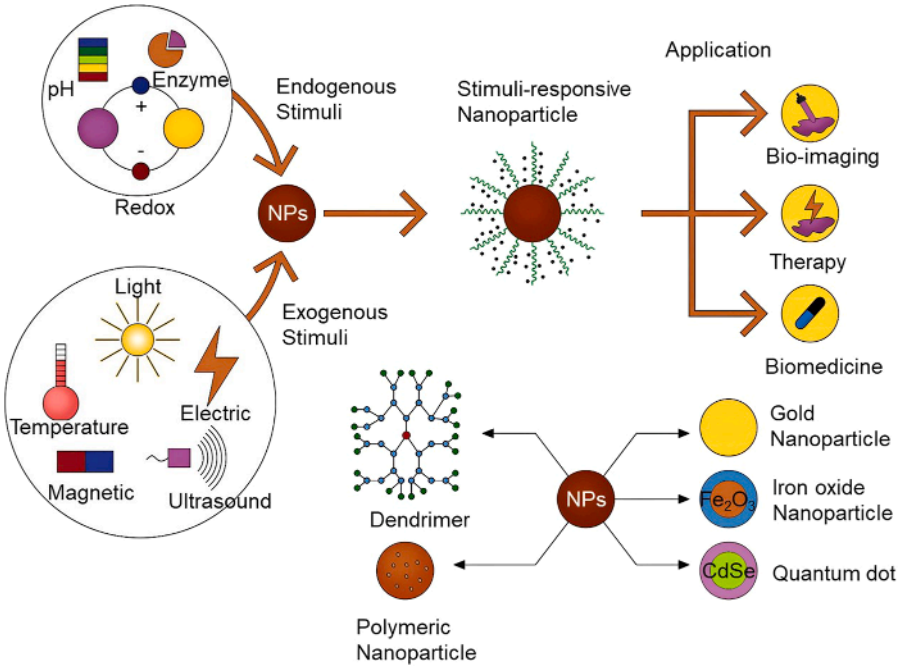


FIGURE 12.2

An illustration of exogenous and endogenous stimulus factors.

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High-energy sound waves, ultrasound (US), may have an impact on NCs for regulated medication release in sick areas (such as tumors). It is possible to modify the ultrasound's intensity to suit various uses. It may be used for imaging at low ultrasonic frequencies (<20 kHz) and for releasing cargo from NCs or increasing the permeability of cancerous cellular membranes at elevated frequencies

(>20 kHz) [2]. US-responsive NCs may find use in tumor ultrasonography, a low-risk, commonly used clinical modality that yields high-resolution pictures. Both gas- and contrast-agent-incorporated NCs (e.g., perfluoropentane) [3] and gas-generating NPs (e.g., CO2) in a biological environment have shown tumor-specific imaging at outstanding clarity and intensity. An alternative approach would include transforming the porphyrin microbubbles (1–10 μm) into nano sized bubbles (5–500 nm) for use in tumor ultrasonic imaging [4]. Additionally, ultrasound may be used to initiate the regulated dissolution of cargos from NCs at specific tumor locations, such as imaging devices or anticancer medicines [5,6].

The benefit of applying a magnetic field resides in the many forms that the magnetic response may take: applying a permanent magnetic field can produce magnetic guidance; using a magnetic field with alternating phases can cause a temperature rise; or applying both at the same time can produce both effects. Hence, a variety of medication delivery pathways are possible with magnetically responsive devices. Additionally, it is possible to use magnetic resonance imaging, which would allow for the integration of therapy and diagnostics into a single system—a concept known as the theranostic approach [7].

Photon illumination has drawn the attention of many researchers amongst the stimuli used in many kinds of DDSs because of its exact tuning, controllability over length of exposure and tissue location, and noninvasive activation. Using the changeable area of the light spectrum, such as the UV-visible (10–400 nm) or near-infrared (650–900 nm) range, one can employ nanocarriers to deliver drugs or release genes. UV radiation with a wavelength between 200 and 300 nm has the ability to harm the tissues that represent their use in therapy. As a result, wavelengths shorter than 650 nm are thought to be helpful for triggering medication release in the modern therapy of pathological disorders [8]. Liposomes and micelles are the most widely used light-responsive NPs, and they have a broad variety of application [9]. The photo-cleavage that occurs in the NP's core is the pathway of drug dispensing [10]. The concurrent absorbance of two photons triggers a chemical response in two-photon NIR-triggered mechanisms, resulting in yet another crucial drug-release approach. Nevertheless, the process's poor efficiency limits its efficacy [11].

1.3 Endogenous stimuli-responsive drug delivery

1.3.1 pH and enzyme responsive NCs

The low pH seen in the organelles of tumor cells and the tumor microenvironment (TME) has led to the widespread use of pH-responsive NCs. The pH of the TME ranges from 6.5 to 6.8, while the pH of the blood, cytoplasm, and regular tissues is typically between 7.0 and 7.4. The pH of endosomal/lysosomal organelles is within the range of 6 and 4 [12]. The pH-responsive nature of the TME presents an opportunity for regulated drug discharge or prodrug stimulation, all the while maintaining the "stealth effect" of nanocarriers in normal areas (such as blood circulation) and preventing cargo leakage. In order to achieve excellent therapeutic efficacy, this would selectively transport hazardous cargos, such doxorubicin, to tumors while reducing the danger of exposure to normal organs, including the heart. In contrast to the practically neutral pH of cytoplasm (pH 7.2), endosomal/lysosomal organelles have a pH of roughly 6 to 4. Typically, endocytosis is the method by which NCs penetrate cancerous cells. To prevent additional damage in late lysosomes that have a poor pH, endosome/lysosome escape is necessary. Many intercellular pH-stimulated NCs were developed recently with the goal of releasing payload from cancerous tumor cells [13].

1.3.2 Redox-responsive nanocarriers (RRNCs)

When viewed in relation to the adjacent normal cells the micro ecosystem of cancerous cells is a strongly reducing environment, also known as a "reductive environment". Glutathione (GSH), a tripeptide with a thiol (-SH) moiety that originates from cysteine residue, is the primary biochemical responsible for its unique reducing tendency [14]. Tumor endothelial cells (TECs) are proangiogenically affected by reactive oxygen species (ROS) produced in the TME as an effect of oxidative stress. TECs have characteristics of chemo-resistance and multiply and grow more quickly than normal endothelial cells (NECs) Additionally, a hypoxic environment produces ROS, which lead to the creation of TECs and genetic defects in NECs [15]. Research has demonstrated a correlation between elevated ROS levels and elevated antioxidant levels [16]. Intracellular GSH functions as a potent antioxidant that occurs spontaneously. The TME has elevated levels of each ROS and GSH. Healthy cells have intracellular GSH levels between 1 and 10 mM, whereas extracellular GSH levels are substantially lower (2–20 μM). Tumor cells, on the other hand, have GSH concentrations that are at least quadrupled than the healthy cells [17]. Because of the significant difference in GSH levels between cancerous and normal cells, medication delivery pathways may use the reduced atmosphere as an endogenous trigger [18]. Furthermore, the cytosol has more than 80% of GSH molecules, whereas the rest of the organelles only have around 10% of such molecules. This is advantageous because it allows anti-tumor medicines to be released straight into the cytosol, such that they could reach their targets more precisely [18]. Thus, an endogenous trigger for tailored medication delivery might be the intracellular GSH levels. Redox hemostasis, which involves several cellular metabolic pathways such as, glutathione production, glycolysis, glutaminolysis, and fatty acid oxidation, is an essential mechanism for cell survival [19]. Nevertheless, the abnormal redox hemostasis in carcinoma cells changed the redox balance and raised ROS levels. ROS levels increased as an outcome of mitochondrial malfunction, NADPH oxidase amplification, and modifications to antioxidant enzymes [20]. As a consequence, the elevated ROS levels altered the phenotypic of CAFs, a kind of fibroblast, which produces more ROS and causes ECM modification and tumor progression [21].

1.3.3 Mechanisms involved in RRNCs

In the course of immunological or inflammatory reactions, inflammatory cells, particularly neutrophils and macrophages, yield reactive oxygen species (ROS) [22,23]. However, there are many other sources associated with ROS generation, for instance, angiogenesis, a process necessary for the development, growth, and spread of tumors is a crucial source of ROS generation. Moreover, the activity of endogenous antioxidant enzymes including NADPH oxidase, superoxide dismutases (SOD), and thioredoxin may be the cause of high ROS levels. When it comes to pharmaceutical nanotechnology, the elevated intracellular GSH levels in tumor cells help create stable nano-disperse drug carriers or Nano drug delivery system (NDDS) amid the challenging conditions of blood circulation. However, these nanodevices may become unstable when exposed to cytosolic GSH, leading to regulated drug release. Employing active-targeted NDDS, that possess selective ligands like aptamer, peptide, or even antibody embellished on their surface to enable them to focus on the excessively expressed surface ligands on cancer cells, is one method of delivering larger doses of medication to the tumor site, as we previously discussed in the preceding section [24,25]. Using pathological alterations in the TME and creating NDDSs that can react to either exogenous or endogenous stimuli like redox potential, light, temperature, pH, or elevated enzyme activity is an additional strategy [26,27]. Research has demonstrated that, at healthy concentrations, ROS levels are essential for controlling signaling pathways linked to cell

development, their existence, movements, and reproduction. Reactive Oxygen Species (ROS) formation involves a variety of cells, such as ECs, inflammatory cells, CAFs, angiogenesis, and hypoxia [28]. According to certain research, oxidative stress causes CAFs to engage in aerobic glycolysis and autophagy/mitophagy, which influence the production of lactate and raise ROS levels [29]. DDS commonly employ redox-responsive compounds that focus on the redox responsive sites, including diselenide bonds (-SeSe-), disulfide bonds (-S-S-), platin conjugation (-Pt-), thioether bonds (-S-), and thiol groups (-SH) [30]. It is possible to pack hydrophobic medications into the innermost part of NPs, into their pores, or covalently bind them onto the outermost layer via a redox-sensitive coupling [31].

1.3.3.1 Reduction mechanism

The transfer of a hydrogen atom from the thiol group to certain moieties or functional groups in the drug delivery NCs is the means of action of GSH as an antioxidant [32]. Redox-responsive chemical entities seen in NCs typically consist of bonds that experience a conformational shift upon acquiring a hydrogen atom (reduction), resulting in breaking and a crack in the framework of the NC [33,34]. Consequently, RRNCs will absorb hydrogen atoms from the elevated GSH level at tumor locations. These breakages will cause the nanocarrier to dissolve and discharge the filled anti-tumor chemicals into the cell. The disulfide links that forms as an outcome of the concurrent transfer of hydrogen oxidizes GSH to glutathione disulfide (GSSG) [32]. The kind of nanocarrier, where the redox-sensitive functional groups are located, and what kind of redox-sensitive functional groups are existing all affect how the nanocarrier disintegrates. By means of this approach, tailored cargo release and NC breakdown may be simply and quickly facilitated by the decreasing environment of malignancies. The GSH – GSSG redox reaction has been illustrated in the figure below (Fig. 12.3).

As we have discussed in the previous section, different functional groups (like disulfide) may be included in NDDS to increase their sensitivity to ROS in tumor locations. As such, knowledge of their structures, toxicity, and responses is essential.

1.3.3.2 Disulfide bonds

Disulfide bonds (S–S), which form between two sulfur atoms, are among the most studied linkers in the design of NDDS for cancer therapies. These bonds are sensitive to glutathione (GSH) and undergo rapid reduction to thiol groups in its presence through a "thiol-disulfide exchange" reaction.

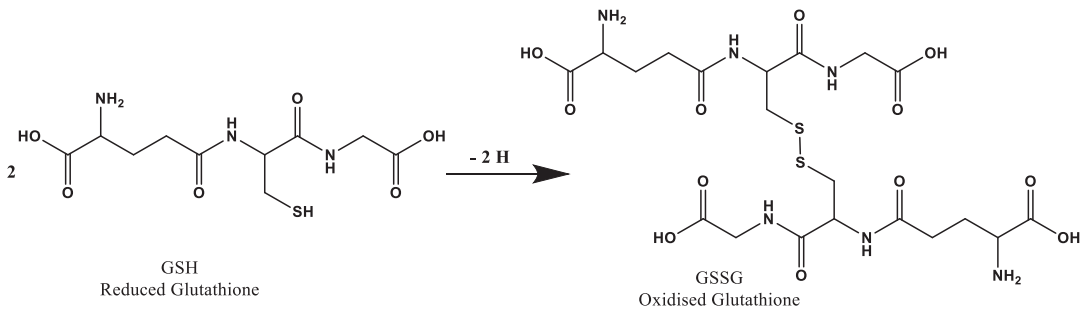


FIGURE 12.3

Structural representation of GSH/GSSG redox reaction.

Reused from H.F. Abed, W.H. Abuwatfa, G.A. Husseini, Redox-responsive drug delivery systems: A chemical perspective, Nanomaterials 12 (18) (2022) 3183, under CC By license.

Specifically, this redox reaction occurs when GSH donates two hydrogen atoms to the sulfur atoms in the disulfide bond, resulting in the bond breaking into two thiol groups (–SH) and the oxidation of GSH to GSSG [35]. This mechanism makes disulfide bonds ideal for use in nanocarriers, as they degrade upon contact with GSH in the TME, triggering the release of the therapeutic payload. Additionally, disulfide linkers are preferred for their stability in circulation, only breaking down in the tumor region, thus enabling targeted drug delivery and minimizing side effects. Furthermore, disulfide linkers' positions within drug delivery NCs have a significant impact on the efficacy and stability of the DDS, enabling the adaptable construction of a wide range of RRNCs. The polymers employed to create the NCs include disulfide linkers as their backbone [33]. Disulfide bonds have also been widely employed as cross linking agents in micelles and nanogels [36] where they have been utilized to crosslink the outer shell [37] or inner core [38] of polymeric micelles.

1.3.3.3 Diselenide bonds

Diselenide bonds are formed by two selenium atoms (Se–Se), and when reduced, they break into selenol groups (–SeH). This process occurs when glutathione (GSH) donates hydrogen atoms to the selenium atoms, resulting in the cleavage of the bond and the formation of selenol groups. Selenium shares similar chemical properties with sulfur, as they are both members of the same group on the periodic table [39]. However, because selenium atoms are larger than sulfur atoms, diselenide bonds and carbon-selenium bonds (C–Se) are weaker and less stable than disulfide bonds due to their longer bond lengths. While this makes diselenide bonds beneficial in the reductive environments of tumors—enhancing drug release—it also results in lower stability, which can cause premature drug leakage in drug delivery systems during circulation [40]. Additionally, materials containing diselenide bonds tend to have poor solubility, further hindering the performance of these drug delivery systems [41]. Nevertheless, diselenide bonds' high sensitivity to the reductive conditions in tumors has made them useful for developing redox-responsive drug carriers. Furthermore, diselenide bonds are adaptable because they can cleave in response to ROS, which tend to exist in TME at elevated levels (up to 1 mM) than in ordinary cells. Additionally, diselenide bonds react to the reducing environment of the TME [42]. Selenic acids (RSeOOH) would result from the oxidation of the diselenide bonds in this situation [42]. The reagent frequently employed for in vitro experiments, hydrogen peroxide (H_2O_2), and hydroperoxy radicals ($\bullet HO_2$) are examples of prevalent ROSs [43].

2. Types of RRNCs

The creation of intracellular environment-sensitive NDDSs that release their contents in response to an inherent biological condition—specifically, cytoplasmic GSH—has garnered significant attention in the field of pharmaceutical nanotechnology [44]. Scientists can create chemically sensitive NP compositions that discharge their therapeutic payload in the wake of intracellular circumstances thanks to the high amounts of GSH seen in the pathophysiological settings of tumors. The past few years have seen the report of several redox-responsive drug delivery systems (RRDDS), comprising prodrug-based systems, liposomes, micelles, NPs, and nanogels (NGs).

2.1 Nanoliposomes

Nanoliposomes are lipid-based carriers that are nanosized particles which are widely employed in drug administration because they are biocompatible, biodegradable, and may exploit tumor

aggregation through the EPR effect [45]. It is possible to functionalize liposomes for an extended half-life (using PEG), localization (using aptamer binding), and stimulus-responsive activity. When reducing agents are present, redox-responsive liposomes assist in dispensing drugs by destabilizing the liposomal barrier or reducing disulfide linkers. For example, Tian et al. [46] created redox-sensitive liposomes preloaded with low molecular weight heparin functionalized (LMWH) doxorubicin (DOX) to delay the spread of tumors. Many researchers have also tried to functionalize liposomal dosage forms with antibodies, peptides, affibodies, or different ligands in order to improve the effectiveness of drug delivery to the site of tumors [24,47,48]. Tumor tissues have greater levels of GSH and ROS than regular tissues, which may aid in the creation of liposomes that are sensitive to reduction or oxidation. Glutathione levels in cancerous cells can increase up to 10 mM, although they are only 0.1 mM in normal cells. Yin X et al. [49] used a disulfide bond to encase liposomes carrying doxorubicin (Dox) with chitooligosaccharides (COS). These liposomes were robust in physiological settings and could penetrate tumor cells because of COS's sticky qualities. Numerous investigations have been carried out using reduction-sensitive liposomes to deliver genes via a liposomal system based on a naphthalimide-sulfonamide group, distribute DNA into HEK293 cells [50], and co-deliver paclitaxel (PTX) and antisurvivin siRNA [51]. According to these research disulfide bonds are the most intriguing linker which are being employed in liposome construction thus far.

2.2 Polymer-based nanoparticles

In biomedical applications, polymer-based both micro-sized and NPs are valuable because they may disintegrate when exposed to specific factors including enzymes, pH, temperature, light, and ROS. A primary cause of their ineffectiveness is the early release of drugs into the bloodstream, despite the fact that many polymeric and macromolecular (protein, polysaccharide) NPs are being expansively investigated for targeted drug delivery for tumor regression [52]. In order to create redox-responsive polymeric NPs, redox-sensitive functional groups are either cross-linked to the NP framework or integrated into the polymeric structure as side groups. One method for creating redox-sensitive polymeric-based NPs that react to ROS species, particularly H_2O_2, is to use arylboronic esters. According to reports, compounds comprising phenylboronic ester undergo oxidation in the presence of H_2O_2, resulting in the production of highly reactive electrophile quinone methides that interact with biological macromolecules [53]. In reaction to ROS, the resultant polymeric NPs would offer enhanced cargo release, cleavable kinetics, and high hydrolytic stability in circulation [53,54]. RRNPs made of different substrates were recently demonstrated to supply drugs to specific cells while preventing early discharge [55]. For example, Yu et al. [56] described mesoporous organosilica nanoparticles (MONs) that are tiny (less than 50 nm) and used a redox-responsive biodegradation process to distribute doxorubicin in a targeted manner. To make the NPs redox-responsive in nature, the disulfide link was included into MONs.

Recently, a key method to improve the bioavailability of water insoluble chemotherapy drugs has been the development of polymeric micelles, sometimes referred to as nanoscopic core/shell structures made by amphiphilic block copolymers. The benefits of polymeric micelles, including increased blood flow, improved drug dissolution in water, and increased drug buildup in the tumor by means of the EPR effect, have been adequately considered [57]. Nevertheless, poor stability of these polymeric micelles in the bloodstream after intravenous injection, is still a problem.

2.3 Nanogels

NPs based on nanogel (NG) have emerged as viable options for effective RRDDS. Typically made of cross-linked polymers, nanogels use the sol-gel process to transform monomers into materials that resemble solids. There are several types of NG structures, including simple, hollow, core-shell, and multilayered. Diffusion, enzymatic and chemical breakdown, and swelling regulate the discharge processes from the NG matrix. Prominent techniques for creating nanogels include polymerization, chemical cross-linking, and physical cross-linking [58]. Weaker van der Waals, hydrophobic, electrostatic, or hydrogen bonding interactions create the physical crosslinks. Covalent bonding creates the chemically cross-linked gels. The inclusion of polymers and disulfide-bonding elements makes NGs highly sensitive to the intracellular reductive environment. For instance, degradable NGs were synthesized using cystamine and a block copolymer of poly(ethylene glycol) combined with poly(2-(hydroxyethyl) methacrylate-co-acryloyl carbonate) (PEG-P(HEMA-co-AC)). These NGs effectively encapsulated proteins such as FITC-labeled cytochrome C (FITC-CC) in a uniform manner [59]. Nguyen et al. [60] demonstrated that disulfide-cross-linked heparin-pluronic (DHP) nanogels could encapsulate RNase A and that their content released more quickly when reducing agents were present. Due to selenium's greater atom radius and weaker disulfide connections, nanogels with diselenide cross-linkers may be more successful than those with disulfide bonds. Cross-linked hydrophilic NG-based polymers with a polyamide structure, such pNIPA (poly(N-isopropylacrylamide)), can soak up a lot of water and alter their volume in accordance with external stimuli. Researchers have looked at poly (N-isopropylacrylamide-acrylic acid) (PNA) nanocapsules for regulated drug discharge and improving targeted anticancer effects. Pickering emulsion (PE) technique, which uses solvent evaporation, produced the capsules. To achieve targeted delivery features, these nanocapsules were crosslinked with cystamine and affixed to the cell surface markers (cRGDfK). DOX encapsulation efficiency was high in nanocapsules.

2.4 Inorganic nanomaterials

Because of their adaptable functionalization biochemical compatibility, carbon-based NMs, such as graphene oxide, carbon nanotubes, and fullerene, have been extensively employed as vectors for cancer treatment throughout the last few decades. Additionally, their distinct thermal, optical, and mechanical characteristics yield them promising candidates for enhancing medicinal results [31]. Carbon NMs can interact with chemotherapeutic cancer treatments by π-π stacking, absorption, hydrophobic, non-covalent interactions, or covalent conjugation because of their distinct sp2 structure and intrinsic hydrophobicity [61]. Using disulfide bonds, Yongdoo Choi and Youngnam Cho [62], connected GO to chlorine6 (Ce6), a second-generation photosensitizer. The discharge of Ce6 occurred when intracellular GSH dissolved the disufide bonds after the cancer cells had absorbed the GO-SS-Ce6 conjugates. Photodynamic treatment and fluorescence imaging can both benefit from the extremely luminous and phototoxic dissociated Ce6.

Photothermal therapy (PTT) can make use of gold nanoparticles (GNPs) since they can take up visible or near-infrared radiation and convert it into heat energy, which causes the sick cells to die. Au-sulfur or Au-N2 bonds, which can be broken in response to reducible substances like GSH or photothermal processes, give GNPs the further benefits of excellent biocompatibility and simple surface alterations. Using a core of 2 nm GNPs and a shell made up of a mixed layer of a cationic quaternary ammoniated (11-mercaptoundecyl) tetra (ethylene glycol) (TTMA) and a fluorescent thiolated

molecule, Vincent M. Rotello's group [63] developed a drug delivery system. Medicinal genetic substances and water-resistant or water insoluble therapeutics can be dual-loaded into the system.

The fabrication of RRNPs for therapeutics has also made extensive use of various other inorganic NMs, such as layered double hydroxides (LDHs), mesoporous hydroxyapatite (MHAp) nanoparticles, and quantum dots (QDs). The Duqiao Ding group [64] discovered that GSH and oxidative hydroxyl radicals may reversibly quench and recover the fluorescence of selenium-doped graphene QDs (Se-GQDs). Using Se-GQDs as nanovectors allowed for the tracking of the therapeutic drugs by their fluorescence, according to this result. With the help of MHAp nanoparticles, Yu-Dong Huang et al. [65] creatively created LA-Col-S-SMHAp, a redox-sensitive nanovector. For drug loading and controlled release, lactobionic acid-conjugated collagen was covalently bonded to the outermost layer of MHAp NPs, serving as both a cap and a targeting molecule.

3. Biomedical applications of RRNCs

3.1 Gene therapy

Numerous human illnesses are associated with genetic alterations. A significant advancement in addressing the management of these genetically modified diseases, gene therapy sets the course for future research into diseases that traditional medicine is unable to address. It is anticipated that gene therapy will soon be widely used in clinical settings because of its progress in children with Duchenne malnutrition [66] and vision disorders. Nucleic acids must be delivered to target cells via safe and efficient vectors because they are readily broken down by enzymes in vivo. For gene delivery, numerous approaches have been devised and put forth. Nonviral vectors do better among these tactics in reducing expenses and negative reactions. Delivery techniques for stimuli-responsive nanoparticles are among the most researched nonviral vectors. By reacting to various internal and external stimuli, they take part in the transfection of nucleic acids. Furthermore, it is challenging for negative-charged macromolecules like DNA to approach negative-charged cellular membranes. As an outcome, target cells do not absorb the supplied nucleic acids very well. Protein/nucleic acid complexes, such as Cas9 (positively charged) paired with synthetic single-guide RNA (sgRNA; negatively charged), are significantly more challenging to deliver compared to DNA or mRNA due to their dual charges. To get to the target cell's cytoplasm or nucleus, a nanocarrier must get past the previously described barriers. Using extracellular exterior triggers for signal activation is one amongst the most efficient ways to increase carrier discharge efficacy [67]. Glutathione is the primary reducing agent in cells, as we have shown in the preceding sections.Glutathione levels in tumor cells are 7–10 times greater than those in regular cells, which are already 100–1000 times greater than those in the extracellular environment. This makes glutathione disulfide a perfect vector-triggering component for gene therapy. The disulfide bond is an exceptionally crucial and frequently utilized redox-sensitive connector for RRDDS (Fig. 12.4).

Under mild reduction settings, this bond remains stable in the extrinsic milieu or circulatory system, but under the severe reduction circumstances, it is reduced to sulfhydryl groups. A cationic block copolymer, poly(aspartic acid-(2-aminoethyl disulfide)-(4-imidazole carboxylic acid))-PEG, designed as a glutathione-sensitive nanocarrier with disulfide bonds and imidazole residues, has been validated in vitro for its capacity to deliver DNA, mRNA, and Cas9/sgRNA ribonucleoprotein complexes. Low cytotoxicity and comparatively high transfection effectiveness are characteristics of the NC [68].

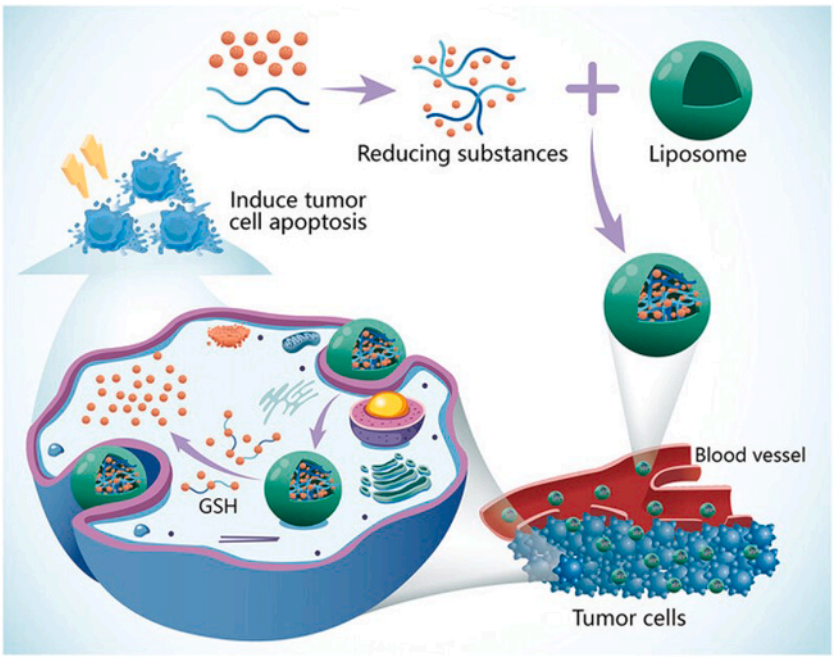


FIGURE 12.4

A redox-responsive nanocarrier being used for gene therapy.

Reused from C. Yu, L. Li, P. Hu, Y. Yang, W. Wei, X. Deng, L. Wang, F.R. Tay, J. Ma, Recent advances in stimulus-responsive nanocarriers for gene therapy, Adv. Sci. 8 (14) (2021) 2100540, under CC by 4.0.

3.2 Cancer treatment

It is well established that cancer is one of the deadliest diseases with second highest fatalities every year, given the generalized chemical action in traditional chemotherapy, toxicity is a significant issue. In addition to toxicity, one of the main reasons why traditional treatment methods don't work is medication resistance. For decades, the innate incentive for efficient intracellular transport has been the reducing conditions in cancer cells. One effective method for achieving low cytotoxicity and effective intracellular medication delivery is the use of RRDDS. Theoretically, RRDDS can satisfy the need for tailored therapy by taking advantage of the variations among the unique microenvironments of tumor cells and healthy cells. The redox-responsive system is heading toward low toxicity and high efficacy with the addition of different reducing components or functional moieties. RRDDS are thought to be effective for attacking tumors among various internal stimuli-responsive systems since the concentration of GSH in cancerous tissue is approximately four times that of regualar tissue [69]. Furthermore, as we have discussed in the earlier sections, the elevated intracellular concentration of GSH (about 2–10 mM) in comparison to the external environment (2–20 mM) makes RRDDS suitable for intracellular administration. The reduction of thiolated polymers under physiological reducing circumstances, however, takes many hours, according to several research [70], which might be an eventual obstacle that has to be addressed. In order to treat laryngopharyngeal cancer, Sun et al. [71]

described using redox-responsive micelles to specifically initiate drug discharge in the TME. Heparosan and deoxycholic acid were created using a disulfide link to create a redox-responsive amphiphilic polymer, which increased the effectiveness of NPs against cancer. The polymer micelles encapsulated DOX and self-assembled with a good drug-loading capacity. Our lab created a reducible disulfide link among the hydrophilic polyethylene glycol (PEG) block and the hydrophobic polymethacrylate-cholesterol block (DOX-encapsulated-SS-NPs) in order to enhance the discharge of DOX from stable, cholesterol-based polymeric NPs [72].

3.3 Neurological disorders

Among the most well-known CNS diseases are neurological illnesses like Parkinson's disease, epilepsy, dementia, Huntington's disease, amyotrophic lateral sclerosis, brain stroke, Alzheimer's disease, multiple sclerosis, and traumas. The existence of blood-central nervous system (CNS) barriers or commonly referred to as Blood-Brain Barrier (BBB) is primarily responsible for the advanced medical care for these illnesses. These barriers are essential partitions between blood in circulation and brain tissue that prevent or limit the migration of immune cells, xenobiotics, and toxic metabolites into the central nervous system [73]. They can regulate ion and molecule dynamics as well as brain homeostasis because of their special defensive qualities. Both neuroinflammation and neurodegeneration may result from the BBB's structural integrity breaking eroded. The BBB can nevertheless present significant obstacles to the transport of therapeutic drugs into the brain, even if it becomes dysfunctional and more permeable in a number of high incidence brain illnesses (such as AD, PD, and MS) [74]. SCA2, a widely preferred RRNC affects extra-cerebellar central routes, the cerebellum itself, fiber connections in the cerebellum, and the peripheral nervous system. An effective clinical diagnostic test for redox balance may be a powerful tool to manage the progression of neurodegeneration linked to an imbalance in oxidative capability in SCA2. Antioxidants such as bioactive compounds may be a possible treatment for these fatal illnesses because oxidative stress is recognized to be a more prevalent feature in the pathophysiology of SCAs despite their variability. To facilitate the targeted delivery of the neuroprotective drug NR2B9C, bioengineered "core-shell" nanoparticles sensitive to reactive oxygen species (ROS), referred to as SHp-RBC-NP, were proposed. These nanoparticles were designed by modifying dextran (DEX) with a ROS-responsive boronic ester and coating them with a red blood cell (RBC) membrane functionalized with a stroke-homing peptide (SHp). In vivo experiments using rat models of middle cerebral artery occlusion demonstrated prolonged systemic distribution of NR2B9C, improved targeting to ischemic tissues, and a reduction in brain injury. Meanwhile, in vitro tests showed its protective action against glutamate-induced cell toxicity in PC-12 cells.

4. Challenges

The foremost challenge when it comes to not just redox-responsive NCs but toward any stimuli-responsive NCs is that they all in-depth research in certain areas or they find out the lack of literature. When treating intracellular infections like Francisella tularensis, redox-responsive NCs that can release the loaded medication intracellularly with selectivity are quite useful [75]. RRNCs for the targeted administration of antibiotics, however, have a very small body of research. It's interesting to note that there is just one ionic microenvironment-responsive NPs with documented antibacterial activity. In 2015, X. Huang and colleagues developed a new biocompatible model of alginate and

nanosilver that has anti-S. aureus action. In this work, the amount of wound exudates controls the alginate composites' swelling, which controls the medication release of nanosilver. In order to prevent cytotoxicity and lower the bacterial load both in vitro and in vivo, the dosage of nanosilver stayed constant. Additionally, these, rapidly responsive, redox-stimulated responsive polymeric NCs have potential uses in tissue engineering, tumor treatment, and antioxidants. The intricate process of creating RRNC elements and their partial breakdown in vivo present additional difficulties for this system, despite the obvious chemical bonding mechanism that enables them to react. It is quite difficult to accomplish a thorough immune activation mechanism with just one NC. In order to create programmable immune-activating nanocarriers, Chen et al. used thioketal chemical bonds and supramolecular assembly [76]. By automatically changing in response to tumor-specific stimuli and completing a series of steps to elicit strong antitumor immune responses, the nanocarriers might function as an in situ cancer vaccination following intravenous administration. Ensuring the safety and effective targeting of nanocarriers within biological systems is a critical priority. The diverse types of nanocarriers, along with their unique physical and chemical characteristics, exhibit therapeutic actions and metabolic patterns in vivo that contrast sharply with those of traditional small-molecule or macromolecular drugs. Many nanocarriers incorporate various elements, including polymers, photosensitizers, fluorescent dyes, metallic components, and inorganic materials. These foreign components are often detected and cleared by the immune system, but their low clearance rates can result in accumulation within the body, potentially leading to toxicity. Therefore, a detailed assessment of their biocompatibility and degradation is crucial. In addition, nanocarriers are influenced by factors such as circulation in the bloodstream and distribution across different organs, which impact their functionality. Their distinct physicochemical, pharmacokinetic, and therapeutic properties require comprehensive investigation to enhance their efficacy. Effective nanomedicine design must consider these unique attributes to address delivery challenges and improve clinical outcomes. To tackle these issues, nanocarrier synthesis must prioritize environmentally friendly approaches aligned with green chemistry principles. The process should be straightforward, safe, and efficient, converting low-cost and readily available materials into nanocarriers. Moreover, strict standards for characterization and quality control must be developed, ensuring consistent structural, compositional, and functional properties such as particle size, dispersion, surface charge, and chemical stability. Inspiration from biological processes and the unique features of the TME could lead to the discovery of diverse chemical response bonds, opening new pathways for innovation in nanomedicine.

5. Advances and future prospect

RRNCs represent a groundbreaking innovation in drug delivery systems, leveraging the unique redox gradients within biological environments to achieve precise and controlled drug release. Tumor tissues and diseased cells often exhibit elevated levels of ROS and an altered GSH concentration, creating an ideal environment for RRNCs to activate. Over the years, advances in material engineering and nanotechnology have enabled the development of RRNCs with enhanced specificity, stability, and responsiveness to these redox triggers.These systems have demonstrated significant potential in improving the therapeutic efficacy of anticancer, anti-inflammatory, and gene therapies while minimizing systemic side effects. As research progresses, the incorporation of smart design elements, such as dual or multi-responsive functionalities and bioinspired materials, is paving the way for next-generation RRNCs. Looking ahead, efforts are focused on overcoming challenges like premature

drug leakage, scalability in manufacturing, and ensuring biocompatibility to enable clinical translation. The future of RRNCs lies in their integration with advanced technologies such as artificial intelligence and personalized medicine to optimize delivery and therapeutic outcomes.

5.1 Dual responsive NPs for combination therapy

Owing to the limits of single intrinsic and external stimuli, the majority of stimuli-responsive nanomedicines still have inadequate drug release characteristics while being circulated and off-target discharge in normal tissues. The limited absorption into tissue of external stimuli restricts the use of external stimuli-responsive nanotherapeutics to superficial tumors, despite the fact that they provide for more flexible controllability of drug release through the simplicity of modification of physical stimuli [77,78]. Moreover, since tumors are heterogeneous, various malignancies have varying ROS and GSH concentrations, and even different parts of the same tumor have varying GSH/ROS levels [79]. GSH or ROS targeting single-responsive DDSs might therefore exhibit poor response effectiveness and specificity [80]. Redox dual-triggered NPs have a greater drug-release efficiency and a quicker reaction rate than single-responsive NPs. Consequently, in a variety of clinical circumstances, DDSs with dual response show greater promise [81]. For the construction of responsive NPs, certain chemical components, such diselenide and a-dicarbonyl thioether, may interact with ROS and GSH at the same time. Because tumor cells proliferate so quickly, the TME exhibits aberrant metabolism, which results in lower pH, greater ROS levels, and higher GSH concentrations in contrast to regular tissues. Consequently, prodrug nanotherapeutics that contain cleavable chemical bonds that conjugate chemotherapeutic pharmaceuticals can react to certain cues in the cancerous microenvironment and speed up drug release without causing early leakage while in circulation [82].

Ionizable groups can exhibit pH sensitivity without forming covalent bonds by becoming protonated or deprotonated under varying pH conditions. By integrating acid-responsive moieties and ROS-reactive components, nanomedicine can enhance the targeted delivery of chemotherapy drugs, achieving tumor-specific retention through charge reversal and triggering drug release via ROS. For example, Zhang's team developed a polymeric nanomicelle system that responds to both pH and ROS for improved cancer treatment [83]. In this formulation, doxorubicin (DOX) was enclosed within micelles composed of methionine-modified dimethylmaleic anhydride (DMA) and methoxyl poly (ethylene glycol)-block-poly(L-lysine)-graft-α-tocopheryl succinate. Within the acidic environment of the tumor, DMA experienced a shift in charge, which facilitated greater nanoparticle absorption by the cancerous cells. Concurrently, reactive oxygen species (ROS) produced by the interaction of α-tocopheryl succinate (TOS) with mitochondrial respiratory complex II led to the oxidation of the thioether groups in poly-methionine segments, converting them into water-soluble sulfoxide moieties. This change initiated the disassembly of the polymeric micelles, amplifying their breakdown and releasing DOX for chemotherapy. Nanosystems that can promote the release of anticancer drugs by lowering circumstances have also gained traction in oncology therapies as a result of the significant alterations in GSH levels in tumors [84]. These NCs, which are responsive to both lysosomal pH and cytoplasmic GSH, have gained significant attention due to their ability to be activated by a "dual-key" mechanism, integrating both redox and pH conditions. To achieve aggregation-induced emission (AIE) for active imaging and redox- and pH-responsive doxorubicin (DOX) release for chemotherapy, Wang's team developed a polymeric micelle system [85]. DOX can be enclosed within self-assembled poly(ethylene glycol) methyl poly(TPE-co-2-azepane ethyl methacrylate) micelles, relying on the

hydrophobic forces and π-π stacking interactions between DOX and TPE. Under low pH circumstances, water-resistant AEMA's polarity reversal depolymerized the micelles, releasing DOX. A figure representing the usage of dual pH-redox responsive NCs for cancer treatment has been provided below, which also encloses a list of stimuluses, usually used for creating dual stimulus responsive NCs.

5.2 Multi-responsive NCs

Designing multi-responsive NCs may be possible through the integration of various endogenous and/or exogenous stimuli. It is theoretically possible to produce more accurate and specific regulated drug dischrge in smart nanomedicine by requiring additional stimuli. Therefore, for safe and efficient cancer treatment, multi-responsive NCs may supply therapeutic medications with more specificity than mono- or dual-responsive nanomedicines.

For tumor-specific multistimulus-initiated cancer treatment, Luo's group reported a nano-aggregate based on a PEGylated polymeric bioconjugate [86]. PEGylated N-(1,3-dihydroxypropan-2-yl)methacrylamide, connected by a peptide linker responsive to cathepsin B and conjugated with DOX via pH-sensitive hydrazone bonds, can spontaneously aggregate to form these nano-aggregates. PEG detachment and the subsequent disintegration of nano-aggregates may result from the combination of cathepsin B-induced peptide linker fragmentation and GSH-mediated disulfide bond reduction in the tumor environmentTo deliver the chemotherapeutic agents DOX and JQ1 under the combined stimuli of GSH, MMP-2, and NIR for cancer therapy, Yu and colleagues developed a sequential prodrug nanovesicle system [87]. The prodrug nanovesicle was composed of DOX, the PEGylated NIR-II dye IR-1061, and a JQ1 prodrug connected by a reduction-sensitive disulfide spacer. To enhance tumor targeting, the MMP-2-sensitive heptapeptide spacer in PEGylated IR-1061 facilitates the removal of the PEG coating. This removal allows GSH to access the disulfide bond in the JQ1 prodrug, triggering the release of JQ1 from the nanovesicles. Under conditions of high intracellular GSH, JQ1 is released, leading to a reduction in PD-L1 expression during transcription.

5.3 Future prospectives

There is a lot of promise for RRNCs in nanotherapeutics. The synthetic approach, water-soluble self-assembly, redox-responsive characteristics, and use as drug, gene, and antigen delivery vehicles in various forms of liposomes, polymeric micelles, NGs, and dendrimers have all been thoroughly studied in relation to NPs. Even though many kinds of inorganic NPs were created for administering active drugs, their clinical utility was limited by their restricted control over loading, distribution profile, and biodegradability. Innovative hybrid NPs are proposed as a way to selectively overcome delivery site obstacles and take use of collective features. It is almost possible to overcome pharmacokinetic constraints and improve medication bioavailability, circulation time, sustained release, and targeted administration with the hybrid RDDS nanoplatform.

6. Conclusion

RRNCs have emerged as a transformative approach in the field of drug delivery, offering unparalleled specificity and control in therapeutic applications. By capitalizing on the unique redox gradients in pathological environments, these nanocarriers enable precise and efficient drug release, enhancing the

efficacy of treatments while minimizing systemic side effects. Over the years, advances in material science and nanotechnology have led to the development of highly versatile RRNCs with tailored functionalities to address complex medical challenges, particularly in oncology, inflammatory diseases, and gene therapy. Despite their immense potential, RRNCs face hurdles such as premature drug release, scalability issues in production, and ensuring long-term biocompatibility and biodegradability. Addressing these challenges through innovative design strategies, green synthesis methods, and integration with cutting-edge technologies like artificial intelligence will be pivotal for their successful translation into clinical settings. Furthermore, the combination of RRNCs with personalized medicine approaches promises to revolutionize therapeutic strategies, offering tailored treatments based on individual redox profiles and disease characteristics.

In conclusion, RRNCs represent a promising frontier in nanomedicine, with the potential to redefine the landscape of drug delivery. Continued research and collaborative efforts are essential to overcome current limitations, optimize their design, and fully realize their clinical impact. With sustained innovation, RRNCs are poised to become a cornerstone of next-generation therapeutic systems, driving advancements in precision medicine and improving patient outcomes worldwide.

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CHAPTER

Enzyme-responsive nanocarriers: Revolutionizing biomedical applications

13

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1. Introduction to enzyme-responsive nanocarriers

1.1 Overview of nanocarriers in biomedical applications

Nanocarriers have emerged as a revolutionary platform in the field of biomedicine, offering unprecedented capabilities for targeted delivery of therapeutic agents [1]. These nanoscale vehicles are engineered to navigate the complex biological environments within the human body, delivering drugs, genes, or other therapeutic substances directly to the diseased cells or tissues, while minimizing off-target impacts [2,3]. This targeted delivery enhances the efficacy of treatments and reduces the risk of side effects, representing a significant advancement over traditional therapeutic methods [4]. Various types of nanocarriers have been developed, including liposomes [3], polymeric nanoparticles [5], dendrimers [6], carbon nanotubes [7], gold nanoparticles [8], and magnetic nanoparticles [9]. Liposomes are lipid-based vesicles capable of encapsulating both hydrophilic and hydrophobic drugs, which can be engineered to release their payload in response to specific stimuli, such as pH or temperature changes [10]. Polymeric nanoparticles, made from biodegradable polymers, can be tailored to release their contents in a controlled manner over time, offering significant flexibility in terms of size, shape, and surface characteristics [11]. Dendrimers are highly branched, tree-like structures, that provide multiple attachment points for drug molecules, enhancing drug solubility, stability, and bioavailability [12]. Carbon nanotubes are cylindrical nanostructures composed of carbon atoms, that can be functionalized to carry drugs, proteins, or genes, and offer high surface area and unique physical properties such as electrical conductivity [13]. Gold nanoparticles possess unique optical and electronic properties, which can be utilized for imaging, diagnostic and therapeutic purposes, and can be functionalized with various molecules to access the target of specific biological processes [14]. Magnetic nanoparticles can be manipulated using external magnetic fields, making them ideal for targeted drug delivery and hyperthermia treatment [15]. These nanocarriers can deliver therapeutic agents through several mechanisms, including passive targeting, active targeting, and stimuli-responsive release [16]. Passive targeting leverages the enhanced permeability and retention (EPR) effect, allowing nanocarriers to accumulate in tumor tissues due to their leaky vasculature and poor lymphatic drainage [17]. Active targeting involves surface modification of nanocarriers with ligands

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such as antibodies or peptides, which enables them to bind specifically to targeted cells or receptors [18]. Stimuli-responsive release directs nanocarriers to release their payload in response to specific internal or external stimuli, such as pH, temperature, enzymes, or light, thereby ensuring the therapeutic agent is released in the right place and at the right time [19]. Nanocarriers have a wide range of applications in medicine, including cancer therapy, where they deliver chemotherapeutic agents directly to tumor cells, reducing systemic toxicity and enhancing therapeutic outcomes [20,21]. In gene therapy, they deliver genetic material, such as DNA or RNA, to specific cells to correct genetic defects, modulate gene expression, protect genetic material from degradation and enhance its uptake by targeted cells [22]. Nanocarriers also improve vaccine efficacy by encapsulating antigens and delivering adjuvants to boost the immune response [23]. In antimicrobial therapy, targeted delivery of antimicrobial agents improves treatment efficacy and reduces resistance risk, while in the case of cardiovascular diseases, nanocarriers deliver drugs to specific sites within the cardiovascular system for conditions such as heart disease and stroke [24]. The field of nanocarriers is rapidly evolving, with ongoing research focused on improving their efficacy, specificity, and safety. Advances in nanotechnology, materials science, and molecular biology are driving the development of new types of nanocarriers and novel drug delivery mechanisms, as well as integrating with personalized medicine approaches and multifunctional capabilities. As research continues to advance, the potential for nanocarriers to revolutionize the treatment of various diseases is immense, paving the way for more effective and personalized medical therapies.

1.2 Importance of enzyme-responsive systems

The integration of enzyme-responsive mechanisms in nanocarriers marks a significant innovation in the field of drug delivery and biomedical applications. Enzyme-responsive nanocarriers are designed to respond to specific enzymatic activities, releasing their therapeutic payload in a controlled and targeted manner. This specificity leverages the unique enzymatic profiles associated with different tissues and pathological conditions, enabling precision medical approaches that enhance therapeutic efficacy, while minimizing side effects [25]. Enzyme-responsive nanocarriers employ enzyme-sensitive linkers, which are chemical bonds or linkers cleaved by specific enzymes to trigger the release of encapsulated drug. The selection of these linkers is crucial, as they must align with the target enzyme's specificity and activity. These nanocarriers are typically constructed from materials such as biodegradable polymers or lipids, which provide structural integrity and biocompatibility, ensuring stability in the bloodstream, while being responsive to enzymatic degradation at the target site [26].

The operational mechanism of enzyme-responsive nanocarriers involves a series of orchestrated steps: targeting and accumulation, enzymatic recognition, and payload release. Initially, the nanocarriers circulate in the bloodstream and accumulate at the target site through passive or active targeting mechanisms. Upon encountering the specific enzymes that are over-expressed or uniquely present in the diseased tissue, these enzymes recognize and cleave the enzyme-sensitive linkers [27]. This cleavage destabilizes the nanocarrier matrix, resulting in the controlled release of the therapeutic payload directly at the site of interest. This localized release maximizes therapeutic efficacy, while minimizing systemic side effects [16].

Enzyme-responsive nanocarriers have demonstrated significant potential across various biomedical applications. In cancer therapy, they exploit the aberrant enzyme expression in tumors, such as matrix metalloproteinases (MMPs) and cathepsins, to selectively release chemotherapeutic agents within the

tumor's microenvironment. This approach enhances drug's cytotoxicity impact to cancer cells, while sparing healthy tissues and reducing systemic toxicity [28]. In inflammatory diseases, these nanocarriers target elevated levels of proteases and other enzymes to deliver anti-inflammatory drugs precisely where needed, mitigating inflammation and tissue damage. For infectious diseases, enzyme-responsive nanocarriers target specific enzymes secreted by pathogens, improving the delivery of antimicrobial agents and enhancing efficacy against resisting strains, while minimizing the impact on beneficial microbiota [29]. In neurodegenerative disorders, they cross the blood-brain barrier and release therapeutic agents in response to enzymes associated with conditions such as Alzheimer's or Parkinson's disease, offering new treatment avenues. Additionally, in regenerative medicine, enzyme-responsive nanocarriers deliver growth factors or other regenerative agents in response to enzymes involved in tissue repair and regeneration, promoting healing and recovery in damaged tissues [30].

The future of enzyme-responsive nanocarriers in biomedicine is promising, with ongoing research focused on enhancing their precision, efficiency, and range of applications [31]. Key areas of development include the creation of multifunctional nanocarriers that combine enzyme responsiveness with other stimuli-responsive features, such as pH or temperature sensitivity, and the tailoring of nanocarriers to the specific enzymatic profiles of individual patients for personalized treatments. Advances in targeting strategies, such as ligand-receptor interactions and biomimetic surfaces, will further enhance the specificity and efficacy of these nanocarriers. Bridging the gap between laboratory research and clinical application involves addressing challenges related to scalability, regulatory approval, and safety [32,33]. In summary, enzyme-responsive nanocarriers represent a cutting-edge approach in biomedicine, offering targeted, efficient and safe delivery of therapeutic agents. By capitalizing on the unique enzymatic landscapes of various diseases, these nanocarriers enhance therapeutic outcomes, minimize side effects, and pave the way for more personalized and effective medical therapies. As research and development continue, the potential for enzyme-responsive nanocarriers to revolutionize the treatment of numerous diseases is immense, heralding a new era in precision medicine [34].

1.3 Historical perspective and evolution

The concept of enzyme-responsive nanocarriers has evolved significantly over the past few decades, driven by advances in nanotechnology, materials science, and a deeper understanding of enzymatic processes in biological systems. The historical perspective and evolution of these innovative systems highlight the progress from basic research to sophisticated clinical applications, underscoring their transformative potential in biomedicine [31]. In the early stages of this evolution, the development of nanocarriers was focused primarily on passive targeting mechanisms, utilizing the enhanced permeability and retention (EPR) effect for accumulation in tumor tissues. However, this approach faced limitations due to the heterogeneity of tumor vasculature and variability in patient responses. The need for more precise and controlled drug delivery systems led researchers to explore stimuli-responsive nanocarriers, which could release their payload in response to specific environmental triggers such as pH, temperature or light [35].

The exploration of enzyme-responsive mechanisms began in earnest in the late 1990s and early 2000s [36], inspired by the recognition that certain diseases, including cancer, inflammatory conditions, and infectious diseases, are characterized by distinct enzymatic signatures. Early research demonstrated the feasibility of using enzyme-sensitive linkers in polymeric drug delivery systems.

These linkers could be cleaved by specific enzymes, resulting in controlled release of drugs at the desired site of action. This marked a significant departure from previous approaches by introducing a new level of specificity and control in drug delivery [37]. As the field progressed, the design and synthesis of enzyme-sensitive linkers became more sophisticated. Researchers began to tailor these linkers to respond to a wider range of enzymes, including MMPs, cathepsins, and other proteases, which are often over-expressed in diseased tissues [38]. This period also saw the development of novel nanocarrier platforms, such as dendrimers, liposomes, and polymeric nanoparticles, which provided versatile and biocompatible matrices for incorporating enzyme-responsive mechanisms [39].

The mid-2000–2010s witnessed significant advances in the functionalization and targeting capabilities of enzyme-responsive nanocarriers [40,41]. Techniques for surface modification that target ligands such as antibodies, peptides, and small molecules, were refined, enabling active targeting of specific cells or tissues. This era also saw the integration of multiple stimuli-responsive features into a single nanocarrier, allowing for even greater control over drug release in response to complex biological environments [42]. Recent years have been characterized by a focus on translating these advanced nanocarrier systems from the laboratory to clinical settings. This involves addressing challenges related to scalability, reproducibility, and regulatory approval. Clinical trials are now exploring the efficacy and safety of enzyme-responsive nanocarriers in various therapeutic areas, including oncology, infectious diseases, and regenerative medicine [43]. Additionally, advances in personalized medicine are driving the customization of enzyme-responsive nanocarriers to match the unique enzymatic profiles of individual patients, further enhancing the precision and effectiveness of treatments. Throughout their evolution, enzyme-responsive nanocarriers have transitioned from a conceptual innovation to a practical tool with significant clinical potential. This journey reflects the broader trends in nano-medicine, where interdisciplinary collaboration and technological advancements continue to push the boundaries of what is possible. As research in this field continues to advance, enzyme-responsive nanocarriers are poised to play an increasingly important role in the development of more effective, targeted, and personalized therapeutic strategies, heralding a new era in the treatment of complex diseases [26,44].

2. Mechanisms of enzyme-responsive nanocarriers

2.1 Types of enzymes utilized in responsive systems

Enzyme-responsive systems have garnered significant attention in the field of targeted drug delivery and biomedical applications due to their ability to leverage the specific catalytic activities of enzymes to achieve precise control over the release of therapeutic agents [45]. Proper selection of enzymes for these systems is crucial, as it determines the specificity and efficiency of the drug release process. The enzymes most commonly utilized in responsive systems include proteases, glycosidases, lipases, and oxidoreductases [31]. Each class of enzymes offer unique advantages based on their substrate specificity and the pathological context in which they are active.

Proteases are among the most extensively studied enzymes for responsive systems [46]. They catalyze the hydrolysis of peptide bonds in proteins and peptides, and their activity is often upregulated in pathological cases such as cancer, inflammation, and infectious diseases. MMPs are a prominent subgroup of proteases used in these systems. MMPs are over-expressed in the tumor microenvironment, and play a crucial role in tissue remodeling and metastasis [47]. By designing

nanocarriers with MMP-sensitive linkers, researchers can achieve targeted drug release, specifically within tumor sites. Cathepsins, as another group of proteases, are lysosomal enzymes involved in protein degradation and are up-regulated in various cancers and inflammatory diseases. Incorporating cathepsin-sensitive linkers into drug delivery systems ensures that the therapeutic payload is released within lysosomes, enhancing the treatment efficacy for intracellular targets [48,49].

Glycosidases are enzymes that hydrolyze glycosidic bonds in carbohydrates, glycoproteins, and glycolipids [50]. These enzymes are particularly relevant in the context of lysosomal storage diseases and certain cancers, where glycosidase activity is aberrantly regulated. β-glucuronidase is a notable example used in responsive systems. It is highly expressed in the necrotic regions of tumors under certain inflammatory conditions [51]. Nanocarriers equipped with β-glucuronidase-sensitive linkers can release their drug payload in response to the enzyme's activity, ensuring targeted delivery to diseased tissues [52]. Another example is α-amylase, which is utilized in the treatment of pancreatic disorders and some metabolic diseases. Incorporating α-amylase-responsive components in drug delivery systems allows for the controlled release of therapeutics in the gastrointestinal tract or specific metabolic environments [53].

Lipases catalyze the hydrolysis of ester bonds in lipids, playing a pivotal role in lipid's metabolism. Their activity is elevated in various pathological conditions, including obesity, pancreatitis, and certain cancers [54]. Phospholipase A2 (PLA2) is a well-studied enzyme in this category. It is over-expressed in inflammatory diseases and certain tumors. Drug delivery systems that respond to PLA2 can release their cargo in inflamed tissues or tumor sites, improving therapeutic outcomes, while reducing systemic side effects [55]. Another example is lipoprotein lipase, which is involved in the metabolism of triglyceride-rich lipoproteins. By designing nanocarriers that respond to lipoprotein lipase activity, researchers can target lipid-related metabolic disorders more effectively [56]. Oxidoreductases catalyze oxidation-reduction reactions, and are involved in various metabolic pathways. Their dysregulation is associated with numerous diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders [57]. One important oxidoreductase utilized in responsive systems is glutathione S-transferase (GST). GST is over-expressed in many tumors and contributes to drug resistance by conjugating glutathione to chemotherapy agents, rendering them inactive. By incorporating GST-sensitive linkers in drug delivery systems, researchers can design pro-drugs that release active therapeutic agents specifically in the presence of GST, thus overcoming drug resistance [58]. Another example is myeloperoxidase (MPO), which is involved in inflammatory responses. MPO-responsive nanocarriers can release anti-inflammatory drugs at inflammation sites, providing targeted therapy for inflammatory diseases [59].

2.2 Enzyme-triggered release mechanisms

The enzyme-triggered release mechanisms in nanocarrier systems are a cornerstone of modern targeted drug delivery, providing sophisticated means of achieving site-specific therapeutic actions. These mechanisms rely on the unique catalytic properties of enzymes to trigger the release of encapsulated drugs in response to specific biological signals. This precision ensures that therapeutic agents are released primarily at the site of interest, thereby maximizing efficacy and minimizing systemic side effects [60]. At the core of enzyme-triggered release mechanisms is the incorporation of enzyme-sensitive linkers or substrates into the nanocarrier design. These linkers are strategically chosen based on their susceptibility to cleavage by specific enzymes, that are either over-expressed or

uniquely present in the target tissue. When the nanocarriers reach the target site, the local enzymatic environment acts upon these linkers, cleaving them and causing the release of the drug payload [26,38]. One of the most common mechanisms involves protease-sensitive linkers. Proteases, such as MMPs and cathepsins, are frequently over-expressed in the tumor's microenvironment and in inflamed tissues [49]. Nanocarriers designed with peptide linkers susceptible to these proteases remain intact during circulation but undergo cleavage and subsequent drug release upon encountering the target protease [61]. For instance, in cancer therapy, MMP-sensitive nanocarriers can release their chemotherapeutic payload directly within the tumor matrix, enhancing local drug concentration and reducing systemic toxicity [62]. Another critical mechanism leverages the activity of glycosidases. Glycosidase-sensitive linkers are particularly useful in targeting lysosomal storage diseases and certain tumors [51]. Enzymes such as β-glucuronidase, which are up-regulated at specific pathological conditions, can cleave glycosidic bonds in the linkers, triggering the release of encapsulated drugs. This method ensures that the therapeutic agents are released in response to the specific enzyme profile of the diseased tissue, thus achieving high specificity [63]. Lipase-sensitive linkers represent another important class of enzyme-triggered mechanisms. Lipases such as phospholipase A2 (PLA2) are over-expressed in inflammatory conditions and certain cancers. Nanocarriers equipped with lipase-sensitive linkers can remain stable during circulation but disassemble in the presence of lipases, releasing their drug payload. This approach is particularly advantageous for treating inflammatory diseases and cancers, where lipase activity has markedly increased [64,65]. Oxidoreductases also play a pivotal role in enzyme-triggered release mechanisms. Enzymes such as glutathione S-transferase (GST), which are often over-expressed in drug-resistant tumors, can cleave specific linkers, thereby releasing active drugs. This strategy not only targets the tumor cells, but also helps in overcoming drug resistance by ensuring the active drug is released in the vicinity of resistant cells [66,67]. In addition to single enzyme-responsive systems, there is growing interest in designing multifunctional nanocarriers that respond to multiple enzymatic stimuli. These advanced systems incorporate linkers that can be cleaved by different enzymes, providing a higher level of control and specificity [32,68]. For example, a nanocarrier might be designed to release its payload only in the presence of both MMPs and cathepsins, ensuring that the drug is delivered only in the highly specific microenvironment of a tumor [69]. The design and optimization of enzyme-sensitive linkers are crucial for the success of enzyme-triggered release systems. Factors such as the linker's stability in the bloodstream, the rate of enzymatic cleavage, and the resulting release kinetics of the drug must be carefully balanced. Advanced synthetic techniques and molecular engineering are continually improving the precision and effectiveness of these linkers, enabling the development of highly efficient enzyme-responsive nanocarriers [70–72]. Another innovative approach in enzyme-triggered release mechanisms involves application of prodrugs. Prodrugs are inactive derivatives of drugs that can be converted into their active form by enzymatic action [73]. By incorporating prodrug strategies into nanocarriers, it is possible to achieve a two-step targeting mechanism: the nanocarrier delivers the prodrug to the target site, and the local enzymatic environment converts the prodrug into its active form, providing a highly localized therapeutic effect [74].

Overall, enzyme-triggered release mechanisms offer a powerful and precise method for targeted drug delivery, capitalizing on the specific enzymatic profiles of diseased tissues to achieve controlled and site-specific drug release. These systems not only enhance the therapeutic efficacy of drugs but also significantly reduce the potential for off-target effects and systemic toxicity. As research in this field advances, the continued development and refinement of enzyme-sensitive linkers and

multifunctional nanocarriers will undoubtedly lead to more effective and personalized treatments for a wide range of diseases. This approach holds immense promise for the future of precision medicine, offering new solutions for some of the most challenging medical conditions [60,75].

2.3 Principles of designing enzyme-responsive nanocarriers

The design of enzyme-responsive nanocarriers is a sophisticated process that integrates principles of nanotechnology, materials science, and biochemistry to achieve precise and efficient drug delivery systems. These nanocarriers are engineered to exploit the specific enzymatic activities present in diseased tissues, ensuring targeted and controlled release of therapeutic agents [76]. Fig. 13.1 shows the key principles of designing enzyme-responsive nanocarriers.

- **Selection of Enzyme-Sensitive Linkers:**

Enzyme-sensitive linkers are pivotal in the design of responsive nanocarriers. These linkers are cleaved by specific enzymes over-expressed in target tissues. The choice of linker is based on enzyme's substrate specificity, catalytic efficiency and pathological context. Common linkers include peptide sequences for proteases (e.g., MMPs, cathepsins), glycosidic bonds for glycosidases (e.g., β-glucuronidase), and ester bonds for lipases (e.g., PLA2). The stability of these linkers in systemic circulation and their rapid cleavage at the target site are crucial for ensuring precise drug release [49,77].

- **Nanocarrier Composition and Structure:**

The composition of nanocarrier's matrix is integral to its functionality. Biocompatible and biodegradable materials such as lipids, polymers, and dendrimers are the ones often used. These materials provide structural integrity during circulation and allow for controlled degradation upon enzymatic action. The surface chemistry of nanocarriers is also tailored to enhance circulation time and evade immune detection, mostly through the incorporation of polyethylene glycol (PEG) [78,79].

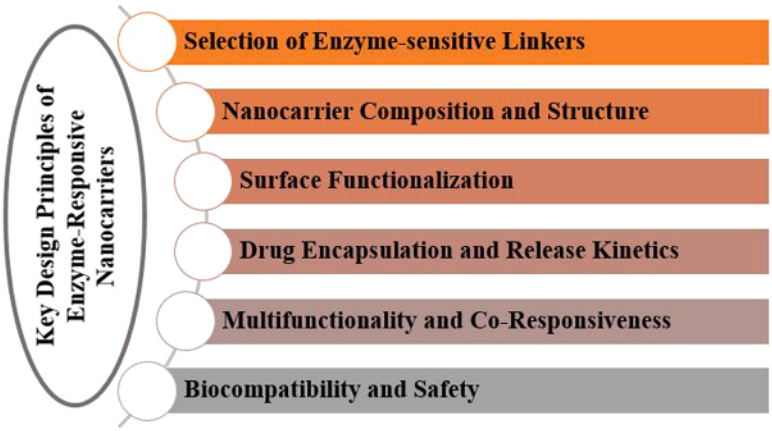


FIGURE 13.1

The important keys in designing enzyme-responsive nanocarriers.

- **Surface Functionalization:**

Surface functionalization with targeting ligands, such as antibodies, peptides, or small molecules, enhances the specificity of nanocarriers for their target cells. These ligands bind to over-expressed receptors on the surface of target cells, facilitating the accumulation of nanocarriers at the desired site. Combining active targeting with enzyme-responsive release, further refines the precision of drug delivery [80].

- **Drug Encapsulation and Release Kinetics:**

The method of drug encapsulation within nanocarriers affects the release kinetics. Drugs can be loaded into the nanocarrier matrix through physical entrapment, covalent attachment, or by forming prodrugs that are activated upon enzymatic cleavage. The release kinetics are designed to align with the rate of enzymatic cleavage, ensuring a sustained and controlled drug release [81].

- **Multifunctionality and Co-Responsiveness:**

Advanced designs incorporate multifunctional and co-responsive capabilities, where nanocarriers respond to multiple stimuli (e.g., pH, temperature, redox conditions) in addition to enzymatic activity. This co-responsiveness enhances the specificity and effectiveness of drug delivery, providing a more comprehensive response to the complex microenvironment of diseased tissues [82].

- **Biocompatibility and Safety:**

Ensuring the biocompatibility and safety of enzyme-responsive nanocarriers is paramount. The selected materials must be non-toxic, and their degradation products should not induce adverse reactions. Extensive in vitro and in vivo testing is conducted to evaluate the safety profile and therapeutic efficacy of these nanocarriers [83].

In summary, the design of enzyme-responsive nanocarriers requires a meticulous balance of material properties, enzymatic specificity, and biological interactions. By adhering to these design principles, researchers can develop highly targeted and efficient drug delivery systems that enhance therapeutic outcomes, minimize side effects and pave the way for advancements in precision medicine.

3. Synthesis and fabrication of enzyme-responsive nanocarriers

(Fig. 13.2) General schematic nanocarrier fabrication methods, functionalization techniques for enzyme responsiveness, and characterization processes for enzyme responsive nanocarrier.

3.1 Nanocarriers' fabrication methods

The fabrication of enzyme-responsive nanocarriers involves a combination of advanced materials science techniques and precise biochemical engineering to create effective systems that can deliver therapeutic agents in response to specific enzymatic triggers. The fabrication methods focus on creating nanocarriers with the desired size, shape, surface characteristics, and functional responsiveness to enzymes. The key methods include emulsion techniques, self-assembly processes, chemical conjugation, and surface modification strategies [31].

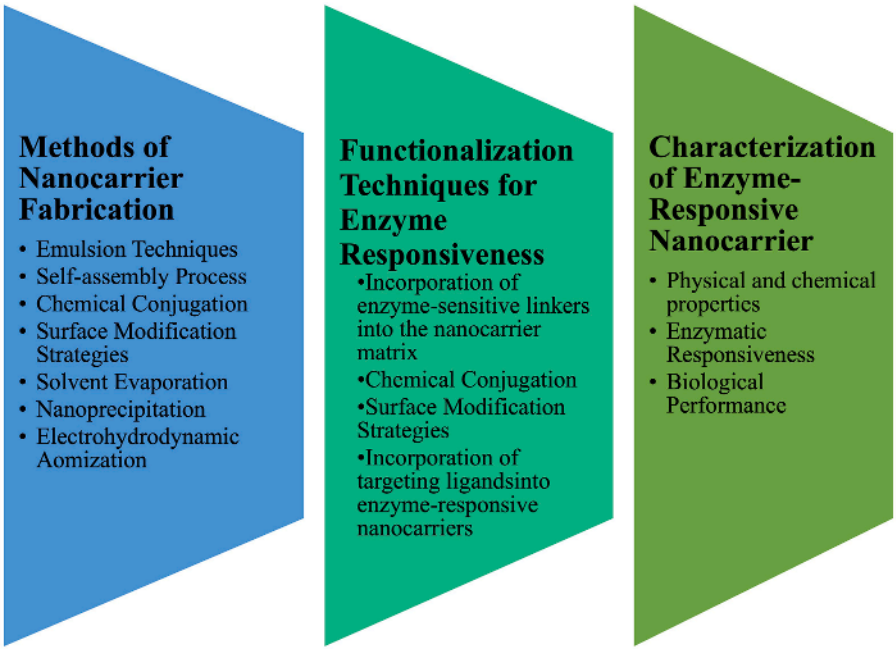


FIGURE 13.2

Displays the summary of important methods and functionalization techniques, which are discussed in the following.

Emulsion techniques, including single and double emulsion methods, are widely used for fabricating polymeric nanocarriers. In these methods, an aqueous solution containing the drug and enzyme-sensitive linkers is emulsified in an organic solvent containing the polymer. The polymer solidifies upon solvent evaporation, forming nanoparticles with the encapsulated drug. Double emulsion techniques are particularly useful for encapsulating hydrophilic drugs. These methods allow precise control over particle size and drug loading efficiency. For example, poly(lactic-co-glycolic acid) (PLGA) nanoparticles can be fabricated using this technique, incorporating enzyme-sensitive peptides for targeted drug release [84,85]. Self-assembly processes leverage the natural tendency of amphiphilic molecules to form nanostructures such as micelles, liposomes, and polymersomes in aqueous environments. Liposomes, for instance, are formed by hydrating a dry lipid film, resulting in bilayer vesicles that can encapsulate both hydrophilic and hydrophobic drugs. Enzyme-responsive elements can be incorporated into the lipid bilayer or attached to the surface to ensure controlled release upon enzymatic action. Similarly, block copolymers can self-assemble into micelles with enzyme-sensitive cores or shells, facilitating targeted drug delivery [86,87].

Chemical conjugation involves attaching enzyme-sensitive linkers to nanocarriers through covalent bonds. This method ensures that the drug is securely bound to the nanocarrier and released only upon enzymatic cleavage [88]. For instance, prodrugs can be chemically conjugated to nanoparticles via peptide linkers sensitive to proteases. This strategy is often used with dendrimers, where multiple drug

molecules are conjugated to the dendrimer surface via enzyme-cleavable linkers, allowing for high drug loading capacity and controlled release [89]. Surface modification strategies are employed to functionalize nanocarriers' surfaces by targeting ligands, protective polymers, or enzyme-sensitive components. Techniques such as layer-by-layer assembly and click chemistry are commonly used for this purpose. In layer-by-layer assembly, alternating layers of oppositely charged polymers which can include enzyme-sensitive layers that degrade upon exposure to specific enzymes are deposited on nanocarrier's surface. Click chemistry, on the other hand, provides a highly efficient and specific way to attach enzyme-sensitive linkers and targeting ligands to nanocarrier's surface, enhancing specificity and functionality [90]. The solvent evaporation method is a straightforward technique, where a polymer and drug are dissolved in a volatile organic solvent. This solution is then emulsified in an aqueous phase containing a surfactant. As the solvent evaporates, nanoparticles form and solidify, encapsulating the drug. Enzyme-responsive linkers can be incorporated into the polymer matrix or on the nanoparticle surface, allowing for controlled drug release upon enzymatic action. This method is particularly useful for fabricating nanoparticles from biodegradable polymers such as PLGA, which can be designed to degrade in response to specific enzymes [91].

Nano-precipitation, also known as the solvent displacement method, involves precipitation of polymers from an organic solvent into an aqueous solution. The polymer, drug, and enzyme-sensitive linkers are dissolved in a solvent that is miscible with water, and this solution is then added to an aqueous phase under stirring. Nanoparticles will form instantaneously as the solvent diffuses into the water, precipitating the polymer. This method allows for a rapid and efficient fabrication of nanoparticles with controlled size and drug loading [92]. Electro-hydrodynamic atomization (EHDA), also known as electro-spraying, is a technique that uses electrical forces to create nanoparticles from a polymer solution. The polymer solution is subjected to a high-voltage electric field, causing it to break up into fine droplets that solidify into nanoparticles. Enzyme-responsive linkers and drugs can be incorporated into the polymer solution before electro-spraying, resulting in nanoparticles that release their payload upon enzymatic cleavage. This method is advantageous for producing nanoparticles with uniform size and morphology [93]. In conclusion, the fabrication of enzyme-responsive nanocarriers involves a variety of sophisticated techniques tailored to achieve precise control over their physicochemical properties and functional responsiveness. These methods ensure that nanocarriers can encapsulate and release therapeutic agents effectively in response to specific enzymatic triggers, enhancing the efficacy and specificity of drug delivery systems. The continuous advancement in fabrication techniques promises to further optimize the performance of enzyme-responsive nanocarriers in clinical applications.

3.2 Functionalization techniques for enzyme responsiveness

Functionalization techniques for enzyme responsiveness play a crucial role in tailoring nanocarriers to respond specifically to enzymatic triggers, enhancing the precision and effectiveness of drug delivery systems. These techniques are designed to incorporate enzyme-sensitive elements into nanocarrier's structure, ensuring that therapeutic agents are released only in the presence of target enzymes. The functionalization process involves various strategies to modify the surface and internal composition of nanocarriers, integrating enzyme-responsive features that facilitate targeted and controlled drug release. One of the primary methods of functionalization is the incorporation of enzyme-sensitive linkers into the nanocarrier's matrix. These linkers are designed to be cleaved by specific enzymes,

resulting in the release of encapsulated drugs. The choice of any linker depends on enzyme's substrate specificity and the desired therapeutic outcome [26,94]. For example, peptide-based linkers that are sensitive to proteases such as MMPs or cathepsins are commonly used in cancer therapy. These linkers are incorporated into polymeric or lipid-based nanocarriers, where they remain stable during circulation but degrade upon encountering the target protease, triggering drug release directly at tumor's site [95,96]. Chemical conjugation is another key technique used in the functionalization of enzyme-responsive nanocarriers. This approach involves covalently attaching enzyme-sensitive groups to the nanocarrier's surface or within its matrix. Chemical conjugation can be achieved through various reactions, such as click chemistry or amidation, which provide high specificity and stability for the attached enzyme-responsive components. For instance, nanocarriers can be functionalized with enzyme-sensitive prodrugs that become activated only when cleaved by specific enzymes, allowing for precise control over drug activation and release [94,97]. Surface modification strategies are also pivotal in enhancing enzyme responsiveness. These modifications often involve the addition of enzyme-sensitive polymers or peptides to nanocarrier's surface. Techniques such as layer-by-layer assembly enable the sequential deposition of enzyme-responsive polymers on nanocarrier's surface, creating a multi-layered coating that responds to enzymatic cleavage. This approach can be used to create a protective outer layer that ensures stability in the bloodstream, while allowing for targeted drug release upon enzyme exposure [98]. Additionally, the incorporation of targeting ligands into enzyme-responsive nanocarriers further refines their functionality [99]. By attaching ligands such as antibodies, peptides, or small molecules that bind to specific cell surface receptors, nanocarriers can be directed to particular tissues or cells. When combined with enzyme-responsive linkers, this dual-functionalization strategy enhances the precision of drug delivery, ensuring that therapeutic agents are released in response to both enzymatic and receptor-specific signals. This is particularly useful in cancer therapy, where nanocarriers can be designed to target tumor-specific antigens and respond to the elevated levels of proteases present in the tumor's micro-environment [100,101]. Another innovative functionalization technique involves the incorporation of prodrugs, which are inactive drug derivatives that become active only after enzymatic cleavage. Prodrugs can be chemically conjugated to the nanocarrier's matrix or surface, ensuring that the drug is released in its active form only upon encountering the specific enzyme at the target site. This approach enhances the localization of the therapeutic effect and minimizes potential side effects associated with the premature release of active drugs [102]. The development of multifunctional nanocarriers represents a significant advancement in enzyme-responsive technology. These carriers integrate multiple stimulus-responsive elements, such as pH, temperature, or light sensitivity, alongside enzyme responsiveness. This multi-stimuli approach allows for more sophisticated control over drug release, addressing the complex and dynamic nature of biological environments. For example, a nanocarrier could be designed to release its drug payload in response to a combination of enzyme activity and pH changes, providing enhanced specificity for targeting disease sites with distinct biochemical profiles [103]. Overall, the functionalization of enzyme-responsive nanocarriers involves a range of techniques aimed at achieving precise and controlled drug release. By incorporating enzyme-sensitive linkers, utilizing chemical conjugation, modifying surfaces, and integrating targeting ligands and prodrugs, researchers can develop highly effective and targeted drug delivery systems. These advanced functionalization strategies enhance the therapeutic efficacy of nanocarriers, while minimizing off-target effects, paving the way for more personalized and effective treatments in various clinical settings [104].

3.3 Characterization of enzyme-responsive nanocarriers

The characterization of enzyme-responsive nanocarriers is a critical aspect of developing effective drug delivery systems, as it ensures that these carriers meet the required specifications for targeted and controlled release. Comprehensive characterization involves assessing several key attributes, including physical and chemical properties, enzymatic responsiveness, and biological performance. Each aspect of characterization provides insights into the nanocarrier's functionality, stability, and suitability for therapeutic applications [105]. The physical and chemical properties of enzyme-responsive nanocarriers are fundamental to their performance and efficacy. Key parameters include particle size, size distribution, shape, and surface charge [64,70]. Techniques such as dynamic light scattering (DLS) and scanning electron microscopy (SEM) are employed to measure particle size and morphology. DLS provides information on hydrodynamic diameter and size distribution in solution, which is crucial for ensuring uniform drug loading and controlled release [106]. SEM offers high-resolution images to evaluate the surface structure and shape of nanocarriers [107]. Surface charge, measured by techniques such as zeta potential analysis, is another important property. It affects nanocarrier's stability, interaction with biological systems, and cellular uptake [108]. A suitable surface charge ensures that nanocarriers remain dispersed in suspension and avoid aggregation, which could impact their effectiveness and safety [109]. Chemical characterization involves analyzing the composition and structure of nanocarriers' materials and functional components. Techniques such as Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy provide information on chemical bonds and molecular structure of polymeric or lipid materials used in nanocarriers. These analyses validate the incorporation of enzyme-sensitive linkers and other functional groups, ensuring that nanocarriers are designed as intended [110].

- **Enzymatic Responsiveness:**

The core functionality of enzyme-responsive nanocarriers relies on their ability to release therapeutic agents in response to specific enzymatic activity. Characterizing enzymatic responsiveness involves several key assessments. First, the rate and extent of enzymatic cleavage of the enzyme-sensitive linkers must be evaluated. This is typically done using in vitro enzyme assays, where nanocarriers are exposed to target enzymes, and the release of the drug or cleavage products is monitored over time [111]. Techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS) are used to quantify the released drug and verify the cleavage of enzyme-sensitive linkers [112]. The stability of enzyme-responsive nanocarriers under physiological conditions is also crucial. This involves assessing the carriers' stability in biologically relevant environments such as simulated body fluids or serum, to ensure they maintain their integrity until reaching the target site. Stability studies often include evaluating nanocarrier's size, surface charge, and drug release kinetics over time [113]. Additionally, the interaction between nanocarriers and target enzymes must be characterized. This involves studying the binding affinity and specificity of the enzyme-sensitive components to the target enzymes. Techniques such as enzyme kinetics assays and fluorescence resonance energy transfer (FRET) can provide insights into the interaction dynamics and efficiency of enzyme-triggered drug release [114].

- **Biological Performance:**

Evaluating the biological performance of enzyme-responsive nanocarriers is essential to determine their therapeutic potential and safety profile. In vitro cell studies assess cellular uptake, cytotoxicity, and drug release profiles. Cellular uptake studies, often performed using fluorescence microscopy or flow cytometry, help determine how efficiently nanocarriers are internalized by target cells. Cytotoxicity assays, such as MTT or CellTiter-Glo assays, evaluate the potential adverse effects of nanocarriers on healthy and target cells [115,116]. In vivo studies provide further insights into nanocarrier's performance within a biological organism. These studies assess bio-distribution, pharmacokinetics, and therapeutic efficacy. Techniques such as imaging (e.g., magnetic resonance imaging, MRI, or positron emission tomography, PET) are used to track the distribution of nanocarriers within the body and monitor their accumulation in target tissues [117]. Pharmacokinetic studies help determine the circulation time, clearance rate, and overall stability of nanocarriers in bloodstream. Furthermore, in vivo studies evaluate the therapeutic outcomes of enzyme-responsive nanocarriers, including their effectiveness in delivering drugs to the target site and their impact on disease progression. Safety assessments are also conducted to identify any potential toxic effects or adverse reactions associated with nanocarriers [118]. Characterizing enzyme-responsive nanocarriers involves a multifaceted approach that integrates physical, chemical, enzymatic, and biological assessments. Each characterization method provides critical information on nanocarriers' design, functionality, and performance, ensuring that they meet the criteria for effective and safe drug delivery. Advances in characterization techniques continue to enhance the development of enzyme-responsive nanocarriers, paving the way for more precise and personalized therapeutic interventions in the treatment of various diseases [31].

4. Biomedical applications of enzyme-responsive nanocarriers

Enzyme-responsive nanocarriers represent a transformative advancement in the field of targeted drug delivery, and have shown substantial promise in various biomedical applications. Their ability to release therapeutic agents in response to specific enzymatic activity allows for a high degree of precision, reducing off-target effects and enhancing therapeutic efficacy. This section delves into the detailed applications of enzyme-responsive nanocarriers across different medical fields, including cancer therapy, cardiovascular diseases, infectious diseases, lysosomal storage disorders, gene therapy and DNA delivery, and targeted drug delivery (Fig. 13.3) [26].

One of the most prominent applications of enzyme-responsive nanocarriers is in cancer therapy [69]. Tumor micro-environments are characterized by the over-expression of various enzymes such as MMPs and cathepsins, which are involved in tumor progression and metastasis [119]. Nanocarriers designed with enzyme-sensitive linkers that degrade in the presence of these specific enzymes can deliver chemotherapeutic agents directly to tumor sites, significantly improving the local concentration of the drug while minimizing systemic toxicity. For example, nanocarriers incorporating MMP-sensitive peptides can release anticancer drugs specifically within the tumor extracellular matrix, where MMPs are overactive. This targeted release not only enhances the effectiveness of chemotherapy, but also reduces the adverse effects on healthy tissues. Additionally, enzyme-responsive systems can be engineered to respond to the typical acidic microenvironment of tumors, further refining the targeting and release process [120]. In the field of cardiovascular diseases, enzyme-

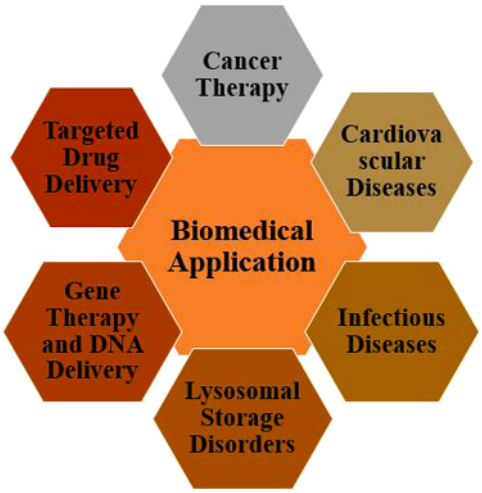


FIGURE 13.3

Biomedical application of enzyme-responsive nanocarriers.

responsive nanocarriers are employed to address conditions such as atherosclerosis and myocardial infarction [121]. Enzymes such as MMPs are involved in the degradation of extracellular matrix components, and play a role in plaque rupture and cardiovascular events. Nanocarriers designed with MMP-sensitive linkers can release therapeutic agents, such as anti-inflammatory drugs or agents that stabilize atherosclerotic plaques, specifically at the sites of enzyme activity [122]. Moreover, enzyme-responsive nanocarriers can be used to deliver drugs that promote tissue repair and regeneration following myocardial infarction. For instance, nanocarriers sensitive to enzymes involved in tissue remodeling can release growth factors or anti-fibrotic agents precisely at the site of injury, facilitating cardiac tissue repair and improving overall cardiac function [123]. Enzyme-responsive nanocarriers have also found applications in treating infectious diseases, particularly in targeting bacterial infections and managing antibiotic resistance. Many bacterial pathogens produce specific enzymes that can be exploited for targeted drug delivery [124]. For example, β-lactamase enzymes produced by resistant bacterias can be targeted by nanocarriers containing enzyme-sensitive linkers that release antibiotics only in the presence of these enzymes [125]. Additionally, enzyme-responsive nanocarriers can be used to deliver antiviral agents in response to viral enzymes such as proteases and polymerases. By designing nanocarriers that release their payload upon encountering these viral enzymes, researchers can develop more effective therapies with reduced side effects and improved targeting [126]. Lysosomal storage disorders, such as Gaucher's disease and Fabry's disease, are characterized by accumulation of unmetabolized substrates due to enzyme deficiencies. Enzyme-responsive nanocarriers offer a promising approach for delivering replacement enzymes or therapeutic agents directly to lysosomes. By incorporating enzyme-sensitive linkers that are cleaved within the acidic lysosomal environment, these nanocarriers ensure that therapeutic agents are released precisely where they are needed [127]. For example, nanocarriers designed with linkers sensitive to β-glucuronidase can deliver enzyme replacement therapies for diseases such as Gaucher's disease [128]. Similarly, nanocarriers

responsive to α-galactosidase can be used to treat Fabry's disease, ensuring that the therapeutic enzymes are released within the lysosomes to correct the underlying metabolic defect [129]. Enzyme-responsive nanocarriers are also being explored for gene therapy and RNA delivery applications. The stability and release of nucleic acids such as DNA, mRNA, and small interfering RNA (siRNA) can be controlled by incorporating enzyme-sensitive elements. These nanocarriers can be designed to release their genetic payload in response to specific enzymes present in diseased tissues or cells [130,131]. For instance, in gene therapy for cancer, nanocarriers can be engineered to release therapeutic genes or RNA molecules only in the presence of tumor-associated enzymes. This approach enhances the specificity of gene delivery, reducing off-target effects and improving therapeutic outcomes [132].

5. Conclusion

Enzyme-responsive nanocarriers have emerged as a highly versatile and innovative platform in the field of modern biomedicine. Their unique ability to respond to specific enzymatic stimuli allows for controlled and targeted release of therapeutic agents, leading to enhanced efficacy and reduced systemic side effects. This capability holds particular promise for treating a wide spectrum of diseases, including cancer, cardiovascular disorders, infectious diseases, and lysosomal storage disorders, where precise localization and timed release of drugs are critical. Enzyme-responsive nanocarriers offer the potential for more personalized and efficient therapeutic interventions through a combination of advanced design principles and innovative fabrication techniques. Their adaptability allows for integration of various enzymes as triggers, enabling the development of nanocarrier systems that can be fine-tuned to match the biochemical environment of specific diseases. This flexibility not only enhances therapeutic outcomes, but also opens new pathways for tackling previously intractable conditions such as multidrug-resistant infections and genetic disorders. As the field continues to evolve, further advancements in enzyme-responsive nanocarrier technology, including improvements in biocompatibility, stability, and targeting specificity are anticipated. Continued research into novel enzyme triggers, coupled with progress in nanocarrier design and functionalization will likely expand the application of these systems to even more complex medical challenges. Ultimately, enzyme-responsive nanocarriers represent a promising frontier in the development of precision medicine, with the potential to transform the treatment landscape for some of the most difficult-to-treat diseases in the coming years.

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CHAPTER

Glucose-responsive nanocarriers for biomedical application

14

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1. Introduction

Diabetes mellitus is a universal metabolic and chronic disease impacting over 415 million humans globally [1]. The population has been anticipated to be 642 million by 2040. Type 1 (T1DM) and Type 2 (T2DM) diabetes mellitus originate when the body is unable to handle insulin or generate enough of it. Insulin, respectively, is characterized by persistently elevated blood glucose levels (hyperglycemia). Continuous Insufficient or inefficient od insulin can result in tissue damage, contributing to severe complications, including cardiovascular disease, nerve damage, renal failure, ketoacidosis, stroke, lower limb amputation, and retinopathy [1].

In the meantime, diabetes is considered intractable with the available glucose-lowering treatments. Selective Therapy should be customized to individual needs grounded in the control of glycemic levels, with enduring advantages, side effects, and tolerability [2]. Type 2 diabetes mellitus (T2DM) is highlighted by reducing insulin secretion and interrupting signaling pathways due to pancreatic beta cells' failure to function satisfactorily, which results in reduced insulin signaling and secretion from the pancreas [3]. The insulin resistance pathways inhibit the functionality of insulin, especially in skeletal muscle, liver, adipose tissue, and kidney. This leads to elevated fatty acid deposits in muscle tissue and peripheral hyperinsulinemia. , which consequently exacerbates insulin secretion and resistance [4].

Both hereditary and environmental factors play significant roles in type 1 diabetes, which differs from type 2 diabetes in that it is an autoimmune destruction of the pancreatic islet beta cells. In patients with Type 1 Diabetes Mellitus (T1DM) who exhibit an absolute deficiency of insulin, the primary treatment for blood glucose management is The self-delivery of exogenous insulin via subcutaneous injection. The injections comprise a blend of rapid-acting human insulin formulations (aspart, glulisine, and lispro) or intermediate-acting insulins (neutral protamine Hagedorn and Lente) to address postprandial increases in blood glucose alongside long-acting insulin (glargine, detemir) administered at bedtime to maintain basal insulin levels throughout the [5,6]. Routine, conventional insulin

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injections and self-monitoring of blood glucose prove ineffective, as they result in damage to the nerve, pain, local infection inconvenience, non-compliance, hypoglycemia, discomfort, and skin necrosis [7]. Numerous nanoparticle-based therapeutic delivery systems have been developed to mitigate the adverse effects to enhance insulin delivery systems.

In type 1 diabetes, subcutaneous insulin injection is the principal treatment method. Maintaining normal blood glucose levels (BGLs) requires patients to self-monitor and inject themselves with insulin multiple times daily. This is commonly linked to pain and insufficient glucose regulation [8]. Research has focused on non-invasive insulin delivery systems, including buccal, oral, nasal, transdermal, rectal, and ocular methods. Besides subcutaneous injections, these methods are recognized as an open-loop insulin release mechanism. The inability of these methods to regulate patients' blood glucose levels arises from the need for direct coupling between glucose sensing and insulin release [9].

Developing a closed-loop insulin release mechanism is essential to enhancing standard glycemic control, decreasing the occurrence of hyperglycemia and hypoglycemia, and preventing the complications that follow in diabetic patients. A system mimics the pancreatic beta cells of natural pancreatic function cells by detecting high glucose levels and provoking insulin release named glucose-responsive systems. These days, glucose-binding molecules such as glucose oxidase (GOx) and phenylboronic acid (PBA) are the main glucose-sensing substances used to create chemically synthetic glucose-responsive insulin delivery systems [10]. Researchers have used these molecular tools to create different modified insulin molecules and delivery systems and formulations, including hydrogels (bulk or micro/nanogel), liposomes, membranes, nanovesicles, microneedle array patches, and cells [11]. Changes in glucose-induced binding affinity, swelling and shrinkage, dissolution, pore size variation, and degradation enhance these formulations' potential to release insulin. This chapter will focus on glucose-responsive systems, their functional groups, and nanocarrier polymers from natural and non-natural materials to create closed-loop insulin and a biomedical application. Such systems depend on the future advancement of innovative glucose-responsive elements and their unification with a compatible device [12]. This perspective explores the progression of key strategies within this research domain, highlighting significant advancements in contemporary medical applications.

2. Mechanisms of glucose responsiveness

Glucose-responsive systems are pivotal in applications like diabetes management, where controlling blood glucose levels is crucial. The development of these systems hinges on creating materials that can detect and respond to shifts in glucose concentration. Various mechanisms and technologies have been explored to design efficient glucose-sensitive systems, including glucose-sensitive polymers, enzymatic reactions, and chemical interactions. Each approach offers distinct advantages and limitations, influencing their application in drug delivery or glucose monitoring. Glucose-sensitive polymers are engineered to respond to changes in glucose levels by altering their physical or chemical properties [13]. These polymers possess the ability to be drug delivery systems such as hydrogels or nanoparticles to administer insulin or other therapeutic agents in a glucose-dependent manner. For instance, glucose-sensitive hydrogels swell or shrink in response to glucose, releasing encapsulated drugs in a controlled manner [11]. Micelles and nanoparticles, on the other hand, undergo structural changes when exposed to glucose, allowing insulin to be released into the bloodstream [14].

Several glucose-sensitive polymers have been designed, including those that interact directly with glucose molecules and respond to secondary glucose metabolism products. Polymer-based systems are advantageous because they are biocompatible and mimic the body's natural glucose regulation mechanisms. Additionally, these systems are tunable, meaning their sensitivity and response time can be adjusted to meet different therapeutic needs. However, challenges remain in ensuring these polymers maintain their functionality over long periods, particularly in dynamic physiological environments.

2.1 Glucose oxidase a sensing enzymes

2.1.1 Mechanism of action Gox

Glucose oxidase (GOx) is the most widely utilized enzyme in glucose sensing systems because it catalyzes glucose oxidation. The enzyme converts glucose to gluconic acid and hydrogen peroxide (H_2O_2), as shown in the following reaction [15].

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{GOx}} \text{gluconic} \quad \text{acid} + H_2O_2$$

The production of H_2O_2 is a crucial trigger for many glucose-responsive systems, leading to subsequent processes such as insulin release or signal generation in biosensors [16]. In drug delivery systems, for example, H_2O_2 can induce changes in the material's structure, allowing the release of insulin in response to rising glucose levels. GOx-based systems are susceptible and widely applied in continuous glucose monitoring devices.

GOx performance is affected by environmental conditions such as oxygen levels and pH. GOx requires oxygen as a cofactor for the oxidation process, and in hypoxic conditions, such as in diabetic patients, the enzyme's activity may be impaired. Additionally, pH changes can affect glucose oxidation efficiency, limiting GOx-based systems' accuracy in specific physiological environments.

However, many limitations are associated with the based system, revealed in its sensitivity to high temperature, pH, and enzymatic action. Thus, to overcome all these challenges, a glucose-responsive noncarrier was used to avoid inactivity and was encapsulated in a hydrogel, nanogels [10], hollow nanospheres [17], and vesicles [18], Liposomes. Wu et al. also used a protective layer of metal-organic frameworks (MOFs) to immobilize GOx, effectively avoiding activity degradation in trypsin or EDTA (1 wt%) solutions.

H_2O_2 was classified as a highly reactive molecule that can cause inflammation by reacting with other tissue; this is another concern for GOx-based innovative insulin delivery systems [10]. Attention has turned to a solution of antioxidants. Hence, Many outcomes are linked to catalase (CAT) [19], peroxidase (HRP) [20], and other artificial peroxidases. For example, Qu et al. [21] Encapsulated hemin within GOx-based glucose-responsive hydrogels to achieve stable H_2O_2 degradation activity.

2.2 Lectin-modified system

Lectins are proteins that bind carbohydrates. They can serve as biosensors in creating glucose-sensitive systems, much like GOx. Their ability to adhere to biological structures is a remarkable feature. Lectin interacts with glycoproteins and glycolipids on the surface of cells to regulate hormones. Brownlee and Cerami first declared that Concanavalin A (Con A) has received the most outstanding research

attention of all the glucose-responsive lectins [22]. Con A usually binds with saccharide residues in these four binding locations throughout the polymer chain. Con A redesigned technology enables a glucose-responsive drug-release and glucose-monitoring mechanism.

Insulin administration systems that use Con A have received more attention than glucose monitoring systems glycosylated polymer, insulin, and Con A fabricated the system. Entrapping and Embedding Con A in the polymer chain by forming bonds with glycosylated moieties along the chain is the primary mechanism for glucose-responsive medication release, resulting in Encasing insulin in a three-dimensional structure; the saccharide residue-Con A combination was broken, triggering a response to glucose presence because free glucose binds more strongly to Con A than the saccharide residue, prioritizing Con A over saccharide residues. Consequently, the gel matrix becomes less dense, and insulin can flow more freely as the unbound glucose competitively replaces the complexed glucose.

Chang and coworkers successfully co-assembled Canavalin A (Con A) and amylopectin to deliver embedded insulin acting as a glucose-responsive biopolymer. Con A or lectin is a protein that binds to glucose and other sugars, thus allowing it to interact with carbohydrates. Amylopectin -one of the two central units of starch completed with amylose - is a highly branched polysaccharide that consists of glucose units linked together in a complex branched structure. Chang et al. (2018) manipulate these two Biomolecules by using them as an insulin-embedded vehicle that interacts with glucose in a synergic pattern. The mechanism of average glucose levels Con A is bound to amylopectin, maintaining the stability of the nanoparticles and keeping the insulin encapsulated; when glucose levels rise, under a competitive binding strategy, Con A preferentially binds to the free glucose in the bloodstream rather than the amylopectin, this competitive binding causes the structure of the nanoparticle to break down releasing the caged insulin in response to the increased glucose. In a process called glucose-induced insulin release [23].

2.3 Phenylboronic acid-based systems

In the previously discussed sections, we discussed two prevalent methodologies for developing glucose-sensitive systems. The process involves the reaction of an enzymatic (GOx) and glucose. The other involves the mechanism of Con A privilege, which relates to the differentiation of glucose upon the chain of the glycosylated polymer when glucose-rich conditions are satisfied. However, these protein-based elements are highly susceptible to environmental changes and may undergo denaturation. Consequently, there is a constraint when the system must be occupied and retained for a long time. In recent advancements in glucose detection mechanisms using non-natural and synthetic components such as the glucose sensor, phenylboronic acid (PBA)–based systems have gained significant attention for their stability, durability, sensitivity to glucose, and versatility in biomedical applications.

Phenylboronic acid (PBA) is a non-enzymatic approach to glucose detection. In 1954, Kuivila and colleagues published the first report on using saccharide with PBA [24]. Additional studies helped to clarify a mechanism by which PBA binds reversibly to the diol groups on glucose molecules, forming cyclic esters. Fig. 14.1 shows the equilibrium of the PBA's two compounds in water. One is an uncharged and relatively hydrophobic form; the other is charged and, consequently, a hydrophilic form. In an aqueous environment, when glucose is provided, the electrically charged form of phenyl borate

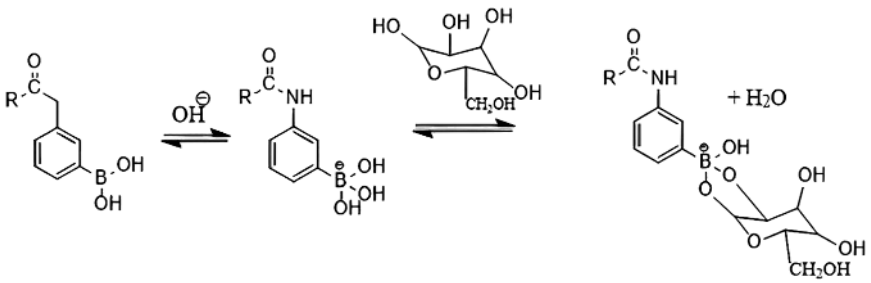


FIGURE 14.1

Reversible binding of glucose to APBA.

forms a stable glucose complex via reversible covalent bonding. Consequently, the equilibrium moves toward the direction of the growing charged (hydrophilic) forms of phenyl borate.

This interaction leads to changes in the physical or chemical properties of the material, such as swelling, solubility, or fluorescence. PBA-based systems are beautiful for glucose-responsive drug delivery due to their simplicity and stability compared to enzymatic systems. Unlike GOx-based systems, PBA does not rely on oxygen or specific environmental conditions, making it a more versatile option for glucose detection [25]. Furthermore, PBA can detect glucose over various concentrations, offering potential for therapeutic and diagnostic applications.

Phenyl boronic acid and its derivatives can reversibly bond to sugars and 1,2 or 1,3 diol molecules. Consequently, They are capable of detecting carbohydrates, including glucose in the bloodstream and sialic acid (SA) in neoplastic cells [26,27]. This property has been extensively examined in glucose-responsive polymer frameworks, sensing devices, diagnostic methodologies, and numerous therapeutic interventions. [28,29]. A glucose-responsive polymer with an incompatible base with phenylboronic acid was fabricated for self-regulated insulin delivery. 3-APBA was conjugated with CS (3APBA-CS) via synthesis using EDC: HCl and hydroxy benzotriazole. Insulin-loaded glucose-sensitive nanoparticles were constructed through a self-assembly technique. The insulin-loaded 3-APBA CS NPs showed a significant encapsulation efficiency (53.6%).

The insulin release pattern proposed that insulin release was glucose concentration-dependent. The study's outcome revealed that the copolymer could modulate insulin release and exhibited glucose responsiveness [30]. Hence, PBA is known for reversibly binding with diols, such as glucose, forming stable boronated esters. This characteristic allows PBA to act as a critical glucose-sensing element in lipid nanoparticles (LNPs) engineered for drug delivery. In PBA-based systems, such as those described in the paper [31], glucose is the sensing element. When glucose levels rise, PBA forms boronated esters with glucose, triggering a reduction in the positive charge of the lipid nanoparticles (LNPs). Upon exposure to high glucose concentrations, these LNPs exhibit a charge-switching behavior, where the initially positively charged nanoparticles become less charged, facilitating the controlled release of insulin. This charge-switching mechanism weakens the electrostatic appeal between the LNPs and insulin, leading to the controlled release of insulin. Thus, PBA ensures insulin is released specifically responsive to high glucose levels, making the system highly effective in managing hyperglycemia.

2.4 Factors influencing the sensitivity and specificity of glucose response

The exploration of nanomedicine in managing diabetes, especially regarding its treatment, involves several important factors that warrant careful consideration. The initial steps in preparing nanoformulations should focus on the following aspects: The choice of materials is defined by their biocompatibility, biodegradability, low immunogenicity, and availability, among other considerations. (ii) formulation in an aqueous medium free from organic solvents, (iii) Surface engineering and electrostatic charge should harmonize the relationship between preferred properties and detrimental effects in vivo effects, (iv) control of size and uniformity as well as consistency across batches, (v) maintenance of long-term stability after lyophilization and consistent size upon reconstitution. Many designs and polymers were integrated to enhance the previous responsive glucose group functionals.

The efficacy of glucose-responsive systems is influenced by multiple factors, such as the concentration of glucose-binding sites, the affinity of the glucose-responsive material, and the physiological conditions present. In GOx-based systems, the accessibility of oxygen is a crucial factor, as the enzyme necessitates oxygen to catalyze the reaction. In hypoxic conditions, the enzyme's efficacy may diminish, impairing the system's capacity to measure glucose concentrations accurately. pH fluctuations in various tissues can influence GOx activity, resulting in changes in glucose detection accuracy [11].

The boronic acid group's pKa impacts the sensitivity and specificity of PBA-based systems. Modifying the pKa enhances the system's ability to bind glucose at physiological pH levels, thus improving its sensitivity to glucose. Nonetheless, a significant challenge lies in preventing PBA-based systems from cross-reacting with other carbohydrates, as such interactions may result in false positives or erroneous glucose measurements. Researchers are refining the molecular design of these systems to improve specificity and minimize interference from other molecules in the body [32]. As a result of all the above, Research efforts have been consolidated to address the barriers hindering the functionality of all functional groups responsive to glucose ratios. This aims to enhance their efficacy by applying various natural and industrial materials, as detailed below.

3. Design and synthesis of glucose-responsive nanocarriers

3.1 Nanomaterials used in glucose-responsive systems

Polymeric nanoparticles, constructed from polymers formed through the covalent bonding of one or more types of monomeric units, serve as highly effective vehicles for the delivery of pharmaceuticals. These nanoscale carriers are formulated to encapsulate and protect therapeutic molecules, enhancing their stability and bioavailability. By facilitating controlled and targeted site-specific drug release, polymeric nanoparticles minimize systemic side effects and improve the therapeutic efficacy of pharmaceutical compounds, making them a pivotal innovation in modern drug delivery systems [33]. Polymeric nanoparticles (NPs), originating from naturally occurring and artificial sources, have proven to be a versatile basis for improving the delivery efficacy of hypoglycemic medicines. These nanoparticles offer many advantages, including the facile modification of chemical structures, regulated and lengthened drug release process in vivo, improved bioavailability, and excellent biocompatibility. These features make them highly versatile and efficient carriers for therapeutic agents, contributing to enhanced treatment outcomes and reduced systemic toxicity in drug delivery applications [34].

3.2 Polymers and glucose-responsive systems

Considering the alarming rise in diabetes cases, expanding interest has been paid to research on glucose-responsive polymers. A wide range of polymers and functional groups have been investigated to evaluate their physical potential to respond to the oscillation of glucose levels. The glucose oxidase-modified system is the first to be classified as a traditional glucose-responsive system, revealed in the past few decades. Historically, the modulation of glucose oxidase as a sensing element with a pH-sensitive hydrogel acting as a structural backbone was considered the first glucose-responsive material. The immobilization of (GOx) can equip the pH-sensitive materials with glucose-responsive properties, a characterization provided by a reaction of (GOx) with glucose forming a gluconic acid. Such a formation results in a lowering of pH, acquiring the swelling characterization of the pH-sensitive polymer chains, and changes in their morphology. Glucose oxidase has been expansively studied within insulin release and glucose-responsive modeling fields [35].

The pioneering discovery of a hydrogel as a responsive glucose vehicle set it apart from other materials and served as the foundation for subsequent research. Therefore, hydrogels and films are fundamental materials that contribute significantly to insulin delivery and release studies.

3.3 Swelling of hydrogel and filming

Hydrogels are cross-linked hydrophilic polymer networks that can swell in response to glucose levels and maintain their insolubility. Their elevated moisture level and biocompatibility make them prime candidates for controlled drug delivery applications. Glucose-responsive hydrogels typically incorporate glucose oxidase to convert glucose into gluconic acid, causing a pH Changning and triggering drug release. The pH-sensitive polymer can be divided into cationic and anionic types. The swelling and deswelling reactions are contingent upon the charge characteristics of the essential backbone monomer. When the surrounding pH medium exceeds the pKa of the cationic groups, the copolymer exhibits hydrophobic characteristics and repels water from the system; conversely, when the pH is below the pKa, the cationic groups transition to a protonated state. Consequently, the system becomes hydrophilic and absorbs water.

Peppas and co-workers researched poly (methacrylic acid-g-ethylene glycol) (P(MAA-g-EG)) hydrogels to serve as a "squeezing gel" with a high glucose concentration [13]. Catalase was included to enhance the GOx-mediated process through its enzymatic activity and address the limitation of GOx activity due to oxygen availability.

$$H_2O_2 \xrightarrow{\text{Catalase}} 1/2 \quad O_2 + H_2O$$

As the reaction reveals, oxygen is generated through the catalase process, resolving the requirement for oxygen. Furthermore, H_2O_2 produced by the GOx enzymatic activity was used, thereby mitigating the peroxide-trigger degradation of the GOx enzyme. Several hydrogel matrices encapsulating and embedding GOx and catalase were made and studied [36,37]. It was demonstrated that introducing catalase caused an increase in the rate of hydrogel's swelling and improved the enzyme's stability.

One of Con A's main limitations is that it still needs better aqueous stability and solubility. Modifying Con A with hydrogel polymer was one method to ameliorate the system's solubility and stability. Con A, designed with poly(ethylene glycol) (PEG) molecules, was researched [36]. As the quantity of grafted PEG chains per Con A reached five, the binding affinity for glucose progressively

intensified to its peak. The fabricated system was formulated by combining PEGylated Con A with glycosylated polymer chains. The findings indicated that PEGylated Con A exhibited superior water solubility, enhanced stability, and increased glucose sensitivity compared to native Con A. Covalent bonding demonstrated superior efficacy in maintaining the system's strength compared to mixing Con A [38]. Tanna and colleagues produced a covalent bond between the amine groups of Con A and carboxylic moieties in several carbomer resins, such as Carbopol 974P NF, 40e Carbopol 934P NF, and 941P NF, 43, using carbodiimide chemistry to make the gel. Using covalent couplings enhanced the gel's endurance and reduced component leakage.

Furthermore, most researchers in this area have selected hydrogel swelling as a more accessible drug-release mechanism due to the system's controllability and reversibility. PBA compounds were also integrated into the three-dimensional polymer hydrogel network. One of the challenges of glucose-responsive provided functions that nanocarriers overcome is that the pKa of APBA is 8.6, which significantly exceeds the pH value found in the human body, rendering the system unsuitable for in vivo applications. Consequently, Hoare and Pelton developed a switching-charge polymer through acrylic acid (AA). AA is the anionic monomer, while N, N-dimethylamino ethylacrylate (DMAEA) is the cationic monomer. APBA also functionalized the polymer [39]. Due to its amphoteric nature, the system reacted to glucose in the physiological milieu, with the nanostructure facilitating a rapid response time. Furthermore, it yielded a significant insulin release at physiological pH and ionic strength.

3.4 Polysaccharide as an integrated polymer in the glucose response system

Biomolecular nanocarriers are increasingly gaining attention as a drug delivery system due to their exceptional characteristics, including abundant availability, inherent biocompatibility, biodegradability, and ease of preparation. These carriers leverage biomolecules as foundational building blocks, enabling the development of a diverse array of glucose-responsive biopolymers. The integration of biomolecules into nanocarrier systems not only enhances their functionality but also aligns with the principles of green and sustainable biotechnology, offering a versatile platform for advanced therapeutic applications of these main biomolecules is polysaccharide, combined with hydrogel to form stimulus-responsive polysaccharide-based hydrogels widely used in the biomedical sector, especially in pharmaceutical delivery [40].

Polysaccharides are a major classifying biomolecule used intensively in biomedical applications due to their large number of active groups on the natural molecular chain structure, a variety that enhances reaction modification [41]. Polysaccharides, including Chitosan, Cellulose, Alginate, Starch, Glucan, and Hyaluronic acid (HA), are rich in sources, biocompatible, biodegradable, and easy to prepare [42]. One of the most critical vehicles for embedding biomolecules is hydrogel. Polysaccharide-based hydrogels represent the most thoroughly investigated category of hydrogels.

One challenge with PBA-based systems is ensuring their selectivity, as other sugars, such as fructose, can also bind to PBA, potentially leading to false signals. Researchers have worked to modify PBA molecules to enhance their specificity for glucose while minimizing interactions with other sugars. The combination of PBA and Chitosan CS -cationic polysaccharide has opened a versatile potential as a glucose-responsive nanocarrier due to their distinct connection. PBA forms covalent bonds with polyol compounds. This modification occurred in the chitosan primary amine group or the

hydroxyl group. Such conjugation enhances the ability to function as a glucose-sensitive polymer, leading to self-regulated insulin release in treating diabetes [43].

3.5 Liposomes as a glucose-responsive nanocarrier

Liposomes are spherical vesicles with an amphiphilic nature and a lipid bilayer associated with a water-containing core. They can encapsulate hydrophilic and hydrophobic drugs, which helps prevent these substances' rapid degradation and promotes their eventual release at the target site [44]. Due to their small size, biodegradability, instituted capabilities for industrial scalability, and reduced immunogenicity and toxicity compared to polymeric and inorganic agents, liposomes are considered promising candidates for the delivery of anti-diabetic agents [45,46]. Furthermore, the physical features of the phospholipid bilayer, including rigidity, fluidity, and surface charge, are substantially determined by its structural constituents. The drug loading capacity and encapsulation efficiency are influenced by vesicle size and the number of bilayers [46]. It is essential to elucidate and utilize the physicochemical properties of liposomes further to enhance the therapeutic efficacy and durability of vehicular systems.

Multivesicular liposomes (MVLs) are designated by multiple nonconcentric aqueous chambers separated by crosslinked lipid layers, with particle sizes ranging from 1 to 100 μm [47]. Because of their enormous size and distinctive structure, MVLs allow controlled and sustained release of hydrophilic and lipophilic medications. In addition, the exterior compartments can be removed without affecting the interior compartments' structure [48].

The primary advantage of multivesicular liposomes (MVLs) formation lies in utilizing distinct neutral lipids, such as triglycerides. These triglycerides are strategically incorporated into MVLs in two specific locations: at the intersections of the bilayers and as intravesicular triglyceride droplets. This unique arrangement contributes to the structural stability of MVLs and enhances their capacity for encapsulating and delivering hydrophobic and hydrophilic therapeutic agents, making them a highly effective drug delivery system [49].

Using triglycerides with alkyl chains of differing lengths allows the trapped drugs to be sustainably released for a long time [48]. Consequently, MVLs have the potential to offer numerous benefits as a medication carrier, such as excellent biocompatibility, high encapsulated volume, excellent biocompatibility, the effect of sustainable-release, various administration methods, and adjustable release rate [38,50]. No glucose-responsive MVLs have been found to stabilize blood glucose levels despite reports of such a phenomenon [47]. The combined benefits of MVLs and glucose responsiveness could be achieved by incorporating the right glucose-sensitive components into these delivery systems. Using these glucose-sensitive components to make MVLs glucose-responsive, however, is challenging.

By using glucose-responsive functional compounds (GOx), Liu et al. (2021) [49] Conducted research on a novel method for glucose-responsive insulin delivery utilizing multivesicular liposomes (MVLs) that exhibit sensitivity to pH and H_2O_2. The MVLs were developed through a double emulsion technique, using a liposomal membrane that included (3-fluoro-4-((octyloxy)carbonyl) phenyl) boronic acid (FOP) and 1,2-distearoyl-sn-glycero-3 phosphoethanolamine (DSPE). Insulin (GOx) and catalase (CAT) were encapsulated within the internal vesicles. This system's primary characteristic is the glucose-triggered interaction of FOP with glucose, creating an acidic and H_2O_2-rich microenvironment. In high glucose environments, the in-situ catalysis by (GOx) produces

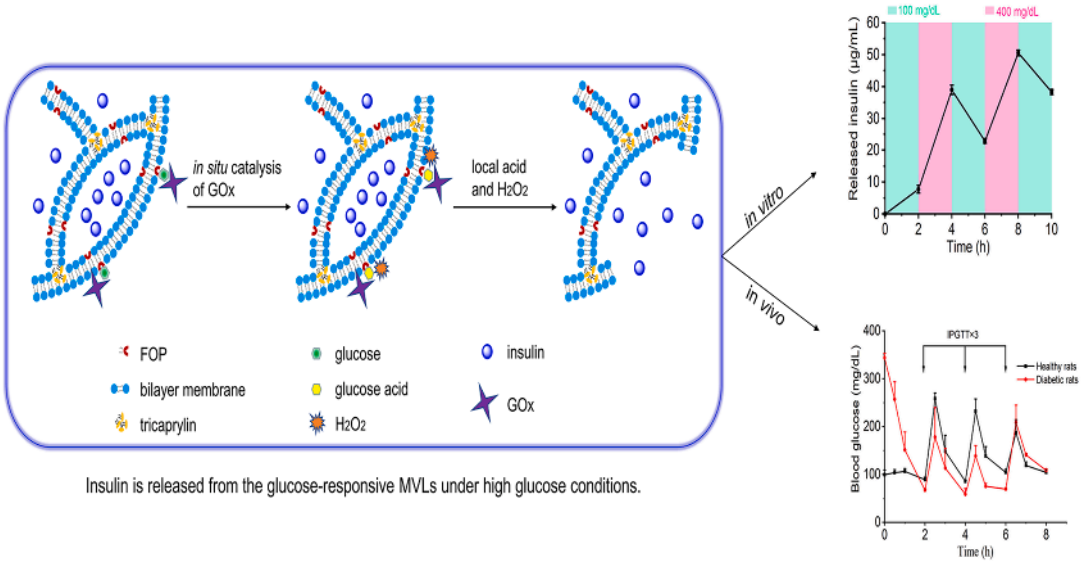


FIGURE 14.2

Reaction mechanisms of GOx entrapped MVLs as a modulated glucose responsive nanocarrier [1].

gluconic acid and hydrogen peroxide H_2O_2, leading to the degradation of the microvesicular lipid (MVL)'s outer membrane, which results in insulin release. In vitro experiments indicated that the glucose-responsive MVLs efficiently regulated insulin release by varying glucose concentrations. The stable structure of the MVLs was preserved at average glucose concentrations, accompanied by minimal insulin release. In vivo experiments demonstrated the system's efficacy in lowering blood glucose levels (BGLs) and sustaining normoglycemia. The findings indicate that biocompatible glucose-responsive MVLs present a viable approach for intelligent insulin delivery, with potential applications in diabetes management owing to their capacity to quickly and effectively respond to fluctuations in blood glucose levels. This is illustrated in Fig. 14.2.

4. Biomedical applications of glucose-responsive nanocarriers

4.1 Diabetes management

4.1.1 Glucose-responsive insulin delivery systems

Diabetes, often referred to as a sugar disease, is a complex metabolic dysfunction characterized by prolonged hyperglycemia resulting from a disruption in the body's glucose regulation mechanisms. This ailment arises from the dysfunction or loss of pancreatic beta islet cells, peripheral tissue resistance to insulin, or a combination of these factors. The result is insufficient insulin production or impaired insulin action, disrupting glucose homeostasis and leading to long-term complications if left unmanaged [51]. In 2022, 14% of persons aged 18 and older had diabetes, up from 7% in 1990. In 2022, over half (59%) of persons aged 30 and older with diabetes were not receiving pharmacological

treatment for their condition. Coverage for diabetes treatment was minimal in low- and middle-income nations. In 2021, diabetes immediately induced 1.6 million deaths, with 47% of all diabetes-related fatalities arising over the next 70 years. Diabetes made up an extra 530,000 deaths due to renal illness, while elevated blood glucose levels contribute to around 11% of cardiovascular lethality. It is projected that diabetes will impact the lives of 629 million individuals by 2045 [52]. The rise in the universal spread of diabetes and impaired glucose tolerance among mature individuals has been notable in recent decades [53–55]. Rapid urbanization and significant shifts toward sedentary lifestyles have accelerated the increase in diabetes prevalence across various countries and regions [56]. Precise assessments of the present and prospective burden of diabetes are essential for allocating community and health resources and devising interventions to mitigate these escalating trends. The glucose-responsive medication administration mechanism is an innovative system with capability applications in diabetes management. It might autonomously administer medications in reaction to fluctuations in sugar levels in the bloodstream to sustain serum glucose inside a healthy range. This is illustrated in the Fig. 14.3.

4.1.1.1 Hydrogels

Glucose-sensitive hydrogels serve as impactful transporters for insulin administration because of their capacity to offer a dual-phase release pattern contingent upon the blood sugar concentrations in the blood circulation. It requires rapid insulin administration, and these hydrogels may autonomously secrete insulin free from human intervention. In contrast, insulin is unnecessary in hypoglycemic

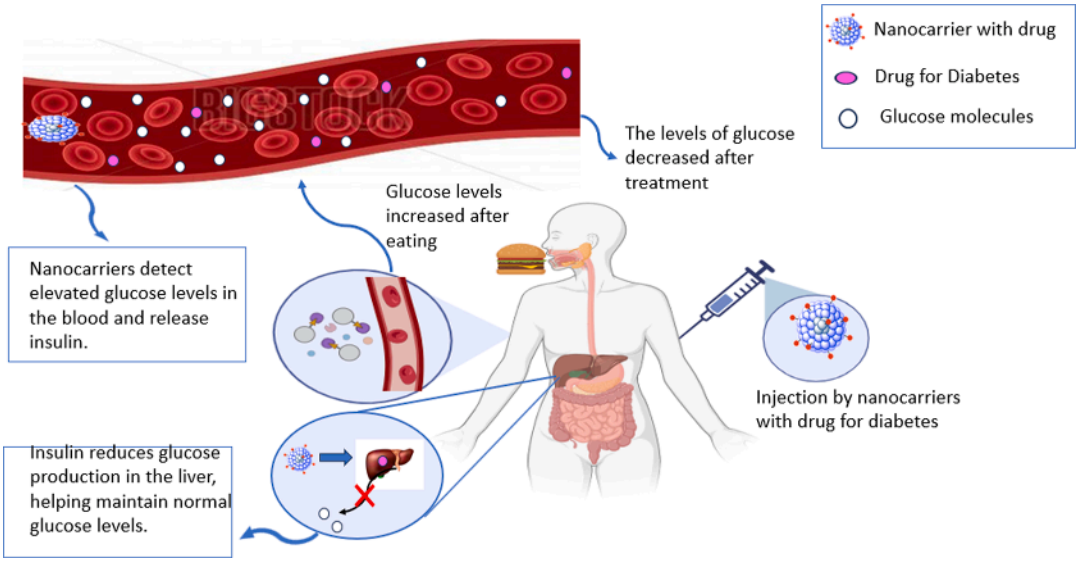


FIGURE 14.3

The mechanism of glucose-responsive nanocarriers in insulin delivery. These nanocarriers detect elevated blood glucose levels and trigger the controlled release of insulin. This process facilitates efficient glucose uptake by cells, reduces blood sugar levels, and maintains them within the normal range, offering a dynamic and targeted approach to diabetes management.

conditions, and the hydrogels could regulate and halt insulin administration. Consequently, hydrogels may be a prospective personal monitoring apparatus for individuals with diabetes. The fundamental component of these hydrogels is a glucose detector capable of measuring glucose levels and their variation rate to determine the necessity for insulin administration. The three primary sugar mentors utilized in these hydrogels are Con A, PBA, and Gox [57,58].

4.1.1.2 GOx

GOx is an extensively utilized glucose response system. It is a redox enzyme that explicitly promotes β-D-glucose. The glucose oxidase pharmaceutical delivery platform is a symmetric dimer composed of Enzo units, with a related compound weight of 80 kDa per unit, requiring flavin adenine dinucleotide enzyme cofactors [59]. GOx converts glucose to gluconic acid in hyperglycemic surroundings when integrated with a pH-responsive polymer substance. This can alter the microenvironment's pH and induce a configuration modification in the vector, facilitating the release of the pre-injection drug. Simultaneously, using O_2 leads to the GOx, creating an inside hypoxic microenvironment, resulting in the depletion of specific carrier components. The secretion of the preloaded medicine can compromise the medication-filling system [60]. Within Gox-facilitated glucose-sensitive drug platforms, blood glucose levels modulate insulin release or other hypoglycemic agents. GOx employs O_2 as an electron acceptor in glucose reaction, rapidly depleting O_2 to produce gluconic acid and H_2O_2. The pH of the microenvironment and O_2 levels decrease, while H_2O_2 concentration increases during glucose oxidation [61]. The alterations in pH, H_2O_2 concentration, and oxygen levels may serve as catalysts for the commencement of medicine discharge originating from the drug release mechanism. The glucose-responsive active activity of glucose oxidase elicits a reaction in the platform. Consequently, GOx was incorporated inside transporters exhibiting pH, O_2, and H_2O_2 responsiveness to develop glucose-responsive drug delivery systems [58].

4.1.1.3 PBA

Glucose-responsive patches utilizing PBA have been created as alternative glucose oxidase ones. PBA is a synthetic compound that functions as a glucose detection device due to its acidic catalyst characteristics, allowing it to temporarily create esters with cis-1,2 or cis-1,3 diols, including glucose [62]. In water-based solution environments, it is balanced between PBA, a neutral trigonal geometry, and a charged tetrahedral boronate framework. Although simultaneously structures may temporarily bind glucose and form cyclic phenyl borate compounds, the anionic boronate structure demonstrates significantly more binding affinity. Due to the elevated pKa of PBA (8.6) at physiological pH (7.4), the balance favors the uncharged form, leading to diminished glucose response. Therefore, the PBA in glucose-responsive systems is compositionally altered to increase acidity and decrease pKa to enhance glucose sensitivity at biological pH. Consequently, both electron-withdrawing groups (such as halogens, nitro, or carboxyl groups) and ortho-substituents capable of forming internal linkage bonds (between B and N or B and O) have been incorporated into the cyclic aromatic compounds of PBA [59,63,64]. To date, innovative insulin delivery systems utilizing PBA encompass electrostatic transition-triggered swelling, glucose substitution competition, and bond cleavage [65].

4.1.1.4 Con A

The tertiary group of glucose-regulated molecules pertains to glucose-associated proteins, encompassing harmful bacteria, inactive enzymes, and sugar-recognizing molecules [21]. Con A is the most

prevalent sugar-recognizing molecule. At physiological pH, every unit has four carbohydrate-binding sites [66]. The carbohydrate-binding sites of Con A can interact with the alcohol groups of α-D-mannopyranose, α-D-glucopyranose, and other nonreducing sugars at C-3, C-4, and C-6 [67]. Consequently, particular bonding actions have been utilized to detect specific sugar [67,68] and specific cells carrying glycoproteins [69]. Engineered a new glucose-based sensor utilizing Con A and dextran. Con A and dextran were disseminated and connected with the probe interfaces, dissociating in the competitive reaction with glucose as the concentration escalated [70].

4.1.1.5 PH sensitive systems

MOFs, porous crystalline structures that self-assemble and are composed of organic ligands and metallic ions, are becoming drug storage and delivery nanocarriers [71,72]. Due to their variable pore dimensions and stiff chemical configuration, MOFs may embed proteins into their interior pores and sustain their bioactivity in harsh environments. However, acid, adenosine triphosphate, glutathione, etc., can destroy several MOFs [9]. Pickup and Keen engineered a glucose-responsive insulin therapeutic release mechanism utilizing zeolitic imidazole framework-8 (ZIF-8), a conventional metal-organic framework nanocrystal, glucose oxidase (GOx), and insulin. ZIF@Ins&GOx nanoparticles form through the autonomous organization of Zn^{2+} and imidazole-2-carboxaldehyde with GOx and insulin, as seen in Scheme 1. Insulin and GOx are enclosed within the inner voids of ZIF-8 to maintain physiological function, minimize protein unfolding, and avert antigenicity [73]. The encapsulated glucose-specific enzyme converts glucose into gluconic acid. When the pH of the cellular surroundings decreases, the metal-organic framework (MOF) disintegrates, releasing insulin. We proved that the ZIF@Ins&GOx-based hydrogel could provide glucose-responsive, prolonged insulin for treating Type 1 Diabetes, utilizing Pluronic-127 (PF127) as a subcutaneous formulation, temperature-sensitive hydrogel scaffold [74].

4.1.1.6 Smart hydrogels

In recent years, increasing focus on tailored precision medicine and pharmacotherapy has driven the development of advanced biological materials [75,76]. Stimuli-responsive hydrogels are considered advanced biomaterials, with external triggers like pH, temperature, electromagnetic fields, photonic energy, and biomolecule levels utilized to initiate drug release [77]. The terms "trigger-sensitive hydrogels and intelligent hydrogels" were first introduced in the research study in 1990 and 1991. In 2021, over 2800 papers were published, with 70% emerging in the past 5 years. This growth is directly associated with the creation and utilization of advanced hydrogels. When subjected to a minor outer stimulus, smart hydrogels exhibit sudden transformations in their physical characteristics and significant macroscopic changes [78]. The distinctiveness of these hydrogels lies in their dynamic reactive adjustment. They can react to stimuli exhibiting switchable, expandable, repeatable, and anticipated state changes and revert to their initial form once the stimulus is eliminated [78,79]. Such changes encompass alterations in physical state, interactions with solvents, changes in shape and solubility, conductivity, and water affinity [80]. Advanced polymeric gels in therapeutic platforms can decrease the periodicity of dosage, sustain the target drug level with an individual administration, and mitigate the adverse effects of pharmaceuticals by inhibiting their build-up in unintended tissues in non-target tissues [81,82]. Furthermore, the preparation process for smart hydrogels is straightforward, making them an excellent choice for drug-included sustained-release systems [83].

4.1.1.7 Glucose-sensitive drug delivery system based on others

Other glucose-reactive systems have been studied along with GOx, PBA, and Con A. Using erythrocyte vesicles, a microneedle patch (MN) exhibited a glucose-sensitive chemical response for insulin release. The MN has glucose transporters (GLUTs) that convert insulin into Glu-Insulin. Due to GLUT binding, Hyperglycaemia may cause tissue fluid glucose to replace glucose insulin. The result was rapid glucose and insulin release and blood sugar regulation. The MN patch simplified blood sugar control for TD1M and TD2M [84].

The long-term purpose of diabetes therapy is to achieve autonomous control and insulin administration that replicates the physiological role of the pancreas. Two main methods to accomplish this are sugar-responsive insulin release and reports suggesting therapeutic cell implantation. A degradable, partially oxidized alginate matrix for delivering glucose-sensitive nanoparticles has been described. The delivery mechanism via injection exhibited a size of 0.5 mm and contained a 2.5% oxygenated microgel. It provided sustained glucose monitoring over 10 days. The results highlighted the biodegradable drug delivery vehicles and cell transport management in managing diabetes [85].

Reaction mechanisms constitute a complex method for the advancement of biotech drugs. Producing lectin analogs represents significant progress alongside the native lectin Con A. The production route for s-Lectin involves undergoing optimization—a glucose-sensitive microgel embedding s-Lectin in a poly (N-isopropyl acrylamide) interconnected framework. The microgel with s-Lectin demonstrated expansion properties about glucose levels from zero to 30 mmol/L. It possesses targeted identification for glucose. This gel shows potential for blood sugar tracking [86].

Fluorescent-based detectors show significant potential for continuous invigorated glucose monitoring (CGM), enabling remote skin-based communication and extended biological function. In current contexts, fluorescent-based sensors encounter difficulties sustaining their location within the implantation region over prolonged periods and adequately reacting to variations within glucose levels in the bloodstream. The research identified hydrogel fibers composed of polyethylene glycol (PEG)-bound polyacrylamide (PAM). A glucose-sensitive linkage was developed utilizing hydrazone and thiazolidine structure. It exhibited in vitro glucose responsiveness. Linear hydrazone and thiazolidine units linked to the LysB29 insulin's molecular side chain via pH-regulated acylation, resulting in GRI responsiveness to thiazolidine in the lab [87].

4.1.2 Blood sugar monitoring via nanocarrier-embedded systems

Regulating postprandial blood glucose concentrations in individuals with diabetes is more challenging than traditional methods. Ongoing tracking and under-skin insulin injections are essential for effective diabetes management [88]. The conventional management of T1DM primarily involves subcutaneous insulin injections, necessitating frequent patient administration. This regimen is associated with physical discomfort and various side effects, including hypoglycemia, lipoatrophy, and delayed absorption, ultimately leading to suboptimal patient adherence. Furthermore, first-line oral medications for T2DM, such as biguanides, sulfonylureas, and glycosidase inhibitors, present drawbacks, including the necessity for high doses, limited biological half-lives, and potential side effects [89]. Despite recognizing hypoglycemic peptides, fast-acting and extended-duration insulin variants, and glucagon-like peptide-1 (GLP-1) activators, such as significant advancements in enhancing patient life standards, the outcomes of prolonged management of blood glucose levels in clinical trials remain inadequate [90].

4.1.2.1 Electrochemical detection

Electrochemical biosensors are extensively utilized for their cost-effectiveness, user-friendliness, enhanced detectability, and superior selectivity. Innovative bioelectronic sensors have been developed to diagnose diabetes by integrating nanomaterials with targeted biomolecules, such as immunoglobulin or biocatalysis. These detectors exhibit rapid charge or particle transfer rates, enhanced adsorption capacities, and biomolecule fixation options [91]. A digital detector utilizing gold nanostructures (Au NFs) adapted carbon printed sensors has been developed for the unlabeled direct quantification of HbA1c. A molecular binding agent, 4-mercaptophenylboronic acid (4-MPBA), was incorporated into the electrode-based biosensors. The PBA of 4-MPBA reacted with the saccharide units of HbA1c. HbA1c was immobilized on the sensor interface via 4-MPBA, acting as a catalyst that reduced H_2O_2 and induced specific electron-driven processes. In ideal settings, the detectors can detect HbA1c in a restricted sample count within a brief timeframe. A dual-function microfluidic sensor was developed to measure HbA1c and total Hb independently, enabling accurate analysis of HbA1c levels about total Hb. The detector probe consisted of electropolymerized poly (2,2′:5′,5″-terthiophene-3′-p-benzoic acid) and a multi-wall carbon nanotube (MWCNT) hybrid coating (pTBA@MWCNT) on a screen-printed carbon electrode (SPCE). Toluidine blue O (TBO) was then immobilized for the total hemoglobin probe and either TBO or an aptamer for the HbA1c probe [92].

4.1.2.2 Fluorescence sensor mechanisms

It can be designed to incorporate a glucose-binding molecule, a donor chromophore, and a nearby receiving molecule for emitted light or charge. Glucose attaching to the receptor triggers a structural shift in the sensor molecule, resulting in an increased gap between donor and acceptor molecules or a reduction in charge movement to the donor. The attachment to the receptor chromophore complex can reduce FRET by allocating the coordinated bonds linking donor and receptor, resulting in diminished electron coupling and boosting fluorescence [93]. Light-emitting donors and acceptors without glucose exhibit enhanced spatial and electronic effects, leading to improved charge transfer to the donor chromophore and reduced fluorescence because of heightened FRET between these entities. The glucose amount is evaluated by assessing the variation compared to the initial emission level from the donor fluorophore linked to the glucose-binding receptor. A rise in FRET that results in reduced emission from the donor fluorophore under light exposure is termed quenching, whereas a drop in FRET that leads to enhancing light emission of a donor fluorophore under light exposure is referred to as excitation [94]. The majority of fluorophores employed in fluorescence-based glucose-sensitive molecules include organic colorants. A supplementary method for measuring local glucose concentration involves utilizing fluorescence with a boronic acid binding unit on sensing agents, which may selectively attach to specific pigments or sugars. In the absence of glucose, targeted luminescent pigments like alizarin red bind to the receptor, resulting in electron migration from the dye to the boronic acid, which induces fluorescence of the dye. Glucose presence releases the dye from the common receptor, resulting in the dye's absence, which, possessing a complete set of electrons, does not exhibit fluorescence [95]. In this indicator displacement assay, in contrast to most FRET-fluorescent dependent tests, an increase in glucose level decreases emitted fluorescence [96].

4.1.2.3 Quantum dots in glucose sensors

Nanomaterials can act in sensor architecture and contain sensor components. Sensors benefit from semiconductor quantum dots (QDs)' sharp luminescent emission lines and low fluorescence fading.

QDs cannot recognize glucose since they do not interact with it. Successful implementation requires recognition—several teams hold combined cadmium telluride (CdTe) QDs with GOx to make detecting devices. The enzyme's hydrogen peroxide in glucose quenches the QDs' luminescence. They directly connect the enzyme to CdTe QD [97] or sequential layering to generate a CdTe QDs-GOx nanolayer [98]. It provides fast glucose optical detection. The nanofilm method accurately quantifies serum samples. The immediate combination association of GOx with manganese-doped zinc sulfide (ZnS) QDs produced a glucose detector for clinical samples [99]. Peroxide suppresses the luminescence of TiO2/SiO2 nanohybrid materials; hence, coupling with GOx provides serum glucose detection comparable to current approaches [100]. These methods work with non-GOx recognition elements. ConA can bind to QDs and recognize glucose. When glucose is identified, no peroxide is produced. Hence, an alternative light-dimming process is needed. For instance, adding gold nanoparticles marked by β-cyclodextrin (β-CD) reduces luminescence via FRET by binding to ConA and moving the nanoparticle near the QD [101]. QD fluorescence increases when glucose displaces β-CD, even in clinical samples. A cadmium selenide/ZnS QD's FRET-based quencher was fluorophore-labeled sugar. Boronic acids can also detect glucose [102]. Use QDs as a reference signal. One example uses a QDs film as a reference, followed by oxygen-responsive colorant and GOx [103]. The dye reactive to oxygen signal fluctuates with the glucose level as GOx action reduces oxygen in the vicinity while the QD response stays steady. This provides pigment-driven glucose measurement in hypoglycemic conditions. QDs help current-based glucose detectors [104]. Cadmium sulfide QDs create charge carriers under UV irradiation. Employing a barrier with platinum nanoparticles to extract the electron, these QDs boost the electrical output from GOx glucose sensing with UV light, making glucose current response more sensitive. Proteins absorb UV light; therefore, most biological samples cannot use it. Finally, silica-luminous europium-modified nanostructures may detect glucose by displacing water molecules, which extinguishes fluorescence [105]. Due to europium's optical characteristics, this method boosts fluorescence with glucose detection and provides accurate quantification in clinical samples [106].

4.1.2.4 Colorimetric detection

Typically, colorimetric glucose sensors comprise many reactants, including enzymes, coenzymes, and chromogenic chemicals. Conjugating these components onto bio-receptors and carriers is difficult due to their instability on specific receptors. Despite the introduction of several solid surface types to address this limitation, the binding speed of these elements on a solid phase still needs to be improved. Ji et al. developed a nanofiber-inspired chromogenic detector incorporating chromogenic chemicals and enzymes to resolve this issue [107]. Their study employed(GOx), (HRP), and color-producing agents (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) or o-dianisidine) for the visual-based measurement of glucose inside a dual enzyme mechanism. With electron stabilization achieved, it was employed to fabricate testing strips composed of hollow nanofiber membranes. The initial coating phase mixture comprises polyurethane (PU) polymer and o-dianisidine. The fundamental early-stage solution consisted of GOx enzyme, HRP enzyme, and ABTS color-inducing reagent in a diluent. The results included hollow PU nanofibers encapsulating GOx, HRP, and ABTS with o-dianisidine. The nanofiber membrane created by electrospinning was submerged in various glucose levels in the mixture for glucose detection, with visual measurement performed by monitoring the changes in hue brightness on the assay strip. The hue strength of the electrospun hollow fiber membrane augmented with higher glucose concentrations, the detector has a minimal measurable threshold

limit of approximately 0.01 mM and a vast sensing capacity spanning from 0.01 to 20 mM. Furthermore, this sensor demonstrated exceptional extended shelf life, remaining steady and viable for up to 4 months. This straightforward electrospinning method and the concurrent in situ co-immobilization benefits facilitate the development of diverse biosensors based on multienzymes, coenzymes, or chromogenic substances [108].

4.1.2.5 Wearable glucose sensors

This device is used for minimally invasive ongoing glucose measurement levels derived from alternative biological fluids developed for advanced systems alternatives to regulate diabetic conditions. The fundamental platforms and detecting methods of contemporary wearable glucose sensors are designed to evaluate bodily fluids such as tears, saliva, and interstitial fluid, including sweat. Many mixtures of these substances facilitated numerous methods to retrieve, gather, and examine these materials' bodily fluids. This section presents the most recent technology facilitating wearable glucose monitoring systems [109].

4.1.3 Glucose-sensitive smart patches and implants

4.1.3.1 Glucose-responsive transdermal patches

Since MNs are put into the skin immediately after interacting with the skin microvascular system, they may detect real-time fluctuations in serum biomarkers [110]. Diabetes self-management requires frequent blood glucose testing and insulin injections [111,112]. For automated insulin delivery, glucose-reactive insulin-loaded MNs are ideal. Yu et al. created a "smart insulin patch" to cure diabetes through insulin emission as a reaction to hyperglycemic situations [113]. MN array tips were packed with insulin- and GOx-containing glucose-responsive vesicles in this investigation. Hypoxia-sensitive hyaluronic acid (HA) and a water-repellent group transformed into water-attracting (2-nitroimidazole) generated these vesicles. Hyperglycemia caused a local hypoxic environment that reduced 2-nitroimidazole to hydrophilic 2-aminoimidazole, disassembled the blisters, and released insulin. A glucose-reactive insulin delivery mechanism was shown to rapidly detect and adjust high chemically triggered blood sugar levels in TD1M mice to normal within half an hour and sustain normal blood sugar levels for hours. Gu group has created an MN patch containing insulin-producing beta cells and glucose enhancers for glucose-responsive insulin administration [114]. Synthetic glucose-signal amplifiers were designed by encapsulating glucose-responsive vesicles with GOx, α-amylase, and glucoamylase instead of direct insulin release. At elevated glucose levels, α-amylase and glucoamylase hydrolyzed α-amylose in MNs into glucose. The exterior beta-cell encased structures on the MN patch secreted insulin to diffuse within the blood and lymphatic capillary systems as this increased glucose signal diffused further. This framework demonstrated more treatment effectiveness than MNs without glucose-signal amplifiers, with one patch controlling blood glucose levels for 6 h in diabetic mice. In addition to enzymatic hypoxia, H_2O_2 from glucose oxidation can stimulate MN insulin release. Hu et al. created a sugar-sensitive insulin delivery system with MNs-array patches and H_2O_2-sensitive polymeric vesicles [115]. Insulin-loaded PVs were autonomously organized from block polymer chains with PEG and PBE-conjugated polyserine. Within this framework, H_2O_2-mediated PBE pendant degradation is disassociated with PVs. In diabetic mice, the patch combining these PVs corrected hyperglycemia and self-regulated blood glucose levels. The Gu group recently designed MNs with hypoxia and H_2O_2 bi-responsive capsules to improve glucose-reactive insulin delivery [116]. Diblock copolymer of poly (ethylene glycol) (PEG) and poly serine altered by 2-nitroimidazole

via thioether produced these dual-sensitive vesicles. Hypoxia can bio-reduce hydrophobic 2-nitroimidazole into hydrophilic 2-aminoimidazole. The thioether also enhanced the copolymer's water solubility when H_2O_2 converted it to sulfone. Elevated sugar levels in the blood and lymphatic capillaries triggered vesicles containing insulin and Gox to dissociate and release insulin due to the copolymer's increased water-solubility due to rapid oxygen consumption and H_2O_2 generation. In transforming thioether to sulfone, H_2O_2 was removed, reducing oxidative stress injury to skin cells and sustaining GOx function. Researchers found that this combined intelligent insulin patch could manage blood sugar concentrations in diabetic rodents over 10 hours and caused no inflammation over 2 weeks. Hyperglycemia can induce the emission of medicines like insulin, yet euglycemic levels can also prolong medication release. For melanoma treatment, Wang et al. documented an anti-PD-1-loaded MN patch for glucose-mediated degradation therapeutic administration [117]. Anti-PD-1 checkpoint inhibitors were incorporated into glucose-reactive nanocarriers. With the GOx/catalase-based enzyme system fixed within NPs, gluconic acid from glucose oxidation helped NPs self-dissociate, releasing anti-PD-1 over 3 days. In vivo, investigations showed significant immunological reactions within a B16F10 mouse melanoma model administered using a PD1 patch relative to control groups receiving non-degradable patches or direct tumor injections free aPD1 with the same dose [118].

4.1.3.2 Subcutaneous implants for continuous glucose monitoring

The glucose biosensor is essential for the efficacy of Continuous Glucose Monitoring Systems (CGMS). An optimal glucose detector is dependable, readily embedded, non-invasive, durable, and, crucially, agreeable to patients. Skin permeating detection using near-infrared (IR) spectroscopy and ex vivo glucose tracking glucose in bodily fluids has been applied for painless glucose evaluation [119]. Despite being pain-free, pleasant, and acceptable to patients, these ex vivo methods lack the requisite accuracy for therapeutic decision-making due to variations in tissue characteristics such as skin pigmentation and body fluid balance [120]. There needs to be more specificity for glucose and an adequate connection between glucose concentrations in blood and bodily fluids [121]. Real-time tracking within the body yields more dependable responses than ex vivo monitoring. Even with certain drawbacks of implanted biosensors, such as biological contamination, the demand for dependable glucose sensors has catalyzed scientific initiatives for their development. IV devices offer immediate glucose level evaluation. This approach has significant surgical risks, including infection and thrombosis [122]. Subcutaneous glucose sensors have garnered heightened interest in continuous glucose monitoring (CGM) as subcutaneous implants mitigate the significant dangers of intravenous implantation [123]. Subcutaneous implants are straightforward, obviating the necessity for complex surgical procedures. With slight invasiveness characteristics, these devices are patient-friendly. The tissue under the skin consists of a highly vascularized pliable tissue that promotes the passage of glucose from the bloodstream into the subcutaneous layer's interstitial fluid (ISF). Sugar levels in the fluid between cells exhibit a 5 to 30-minute lag relative to the blood glucose concentration [124,125]. This delay arises from the duration necessary for glucose in the bloodstream to permeate into the interstitial fluid. The time delay differs based on the glucose administration methods and species. The inherent characteristics of inferred sugar level readings need to calibrate sensor signals according to the "true" blood glucose values. Moreover, biological obstruction by cells or proteins and the creation of fiber-rich capsules surrounding the detectors post-inserting implants may lead to diminished device

precision and, eventually, in-body device malfunction. Consequently, the biocompatibility of sensors is a critical concern in developing insertable biological detectors [126].

4.2 Cancer therapy

4.2.1 Targeted drug delivery in cancer cells with abnormal glucose metabolism

Metabolism may affect the start and progression of cancer. Clinical investigations have associated modified systematic metabolic activity with tumor onset, advancement, and unfavorable therapy results. Adiposity, hyperglycemia, and reduced insulin sensitivity are correlated with elevated cancer susceptibility development and are linked to poorer patient results in cancer cases [127,128]. Nonetheless, how alterations in organismal metabolism affect cellular metabolism to facilitate cancer remains contentious. Increased insulin levels in the bloodstream and insulin-like growth factors (IGF) are linked to malignancy growth. This indicates that excess weight and reduced insulin sensitivity may contribute to tumors by activating signaling pathways that stimulate cellular proliferation [129]. The same signaling pathways facilitate nutrition absorption into cells and modulate glycolytic enzymes, indicating that hormone alterations might significantly influence cancer cell metabolism. Moreover, heightened glucose levels may enhance glucose absorption in specific cells, while reduced circulating glucose levels correlate with improved cancer therapy results [130]. Consequently, antidiabetic medications are being investigated for their antitumor properties, and historical clinical investigations have indicated a decrease in cancer-induced fatality among diabetic cases administered Glucophage [131]. This impact seems unrelated to sugar concentration, as diabetic people whose glucose levels are managed through alternative methods do not have the same anti-cancer benefits as those using Glucophage [131]. Glucophage is extensively utilized for managing TD2M and functions by blocking cellular energy complex I in hepatic tissue, disrupting ATP synthesis [132]. This induces cellular energy strain, enhances AMP-activated protein kinase (AMPK) activity, and suppresses glucose synthesis, reducing blood sugar concentration, better responsiveness to insulin, and reducing insulin levels [133]. Consequently, it remains contentious whether metformin aids cancer patients through direct action on the tumor or indirectly via reducing insulin and IGFs. Alternative antidiabetic treatments that elevate insulin levels might consequently result in poorer clinical results for cancer patients. Dietary restriction, renowned for extending longevity in cancer models, does not influence tumors that develop without IGF signaling [134]. These data indicate that metformin offers a secondary advantage to cases with elevated bloodstream concentrations of IGF. Nonetheless, elevated prescriptions for metformin are detrimental to tumor-initiating cells [135]. Women administering metformin exhibit an enhanced tumor reaction to preoperative breast cancer therapy, a phenomenon that may also scope cancer cases without diabetes [136]. Metabolism is an established target for effective medicines. Since all cancer cells depend on metabolic alterations for their growth and survival, addressing metabolism could impact malignancies from various tissues [137]—the potential efficacy of medicines targeting cellular metabolism across many tumor categories with historical examples. Antifolate medicines were created before comprehending folic acid's role in a metabolic cycle facilitating single carbon transfer events, essential for nucleic acid synthesis. As a result, the efficacy of antifolate medicines prompted the investigation of additional metabolite analogs as prospective anticancer treatments that interfere with nucleotide synthesis [138]. Currently, the antimetabolite category of nucleoside analogs—encompassing 5-fluorouracil, gemcitabine (Gemzar; Lilly), and fludarabine (Fludara; Bayer/Genzyme), in addition to hydroxyurea and a novel family of folate

inhibitor—indicates that tumors may exhibit sensitivity toward arginase. Arginine deiminase linked to polyethylene glycol (PEG) is a compound that reduces arginine levels external to the cell and is presently undergoing clinical studies for many solid tumors. Initial Phase I/II trials indicate that this medicine can be safely administered, with some favorable results noted in hepatocellular cancer and melanoma [139]. This is illustrated in Fig. 14.4.

4.2.2 Tumor metabolism can be safely targeted

Central metabolic pathways may occur as safely targeted in patients. Dichloroacetate (DCA), a tiny chemical, is utilized in treating cases involving lactic acid buildup caused by uncommon genetic defects of cellular energy metabolism. The single goal of DCA is pyruvate dehydrogenase kinase (PDK). The production of PDK is elevated in numerous malignancies due to heightened initiation of the transcriptional regulation hypoxia-inducible factor (HIF) [140]. PDK is a negative regulator of the pyruvate dehydrogenase complex (PDH) [141]. PDH facilitates the oxidative decarboxylation of pyruvate to acetyl-CoA, enabling pyruvate's entry into the tricarboxylic acid (TCA) cycle and diverting it from lactate synthesis. Consequently, DCA-induced inhibition of PDK results in the activation of PDH, enhanced conversion of pyruvate to acetyl-CoA, and reduced lactate synthesis. DCA can modify the mitochondrial membrane potential and suppress lactate generation in cancer model systems, and it has been demonstrated to affect mitochondria in individuals with glioblastoma. DCA is well tolerated by patients, even at levels that affect the mitochondrial membrane potential. While data are inadequate to ascertain the clinical efficacy of DCA, these findings indicate that a viable therapeutic window may exist for targeting cancer cell metabolism in patients [139].

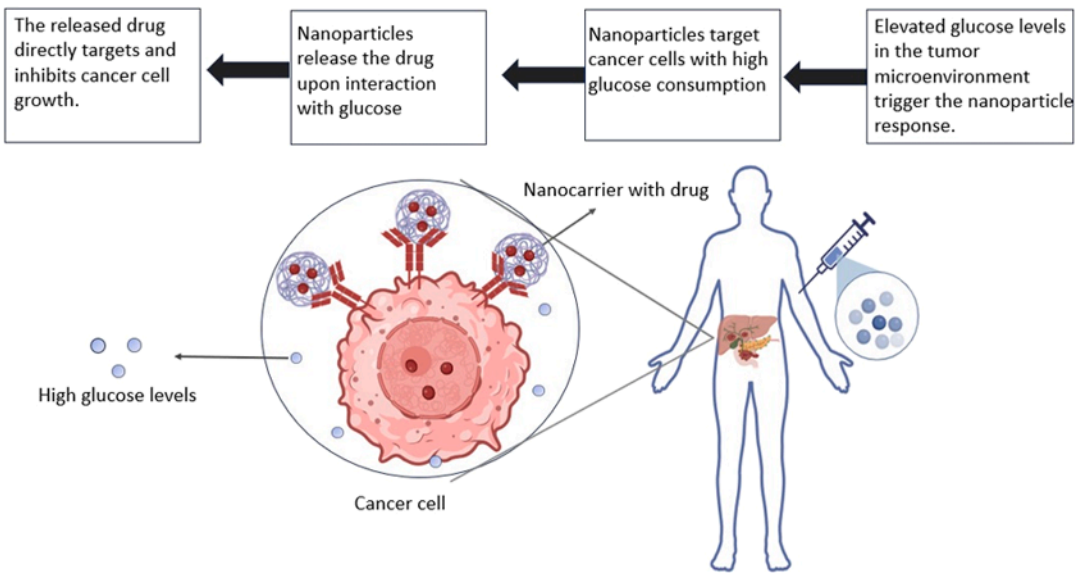


FIGURE 14.4

The drug delivery mechanism of nanocarriers to cancer cells in response to high glucose levels.

4.2.3 Hypoxia-induced glucose-responsive systems in tumor microenvironments

Hypoxia is when cancer cells are exposed to oxygen-deficient conditions, leading to the production and degradation of crucial transcription factors, such as HIF-1a and HIF-2a [142]. Genetic variables can stabilize these transcription factors, including epidermal growth factor and insulin receptor activation, nitric oxide, Akt (kinase B) activity, and ras/MEK activity [143]. Physiological stressors or heritable modifications, such as activated oncogenes, can reversibly elevate HIF [144]. HIF proteins are expressed in cancer, and their prevalence correlates with disease severity [145]. Their pivotal role in glucose metabolism regulation has led to the idea that it alone can account for the Warburg or Pasteur effects [146]. Hypoxia in solid tumors is induced by aberrant angiogenesis, impaired blood perfusion, and unrestrained cancer cell proliferation [142,147]. The tumor microenvironment (TME) is characterized by the interactions between malignant and non-transformed cells, with hypoxia being a joint microenvironment observed in solid tumors [148,149]. This hypoxia is a challenge in cancer treatment as it enhances the metastatic and angiogenic capabilities of cancer cells [150] and may resist radiation and chemotherapy [151,152]. The tumor microenvironment includes immune system cells, tumor vasculature, lymphatics, fibroblasts, pericytes, and occasionally adipocytes. Tumor cells manipulate their environment through intercellular communication mechanisms [153]. Including direct intercellular interactions and the secretion of growth factors, cellular framework metalloproteinases, extracellular matrix proteins, and extracellular vesicles [154]. Tumor cell-driven stromal alterations include suppression of anti-tumoral immune responses, deposition and degradation of ECM components, promotion of vascular network creation, and recruitment of stromal cells and tumor-promoting immune cells. The diverse components of the tumor microenvironment foster conditions conducive to cancer expansion and spread [155].

4.2.4 Glucosamine polymer as a new targeting theory

Chitosan is a general term for a random linear copolymer of β-(1–4) linked D-glucosamine. It is characterized by a straight-chain backbone connected by glycosidic linkages in its chemical structure [156]. Advanced nanotechnology-based delivery methods target the release of active compounds at designated tissues throughout organized administration. Smart polymers are increasingly vital and beneficial in controlled drug delivery, tissue engineering, and medicinal applications [157]. This section discusses many research reports. Researchers have pH-sensitive hydrogels made of chitosan, guar gum, and polyvinyl pyrrolidone. The hydrogels' pH-sensitive characteristics enable their use in intelligent drug-delivery applications [158]. Chitosan and poly (allylamine hydrochloride) composite films have been created as intelligent drug delivery systems. Prolonged drug delivery has been noted in phosphate buffer, which is attributed to the films' inadequate durability. These results illustrate the remarkable potential of composite films in delivering a sustained release for hydrophilic or unstable pharmaceuticals [159]. A separate investigation revealed that the chitosan and polyvinyl pyrrolidone composite hydrogels are appropriate for usage as structured drug delivery systems and intelligent drug delivery scaffolds [160]. Researchers have examined the chemical and mechanical characteristics of cotton cellulose nanofiber nanoformulations, their cytocompatibility with human kidney cells, and the development of surface roughness in chitosan film [161]. Researchers discovered that the formulation of chitosan/corn starch mixed with hydroxyurea, utilizing potassium persulfate as an innovative

material, was appropriate for medicinal applications in drug delivery systems [162]. In a separate study, researchers experimented on innovative pH-responsive hydrogels that integrated neomycin sulfate with silane by combining chitosan and alginate with PVA for controlled drug administration via injection in biomedical applications. The resultant hydrogel displayed the most significant expansion at an acidic pH and minimal swelling at a primary and neutral pH. Furthermore, it revealed a drug emission in 30 min. Consequently, it can be asserted that these hydrogels serve as sophisticated substances for injection-controlled drug delivery [163]. The present study developed indomethacin-loaded, cyclodextrin-modified chitosan nanofibers via the electrospinning technique. FTIR spectroscopy, nuclear magnetic resonance, and XRD analysis validated the graft's preparation. The nanofibers were confirmed to be at the nano size via SEM, and their size enhanced cell division. The drug's regulated release was detected in a phosphate buffer solution, concluding its suitability for intelligent drug delivery systems in tissue repair and regeneration within bone applications [164]. Numerous other studies have been conducted on intelligent drug delivery systems utilizing medicines like ciprofloxacin [165]. Mesalamine [166] Dasatinib, and doxorubicin [167].

4.3 Antimicrobial and antiviral applications

4.3.1 Role of nanocarriers in antimicrobial drug delivery

Antimicrobial Nanomedicine Drug Delivery Systems The advancement of (NDDSs) offers innovative disease prevention and therapy approaches. Recently, lipid vesicles, polymersomes, nanoparticle gels, and inorganic and hybrid (core/shell) nanoparticles have been widely utilized as novel pharmaceutical delivery mechanisms in the early stages of clinical studies and pre-clinical trials [168]. These technologies have the potential to enhance medications' medicinal and medicinal attributes. Three of 27 prolong the effectiveness period, improving effectiveness and specificity, mitigating drug resistance, and decreasing immune response and harmful effects. An increasing number of NDDS are utilized in the early and clinical trial phases. Nanoparticles frequently exhibit significant bactericidal efficacy when evaluated against harmful bacterial strains. Metal nanoparticles can exhibit either bactericidal or bacteriostatic properties, contingent upon their particle size, coating technique, and level. An extensive review of evidence indicates that nanoparticles exhibit antibacterial action, targeting gram-positive and harmful microorganisms. The cell wall comprises a thin peptidoglycan structure (~7–8 nm) with a negatively charged surface [169].

4.3.2 Role of nanoparticles in antiviral drug delivery

Viruses are entities that are typically less than cellular size. They are often below bacterial size and are the primary causative agents of various mild diseases [170]. Virucidal drugs mainly function by directly disrupting the initial phase of viral propagation [171]. Iron oxide nanoparticles are proposed to alter the viral RNA copies of H1N1 Influenza, a virus with a MIC value varying from 4.25 to 0.2 pg within 24 h. Zinc oxide (16–20 nm) inhibits the multiplication of the H1N1 influenza virus, demonstrating antiviral efficacy [169]. The diminutive dimensions of nanoparticles may enhance efficacy owing to the influence of zeta potential. AgNPs ranging from 10 to 80 nm have antiviral action toward the Monkeypox virus, with 10 nm particles demonstrating superior efficacy as a therapeutic antiviral agent. They have documented the effectiveness of diminutive AgNPs that may readily adhere to viral cells, obstructing their subsequent binding to host cells and ultimately diminishing viral reproduction [172].

5. Medicinal plants in designing glucose-sensitive nanocarriers

5.1 Role of medicinal plants in nanocarrier design

The healthcare sector is witnessing a growing need for efficient, secure, and eco-friendly therapy modalities [173]. Therapeutic plants, an essential element of traditional medicine, have rich historical usage and significant healing properties. WHO indicates that 80% of people in underdeveloped nations rely on relating to natural remedies for everyday healthcare requirements. About 11% of the pharmaceuticals in the WHO List of Essential Drugs are exclusively from therapeutic plants [174]. The worldwide demand for plant-based medicine is significant, growing annually at 13%; by 2050, the need to exceed $five trillion is anticipated [175]. Therapeutic plants are those utilized for illness prevention and treatment, with all or part of the plant serving therapeutic aims or as an active ingredient in the pharmaceutical sector [176]. Typically, there are two primary advancements in the nanoformulation of active pharmacological compounds derived from medicinal plants: Nanostructured ultrafine technologies and pharmaceutical nanocarriers [177]. Utilizing nano ultrafine technology, including recrystallization and powdering techniques (e.g., ball milling and air stream powdering), traditional Chinese medicines (TCM) that are not appropriate for commercial extraction, like mineral or toxic substances, as well as those with labile bioactive compounds, are meticulously ground. As the drug particle surface increases, a substantial quantity of active components endows diverse material characteristics [178,179]. This technique pulverizes TCM particles to the nanoscale scale, signifying the foundational technology for nano TCM. Encapsulation of active ingredients in nano-carrier pharmaceuticals utilizes advanced technology to modify medication distribution, regulate release rates, and improve the bioavailability of insoluble compounds [180]. Nanoparticle drug carriers facilitate nonspecific targeting via improving uptake and retention properties. At the same time, ligands affixed to their surface enable the delivery of medications targeting particular organs or cells by realizing targeted delivery [181,182].

5.2 Polysaccharides from medicinal plants for glucose sensitivity

Polysaccharides are a category of carbohydrates consisting of more than 30 monomeric units. This category comprises divers-saccharide chains, like starch and cellulose, and heteropolysaccharides, such as glucosamines. The Treatment effects of polysaccharides on hyperglycemia have been studied in medical research (Table 14.1).

A study assessing the effectiveness of nutritional intake on diabetes individuals found that three distinct diets were effective: a reduced carbohydrate regimen, a high-starch regimen, and a standard American meal. The study found that 24-hour blood sugar levels and insulin amounts were considerably reduced in reduced-carbohydrate meals compared to high-carbohydrate and American diets. High-fiber carbohydrate (HCF) reduces overall fat, and low-solid fat diets could be beneficial in preventing diabetes-related issues. A high-carb meal significantly improved glycemic response and reduced postprandial lipids in patients with T2DM. A diet rich in carbohydrates and fates did not affect daily urine elimination of calcium and trace elements, suggesting that diabetes sequelae, including kidney disease and bone fragility, might not occur under hyperglycemic settings. A study on 10 young,

Table 14.1 The Treatment effects of polysaccharides on hyperglycemia in medical research.

Polysaccharide	Physiological effect	References
Arabinoxylan (AX)	Reduced after-meal blood sugar with elevated insulin secretion.	[183]
Guar gum	Decreased blood glucose levels, improved insulin sensitivity, reduced glycated hemoglobin, low fasting blood sugar, decreased LDL and LDL/HDL ratio, reduced after-meal glucose, lower total cholesterol, minimal cardiovascular risk markers, and no changes in mineral levels.	[184]
Flaxseed gum	Decreased blood glucose and insulin concentrations.	[185]
Starch	Reduced after-meal blood sugar and insulin levels.	[186]
Amylose	With rice noodles, elevated blood sugar and insulin secretion levels.	[187]
Resistant starch	Increased short-chain fatty acids, enhanced gut microbiota, elevated after-meal glucose and insulin sensitivity, higher esterified fatty acids, beta-hydroxybutyrate, and reduced postprandial glucose and insulin.	[188]
PPB-203 (A new resistant starch)	Reduced spikes in after-meal blood sugar levels.	[189]
Resistant dextrin	Improved insulin sensitivity with reduced inflammatory responses.	[190]
Nutriose®FB06 (Resistant dextrin from wheat) Nutriose®FM06 (Resistant dextrin from maize	Reduced baseline insulin, decreased oxidative stress marker (malondialdehyde), and lower inflammation markers such as interleukin-6 and tumor necrosis factor-alpha.	[190]
Icodextrin	Reduced blood sugar reaction and decreased survival probability.	[191]
β-glucan	Improved insulin sensitivity with an elevated blood sugar response.	[192]
Konjac glucomannan (KGM)	Improved insulin sensitivity with an elevated blood sugar response.	[193]
Polysaccharide from lyceum Barbarism	Decreased blood sugar, reduced insulin secretion indicators, and lower high-density lipoprotein cholesterol.	[194]

fit, healthy participants found that a high-carbohydrate diet resulted in NF-κB levels three times higher than a standard food intake, suggesting a correlation between variations in the glycemic values and the inflammatory system's threat [195]. Extensive data indicates that the thyroid-related hormone, 3,5,3′-triiodothyronine, correlates with dietary carbohydrate levels, and its metabolic effects are contingent upon carbohydrate consumption. Among thin patients with T2DM, the T3 reaction to glucose intake is

influenced by carbs consumption, hence strongly correlating with glucose tolerance [196]. A longitudinal clinical trial examining an HCF diet in diabetic males indicated that hospital HCF menus containing 65 mg of fiber and 70% carbohydrates correlated with decreased insulin and circulating lipid profile components levels—a home HCF diet including 50 mg of roughage and 60% carbohydrates enhanced glycemic response. An analysis of dietary adherence revealed that 9 cases exhibited high commitment, 1 showed poor commitment, and one was unsuccessful [197].

5.3 Phytochemicals enhancing nanocarrier efficacy

Phytochemicals or phytonutrients are primarily plant-derived secondary compounds that possess a broad spectrum of molecular composition, encompassing indole, saponins, alkaloids, plant sterols, phenolic compounds, sulfur-containing isothiocyanates, and phytoprostanes/heterocyclic oxygen compounds [198]. Such compounds are not crucial for the plant's expansion and progress; they are advantageous for human wellness and disease management [199]. Phytochemicals, whether in their original form or as biological compounds, confer health benefits through direct free radical scavenging, metal chelation, microtubule and microfilament construction, and protease inhibition [200]. A crucial aspect to evaluate from a metabolic perspective is the pathway following oral ingestion, wherein the body identifies and processes these phytochemicals as xenobiotics. The phytochemicals undergo catabolism and metabolism in the oral cavity, gastric chamber, and intestine and are subsequently absorbed into the bloodstream or lymphatic system. They are then distributed through diffusion or transport to the body's circulation, metabolized in tissues through metabolic transformation or catabolism, and ultimately excreted through kidney, bile, or lung routes [201].

6. Challenges and future directions

6.1 Challenges

6.1.1 Limitations of glucose-responsive components

A significant problem in improving glucose-regulated medication release systems is maintaining the functionality of glucose-regulator molecular components in natural settings to ensure sustained glucose reactivity. While the function of GOD is maintained throughout carrier synthesis, it is unsuitable for continuous glucose sensing because of the inhibition of the enzyme-mediated reaction by various outcome compounds. It limits the possibility of use within autonomous drug delivery. Con A, a carbohydrates-binding protein, exhibits instability in creating glucose-sensitive systems, constraining its utility within glucose-regulated medication delivery platforms. PBA and its compounds, exhibiting superior consistency and extended shelf life compared to GOD and Con A as glucose-sensitive agents, have recently garnered significant focus on glucose-responsive drug systems. The pKa of PBA (8.2−8.6) exceeds physiological pH (7.4), presenting a substantial barrier in adjusting its pKa to 7.4 while maintaining optimal glucose sensitivity [202].

6.1.2 Biocompatibility and safety of nanoparticles

The biocompatibility and security of nanoparticles may be effectively validated by their toxicity assessments in various cell lines and through histology and serum biochemical evaluations. Numerous research has confirmed the compatibility of multifunctional nanoparticles with L929 cell cultures,

dermal connective tissue cells, and histopathological assessment of critical organs like the hepatic, cardiac, pulmonary, splenic, and renal systems [203].

6.1.3 Challenges in stimuli-responsive control

A notable problem is attaining exact regulation of their reaction toward outer stimuli like pH, temperature, or molecular cues, which may fluctuate significantly inside living organisms [204].

6.2 Future directions

The current research encompasses various fields, incorporating regulated drug release, biological catalysis, optical detection, visualization, cellular embedding, and biomedical tissue construction. In the future, cooperative treatment strategies (i.e., localized and generalized) to modulate extended medication delivery through specific stimuli-sensitive hydrogels may represent a novel model in oncology therapy. Prospective studies should emphasize enhancing the use and efficacy of stimulus-responsive hydrogels across these several domains [205].

7. Seven conclusions

Glucose Glucose-responsive systems have great promises for enhancing treatment effectiveness and diabetic patients' quality of life by circumventing the drawbacks of conventional insulin delivery. There have been many GR system developments, but many unanswered questions and significant obstacles prevent GRS formulations from being clinically relevant and widely used in healthcare. One of the main challenges in designing GR systems is ensuring insulin is released and stopped at the exact correct times based on clinically relevant blood glucose levels. Another challenge is ensuring that the system responds quickly to changes in blood glucose levels, such as when to release or store insulin. Lastly, it must be reversible enough to avoid insulin release that is too high when the blood sugar is low or too high. Achieving all three desirable characteristics is necessary to create novel GR systems that regulate glucose levels as effectively as a natural pancreas. This chapter traces their origins and describes their manufacturing strategies. Furthermore, a range of representative biomolecule-responsive polymers is summarized according to their Biomedical applications.

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Further reading

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CHAPTER

15

microRNA responsive nanocarriers for biomedical applications

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1. Introduction

Nanomedicine is a more specialized concept than medical nanotechnology, which was first proposed in 1993 by Robert A. Freitas. Nanomedicine is the observation, control, and treatment of biological systems of the human body at the molecular level by using nanostructures and nanoscale tools [1]. In the realm of targeted therapies, the field of nanomedicine has achieved significant advancement in combating diseases, particularly cancer, by drawing inspiration from natural nanostructures and their intricate designs [2]. The exploration of natural molecules for developing stimulus-responsive systems (SRS) has garnered extensive attention in recent years, as these molecules possess unique properties that allow them to adapt predictably under various conditions [3]. These responsive systems can dynamically react to various physical stimuli, including light, pH variations, temperature changes, and electromagnetic fields, as well as chemical and biological signals such as enzymes, biomolecules, and reducing agents. A wide range of smart nanocarriers has been meticulously designed to enable precise regulation of the timing and location of therapeutic payloads, including drugs and genes. By ensuring targeted delivery to specific affected areas within the body, these advanced carriers enhance treatment efficacy for both genetic and acquired diseases while reducing potential side effects [4]. Notably, recent research efforts have delved into exploring nucleic acids as versatile components in crafting innovative stimulus-responsive systems with promising potential for revolutionizing medical interventions [5].

Nucleic acids, the essential molecules of life, are intricately constructed using nucleotides as their fundamental building blocks. Each nucleotide consists of a pentose sugar, a nitrogen-containing heterocyclic base, and a phosphate group, all intricately bonded together to form the complex structure that is vital for genetic coding and cellular functions [6]. Beyond the iconic double helix formation famously associated with deoxyribonucleic acid (DNA), nucleic acids have the fascinating ability to

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adopt various other configurations. These include ribonucleic acid (RNA) sequences, intricate hairpin loops, bulging structures, three-way and four-way junctions, unique i-motifs, A-form patterns, left-handed Z-form twists, as well as triplex and G-quadruplex structures. Each of these distinct formats possesses its own set of remarkable characteristics: from robust physiochemical stability to configurability and conformational flexibility [7]. As a result, nucleic acids have emerged as highly desirable biomolecules for creating self-assembled nanomachines based on NA, as well as intelligent molecular robots tailored for specific tasks. These structures boast precise design, versatile capabilities, and a diverse array of potential uses [8].

Within the field of nanotechnology, a class of nanocarriers exhibits responsiveness toward specific nucleotide sequences known as microRNA-responsive nanocarriers. These unique carriers are designed to interact with microribonucleic acids (miRNAs), which are diminutive, non-coding RNA molecules composed of 22 nucleotides [9]. Through binding with the untranslated regions of target messenger RNAs (mRNAs), miRNAs play a pivotal role in regulating gene expression by functioning as antagonistic transcriptional agents. Their influence extends across a spectrum of biological processes, including cell viability, programmed cell death (apoptosis), cancerous metastasis, and cellular proliferation. Furthermore, it has been observed that alterations in miRNA expression levels can be intertwined with the onset and progression of various metabolic disorders. Consequently, there has been an upsurge in interest surrounding the utilization of miRNAs within the domain of nanomedicine for both diagnostic and therapeutic purposes aimed at combating such ailments [10]. In recent times, there have been considerable strides made in the development of miRNA-responsive intelligent delivery systems, aimed at providing customized and efficient delivery solutions. Despite these advancements, significant hurdles remain to be surmounted. The overarching goal of crafting innovative smart delivery platforms is to create products that not only excel in live experimentation but also pass rigorous human clinical trials. Thus, it is imperative that these miRNA-responsive delivery vehicles undergo thorough assessments for both in vivo and clinical specifications. These evaluations encompass a spectrum of crucial parameters such as clearance mechanisms, pharmacokinetics, circulation lifespan, dose-dependent toxicity effects, compatibility with biological systems, degradability factors, capacities to evade host immunity responses, minimal immunogenicity risks, and stable colloidal properties [11,12].

2. Basics of microRNA and its biological role

The intricate world of microRNA, a significant RNA molecule devoid of coding, yet possessing immense regulatory power over gene expression, was first investigated in scientific exploration through the observation of a nematode worm [13]. These diminutive strands of genetic material, typically spanning a mere 20–24 nucleotides in length, wield substantial influence over messenger RNA molecules by adeptly binding to complementary sequences found predominantly within the enigmatic 3′ UTR region. Through this binding process, they orchestrate either the obliteration or inhibition of mRNA translation into essential proteins. This nuanced post-transcriptional regulation endows miRNAs with the potent ability to sway an array of biological processes pivotal for life's sustenance - be it development, differentiation, proliferation, or apoptosis. Intricately intertwined within countless cellular pathways, these non-coding RNAs have been implicated in pathology when left unchecked and unbridled by regulatory forces [14,15].

2.1 Biogenesis of miRNA

The process of miRNA synthesis involves multiple steps. According to Fig. 15.1, miRNAs are initially transcribed in the nucleus of the cell by RNA polymerase II from intergenic or intragenic regions. This transcription results in the formation of primary miRNAs that are typically 1–3 kilobases in length. Primary miRNAs are processed in the nucleus by the Drosha enzyme and Pasha protein, forming stem and loop structures known as pre-miRNAs with a length of 70–100 nucleotides. These pre-miRNAs are exported to the cytoplasm by exportin-5, where they are further cleaved by the Dicer enzyme into 18 to 24 double-stranded oligonucleotides, generating mature miRNAs. Once the two strands are separated, one strand becomes a mature miRNA molecule and integrates into the silencing complex

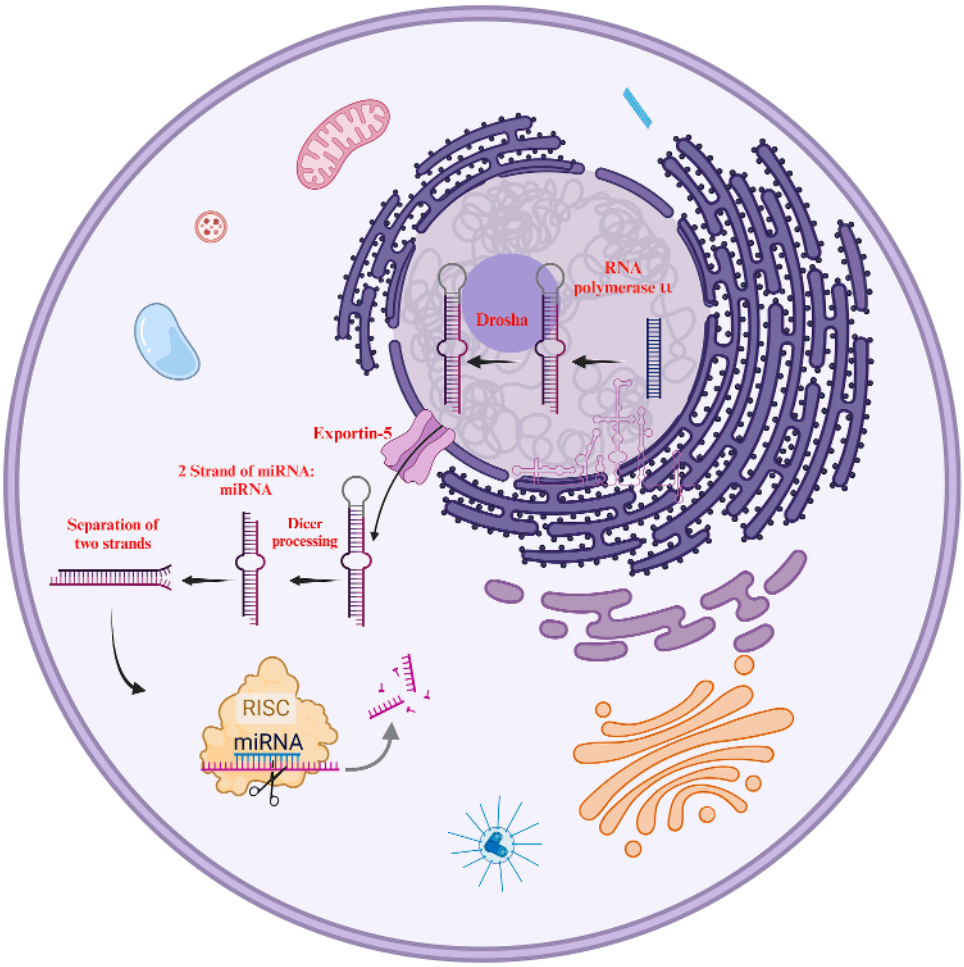


FIGURE 15.1

Synthesis of miRNA.

triggered by RNA-induced silencing complex (RISC) 1 [14]. The other strand, known as strand 2, is typically either degraded or involved in regulating miRNA homeostasis. The RISC complex interacts with its target mRNA through either complete or incomplete binding, leading to mRNA degradation, translation inhibition, or detachment from the translation machinery. Many animal miRNAs only partially match with their target mRNA, leading to translational inhibition for numerous miRNAs. This binding mechanism reduces protein levels, significantly impacting cellular homeostasis. Understanding the role of miRNAs as tumor suppressors and biomarkers hinges on this crucial issue. Over 2600 miRNAs have been discovered in humans, capable of targeting over 30% of the human genome [14,16].

It is of significance to observe that a single gene can be governed by multiple microRNAs, just as a microRNA may have numerous targets due to its imperfect match with the target sequences. A mounting body of evidence suggests that microRNAs exert a crucial influence in regulating diverse biological processes encompassing early developmental stages, cellular differentiation, proliferation, apoptosis, and beyond. Consequently, variations in miRNA expression levels are intricately linked to the onset and progression of various diseases [17]. A plethora of research has unveiled the intricate connection between the irregular expression of miRNAs and the initiation and advancement of various human afflictions, hereditary anomalies, and alterations in immune system functionality [18]. These small regulatory molecules hold a dual role as both promoters and inhibitors of tumorigenesis, underscoring their pivotal significance in cancer pathogenesis. Consequently, the distinctive expression pattern of miRNAs emerges as a promising biomarker for disease onset [19]. The versatile potential of miRNAs extends beyond mere genetic regulation, encapsulating a promising avenue in gene therapy for addressing hereditary disorders. Concurrently, extensive endeavors have been undertaken to devise therapeutic modalities centered on miRNA manipulation, aimed at rectifying aberrant expression levels within cancerous cells. The pervasive influence of miRNAs beckons the imperative for the refinement of diagnostic tools tailored toward unraveling their intricate roles across various regulatory pathways. The expanding miRNA field demands efficient and reliable methods to investigate their complex functions. In recent years, strides have been made in swiftly advancing techniques for detecting and analyzing these diminutive yet potent molecules; nevertheless, such endeavors are compounded by the stringent requisites demanded by their unique attributes, including minuscule dimensions, infrequent occurrence, and noteworthy sequence homologies among closely related members within analogous families [19].

The diminutive size of miRNAs poses a challenge to the various methods utilized for their detection. Techniques such as PCR and hybridization are commonly employed, yet face limitations due to the similarity in length between miRNAs and the primers used in PCR. This results in the generation of very short primers that may hamper the efficiency of PCR amplification. Moreover, labeling short probes for diagnostic purposes proves to be a formidable task, given the difficulty in identifying miRNAs during hybridization. The low melting temperature of double-stranded probes further complicates matters by diminishing the extent of hybridization and promoting crossover events. In light of these challenges, it is imperative to devise novel strategies that enhance specificity when measuring miRNA profiles. Despite constituting only a fraction of total RNA samples, miRNAs exhibit concentrations within cells that can even extend to mere molecules. Therefore, innovative approaches are needed to accurately quantify these minuscule entities within complex biological matrices [20].

2.2 Biological functions of miRNAs

The level of complementarity between miRNA and its target is crucial for understanding how miRNA interacts with mRNA (Fig. 15.2). When miRNA is highly paired with its target mRNA, the mRNA is typically degraded, while partial pairing can inhibit translation. Although miRNA typically binds to the 3′-UTR region of the target gene, research suggests that miRNAs can also bind to the 5′-UTR and coding sequences, although this area is less studied. With over 2600 miRNAs encoded in the human genome and approximately 30,000 mRNAs, one miRNA has the potential to regulate numerous mRNAs, impacting gene expression networks [21]. The complexity of miRNA biology is evident in its intricate organization to regulate gene networks. MiRNAs are crucial in numerous biological

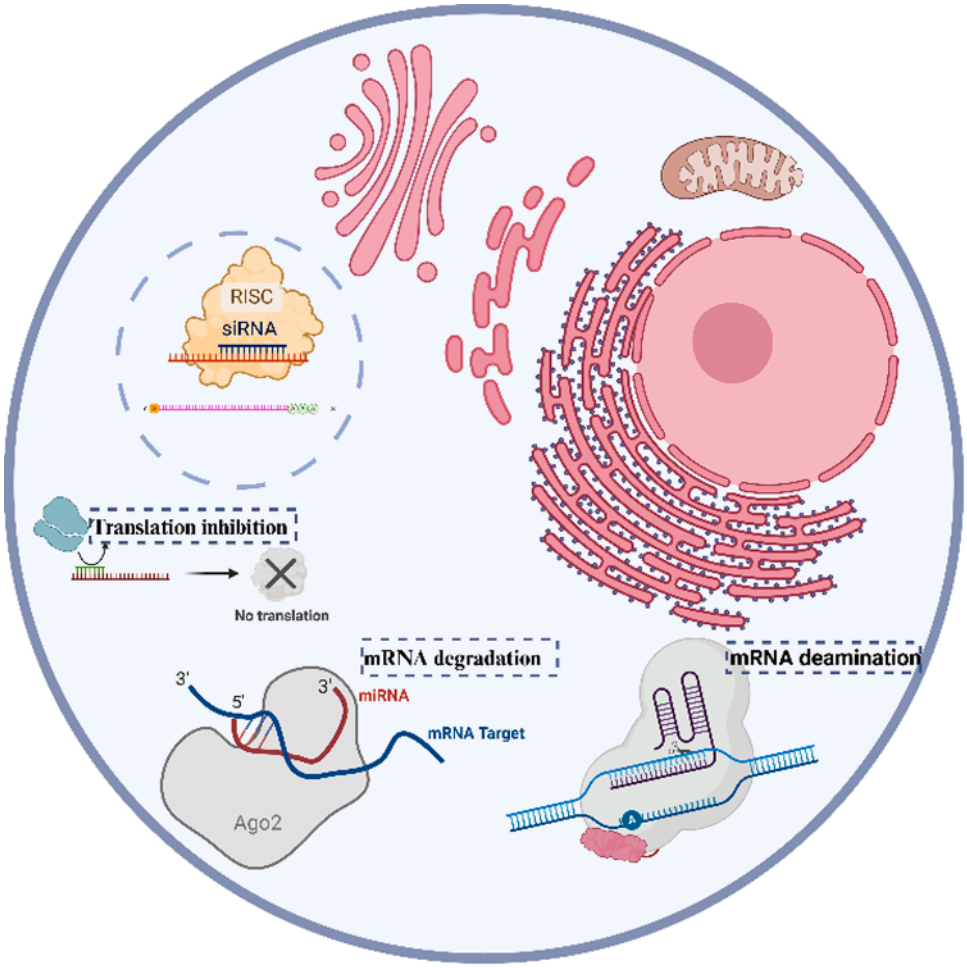


FIGURE 15.2

Regulation mechanism of miRNA gene expression.

processes, such as cell fate determination, proliferation, and cell death. Additionally, they play a role in various cellular activities like immune response, insulin secretion, neurotransmitter synthesis, and circadian rhythm. miRNAs can also influence oncogenic or tumor suppressor pathways, with some functioning as oncogenes or tumor suppressors themselves [22].

2.3 Regulation of miRNAs in various physiological processes

The discovery of miRNAs, particularly lin-4 and let-7, has shed light on their essential role in regulating larval development time within C. elegans. Through various studies, it has become evident that these small RNA molecules also exert significant influence over the processes of neural, muscle, and reproductive development. Notably, miRNA-124 stands out in vertebrate neural development, with its increased expression prompting the initiation of neural developmental pathways while decreased levels serve to impede them. Abundantly expressed in both neural progenitors and mature neurons, miRNA-124 functions as a promoter of neurogenesis, contributing to the intricate orchestration of this fundamental biological process. MiRNAs have been extensively studied for their role in the development of the heart, vascular tissue, and blood. They are crucial in various cardiovascular pathologies, including arrhythmia (miRNA-1 and miRNA-133), fibrosis (miRNA-29), and metabolic disorders (miRNA-33). Among these, miRNA-1 is the most abundant in cardiac muscle cells and plays a key role in heart development. Additionally, miRNA-218, miRNA-143, and miRNA-145 have been identified as important regulators of blood vessel growth. The function of miRNAs is also essential for the development of blood cells [22,23].

2.3.1 miRNAs and cell differentiation

miRNAs are known to wield a significant influence in the intricate process of embryonic stem cell differentiation. As these versatile molecules orchestrate a delicate symphony within the cellular realm, particular miRNAs such as miRNA-296 and members of the 290–295-miRNA class have been noted to witness a decrease in expression levels amidst stem cell differentiation [24]. Conversely, counterparts like miRNA-21 and miRNA-22 exhibit an augmentation in their expression during this transformative journey. The hindered growth of Dgcr8 mutant mouse embryonic stem cells experiences some improvement with the introduction of the 290–295/302 miRNA family, known as the embryonic stem cell-specific cell cycle regulatory miRNAs. Meanwhile, an array of microRNAs intricately mediates the suppression of proliferation within fully specialized stem cells [25]. Notably, miRNA-145 orchestrates a decrement in the presence of OCT4, SOX2, and KLF4 genes during the differentiation process. Furthermore, these small but potent RNA molecules exhibit remarkable efficacy in managing cancer stem cells as well. An example among them is Let-7 miRNA, which stands out for its dual impact on both normal and cancer stem cell populations. Research findings have unveiled the pivotal role played by Let-7 in modulating essential characteristics inherent to breast cancer-initiating cells [26].

2.4 Aberrant expression of miRNAs in cancer

Given the indispensable role that miRNAs play in orchestrating crucial biological functions, it appears evident that these tiny molecules are intricately tied to the prediction, advancement, and etiology of cancer. An observed anomaly in the expression patterns of miRNAs is a recurring phenomenon across

all forms of human malignancies. Notably, key miRNAs such as miRNA-126, miRNA-143, and miRNA-145 exhibit significantly diminished levels in cancerous tissues when juxtaposed with their normal counterparts. Conversely, the expression of miRNA-21 shows a marked elevation in numerous cancer specimens. Such fluctuations underscore the significant impact that miRNAs wield within oncogenic processes [27,28]. Henceforth, miRNAs are employed as biomarkers in the realm of oncology to foreshadow, detect, categorize, monitor progression, and evaluate response to therapeutic interventions [29].

Many pieces of evidence have shown that miRNA-15a and miRNA-16–1, which are located in the 13q14 locus, have decreased expression or been deleted in most cases of CLL1. These two miRNAs act as negative regulators of the BCL2 protein, which can indicate the molecular mechanism that can lead to CLL as a result of the deletion of miRNA-15a and miRNA-16–1 [30]. The diminished manifestation of miRNAs in cancerous cells may stem from a myriad of distinct mechanisms, encompassing chromosomal aberrations, genomic mutations and polymorphisms, epigenetic modifications, and alterations in miRNA biogenesis. Diverse malignancies exhibit shared characteristics concerning the regulation of miRNAs. Foremost among these is the discernible disparity in the miRNA profiles between tumor cells and their healthy counterparts within the same tissue milieu, reflecting the pivotal role played by miRNAs in driving oncogenic progression. While certain miRNAs demonstrate heightened levels of expression, a majority are found to be markedly underexpressed in cancerous tissues relative to their non-neoplastic counterparts. In addition, it is noted that the alteration in the pattern of gene expression is not solely influenced by a singular miRNA, indicating that the onset and advancement of cancer are intricately connected to the collective presence of miRNAs within the cellular framework, commonly referred to as the miRNAome. Moreover, there is a consistent observation in tumors originating from similar evolutionary paths displaying parallel shifts in their miRNA profiles, thereby suggesting promising potential for utilizing these alterations as fundamental markers for both prognosis and diagnosis of cancerous conditions. Furthermore, certain specific miRNAs exhibit frequent aberrations across various forms of malignancies, implying their pivotal roles within crucial signaling cascades that undergo modification in numerous types of cancers [31].

2.4.1 The function of miRNAs as oncogenes

The balance of microRNA expression can have profound implications in the complex landscape of oncogenesis, acting either as a potent oncogene or a vigilant tumor suppressor [32]. Within this intricate system, miRNA-155 emerges as a formidable force, capable of single-handedly instigating tumorigenesis. Through meticulous studies and transgenic mouse models, it has been revealed that heightened levels of miRNA-155 pave the path to high-grade lymphoma and are frequently implicated in various B-cell malignancies such as Chronic lymphocytic leukemia (CLL), aggressive Hodgkin's lymphoma, and select Burkitt's lymphomas [33,34]. Amongst the pioneers in unraveling the orchestration of oncogenic microRNAs stands the miRNA-17–92 cluster, nestled within the genetic tapestry on chromosome 3 at locus 13q31. This cluster orchestrates a symphony of seven distinct microRNAs, including miRNA-92–1, miRNA19b-1, miRNA20a, miRNA19a, miRNA18a, miRNA17-3p, and miRNA-17-5p, which collectively contribute to cancer progression across various fronts, ranging from lymphomas to lung carcinomas [35]. In addition to these formidable players is another noteworthy contender: miR-21, whose menacing presence looms large with augmented expression documented across several types of cancers [36].

2.4.2 The function of miRNAs as tumor suppressors

In contrast to oncogenic miRNAs, microRNAs with reduced expression in malignant cells can act as tumor suppressors. These miRNAs exert their effects by inhibiting oncogenes or genes that suppress cell differentiation and apoptosis. Notable tumor-suppressive miRNAs include let-7, which targets Ras and HMGA2; miRNA-15a and miRNA-16–1, which suppress the anti-apoptotic BCL2 protein; and miRNA-34, which is activated in response to DNA damage and oncogenic stress in a P53-dependent manner, ultimately promoting apoptosis or cellular senescence [37]. Let-7 is among the earliest discovered miRNAs and is one of the most abundant in humans, with 12 paralogs. Studies have shown that let-7 is expressed at lower levels in lung tumors compared to normal lung tissue. Additionally, increasing the expression of let-7 has been found to inhibit the growth of cancer cells both in laboratory settings and in living organisms. Furthermore, the decreased expression of let-7 has been linked to shortened post-surgery survival in numerous cancer cases. These aforementioned studies underscore the significance of miRNA genes in the development and advancement of human cancers. Additionally, multiple research works have demonstrated the efficacy of miRNAs either independently or in conjunction with anticancer medications for treating cancer. Notably, miRNAs can be particularly useful in addressing drug resistance, a primary factor contributing to the ineffectiveness of cancer treatments [19,38].

2.5 miRNAs and their role in other pathological processes

In the field of genetic regulation, a solitary miRNA possesses the remarkable ability to target an array of genes that bear resemblance to their seed sequences. Conversely, siRNA operates with the specific aim of diminishing the expression of a singular gene by precision cleavage of perfectly matched mRNA strands. The disruption of miRNA equilibrium emerges as a prevalent characteristic within cancer manifestations, wherein the absence of tumor-suppressing miRNAs and the rampant presence of oncogenic variants (referred to as oncomiRs) are pervasive across diverse forms of malignancy. Consequently, numerous oncogenic pathways evade suppression enacted by tumor suppressors and instead assume heightened functionality, eliciting pivotal contributions to the progression and onset of cancerous growths [19]. Through the manipulation of tumor-suppressive miRNAs or the depletion of oncomiRs within cancerous cells, the utilization of miRNA-based therapies emerges as a promising approach for combating cancer. This article delves into the intricacies of miRNA-based therapeutics, outlining two distinct strategies: firstly, the restoration of tumor suppressive miRNAs and secondly, the eradication of oncogenic miRNAs [39].

2.5.1 miRNA restoration

The objective of restoring tumor-suppressive miRNAs is to revive the innate capabilities of these small RNA molecules, namely impeding cellular growth, facilitating programmed cell death, and thwarting the spread of cancerous cells. Efforts are underway to devise methods for combating the suppression of epigenetic regulation or enhancing the generation of microRNA. In a different approach, the diminished or eliminated levels of microRNAs can be rejuvenated through the introduction of genes encoding microRNAs or their mimics [40]. The identification of miR-15a and miR-16–1 represented a significant breakthrough in cancer research, as they were the first microRNAs recognized for their direct involvement in tumorigenesis. Their loss or downregulation is commonly observed in chronic lymphocytic leukemia, prostate cancer, non-small cell lung cancer, and multiple myeloma. Restoring

these microRNAs has been demonstrated to induce apoptosis, arrest cell cycle progression, and inhibit tumor growth [41]. In addition to their regulatory effect on the anti-apoptotic protein BCL-2, the miR-15 16 cluster also targets other key proteins such as MCL-1, RAD51C, PDCD6IP, CCND1, and VEGF at a post-transcriptional level [42]. Let-7, the pioneering human miRNA to be unearthed, possesses a pivotal role as a tumor suppressor, notably exemplified in non-small cell lung cancer (NSCLC). The diminishment of let-7 expression in lung cancer is closely tied to an unfavorable prognosis for patients. Through experiments conducted on NSCLC mouse models, it has been evidenced that the reintroduction of let-7 markedly diminishes the overall tumor burden [43]. This decline in let-7 levels has also been observed in other types of cancers, such as breast, ovarian, and prostate; nonetheless, its deregulation shows variability contingent upon the specific disease and cellular environment at hand. The potent capabilities held by let-7 extend beyond mere suppression of tumorous growth; this microRNA effectively hinders malignant cell proliferation and arrests cell division by honing in on key targets like RAS, MYC, CCND1, CCND2, CDK6, and CDC25A. Furthermore, it exerts control over metastasis and stemness by directly targeting HMGA-2 [44].

All three members of the miR-34 family are regulated by p53: miR-34a, miR-34b, and miR-34c. Among them, miR-34a is generally the most abundant in normal tissues, though in certain organs like the lung and ovary, miR-34b/c is more dominant. Many prevalent cancers, including melanoma, breast, lung, colon, kidney, and pancreatic cancers, exhibit reduced miR-34a levels, while lung, colorectal, and ovarian cancers often lack miR-34b/c expression. This downregulation is primarily attributed to hypermethylation of the CpG island within their promoter regions. Functionally, miR-34 plays a crucial role in promoting apoptosis, arresting cell cycle progression, and suppressing tumor growth by targeting key genes such as CDK4, CDK6, CCND1, SIRT1, BCL-2, MYC, and MET. Inhibiting prostate cancer stem cells by restoring miR-34a expression has been shown to reduce CD44 levels. Furthermore, it suppresses epithelial-to-mesenchymal transition (EMT) in malignant cells by directly inhibiting SNAIL expression and modulating IL-6R/STAT3/miR-34a. In vivo studies have demonstrated that reintroducing miR-34a in murine models of NSCLC, lymphoma, prostate cancer, and pancreatic cancer significantly curtails tumor growth and improves overall survival rates [45,46]. A notable advancement in miRNA-based therapeutics is MRX34, a liposomal formulation designed to mimic miR-34a. It represents a significant milestone as the first miRNA replacement therapy to enter Phase I clinical trials for patients with advanced hepatocellular carcinoma (HCC) [47].

2.5.2 miRNA ablation

Within the intricate world of cancer cells, there exists a phenomenon where oncomiRs reign supreme in their overexpression, ruthlessly targeting tumor suppressor genes while the levels of protective tumor-suppressive miRNAs dwindle into insignificance. To combat this imbalance, modern science has developed a repertoire of sophisticated methods to silence these oncomiRs. These include the utilization of antisense oligonucleotides known as ASOs or anti-miRs, such as the potent antagomirs which are cholesterol-conjugated anti-miRs, alongside locked nucleic acid (LNA) anti-miRs and diminutive LNA anti-miRs. Furthermore, ingenious creations like miRNA sponges containing multiple binding sites for specific miRNAs and small-molecule inhibitors termed SMIRs have emerged as promising weapons against the malignant cellular fabric. AS oligonucleotides, abbreviated ASOs, are elegant single-stranded RNA designed to impede the interaction between microRNAs and messenger RNAs through their function as competitive inhibitors. LNA anti-miRs are distinguished by a methylene bridge that enhances their thermal stability and improves hybridization efficiency. Notably, tiny

LNA anti-miRs are 8-nucleotide-long antisense oligonucleotides (ASOs) specifically designed to target the critical 5′-seed region of microRNAs. Additionally, miRNA sponges utilize transgenic RNA molecules engineered to effectively inhibit specific microRNAs or entire families within this complex regulatory network [48,49]. The miR-17–92 cluster was the first group of oncomiRs to be identified, encompassing several microRNAs, including miR-17. This cluster is activated by c-Myc, a transcription factor frequently overexpressed in cancer, and collaborates with c-Myc to accelerate B-cell lymphoma progression. It is also closely linked to E2F transcription factors, which regulate cell cycle progression and apoptosis. Specifically, E2F1, E2F2, and E2F3 are directly modulated by this cluster, with E2F1 and E2F3 also activating these miRNAs, forming a negative feedback loop. The cluster's ability to inhibit apoptosis is largely due to its suppression of pro-apoptotic factors such as Bim, PTEN, and p21, while its role in promoting tumor angiogenesis is mediated through the regulation of thrombospondin-1 and connective tissue growth factor. Many cancers, including B-cell lymphoma, small-cell lung cancer, pancreatic cancer, and renal cell carcinoma, exhibit miR-17–92 amplification. It has been demonstrated that suppressing miR-17 and miR-20a induces apoptosis and impedes cell proliferation in lung and renal cancer cells [50].

MiR-21 is an oncomiR that is greatly elevated in blood cancers and various common solid tumors like glioblastoma [51], HCC [52], breast [53], lung [43], prostate [54], colorectal [55], pancreas [56], ovaries [57], and stomach cancers [58]. Its primary target is the tumor suppressor PDCD4, which helps prevent cancer development and progression [59]. In a mouse model of NSCLC, miR-21 promotes tumor growth by directly targeting inhibitors of the Ras/MEK/ERK pathway and enhancers of apoptosis [60]. In glioblastoma cells, lowering miR-21 levels leads to cell cycle arrest and apoptosis by releasing repression on key components of the p53 and TGF-β pathways [51]. Other important targets of miR-21 include TPM1, PTEN, RECK, maspin, and NFIB. Decreasing miR-21 through genetic methods or anti-miR-21 transfection significantly hinders tumor growth in mouse models of NSCLC, B-cell lymphoma, breast cancer, and multiple myeloma [61].

MiR-155 is an oncomiR initially associated with leukemia/lymphoma tumorigenesis, but it is also present in elevated levels in breast [62], lung [63], colon [64], and pancreatic cancers [65]. Elevated miR-155 expression is associated with unfavorable prognoses in pancreatic and breast cancers. In B lymphocytes, it directly regulates SHIP and C/EBPβ, key modulators of the interleukin-6 signaling pathway. In pancreatic cancer cells, miR-155 targets TP53INP1, a pro-apoptotic gene activated by p53 in response to cellular stress [66]. In breast cancer cells, miR-155 promotes TGF-β-induced epithelial-to-mesenchymal transition (EMT) by interacting with key proteins such as RhoA, C/EBPβ, and ZNF652, a repressor of the EMT gene network. Additionally, miR-155 targets several regulators, including PPP2CA, SOCS1, FOXO3a, VHL, SMAD5, and CK1a, all of which contribute to tumor growth, apoptosis resistance, and angiogenesis. It is noteworthy that miR-155 acts as a link between inflammation and cancer by boosting immune cell production of cytokines, chemokines, proteinases, growth factors, and angiogenesis factors, resulting in a pro-inflammatory tumor microenvironment [67].

3. microRNA responsive nanocarriers in modern medicine

Personalized medicine is a customized approach to healthcare that aims to find the most effective drug and dosage for each patient. This approach has been developed in response to the high occurrence of

adverse drug reactions and varying levels of drug efficacy among different patients. Personalized medicine incorporates the study of proteomics, genomics, and epigenetics, as well as consideration of individual health conditions and environmental factors [68]. Nanotechnology shows great potential in drug encapsulation and delivery due to the unique properties of nanoparticles, such as their large surface area and porosity. These nanoscale molecules can engage with cells at a subcellular and molecular level, allowing for interactions that are not achievable on a larger scale, exceeding 1 μm. Nanotechnology offers significant advancements in the diagnostic field, particularly in the exploration of specific drug targets, pharmacogenetic testing, and the ability to conduct both in vitro and in vivo testing. In the therapeutic area, nanomedicine allows for the customization of drugs to target specific diseases in individual patients. Additionally, nanomedicine's targeting capabilities enable the administration of higher doses than the maximum tolerated dose for traditional drugs, with the added benefit of tailoring doses to each patient's unique conditions [69,70]. Nanomedicine also addresses challenges related to individualized drug responses, such as variability in cytochrome P enzymes (CYP) and drug transporters among different populations. Nanomedicine drug formulations have the potential to make drugs more resistant to metabolizing enzymes and facilitate their entry into cells through the endocytic process without the need for transporters. Originally, nanodrugs were created to enhance the properties of existing drugs or diagnostic agents [71]. In our modern era, nanoparticles have been meticulously designed with precision engineering to mitigate the occurrence of adverse effects both locally and systemically. They are tailored to amplify the effectiveness of orally administered medications by increasing their bioavailability, thereby enhancing their overall pharmacokinetic and pharmacodynamic properties. Moreover, nanoparticles possess the capability to decrease the frequency of drug doses, ultimately improving patient adherence and clinical outcomes. When compared to traditional methods of drug delivery, nanodrugs boast a plethora of advantages in terms of pharmacokinetics. These benefits include heightened solubility and absorption rates, regulated release mechanisms, bolstered stability and metabolic processes, diminished side effects, prolonged bloodstream circulation, and more targeted and efficient distribution throughout the body [72]. In nanomedicine, drug delivery can be accomplished through either passive or active targeting strategies. Passive targeting depends on nanoparticle size and the permeability of endothelial fenestrations. Tumor vasculature and inflamed tissues often exhibit leaky blood vessels with pore sizes ranging from 200 to 800 nm, a consequence of abnormal vascular architecture and rapid angiogenesis. This allows nanoparticles meeting these size criteria to passively accumulate at the target site, enhancing therapeutic efficacy while minimizing side effects. However, the effectiveness of this enhanced permeability and retention (EPR) effect may be limited in non-solid tumors like leukemia, and different tumor types may have varying pore sizes, potentially leading to incomplete drug delivery and suboptimal therapeutic outcomes. Although the EPR effect allows nanoparticles to selectively accumulate in the tumor interstitium, it does not significantly improve cellular uptake. Functionalizing nanoparticles with ligands that target specific cancer cells or subcellular sites can mitigate the limitations of passive targeting while enhancing nanoparticle internalization. This approach leverages the presence or overexpression of specific receptors on the cell surface, enabling precise interaction with a tailored binding moiety. Moreover, active targeting plays a crucial role in personalized medicine by facilitating the precise delivery of an optimal ligand to the intended site, ensuring the accurate administration of nanodrugs at the correct dosage [73].

One of the most sophisticated methods in the realm of nanomedicine is targeted drug delivery, a process that hinges on the unique characteristics of cell surfaces or delves into genetic factors and

post-translational modifications of proteins and signaling pathways. Within this arena, microRNAs emerge as pivotal components for personalized therapy and precision medicine. These minute molecules play a crucial role in regulating gene expression at the post-transcriptional level, wielding their influence by pinpointing specific messenger RNAs for degradation or inhibiting translation. In doing so, they have the power to impact a myriad of cellular processes and participate in the development of numerous diseases. In today's landscape, with advancements in nanotechnology utilizing nanoparticles (NPs), non-coding RNAs like microRNAs (miRNAs) are assuming critical roles as tumor suppressors or oncogenic inhibitors through targeted drug delivery methods [74]. This innovative approach not only targets cancerous cells more effectively but also mitigates adverse effects on healthy tissues and organs. Up to the present time, scholars have utilized cellular and molecular elements implicated in various forms of cancer, such as Colorectal Cancer (CRC) and breast cancer. The genesis and advancement of these cancers are driven by the progress of contemporary personalized medicine, which relies on the utilization of miRNAs delivered to targeted cells through nanocarriers. These miRNAs can function both as tumor suppressors and oncogenes, enabling their modulation for purposes such as DNA repair, cancer stem cell regulation, epithelial-mesenchymal transition, extracellular vesicle generation, drug delivery mechanisms, and autophagy [75]. Notably, specific miRNAs like miR-10b, miR-34a, and miR-155 have been identified in circulating blood samples from breast cancer patients as reliable independent prognostic indicators for assessing cancer risk levels [76]. Additionally, the downregulation of miR-148 and miR-29b in individuals with high-grade prostate cancer suggests a potential tumor-suppressive role for these particular molecules within this context [77].

3.1 Organic nanoparticles for microRNA responsive nanocarriers

The initiation of personalized medicine requires the initial undertaking of pharmacogenetic testing. Within this realm, a variety of organic nanoparticles, including lipid-based ones like niosomes, liposomes, and exosomes, as well as polymers such as dendrimers, protein nanoparticles, and carbohydrates have been carefully studied. These unique particles are investigated for their ability to compile an individualized molecular profile for patients in order to enable precise identification of specific targets or genes. These minuscule particles serve as a groundbreaking tool in the examination of a vast array of patient-specific genes, ultimately leading to precise diagnosis and tailored treatment strategies [78]. By utilizing nanosystems and devices, it becomes feasible to construct individual molecules from nucleic acids owing to the minute dimensions of nanoparticles, which serve as ideal targets for developing drug delivery systems responsive to nucleic acids. In addition to facilitating specific diagnostic procedures, nanoparticles also offer an invaluable means of pinpointing genetic abnormalities with precision. Presently, medical professionals are equipped to evaluate numerous gene panels for an individual patient and make informed decisions regarding suitable medications [79,80]. Below is an overview of the use of organic nanoparticles in personalized medicine in both diagnostic and therapeutic aspects.

Lipid-based drug delivery systems are distinguished by the presence of a lipid bilayer enveloping a central aqueous core, facilitating their precise targeting of specific cells through both active and passive mechanisms. The optimal size for these vesicular systems typically falls within the range of 80–150 nm, ensuring efficient cellular uptake. Liposomes and niosomes can be engineered to seek out cancer cells by leveraging the enhanced permeability and retention effect (EPR) [81,82]. This

approach is further refined through the use of ligands or antibodies to interact with cancer cells directly. Various ligands, such as estrone, folate, and transferrin, have been extensively studied in the context of targeted drug delivery using liposomes in cancer treatment [83]. Moreover, monoclonal antibodies and miRNA-7 have shown promise in breast cancer therapy by effectively impeding tumor growth, invasion, and metastasis. For instance, experiments utilizing a mouse model of ovarian cancer demonstrated successful transfection of liposome-encapsulated miR-7 into ovarian cancer cells at the tumor site with satisfying results [84].

Yan et al. [85] meticulously developed a precise sequence of miRNA, comprised of 25 nucleotides intricately enclosed within functional liposomes that were skillfully enhanced with modification using DSPE-PEG-2000-tLyp-1 peptide to silence the Slug gene in therapeutic endeavors. These tailored liposomes not only heightened the effectiveness of adjunct chemotherapy in experimental mice but also impeded the TGF-β1/Smad pathway while diminishing secretin expression in triple-negative breast cancer cells. In fact, according to studies, the Slug gene is expressed in cancer cells and leads to invasion and drug resistance, so silencing this gene in cancer cells could be a target for gene therapy. Remarkably, when administered to TNBC-afflicted rodents, these innovative miRNA-loaded liposomes showcased a more potent anti-cancer capacity compared to vinorelbine-loaded counterparts. Moreover, when combined with vinorelbine liposomes, the specialized miRNA carriers virtually halted tumor progression altogether. Initial safety assessments revealed no adverse effects on body weight or essential organs in treated mice. Thus, it can be unequivocally stated that functional miRNA liposomes proved highly efficacious in combating TNBC by selectively targeting Slug through post-transcriptional means and curtailing the TGF-β1/Smad signaling cascade effectively.

Polymeric nanoparticles can be categorized into two distinct yet complementary types: nanocapsules, serving as discreet reservoir systems, and nanospheres, functioning as intricate matrix systems [86]. These intricately crafted nanoparticles are meticulously synthesized from a plethora of natural materials such as protein-based polymers like albumin, gelatin, and collagen or polysaccharide-based polymers including hyaluronic acid and chitosan [87]. Alternatively, synthetic polymers like dendrimers, PLGA, PGA, PAA, and PLA make up another classification within this diverse group. The myriad advantages of utilizing polymer-carriers in drug delivery systems far surpass those offered by alternative materials. They boast qualities such as effortless synthesis methods and biodegradability characteristics that align with sustainable practices in pharmaceutical research and development efforts. Their tailor-made design ensures optimal compatibility with diverse drug molecules for enhanced therapeutic outcomes. Moreover, these innovative polymer-carriers exhibit exceptional ability to navigate through complex biological barriers to extend circulation time within the body while increasing cellular assimilation rates and facilitating escape from intracellular compartments for efficient drug delivery purposes [88]. The most commonly researched delivery vehicles for miRNA mimic delivery are polyplexes, PLGA NPs, and dendrimers [89]. Their structures can be seen in Fig. 15.3.

The complex nanocarriers known as polyplexes are made up of a mixture of negatively charged nucleic acids that are intricately connected to positively charged polymers called polycations through the fascinating force of electrostatic interactions [90]. The effectiveness of a variety of cationic polymers, including PEI, chitosan, and N-(2-hydroxypropyl) methacrylamide (HPMA), in delivering different miRNAs intracellularly has been thoroughly studied. Polyplexes (Fig. 15.4(a)) have notable advantages over the use of viral vectors, such as easy production procedures and low immunogenic reactions [91].

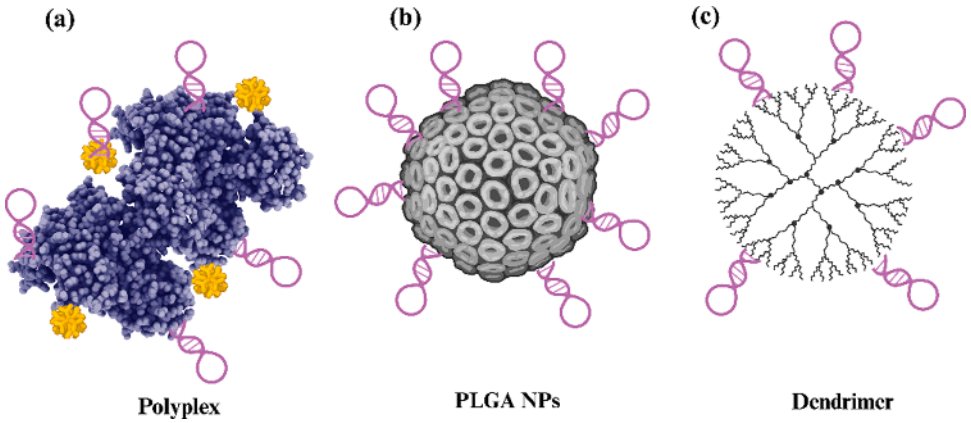


FIGURE 15.3

Polymeric nanoparticles for miRNA mimic delivery. (a) Polyplex-miRNA delivery, (b) PLGA-miRNA nanocarrier, and (c) Dendrimer-miRNA delivery.

Polyethyleneimine (PEI) stands as a stalwart amidst the myriad of polycations utilized for the delivery of microRNA. Crafted from a symphony of CH2CH2NH repeating units, this versatile compound manifests in two distinct forms - one linear and the other branched [92]. In a groundbreaking endeavor, Ibrahim and colleagues harnessed the branched PEI to transport miR-33a and miR-145, both renowned for their propensity to suppress tumors, into a murine model of colorectal carcinoma. Through intraperitoneal administration, nude mice harboring subcutaneous LS174T colorectal tumors were treated with PEI/miR-145 or PEI/miR-33a complexes. The outcome was awe-inspiring; tumor growth ebbed whilst the expression levels of key target genes such as c-Myc and ERK5 underwent a significant reduction. This response stood in stark contrast to those bestowed with mere control of PEI/miRNA concoctions [93].

Chitosan is a commonly used polycation for delivering miRNA, derived from a linear disaccharide made up of N-acetyl-D-glucosamine and D-glucosamine [94]. Studies have shown that chitosan/miRNA polyplexes have been effective in treating various conditions, such as spinal cord injury and cancer [95]. For instance, administering miR-124 through chitosan polyplexes reduced neuroinflammation in rats with spinal cord injury. Additionally, transfecting rat microglia with chitosan/miR-124 polyplexes in vitro led to a decrease in pro-inflammatory factors and major histocompatibility complex II expression. Injured rats treated with chitosan/miR-124 polyplexes also showed reduced macrophage activation compared to those treated with chitosan/miR-CTRL polyplexes. These findings suggest that chitosan nanocarriers could be valuable tools for delivering miRNA in brain disorders [96].

PLGA NPs, illustrated in Fig. 15.4(b), are highly desirable for drug and gene delivery due to their biodegradability, biocompatibility, and ability to protect both hydrophobic and hydrophilic cargo. Comprised of lactic and glycolic acid monomers linked by ester bonds, the composition of PLGA determines its properties (e.g., 50:50 PLGA, containing equal parts lactic and glycolic acid, is commonly used in nanomedicine) [97]. To enhance miRNA delivery, PLGA NP formulations often

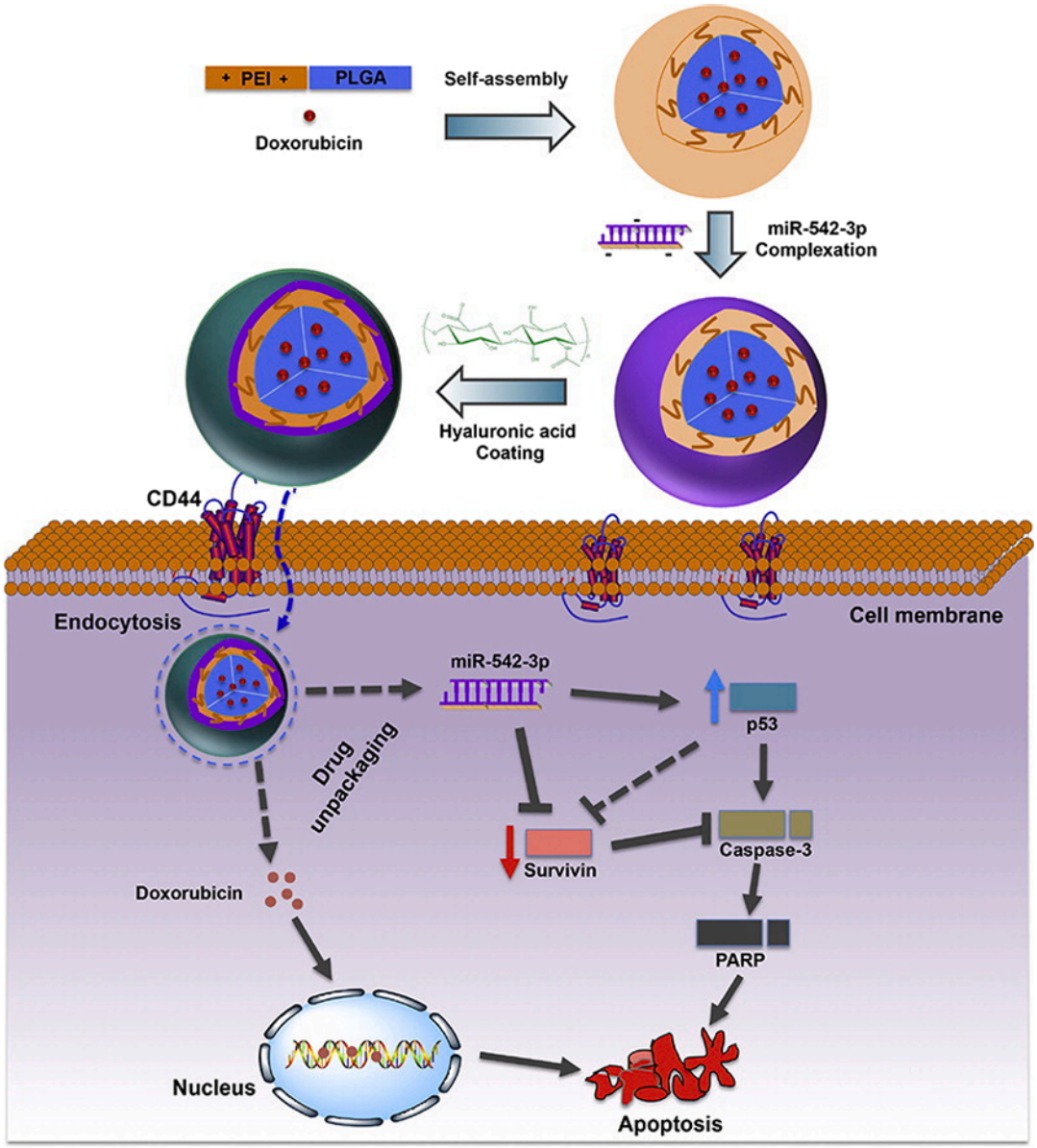


FIGURE 15.4

Schematic representation of PLGA NPs-miR-542-3p delivery for triple-negative breast cancer.

Reproduced with permission from S. Wang, J. Zhang, Y. Wang, M. Chen, Hyaluronic acid-coated PEI-PLGA nanoparticles mediated co-delivery of doxorubicin and miR-542-3p for triple negative breast cancer therapy. Nanomed. Nanotechnol. Biol. Med. *12 (2) (2016) 411–420.* https://doi.org/10.1016/j.nano.2015.09.014, *Copyright 2016 Elsevier.*

incorporate positively charged molecules like PEI or chitosan to aid in condensing nucleic acids through electrostatic interactions. This can result in RNA being loaded either within the core or on the surface of the PLGA NP. Alternatively, copolymers such as PEI-PLGA, PLL-PLGA, or PLA-PLGA can be utilized to create miRNA-loaded NPs. Like other miRNA carriers, PLGA NPs are predominantly researched for their potential in cancer treatment [98].

Wang et al. [99] demonstrated that miR-542-3p delivery to TNBC cells could induce apoptosis by activating p53 and suppressing Survivin expression (Fig. 15.4). Additionally, they encapsulated doxorubicin within hyaluronic acid-coated PLGA nanoparticles to target CD44 receptors on TNBC cells, showing enhanced drug uptake and cytotoxicity in MDA-MB-231 TNBC cells compared to MCF7 cells, which exhibit low CD44 expression. Notably, several other PLGA nanoparticle-based miRNA delivery systems have also achieved promising in vivo results.

For example, one application of PLGA NPs is in the treatment of multiple myeloma through the delivery of miR-34a [100], hepatic carcinoma via miR-99a delivery [101], and colon cancer via miR-204 delivery in murine models [102].

Dendrimers are highly branched polymers with tree-like structures, as shown in Fig. 15.4(c). They all have a central core surrounded by three or more branches made up of repeating monomer units. The terminal functional groups at the ends of the branches make them easy to conjugate with other molecules [103]. Dendrimers stand out as miRNA delivery vehicles due to their unique properties compared to other polymer systems. When they form complexes with biological molecules, they are called dendriplexes. Cationic dendrimers, in particular, are effective carriers for miRNA because they can easily bind to negatively charged nucleic acids. Polyamidoamine (PAMAM) dendrimers, in particular, have gained attention for delivering siRNAs, antagomiRs, and miRNA mimics, as they have low toxicity compared to other compounds. To enhance the targeting of miRNA to specific cells, PAMAM dendrimers are often modified with specialized ligands [104,105].

For instance, in a groundbreaking study by Duan et al. [106], the fusion of chondroitin sulfate (CS) with PAMAM dendrimers gave rise to the powerful CS-PAMAM compound (Fig. 15.5). This innovative creation was designed to effectively transport miR-34a specifically to pancreatic cancer cells that possess an overabundance of CD44. When exposed to the CS-PAMAM/miR-34a dendriplexes, MiaPaCa-2 human pancreatic carcinoma cells underwent a remarkable transformation. The cells exhibited a significant increase in apoptosis (21.3%) and G1 phase cell cycle arrest (75.4%) compared to their counterparts treated with non-targeted dendriplexes, showing only 17.5% apoptosis and 70.7% G1 arrest. In stark contrast, untreated control cells showed minimal levels of apoptosis (6%) and G1 phase arrest (55.2%), highlighting the potent impact of this revolutionary delivery system on combating pancreatic cancer at its core.

3.2 Inorganic nanocarrier for microRNA responsive nanocarriers

In recent times, there has been a surge of interest in utilizing inorganic materials as nano-carriers for drug delivery systems. These minute carriers act as the backbone of the systems, enabling them to store and release drugs while maintaining their structure during circulation in the bloodstream. Moreover, they exhibit excellent biocompatibility and pharmacological characteristics. Some of the inorganic nano-carriers that have been thoroughly researched include mesoporous silica nanoparticles (MSNs), graphene oxide (GO), metal-organic frameworks (MOFs), and gold nanoparticles (GNPs) [107]. A successful nanocarrier must shield nucleic acids from degradation by enzymes, efficiently penetrate

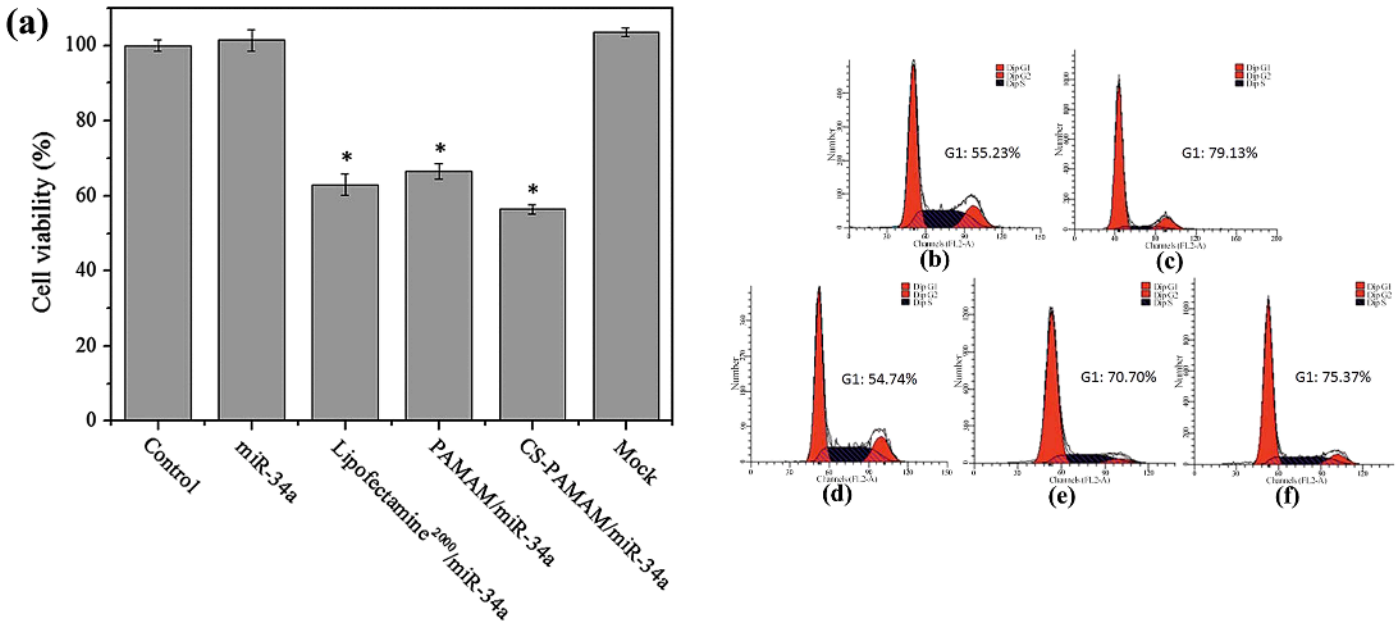


FIGURE 15.5

(a) The viability of MiaPaCa-2 cells was assessed after transfection with miR-34a nanocomplexes. (b–f) The cell cycle of MiaPaCa-2 cells was analyzed following transfection with miR-34a using PI staining. The experimental groups included: (b) untreated cells; (c) cells transfected with lipofectamine2000/miR-34a; (d) cells treated with CS-PAMAM/mock; (e) cells transfected with PAMAM/miR-34a; and (f) cells transfected with CS-PAMAM/miR-34a.

Adapted with permission from Y. Duan, Z. Xing, J. Yang, Y. Wang, J. Chen, Y. Zhang, W. Shi, Q. Li, Chondroitin sulfate-functionalized polyamidoamine-mediated miR-34a delivery for inhibiting the proliferation and migration of pancreatic cancer. RSC Adv. *6 (75) (2016) 70870–70876, https://doi.org/10.1039/C6RA15716E, Copyright 2016 Royal Society of Chemistry (RSC).*

cell membranes for cellular entry, and deliver the nucleic acids intact into the nucleus where they can fulfill their intended function. In the realm of harnessing the potential of inorganic nanoparticles for gene delivery and creating nanosystems attuned to specific nucleotide sequences, three overarching strategies emerge. One way to start is by using positively charged inorganic nanoparticles to form connections with negatively charged genetic material. Another option is to directly attach genetic material onto inorganic nanoparticles using a responsive linker. Finally, a third method involves using a cationic amphiphilic polymer derived from the nanoparticle to help create a stable complex between the nanoparticle and genetic material [108,109].

Gold nanoparticles (AuNPs) have captivated the attention of researchers as versatile vehicles for genetic material, owing to their straightforward creation, exceptional compatibility with biological systems, precise chemical makeup, and the ability to visualize molecules through fluorescence resonance energy transfer (FRET) [110,111]. A common approach in producing AuNP-based carriers for genes or microRNA analysis involves modifying the nanoparticle surface with positively charged substances like amino acids, cationic peptides, and tertiary amine-containing molecules. This process involves creating a scaffold with a positive charge that allows DNA or miRNA to bind together to form complexes that can be taken up by cells. Additional methods involve utilizing gold-thiol chemistry for directly attaching genetic materials onto AuNPs, as well as employing layer-by-layer assembly of PEI and miRNA to fabricate gene nanocarriers. [112]. Vijuan Kai and colleagues developed a miRNA delivery system using gold nanoparticles through an in situ self-assembly method, where microRNAs like let-7a, miRNA-34a, and miRNA-200a were linked to the nanoparticle surface. Research in the lab showed that Au-miRNA properties could potentially inhibit the proliferation of human hepatocarcinoma and HeLa cancer cells [111].

In a pioneering study, Goyal and his team unveiled the utilization of silica core/gold nanoshells as vehicles for delivering miR-34a into triple-negative breast cancer cells (Fig. 15.6). Employing a meticulous layer-by-layer approach, they cloaked the negatively charged gold nanoshells with layers first consisting of positive poly-L-lysine (PLL) followed by the negatively charged miRNA. The intricate design not only facilitated cellular uptake but also shielded the fragile miRNA from potential

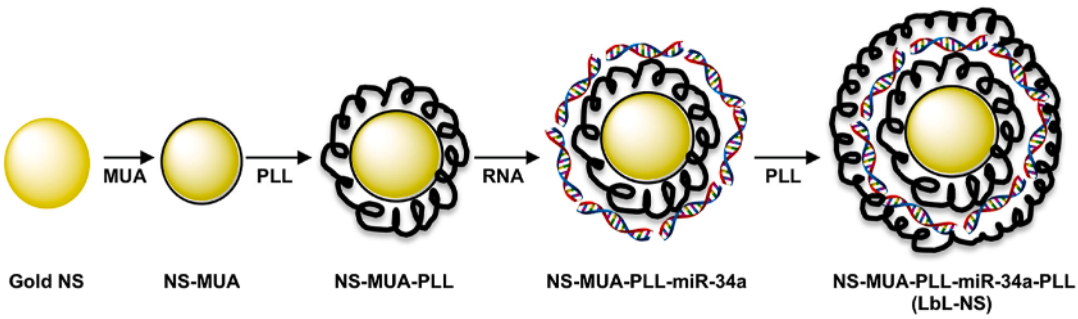


FIGURE 15.6

Schematic of layer-by-layer assembled silica core/gold nanoshells.

Reproduced with permission from R. Goyal, C.H. Kapadia, J.R. Melamed, R.S. Riley, E.S. Day, Layer-by-layer assembled gold nanoshells for the intracellular delivery of miR-34a. Cell. Mol. Bioeng. *11 (2018) 383–396, https://doi.org/10.1007/s12195-018-0535-x; permission conveyed through Copyright Clearance Center, Inc.*

degradation, thanks to an outermost layer of PLL. The profound impact of this sophisticated delivery system was evident in its ability to quash cell proliferation through targeted suppression of SIRT1 and Bcl-2, two key adversaries regulated by miR-34a [113].

Ramchandani et al. [114] developed a sophisticated creation using gold nanoparticles at its core, decorated with positively charged polyelectrolytes (PLLs). Within these PLL layers, a negatively charged mimic of miR-708 was carefully placed. This intricate layer-by-layer structure not only allowed for the gradual breakdown of the PLLs by cancer-related proteases like cathepsin B but also protected the miRNA mimic from degradation by natural enzymes. This strategic design enabled the controlled release of therapeutic agents, increasing their effectiveness and allowing for less frequent dosing. It is worth noting that miR-708 has been identified as a metastasis suppressor and, mechanistically, miR-708 targets the endoplasmic reticulum membrane protein neuronatin (NNAT) to reduce intracellular calcium levels, thereby reducing ERK and FAK activation, which is associated with reduced cell migration and impaired metastasis. The formulation was tested in experimental models of triple-negative breast cancer, both in lab settings and in living organisms, demonstrating its ability to restore miR-708 levels and trigger tumor-suppressing effects. Additionally, treatment with this novel nanoconjugate showed promising results in reducing the formation of lung metastases.

In their groundbreaking study, Grafals-Ruiz et al. [115] employed the innovative approach of utilizing peptide apolipoprotein E-conjugated liposome-encapsulated gold nanoparticles to target miRNA-92b within brain cells. It is worth noting that ApoE is a specific ligand for low-density lipoprotein (LDL) receptors, which are highly expressed in brain vascular endothelial cells and GBM cells. By interacting with LDL receptors on brain capillary endothelial cells, this peptide enables receptor-mediated transcytosis (RMT), the transport of molecules into the circulation across the blood brain barrier (BBB). Through the strategic functionalization of these nanoparticles with an oligonucleotide miRNA-92b inhibitor, they were able to enhance their ability to specifically target and inhibit this aberrant miRNA expression. This sophisticated conjugation to apolipoprotein E not only facilitated enhanced systemic delivery but also promoted accumulation within brain tumors in murine models. The results of their study demonstrated a significant reduction in cell viability as a direct result of the inhibitory effects exerted by these particles on aberrant miRNA expression. Such findings signify a remarkable advancement in the realm of targeted drug delivery for neurological disorders and pave the way for future innovations in this field.

The complex design of porous silica nanoparticles unveils a meticulously structured porosity that bestows upon them a vast surface area, significant pore volume, graceful biocompatibility, strong thermal stability, and endless possibilities for surface alterations. These characteristics render them perfectly suited for the simultaneous release of bioactive substances or tiny nucleotides [116]. Within the realm of scientific inquiry, MSNs were specifically selected as a conduit for effortlessly encapsulating miRNA through uncomplicated physical diffusion techniques. Enriching this core further was the application of a basic side chain cationic spacer to the MSN surface. Moreover, MSNs offer versatility in being coated with various cationic polymers like PEI, PEI cyclodextrin, and PDMAEMA to enable cellular entry and facilitate biochemical interactions [117].

In a groundbreaking study conducted by Maryamsadat Shahidi et al., they unveiled the development of MSNs, which were ingeniously modified with c(RGDfK)-PLGA-PEG [c(RGDfK)-MSN] NPs. This innovative creation aimed at co-delivering miR-34a and siPD-L1 for the treatment of bladder cancer. Their meticulous research uncovered astonishing revelations within bladder cancer cells and tissues, indicating a significant downregulation of miR-34a coupled with upregulation of

PD-L1 in both cell lines and animal models. Furthermore, the biocompatibility of this nanocarrier within serum environments proved to be exceptional, effectively shielding miR-34a and siPD-L1 from degradation. Notably, these c(RGDfK)-MSN nanoparticles exhibited an ability to concurrently decrease PD-L1 expression while elevating levels of miR-34a in T24 cells as well as the corresponding mouse model. A remarkable enhancement in antitumor effects was observed both in vivo and in vitro. In sum, the discoveries made by this group shed light on novel avenues for refining targeted therapeutic strategies geared toward combating bladder cancer through molecular precision [118].

In a groundbreaking study by Manisha Ahir and colleagues, a revolutionary therapeutic strategy for combating triple-negative breast cancer (TNBC) is unveiled (Fig. 15.7). This innovative approach involves the precise delivery of microRNAs (miRNAs) that modulate post-transcriptional gene regulation, leading to targeted modifications in specific miRNA profiles within the tumor microenvironment. By employing miRNA mimics or antagonists, this technique aims to disrupt the aberrant cellular processes that enable cancer cells to thrive. This study is the utilization of MSNs as carriers for the co-delivery of two key miRNAs: miR-34a-mimic and antisense-miR-10b. These custom-designed MSNs with cationic bases are engineered to simultaneously overexpress miR-34a while downregulating miR-10b, effectively rewiring the genetic landscape within TNBC cells. To ensure precise targeting, these loaded MSNs are further encapsulated in a protective coating composed of PEG-PLGA polymer and hyaluronic acid. The investigation delved into the cellular uptake, release kinetics, and resultant impact within Triple-Negative Breast Cancer (TNBC) cells. Through meticulous in vitro and in vivo analyses, an exceptional degree of specificity was demonstrated in targeting TNBC tumors, yielding a striking reduction in tumor proliferation and a notable delay in metastasis. This promising outcome suggests a profound potential for clinical application through the refinement of nanoparticles designed for the protracted delivery of miRNAs within cancerous cells and tissues. Furthermore, the experimental outcomes observed in vivo unveiled a conspicuous depletion of tumor cells within tissue samples alongside marked alterations in pathological staining patterns, indicative of a substantial decrease in malignant cellular presence as verified by histological assessment using Hematoxylin and Eosin staining techniques [119].

Metal-organic frameworks (MOFs) are porous materials created through the self-assembly of metal ions and organic ligands. They have shown great potential for biomedical uses due to their porous structures, biocompatibility, and ability to degrade naturally. The open porous design and numerous interaction sites allow for the incorporation of biomacromolecules like proteins and nucleic acids, resulting in the formation of MOF-biomacromolecule composites [120].

Huaixin Zhao and colleagues unveiled an innovative approach utilizing MOF nanoparticles, specifically ZIF-8, as carriers for miRNA delivery in conjunction with chemodynamic therapy (Fig. 15.8). The intricate study revealed the remarkable cellular uptake and controlled release of miRNA in response to lysosomal cues within the complex formed by miR-34a-m@ZIF-8. The inclusion of zinc ions within the structure of this nanocarrier triggered the generation of reactive oxygen species, ultimately inducing apoptosis in triple-negative breast cancer cells. Upon liberation of miR-34a-m from its carrier, a profound reduction in Bcl-2 expression at both mRNA and protein levels was documented alongside escalated cancer cell death rates. Encouragingly, animal experiments corroborated the efficacy of employing miR-34a-m@ZIF-8 to combat tumor progression through synergistic gene therapy combined with chemodynamics within a mouse model mimicking triple-negative breast cancer. Such pioneering utilization of MOFs as a potent nanoplatform for gene therapy/chemodynamic

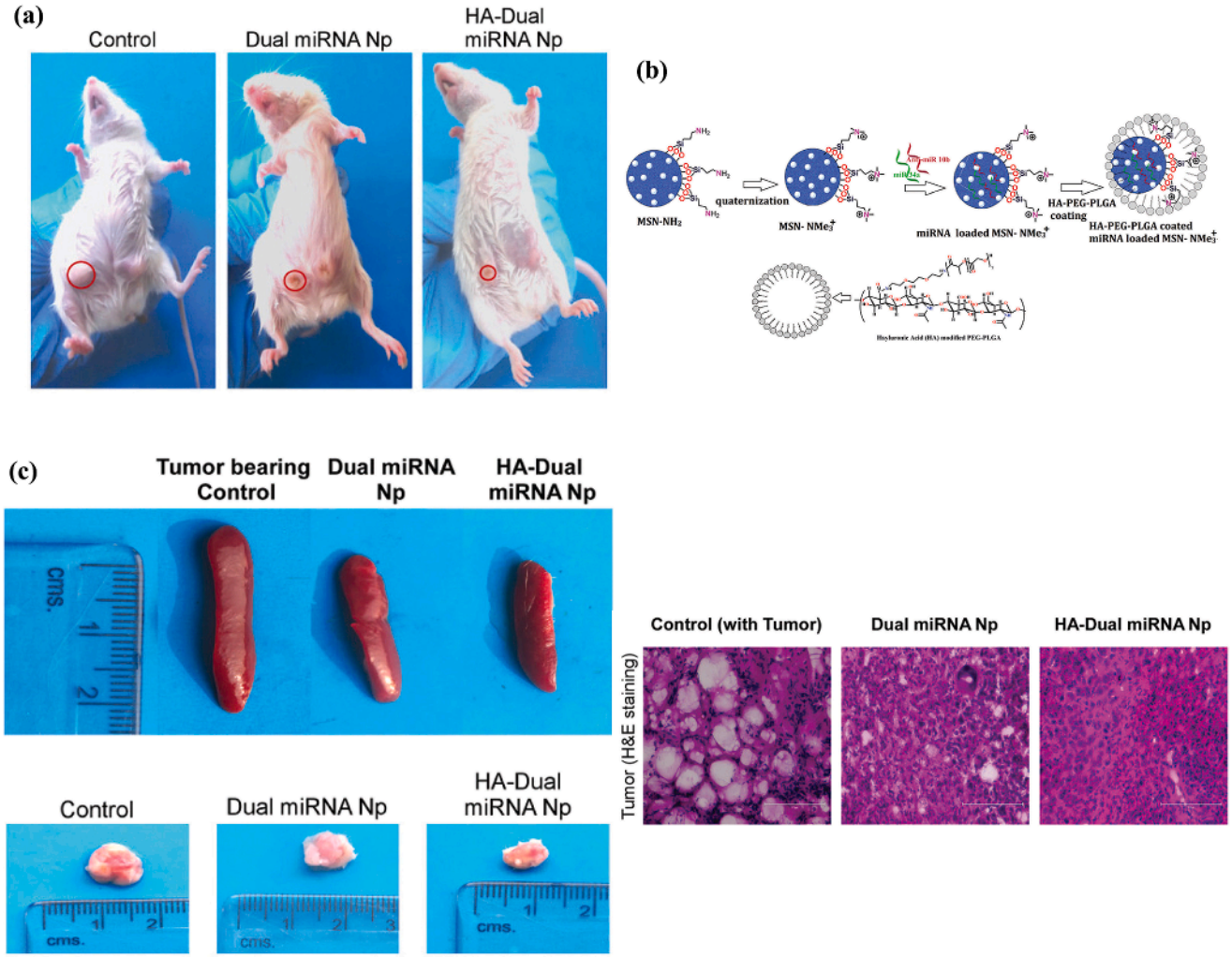


FIGURE 15.7

(a) The volume of tumors in mice and the weight of tumors that were removed from the control group, as well as from the groups treated with dual miRNA NPs and HA-Dual miRNA NPs, were measured and analyzed. (b) Scheme of synthesis and modification of MSNs with PEG-PLGA polymer and hyaluronic acid. (c) Tumors excised from the control group, as well as those treated with dual miRNA NPs and HA-Dual miRNA NPs, were stained with hematoxylin and eosin staining.

Adapted with permission from M. Ahir, P. Upadhyay, A. Ghosh, S. Sarker, S. Bhattacharya, P. Gupta, S. Ghosh, S. Chattopadhyay, A. Adhikary, Delivery of dual miRNA through CD44-targeted mesoporous silica nanoparticles for enhanced and effective triple-negative breast cancer therapy. Biomater. Sci. *8 (10) (2020) 2939–2954, https://doi.org/10.1039/D0BM00015A, Copyright 2020 Royal Society of Chemistry (RSC).*

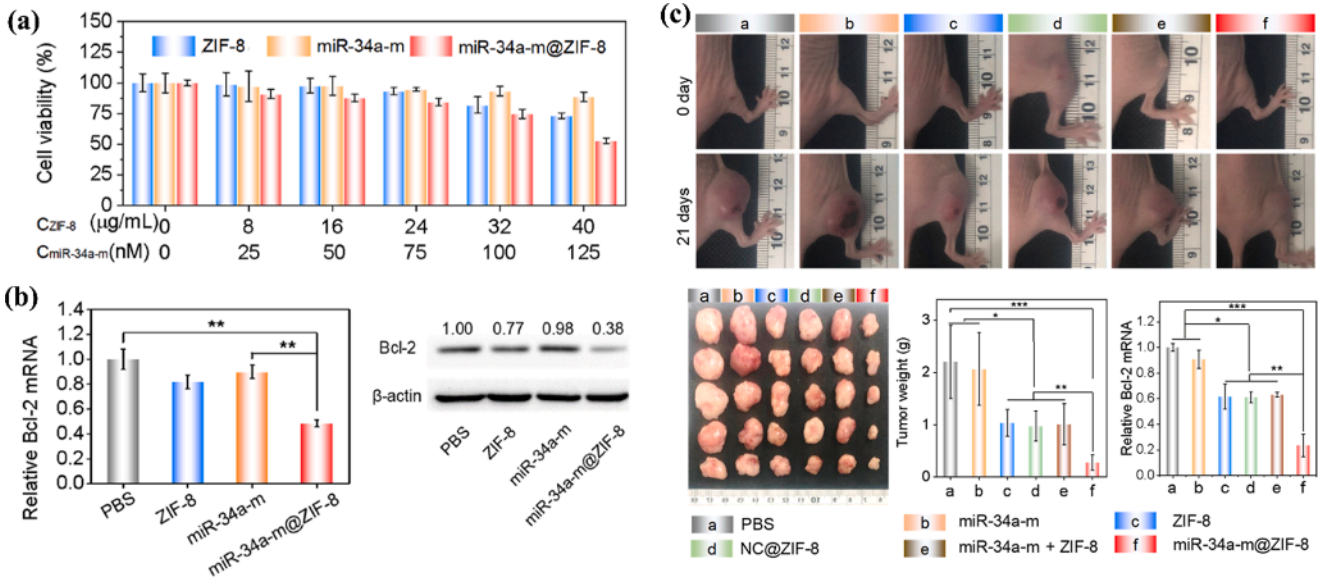


FIGURE 15.8

(a) The MTT assay was used to assess the cell viability of MDA-MB-231 cells after incubation with ZIF-8, miR-34a-m, and miR-34a-m@ZIF-8. (b) The levels of Bcl-2 mRNA were measured using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), while the Bcl-2 protein was analyzed through western blot in MDA-MB-231 cells following treatment with PBS, ZIF-8, miR-34a-m, and miR-34a-m@ZIF-8. (c) Pictures of mice with tumors, taken on the 21st day after the initial treatment, along with the corresponding tumor weights. Additionally, the Bcl-2 mRNA levels in the isolated solid tumors were analyzed using RT-qPCR.

Adapted with permission from H. Zhao, T. Li, C. Yao, Z. Gu, C. Liu, J. Li, D. Yang, Dual roles of metal–organic frameworks as nanocarriers for miRNA delivery and adjuvants for chemodynamic therapy. ACS Appl. Mater. Interfaces *13 (5) (2021) 6034–6042, https://doi.org/10.1021/acsami.0c21006, Copyright 2021 American Chemical Society (ACS).*

interventions exemplifies an auspicious avenue that holds tremendous potential for fostering efficient therapeutic synergy [121].

Mokri and colleagues embarked on a pioneering endeavor in the realm of nanotechnology, crafting a groundbreaking zinc-based nanoscale metal-organic framework coated with folic acid functionalized chitosan (Fig. 15.9). This innovative creation was meticulously designed to serve as a proficient carrier for locked nucleic acid-antisense MiR-224, aimed at targeting colon cancer cell lines. The therapeutic potential of antisense miR-224 LNA was harnessed to effectively inhibit the overexpression of miR-224 and impede the proliferation of malignant cells. Characterized by a size range encompassing approximately 200 nm, the nano complex comprising miR-224 NMOF–CS–FA-LNA antisense exhibited exceptional entrapment efficiency at $72 \pm 5\%$. Noteworthy findings included an initial burst release rate of 50% within the first 6 h, underscoring the dynamic nature of this cutting-edge delivery system. After this, experiments in vitro were conducted on HCT116 (a line of colon cancer cells that expresses the folic acid receptor) and CRL1831 (a normal colon cell line) to assess the potential therapeutic potency of the MCFL224 nanocomplex. The findings revealed a notable reduction in cell viability ($14.22 \pm 0.3\%$ after 72 h), alongside an augmented expression of genes associated with apoptosis and autophagy (such as BECLIN1: showing a 34-fold increase, BAX: exhibiting a rise by 36 times, mTORC1 displaying enhancement by 10 times, and caspase-9 revealing growth by 9 times compared to the control). Additionally, higher levels of cell cycle arrest in the sub-G1 phase were observed (with approximately 19.53% of cells residing within this phase), along with more pronounced signs of apoptosis found during analysis (attributable mostly to late apoptosis, making up around 67.7%). These outcomes serve as compelling evidence for the hindrance of colon cancer cell proliferation and instigation of cellular programmed death when treated with MCFL224 [122].

3.3 Co-delivery nanocarriers that demonstrate efficacy in vitro and in vivo

In the realm of therapeutics, miRNA mimics and anti-miRs stand as a promising class of treatments that have the potential to either replenish or diminish levels of specific microRNAs within cells. Yet their efficacy relies heavily on successful delivery to their intended targets within diseased tissues. These oligonucleotides, with miRNA mimics typically consisting of 22 nucleotides in a double-stranded RNA form weighing around 15 kDa, and anti-miRs being single-stranded RNA molecules no larger than 8 kDa, possess hydrophilic and anionic properties due to their phosphate backbones [123]. This characteristic impedes their ability to passively traverse cell membranes. Their internalization into cells occurs primarily through endocytosis, a process known for its sluggish nature and inefficiency. Once ensconced within endosomes and lysosomes after uptake, these oligonucleotides are at risk of succumbing to acidic degradation before they can reach their intended targets within the cytosol. Moreover, unmodified miRNA mimics and anti-miRs are rapidly eliminated from the bloodstream due to their susceptibility to nuclease degradation and rapid renal clearance, as their molecular weights fall below the glomerular filtration threshold of 60 kDa [124]. To address delivery challenges, methods initially developed for siRNA and ASO transport have been adapted for miRNA mimics and anti-miRs. Chemical modifications that enhance the stability and efficacy of siRNAs and ASOs—such as the incorporation of 2′-O-methyl, 2′-O-methoxyethyl, 2′-F groups, phosphorothioate linkages, cholesterol conjugation, and locked nucleic acid (LNA) modifications—have also been shown to improve the performance of miRNA mimics and anti-miRs. Furthermore, the use of biodegradable and biocompatible nanocarriers has emerged as a crucial strategy for enhancing the

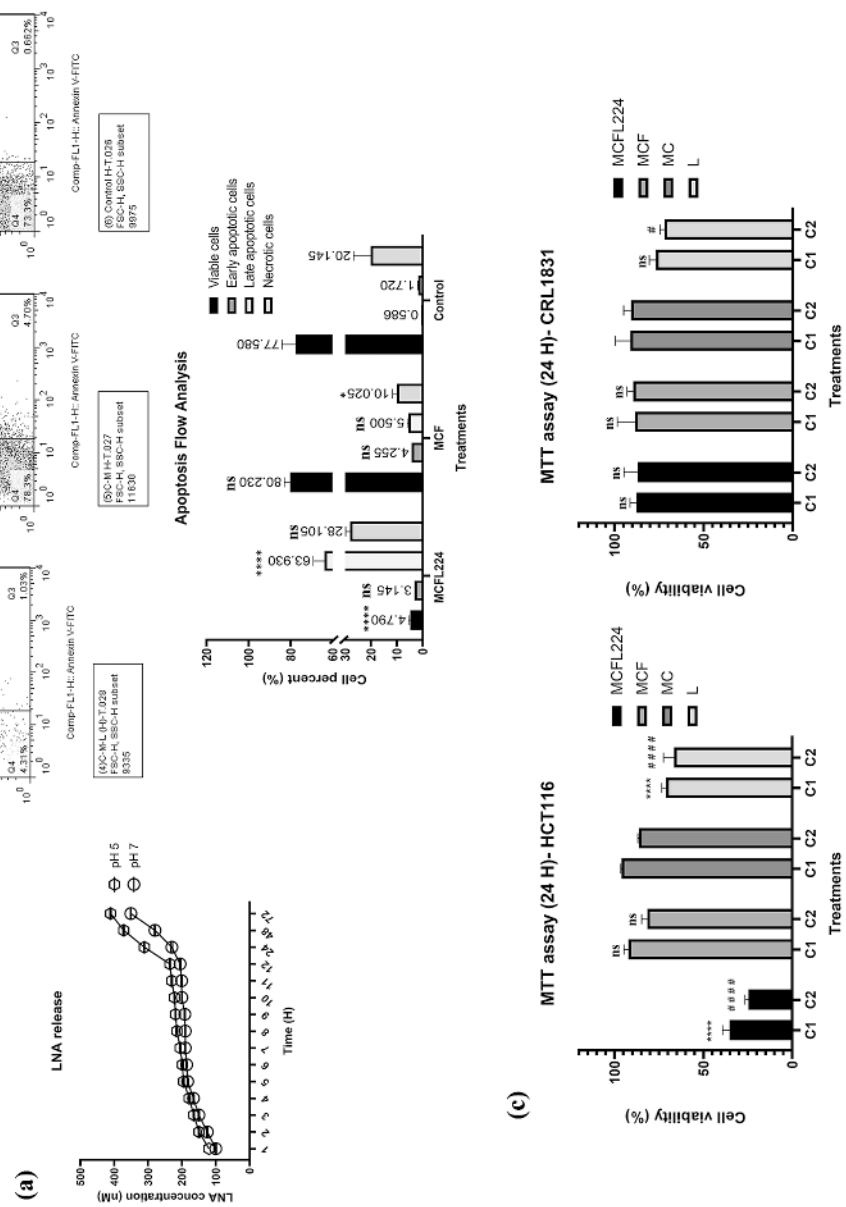


FIGURE 15.9

(a) LNA release in two specific pH, (b) flow cytometry-based apoptosis detection, and (c) cell viability assessment of nanocomposites by MTT assay after 24 h.

Adapted with permission from N. Mokri, Z. Sepehri, F. Faninam, S. Khaleghi, N. Motakef Kazemi, M. Hashemi, Chitosan-coated Zn-metal-organic framework nanocomposites for effective targeted delivery of LNA-antisense miR-224 to colon tumor: In vitro studies. Gene Ther. *29 (12) (2000) 680–690, https://doi.org/10.1038/s41434-021-00265-7, Copyright 2022 Springer Nature.*

pharmacokinetics and intracellular delivery of miRNAs. These nanocarriers are being developed to respond to multiple stimuli, including pH, temperature, redox potential, and magnetic fields, to provide intelligent and sensitive drug delivery systems. This multi-responsive approach allows for precise control over drug release at specific sites, offering the advantages of individual stimulus-based methods. Multi-responsive designs not only respond to single stimuli for targeted drug delivery but also exhibit enhanced release when multiple stimuli are simultaneously triggered [125,126].

In their scholarly investigation, Pu Zhang and colleagues unveiled an ingenious metal-organic framework nanosystem known as UiO-66 that exhibited remarkable responsiveness to both pH levels and miRNA molecules specifically for the treatment of breast cancer (Fig. 15.10). Delving into the intricate details of their research, they introduced two distinct stimuli-responsive Nanoporous Metal-Organic Frameworks (NMOFs). One such system entailed encapsulating drugs within NMOFs that were intricately secured by pH-responsive DNA tetrahedra. When exposed to acidic environments, these molecular gates would disengage from the NMOFs due to the formation of i-motif structures, thereby facilitating drug release. Moreover, these tetrahedral gates were strategically outfitted with AS1411 aptamer moieties, which honed in on nucleolin receptors highly prevalent in malignant cells. A parallel approach involved loading NMOFs with therapeutic agents or dyes and securing them using microRNA-responsive Tetrahedra, allowing targeted delivery based on specific miRNAs such as miRNA-21 or miRNA-155. In comparison to the traditional analog DNA duplex-modified NMOFs, the permeation of drug-loaded NMOFs gated by DNA tetrahedra into the malignant MDA-MB-231 breast cancer cells showcases a heightened efficacy in cellular penetration. This enhanced capability results in more potent and specific cytotoxicity toward the malignant cells when juxtaposed with nonmalignant epithelial MCF-10A breast cells. Such selectivity is attributed to either the acidic pH characteristic of cancerous cells or the presence of miRNA-21 within the MDA-MB-231 malignancies [127].

Juang et al. [128] have meticulously crafted pH-sensitive and peptide-modified liposomes and solid lipid nanoparticles (SLN) for encapsulating irinotecan and miR-200, respectively (Fig. 15.11). These nanoparticles feature a variety of peptides, each serving a unique purpose - from facilitating cellular penetration to targeting tumor neovasculature engaged in angiogenesis to directing delivery specifically to mitochondria. Adding another layer of sophistication, the nanoparticles are coated with a pH-responsive PEG-lipid derivative sealed with an imine bond. Through this innovative design, these particles demonstrate remarkable responsiveness within the acidic environment of colon cancer HCT116 cells: releasing their cargo precisely where it is needed most. Notably, these nanoparticles exhibit minimal toxicity toward healthy blood cells and noncancerous intestinal cells. Moreover, the inclusion of miR-200 in SLN enhances the effectiveness of irinotecan-loaded liposomes against colorectal cancer (CRC) cells by inducing apoptosis and suppressing vital pathways such as RAS/β-catenin/ZEB/multiple drug resistance (MDR). The in vivo results from studies using CRC-bearing mice show that irinotecan and miR-200 delivered in pH-responsive targeting nanoparticles have demonstrated promising therapeutic effects. These nanoparticles have been successful in inhibiting colorectal tumor growth and reducing systemic toxicity. The delivery of miR and chemotherapy through multifunctional nanoparticles has the potential to impact various signaling pathways related to cancer progression, such as β-catenin/MDR/apoptosis/metastasis, leading to programmed cancer cell death. This suggests that pH-responsive targeting nanoparticles could offer a promising treatment option for colorectal cancer.

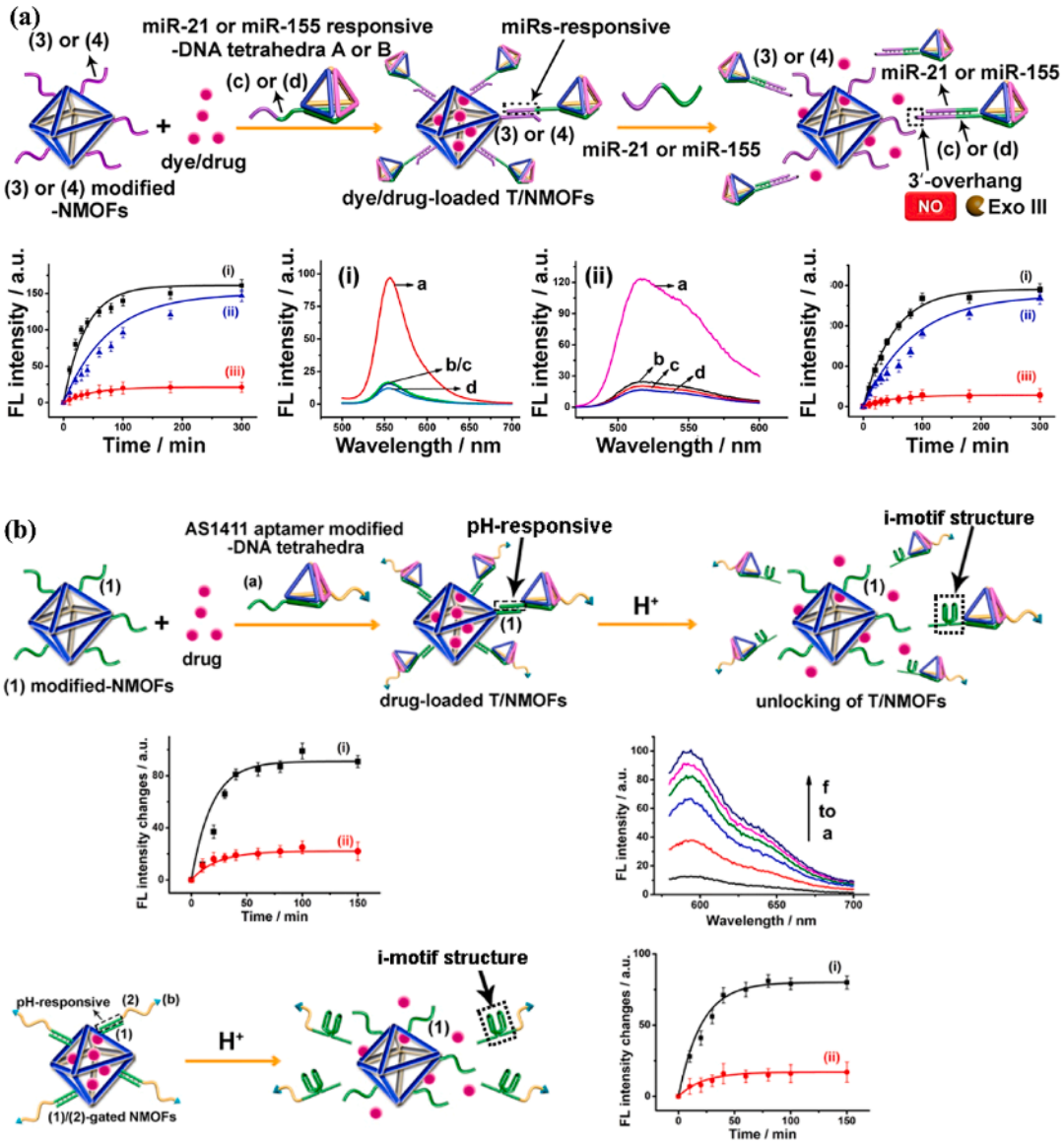


FIGURE 15.10

(a) The DOX-loaded T/NMOF hybrids are assembled, and the release of the loads is triggered by pH-induced i-motif structure formation. (b) The creation of hybrid T A/NMOFs or T B/NMOFs that respond to miRNA, loaded with either a dye or anticancer drug, and the illustration of how the loads are released when triggered by miRNA.

Adapted with permission from P. Zhang, Y. Ouyang, Y.S. Sohn, R. Nechushtai, E. Pikarsky, C. Fan, I. Willner, pH- and miRNA-responsive DNA-tetrahedra/metal–organic framework conjugates: Functional sense-and-treat carriers. ACS Nano *15 (4) (2021) 6645–6657, https://doi.org/10.1021/acsnano.0c09996, Copyright 2021 American Chemical Society (ACS).*

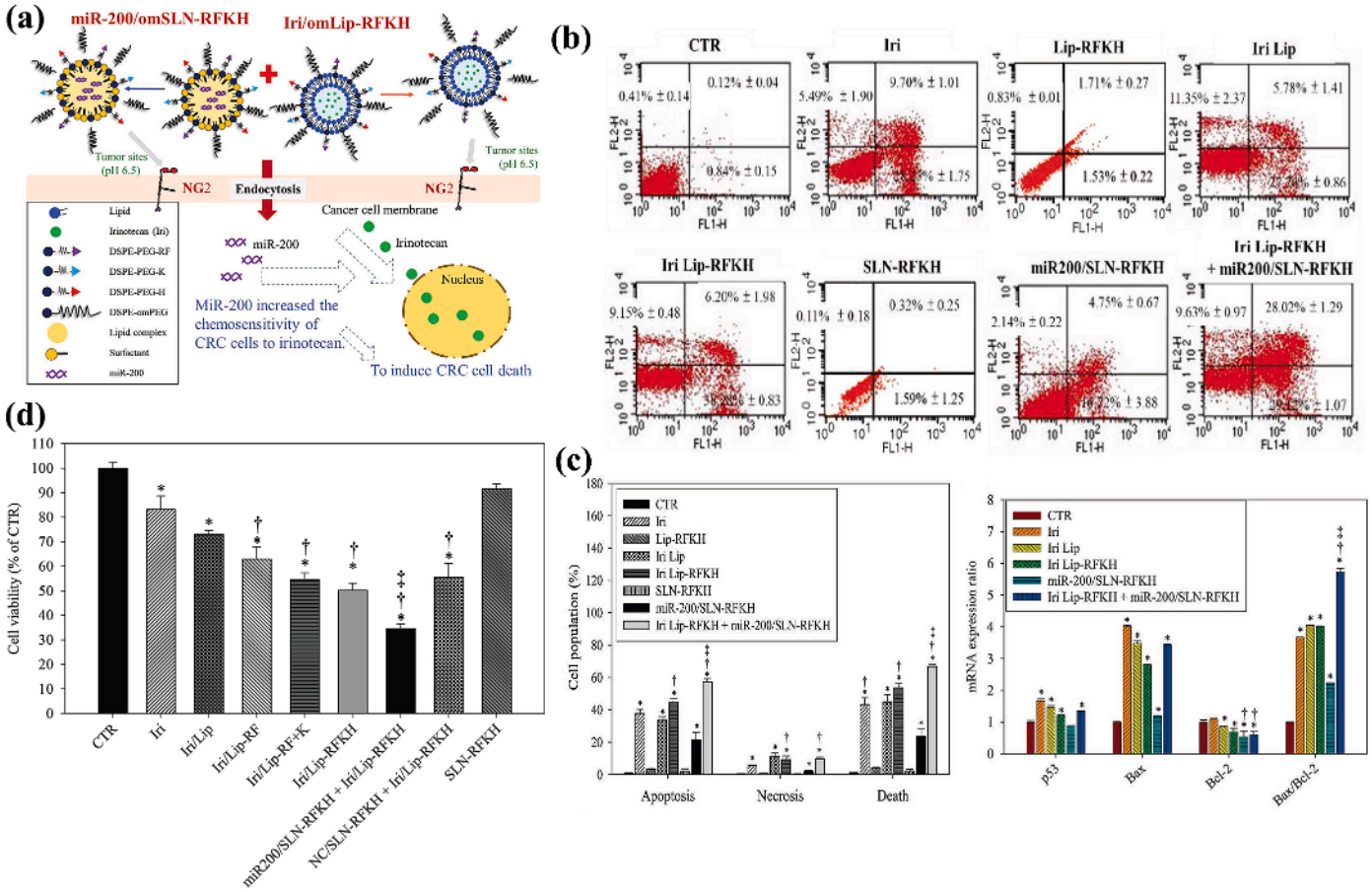


FIGURE 15.11

(a) A diagram illustrating the design of pH-responsive and peptide-conjugated PEGylated liposomes. (b) Flowcytometry assay by V/PI. (c) The relative percentage of apoptosis and mRNA expression levels of p53, Bax, Bcl-2, and the ratio of Bax/Bcl-2. (d) Cell viability assay by MTT test.

Adapted with permission from V. Juang, C.H. Chang, C.S. Wang, H.E. Wang, Y.L. Lo, pH-Responsive PEG-shedding and targeting peptide-modified nanoparticles for dual-delivery of irinotecan and microRNA to enhance tumor-specific therapy. Small 15 (49) (2019) 1903296, https://doi.org/10.1002/smll.201903296, Copyright 2019 Wiley.

4. miRNA-based approaches in tissue engineering

Regenerative medicine focuses on restoring the normal function of damaged cells, tissues, or organs and holds great potential for treating trauma, burns, degenerative diseases, and other conditions leading to organ failure. Cytokines and growth factors are commonly employed to enhance cell-mediated tissue regeneration. However, protein-based therapies face significant challenges, including limited stability, high production costs, and a short biological half-life. As an alternative, gene therapy has gained attention, with growing interest in microRNAs (miRNAs) over traditional plasmid DNA approaches. MiRNAs regulate a broad spectrum of cellular processes, such as proliferation, differentiation, apoptosis, and metabolism, by modulating the expression of target genes. Notably, miRNAs influence more than 60% of gene expression in mammals, underscoring their crucial role in cellular regulation [129].

There have been recent developments in tissue engineering using miRNA-based techniques. According to the miRBase database, a total of 2588 mature miRNAs have been identified in the human body, and this list is rapidly being updated. Considering the impact of miRNAs on the fate of mRNA and cell reprogramming and translation inhibitions, extensive studies are being conducted in the field of tissue engineering and repair [130].

miRNAs play an important part in modulating stem cells, altering their destiny. By targeting particular genes associated with proliferation, differentiation, and pluripotency. Loss-of-function miRNA studies, such as locked nucleic acid (LNA) and miRZip inhibitors (research tools used to block the function of specific microRNAs, or miRNAs), are employed in modern research; however, these techniques have limitations. There are no straightforward methods for knocking out or knocking down miRNA clusters due to the small size and extremely specialized architectures of miRNAs [130,131].

The let-7 family, the miR-302/367 cluster, and other miRNA families play pivotal roles in regulating stem cell function. Zhang et al. explored the suppression of the miR-302/367 cluster by targeting its promoter using a Kruppel-associated box repressor domain combined with transcription activator-like effectors (TALEs). To further achieve complete elimination of the miR-302/367 cluster in primary human fibroblasts, they designed two pairs of TALE nucleases (TALENs). Their findings highlighted TALENs and TALE-based transcriptional repressors as promising tools for investigating the loss-of-function effects of miRNA clusters in both somatic and pluripotent stem cells [131,132].

Tu et al. performed research on neuron development and related disorders employing iPSCs. In neuropsychiatric disorders like autism, cortical interneurons play a crucial role, and Neural differentiation is fundamentally regulated by miRNAs. They identified these miRNAs using a prior model for distinguishing human stem cells from cortical interneurons, particularly the let-7 family exhibiting the greatest TSI value. The vast majority of this family's members are widely distributed throughout neurons. They further found that the hiPSC stage is characterized by strong expression of the miR-320 family and its subfamilies -3p and -5p [133].

Worringer et al. and Chen et al. revealed how the let-7 family suppresses the reprogramming process through a regulatory mechanism that involves pro-differentiation proteins, such as EGR1. In human cells, the let-7 family suppresses reprogramming to a similar level as c-MYC when associated with OCT4, SOX2, and KLF4, and let-7 persistence hinders the reprogramming. The let-7 target LIN-41/TRIM71 is raised when let-7 is inhibited during the reprogramming, which enhances reprogramming and is crucial for removing the let-7 barrier to reprogramming. Furthermore, they confirmed that

LIN-41 blocks the translation of EGR1 by binding to its corresponding mRNA. It also regulates a wide range of differentiation genes like NGFI-A, KROX-24, ZIF268, and TIS8 [134,135].

Zhu et al. investigated the impact of miRNAs on bone tissue engineering, emphasizing their regulatory role in osteoblast differentiation, which has garnered significant attention over the past decade. However, challenges such as the negative charge of cell membranes, nuclease degradation, and immunotoxicity hinder the direct application of miRNAs in the human body. To address these limitations, appropriate carriers are required for efficient intracellular miRNA delivery. Compared to viral vectors, nanomaterial-based vectors offer superior efficiency due to their lower immunogenicity and toxicity, along with their ability to transport miRNAs of higher molecular weight. Their study also explored the role of various miRNAs and anti-miRNAs in bone tissue engineering, highlighting the signaling pathways involved in promoting osteogenic gene expression and facilitating osteogenic differentiation. Additionally, they provided a comprehensive overview of non-viral miRNA-transfected biomaterials, including bioactive glasses, calcium phosphates, liposomes, nanosystems, nucleic acids, silk-based biomaterials, cell-penetrating peptides, and PEI. These biomaterials enable the efficient delivery of miRNAs, enhancing osteogenic gene expression and extracellular matrix mineralization. Key parameters observed in in vitro studies include Runx2, alkaline phosphatase (ALP), osteoblast-specific microRNAs, and their respective target mRNAs and proteins [136,137].

Diez-Cunado et al. investigated the miRNAs associated with the mechanisms regulating the proliferation of cardiomyocytes and identified 96 miRNAs involved in the DNA synthesis and cytokinesis of cardiomyocytes derived from hiPSCs. Chemical results and computational findings indicated that most of these 67 miRNAs are connected to various components of the Hippo pathway, and therefore, their activity depends on the nuclear translocation of YAP (the Hippo transcriptional effector). The majority of these 67 miRNAs are linked to different elements of the Hippo pathway, according to chemical and computational research; as a result, their activity is dependent on the nuclear translocation of YAP (the Hippo transcriptional effector). Additionally, research on anti-miRNAs revealed that none of these miRNAs are required for cardiomyocyte proliferation on their own, even though YAP is crucial for proliferation. Lastly, they put forth a hypothesis in which these miRNAs prevent immature cardiomyocytes from exiting the cell cycle by blocking the Hippo signaling pathway [138].

Naresh Kumar et al. described the role of miRNAs in the maturation and differentiation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) for cardiac regeneration. The article highlights that the functionality and structural organization of hiPSC-CMs improve over a 4-week culture period, showing more advanced synchronization, metabolic maturity, and ultrastructural resemblance to non-failing adult heart tissue. The study identifies the upregulation of specific miRNAs, such as members of the let-7 family, which are linked to the enhanced maturation of hiPSC-CMs. hiPSC-CMs cultured for 4 weeks exhibited upregulation of five let-7 family miRNAs (let-7f-5p, miR-98-5p, let-7b-5p, let-7d-5p, and let-7i-5p) compared to those cultured for 1 week, indicating their involvement in the cardiac maturation process. These findings underscore the importance of miRNA regulation in promoting cardiomyocyte differentiation and suggest that mature hiPSC-CMs could be more effective in clinical applications, such as cell transplantation for heart repair [139].

A recent publication describes a study focused on using miRNA-based therapies to promote tissue regeneration and repair in the context of myocardial infarction (MI). The researchers identified that miR-199a-5p, which is usually downregulated after MI, has protective effects on heart tissue by targeting specific genes involved in oxidative damage and myocardial contractility (AGTR1 and MARK4). To efficiently deliver miR-199a-5p to ischemic heart tissue, they developed a targeted

nanoparticle system using MSNs coated with a peptide for enhanced selectivity to damaged myocardial regions. The results showed that administering these nanoparticles improved cardiac function, reduced cell death, and enhanced heart tissue repair, positioning miR-199a-5p as a promising therapeutic tool for long-term myocardial recovery [140].

Piotto et al. focused on the importance of immunity regulation by miRNAs on tissue regeneration, Therefore, the topic of enhancing the immune regulation of tissue healing through modifications in immunity is a highly relevant subject for research. Since miRNAs control immunity by adjusting post-transcriptional genes in immune cells, targeting them is an attractive alternative. miRNAs play a key role in inflammation by regulating the phenotype and function of immune cells. Also, they studied the function of miRNAs in the tissue homeostasis phase after injury, with a particular emphasis on neutrophils, macrophages, and T cells. The immunoregulatory roles of different miRNAs in the regeneration and repair of liver, skeletal muscle, skin, and cardiac tissues are shown in Fig. 15.12 For example, heart tissue exhibits high expression levels of miR-146a and miR-126, but skin tissue restoration is inhibited by miR-155 [141].

Qiaomei Tang et al. describe a novel therapeutic approach for corneal regeneration using exosomes derived from iPSC-MSCs incorporated into a thermosensitive hydrogel. The exosomes contain miRNA, specifically miR-432-5p, which plays a crucial role in tissue regeneration by suppressing TRAM2, a gene that regulates collagen biosynthesis. This suppression prevents excessive collagen production and ECM deposition, reducing scar formation and promoting the healing of the corneal epithelium and stroma. Overall, the system facilitates controlled miRNA delivery to modulate cellular responses and enhance corneal tissue regeneration [142].

Recently, an innovative strategy was published to promote tissue regeneration in diabetic wounds, which are often difficult to heal due to chronic inflammation, poor vascularization, and impaired collagen remodeling. The approach uses engineered exosomes derived from human placenta-derived mesenchymal stem cells (PMSCs), which are modified to efficiently load miR146a, a microRNA with known anti-inflammatory and regenerative properties. These exosomes are then attached to a silk fibroin patch (SFP) using a specially designed silk fibroin binding peptide (SFBP) that enhances the exosome binding rate and stability on the patch. This engineered exosome system, called SGM-miR146a-Exo@SFP, delivers miR146a in a sustained and targeted manner to the wound site. The design includes real-time tracking capabilities through the integration of Gluc signals and ensures high miRNA loading efficiency using MS2 capsid protein technology. Compared to other treatments, such as exosome-only or patch-only applications, SGM-miR146a-Exo@SFP shows superior therapeutic effects by reducing inflammation, enhancing angiogenesis, and improving collagen deposition, which collectively accelerates wound healing. In essence, this study highlights an exosome@biomaterial-based miRNA delivery platform that could transform the treatment of diabetic wounds. By addressing key challenges in wound healing through sustained and efficient miRNA delivery, this approach offers a promising solution for chronic wound care, significantly improving skin tissue regeneration outcomes [143].

There is an increasing interest in the role of miRNAs in regulating the maintenance and differentiation of stem cells and tissue regeneration. This suggests that altering miRNA signaling in endogenous stem cell populations or utilizing it in vitro as part of a tissue engineering approach could serve as an effective method for guiding tissue regeneration (Table 15.1).

Delivering miRNA effectively and safely is crucial for the advancement of miRNA-based therapies. Both viral and nonviral delivery methods have been explored, each with its benefits and

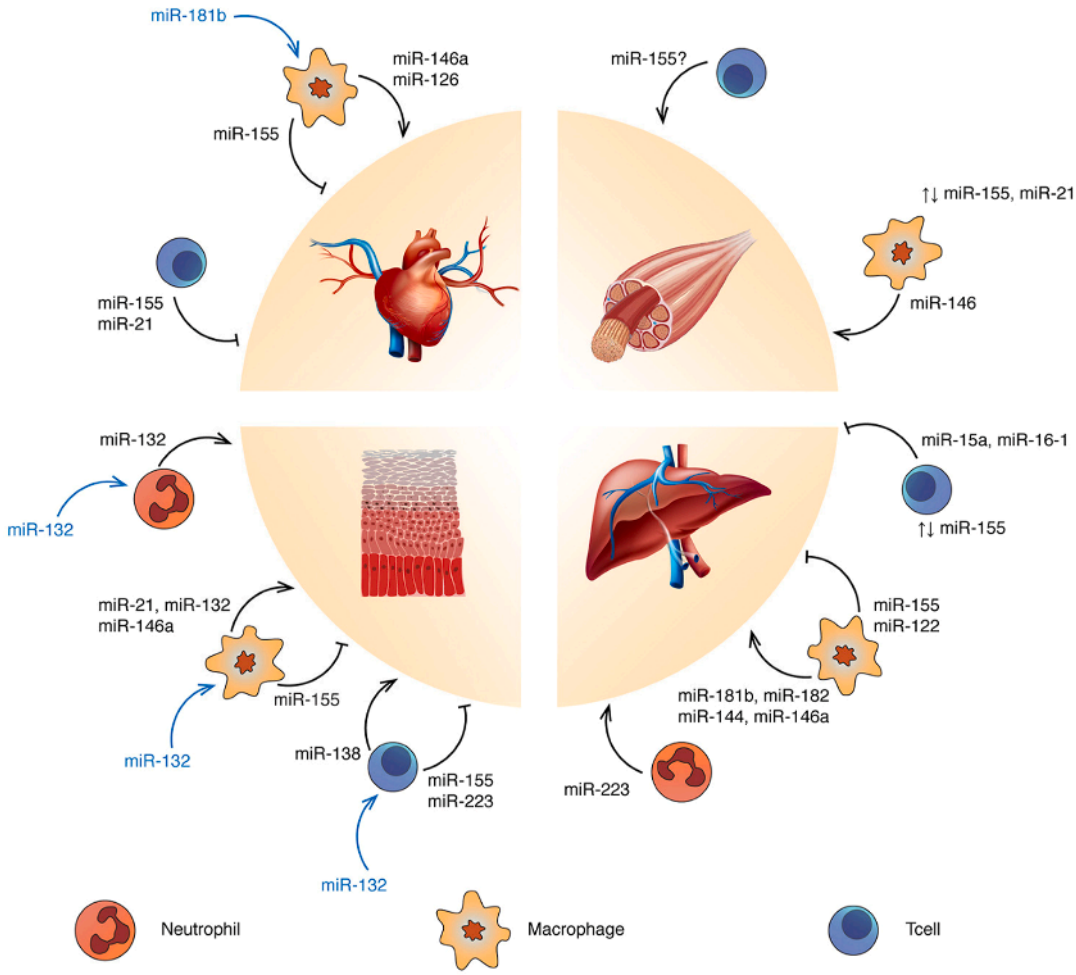


FIGURE 15.12

Targeting miRNA regulation to promote tissue repair and regeneration: ↑overexpression ⊥, inhibition ↑↓, balanced expression. Blue and yellow cells represent T cells and macrophages, respectively. Refer to the text for the specific immune cell subtypes. miRNAs reported in blue refer to tissue-derived miRNAs affecting the related immune cell functions.

Reproduced with permission from C. Piotto, Z. Julier, M.M. Martino, Immune regulation of tissue repair and regeneration via miRNAs—New therapeutic target. Front. Bioeng. Biotechnol. *6 (2018) 98, https://doi.org/10.3389/fbioe.2018.00098, Copyright 2018 Frontiers.*

limitations. Numerous studies have successfully demonstrated miRNAs' potential in enhancing bone formation, improving heart tissue regeneration, and minimizing fibrosis, among other tissue engineering applications. A scaffold-based delivery system is particularly promising for the localized and sustained release of miRNA, as the structural cues from the scaffold can complement the biochemical

Table 15.1 Role of microRNAs in regulating stem cell fate and tissue regeneration.

miRNA name	Focused tissue/Cell line	Function	Ref
miR-302/367	Human fibroblasts	Overexpression of this cluster promotes Cellular reprogramming and maintains the stemness of human embryonic stem cells	[131–133]
miR-302 miR-9	Neuron/iPSCs	Involved in CNS development and neural differentiation	[133]
Let-7 family	iPSCs/Megakaryocytes	Acts as an inhibitory influence on the reprogramming process through a regulatory pathway involving pro-differentiation factors	[134]
miR-135 miR-21	Bone tissue engineering	It can negatively regulate gene expression at the posttranscriptional level. Promotes osteogenic differentiation of mesenchymal stem cells (MSCs) and inhibits osteoclastogenesis	[136,137]
miR-302 Let-7 family miR-199a-5p	Induce human cardiomyocytes	Enhance the differentiation of pluripotent stem cells into cardiomyocytes. Enhanced heart tissue repair	[138–140]
miR-155	Heart, skeletal muscle, skin, and liver	Promotes the differentiation of B and T cells and is involved in the immune response to infections and inflammation	[141]
miR-432-5p	Scar healing of the corneal epithelium and stroma	Regulates collagen biosynthesis. This suppression prevents excessive collagen production and ECM deposition, reducing scar formation and promoting the healing of the corneal epithelium and stroma.	[142]

Table 15.1 Role of microRNAs in regulating stem cell fate and tissue regeneration.—cont'd

miRNA name	Focused tissue/Cell line	Function	Ref
miR146a	Skin wound healing	Reduce inflammation, enhance angiogenesis, and improve collagen deposition, which collectively accelerates wound healing.	[131]

signals provided by miRNA. By mimicking processes seen in tissue development, miRNAs can be strategically used to enhance tissue repair and regeneration. Cellular changes, such as those seen in dedifferentiation, transdifferentiation, or reprogramming, are closely regulated by miRNA activity. Although miRNA delivery using scaffolds for regenerative purposes is still in the early stages, it is generating significant interest. These scaffolds not only act as platforms to support cell growth, differentiation, and tissue formation but also supply crucial factors that help guide stem cells into specific tissue types. Early studies on scaffold-based miRNA delivery have shown great promise for applications like wound healing, cardiac repair, bone regeneration, and corneal repair. As innovative approaches to miRNA therapy continue to emerge, the use of miRNA delivery systems is likely to expand, significantly impacting the field of regenerative medicine.

4.1 Mechanisms of microRNA-responsive nanosystems

DNA and RNA are fundamental nucleotide-based polymers that constitute the genetic framework of all living organisms. DNA exists in both double-stranded (dsDNA) and single-stranded (ssDNA) forms, primarily serving as the genetic blueprint for cellular functions. In contrast, RNA is a more diverse class of molecules, performing a wide range of regulatory and functional roles. RNA includes messenger RNA (mRNA), which conveys genetic instructions from DNA to ribosomes for protein synthesis, transfer RNA (tRNA), which facilitates amino acid transport during translation, and ribosomal RNA (rRNA), a key structural component of ribosomes. Additionally, regulatory RNAs such as microRNA (miRNA) modulate gene expression post-transcriptionally, while small interfering RNA (siRNA) and Piwi-interacting RNA (piRNA) are involved in gene silencing and transposon regulation. Other specialized RNA types include small rDNA-derived RNA (srRNA), small nucleolar RNA (snoRNA), and tRNA-derived small RNA (tsRNA), each playing crucial roles in cellular processes such as rRNA modification, epigenetic regulation, and stress response [144]. DNA and RNA play fundamental roles in genetic information transfer, organism reproduction, cellular functions, development, and growth across all living organisms. Beyond their biological significance, these nucleic acids also serve as promising candidates for smart triggered release systems due to their highly specific nucleotide base sequences, which enable precise complementary base pairing. The incorporation of RNA structures into nucleic acid (NA)-responsive nanoplatforms offers a unique advantage in enhancing targeted delivery and controlled release mechanisms. RNA's ability to form both canonical (Watson-Crick) and non-canonical base pairs allows for the construction of intricate three-dimensional

(3D) structures with diverse functionalities. Additionally, RNA exhibits high thermodynamic stability and can interact with other RNA molecules through stacking interactions, further broadening its potential applications. These properties make RNA-based nanoplatforms highly effective in biomedical fields, including gene therapy, biosensing, and nanomedicine, where precise control over molecular interactions is essential [145]. miRNAs are the focus of interest in NA-responsive nanosystems among various types of RNAs because the RNA strands can connect to their target molecule through different structures and lead to target responses through changes in the strand's structure or by creating a signal in the target molecule. In addition, the amount of a specific miRNA can be up- or down-regulated in various biological conditions. The way of connecting RNA strands is mentioned in several ways, such as the hybridization of DNA strands serving as locks with various RNA structures serving as keys, the structure of the rolling circle, bulge loops, inner loops, multi-branched loops, single-stranded regions, and helices and pseudoknots, which will be explained in detail below [146].

For instance, Ren et al. developed a DNA nanomachine that utilized a DNA scaffold and two DNA/RNA hybrids to form a bulge loop structure through rolling circle amplification. Upon entering target cells, this nanomachine detected intracellular miR-21 and hybridized with a complementary sequence, triggering a cascade reaction. This reaction led to the opening of the bulge loop, facilitating the controlled release of siRNA while simultaneously regenerating miRNAs. Hairpin structures, which are unpaired loops formed when RNA or DNA folds back on itself, play a crucial role in nucleic-acid-responsive delivery systems. These hairpins can function as molecular locks, ensuring that therapeutic cargo remains securely encapsulated until a specific nucleic acid trigger induces their unfolding. Such smart nanoplatforms enhance the precision and efficiency of gene therapy and RNA-based therapeutics, offering highly selective and responsive drug delivery mechanisms [145]. Prinzen and his colleagues introduced an innovative approach in which a spherical nucleic acid (SNA) was functionalized with multiple hairpin structures via a hybridization chain reaction (HCR). This strategy enabled the SNA to efficiently encapsulate small-molecule cargos, leading to the development of novel nucleic acid (NA)-responsive nanomachines. By leveraging the self-assembling properties of HCR, the system allowed for precise and controllable cargo loading and release. The incorporation of hairpin structures ensured that the nanomachines remained stable until they encountered specific nucleic acid triggers, which initiated the unfolding of hairpins and the subsequent release of therapeutic agents. This methodology represents a significant advancement in smart drug delivery systems, offering improved targeting, responsiveness, and biocompatibility [147].

The intricate design of the nanomachine's hairpin consisted of a toehold domain, stem domain, and loop domain, all delicately intertwined. Despite the perfect matching of the toehold and loop domains adjacent to each hairpin on the spherical nucleic acid nanocarrier's surface, their union remained elusive due to being "kinetically trapped". A breakthrough arose when an initiator strand was introduced, possessing the remarkable ability to bind specifically with the toehold domain of one hairpin. This interaction led to a chain reaction - displacing the stem domain and uncloaking the hidden loop domain. With newfound freedom, the exposed loop swiftly connected with its counterpart on another hairpin, triggering a cascade effect that unraveled all remaining hairpins. Through this meticulously orchestrated process, every lock was undone and finally set free, releasing the encapsulated small molecules within. The DNA-mediated connection between the nanoparticles possessed numerous "protecting groups" in the shape of a hairpin located adjacent to the adhesive ends within the linker's sequence. This intricate design aimed to regulate the interaction dynamics of the particles. The hindrance caused by steric factors effectively managed how the particles associated with one another.

At each end of the hairpin structure, there was a hybridization that occurred when in its "protected" state, enveloping and concealing the adhesive ends within a thick layer of dense DNA. Introducing a brief complementary oligonucleotide into this complete hairpin arrangement initiated an unlocking process, transitioning it into an active phase where the sticky ends were guided toward the outer edges of the nanoparticles. This adjustment allowed for complementary sticky ends to find their mates and bind together securely. Furthermore, a slight protruding sequence in this complement strand remained unbound post-hybridization process, enabling potential reshaping and refolding of these protective hairpins. Upon encountering miRNAs or other forms of RNA, nucleic acid-based CDNs can facilitate not merely the regulated administration of medications, but also the initiation of the nicking/replication apparatus for genetic construction, heightened transcription of diverse aptamers, and the synthesis/interpretation of numerous proteins [148,149].

The phenomenon of gene silencing unfolds as a nuanced posttranscriptional mechanism orchestrated by a specialized RNA switch triggered by the binding of miRNA [150]. For instance, the 3′-untranslated region of p27 messenger RNA acts as the locus where this intricate mechanism governs gene expression. Here, a delicate unfolding occurs as the Pumilio-recognition element (PRE) detaches from its binding partner, the esteemed Pumilio RNA-binding family member 1 (PUM1) co-factor, thereby permitting microRNA to bind. This interplay between PRE and PUM1 sets in motion the unfolding of a secondary structure that unveils the mRNA's target site, ultimately facilitating microRNA-induced silencing [151]. The transformation of Pre-miRNA-1229 into the refined miR-1229-3p occurs through an unconventional secondary configuration, specifically in the form of a G-quadruplex structure, which harmonizes with traditional hairpin loops [7].

The tertiary structures of RNA are intricately developed three-dimensional units that emerge from the fusion of multiple secondary structures (Fig. 15.13). These formations come into being through a complex interplay of factors such as base stacking, unorthodox base pairings, the creation of triplex structures with base triples, the involvement of metal ions and metabolites, and the interactions between free bases and the ribose-phosphate backbone. Through these intricate connections between secondary structures and various cofactors, higher-level tertiary RNA configurations are shaped with specific purposes like providing structural support, overseeing biological processes, and catalyzing reactions. One group of primary microRNAs (pri-miRNAs) contains six potential sequences for microRNAs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a. Among these pri-miRNAs, the efficiency in producing pre-miRNA strands varies due to their placement within an RNA tertiary structure—distal microRNAs are liberated more effectively than those located at the core proximal region within a compact spherical tertiary form. The intricate and nuanced display of miRNAs from the miR-17–92 cluster, exhibiting a conformation specific to three dimensions, is intricately intertwined with both developmental processes and the onset of tumorigenesis [7].

5. Challenges and limitations in current technologies

RNA oligonucleotides exhibit characteristics that complicate drug design and efficacy. These challenges include degradation by nucleases in biological environments, poor penetration of cell membranes, entrapment in endosomes, weak binding affinity to complementary sequences, insufficient delivery to target tissues, off-target effects and unintended toxicities, and activation of innate immune responses. As miRNA delivery emerges as a promising therapeutic approach, various strategies are

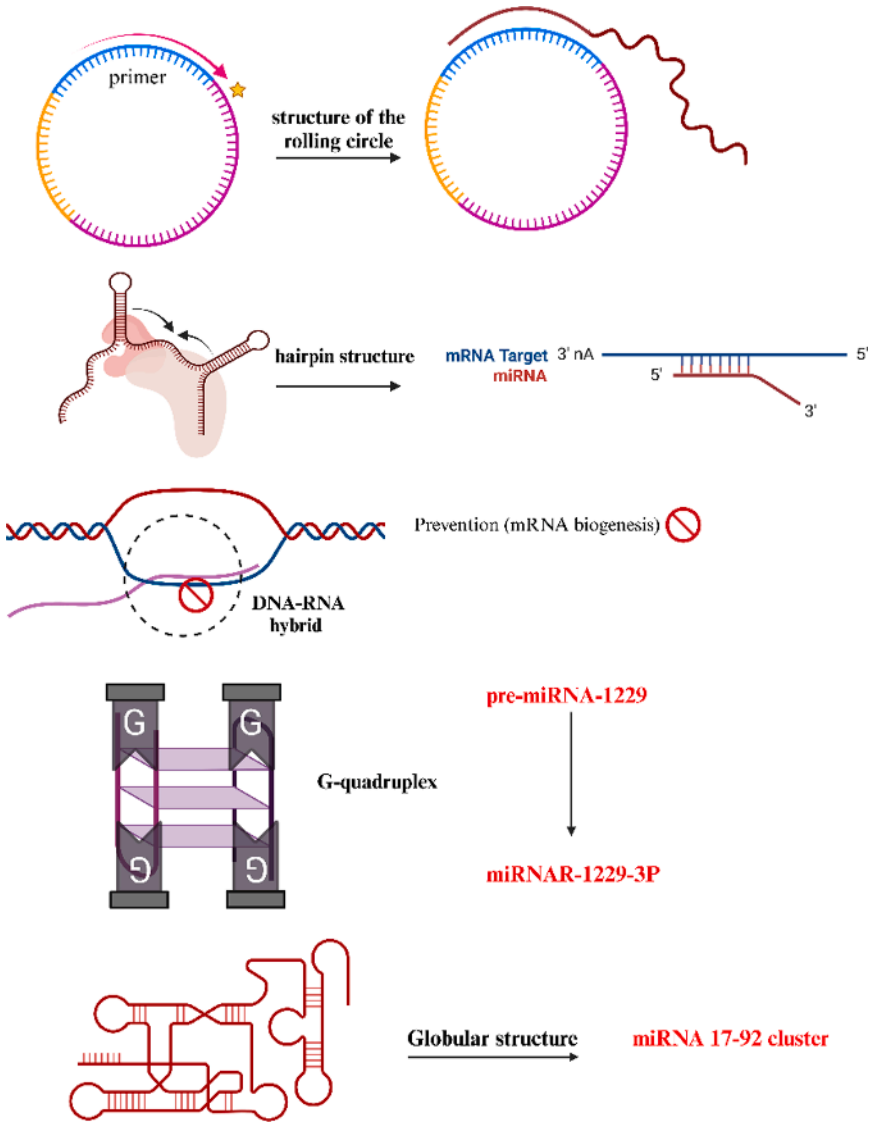


FIGURE 15.13

The miRNA structure.

being developed to overcome these obstacles. For instance, chemical modifications to oligonucleotides can prevent rapid degradation by nucleases, while different delivery systems can help circumvent the hydrophilic nature, negative charge, and high molecular weight of nucleic acids that hinder their passage across cell membranes [152,153].

5.1 Challenging microRNA responsive nanocarriers in blood circulation

When a nanocarrier's surface is engineered with a nucleotide or miRNA sequence for targeted drug delivery, or when this sequence is encapsulated within the nanocarrier, unmodified miRNAs with a 2′ OH group in the ribose can be quickly degraded by nucleases like serum RNase A in the blood. Additionally, these unprotected miRNAs are swiftly removed through renal excretion, leading to a short lifespan in the systemic circulation. To address this issue, chemical modifications can be made to the miRNA's phosphodiester and 2′ ribose backbones to shield it from degradation and enhance its long-term effectiveness (Fig. 15.14). Various chemical modifications have been developed to enhance oligonucleotide stability and improve their target-binding affinity. These modifications include alterations in phosphodiester linkages, ribose backbone, 2′-O-(2-methoxyethyl), 2′-O-methyl, 2′-locked nucleic acid, and 2′-fluoro. These changes not only increase the stability of oligonucleotides but also facilitate their loading into the miRNA-induced silencing complex, thereby enhancing their function [154,155].

5.2 The challenge of miRNA-responsive nanocarrier penetration in vitro and in vivo

Numerous miRNAs play a crucial role in disease development and progression, particularly in cancer. Despite advancements in miRNA delivery strategies, achieving efficient tissue-specific delivery with effective cellular penetration remains a significant challenge. The leaky nature of tumor vasculature results in poor blood perfusion, which limits the delivery efficiency of unmodified miRNAs. To overcome this, various delivery vehicles have been developed. Nanocarrier-based methods, such as liposomes and polymer nanoparticles, utilize the enhanced permeability and retention (EPR) effect to facilitate tumor targeting. Conjugation-based approaches involve covalent attachment of sugars, peptides, or lipids to the 3′-end of the miRNA passenger strand to enhance tissue specificity. Other strategies include exosome-based delivery and the use of viral vectors, particularly adenovirus-based systems, which offer both short-term and long-term expression options. In some cases, nanoparticles are employed to deliver plasmid vectors expressing miRNAs, where cationic polymers

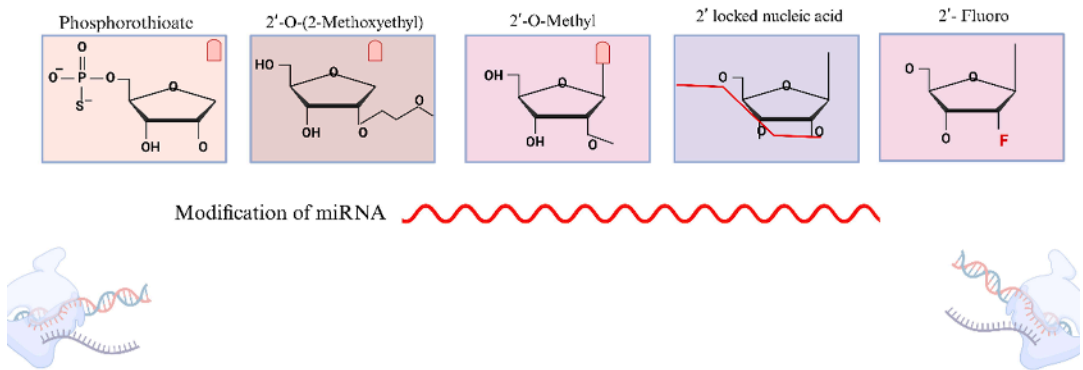


FIGURE 15.14

miRNA modification.

electrostatically bind the plasmid and are subsequently loaded onto nanoparticles. Lipid-based nanoparticles, including liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs), provide controlled release and enhanced stability. Since miRNA molecules are negatively charged, their direct diffusion into tissues is limited. Nanocarriers effectively improve transfection efficiency and stability, enabling the upregulation of downregulated miRNAs or the inhibition of overexpressed miRNAs. These approaches have demonstrated promising results in clinical trials, highlighting the potential of nanotechnology in miRNA-based cancer therapy [152].

5.3 The challenge of protecting the miRNA-responsive nanosystem to run away from endosomes

The journey of miRNAs within cells via the cationic lipid-based nanocarrier, nanoparticles, or specialized vehicles commences within the realms of early endosomes. These vesicles subsequently converge with their late-endosomal counterparts and relinquish their cargo to these acidic vesicles, characterized by a pH range of 5–6. The subsequent course leads this content toward lysosomes, which boast an even more pronounced acidity at approximately pH 4.5 and harbor various enzymes poised to break off oligonucleotides and miRNA mimics. The pivotal juncture in this intricate voyage lies in circumventing the perilous fate awaiting these molecules in lysosomal degradation. Only upon successful escape from endosomes into the cytoplasm can they come face-to-face with the RNA interference apparatus ready to execute their intended function. This feat of endosomal egress stands as a formidable obstacle in achieving efficient delivery of miRNAs. An array of strategies has emerged to facilitate this crucial release - ranging from formulations that respond sensitively to pH variations such as lipoplexes and polyplexes, to innovative approaches involving photosensitive molecules or employments utilizing cationic nanoparticles. Through such diverse methodologies, science endeavors to navigate through the intricate labyrinthine pathways surrounding cellular delivery mechanisms for these potent biological messengers known as microRNAs (miRNAs) [152].

5.4 The challenge of off-targeting effect in miRNA-responsive nanosystem

After miRNAs are transported into the cytoplasm and released from the endosome, a significant concern with miRNA therapy is the off-target effect they can have. MiRNAs are designed to target different pathways by partially matching with 3′ UTRs, potentially causing unintended gene silencing of other genes. This off-target gene silencing can lead to harmful side effects and diminish the desired therapeutic outcomes. The discovery that a single miRNA can impact multiple mRNAs highlights the need for caution due to the potential for unexpected consequences. Even when a specific miRNA is successfully targeted, there may still be undesired on-target effects. One strategy to address this challenge is to administer low doses of multiple miRNAs that work together to regulate the expression of the same target gene. For instance, in cancer treatment, combining miR-34a and miR-15a/16 has been shown to enhance cell cycle arrest in NSCLC by specifically reducing the levels of CCNE1 and CCND3. This coordinated gene regulation complements the cell cycle control by miR-34 [156,157].

5.5 The challenge of stimulating the immune system by miRNA-responsive nanosystems

Double-stranded RNAs can be recognized as harmful by the host immune system, triggering innate immune responses. When miRNAs are delivered systemically, they, like other nucleic acids, can activate the immune system, leading to toxicities and unwanted side effects. This activation is primarily mediated by Toll-like receptors (TLRs) 3, 7, and 8, which detect single- or double-stranded RNAs in endosomal and lysosomal compartments. Once activated, these receptors initiate the secretion of inflammatory cytokines and type I interferons, driving both innate and adaptive immune responses. Ongoing research aims to better understand immune responses associated with miRNA delivery, with evidence suggesting that certain chemical modifications can reduce immune recognition. However, a significant concern lies in the delivery vehicle itself, as nanoparticles with highly positive charges may induce toxicity and immune activation. Upon administration, nanoparticles inevitably interact with immune cells, and factors such as size, shape, hydrophobicity, and surface modifications influence these interactions. Therefore, both the miRNA and its carrier must be carefully optimized. By designing targeted delivery systems, it may be possible to reduce required dosages and minimize immune activation, enhancing the safety and efficacy of miRNA-based therapies [158,159].

5.6 Challenges in simultaneous delivery of miRNAs and small-molecule anticancer drugs

One of the main challenges in developing nanocarriers for miRNAs and drugs is achieving targeted delivery to tumors. While intratumoral injection can be effective in inaccessible tumors, systemic administration is necessary for late-stage metastatic cancers. These nanoassemblies must be designed to stably carry both cargos, avoid being cleared by the body's immune system, and preferentially target tumor tissue. MiRNAs and drugs are typically housed in the polymeric matrix of nanocarriers through non-covalent interactions, which can be disrupted in the bloodstream, leading to premature release [160]. Additionally, while nanoassemblies are expected to accumulate in tumors through the EPR effect, they often accumulate in the liver instead. The development of novel nanomaterials and nanoconstructs with tumor-specific targeting in non-liver tissues is highly sought after. However, a significant challenge lies in assessing and mitigating potential off-target effects and toxicities induced by miRNAs and nanocarriers [161]. Double-stranded RNA sequences of 21 base pairs or more can induce an interferon response regardless of sequence specificity, resulting in the inhibition of protein synthesis and the arrest of cell growth. Moreover, circulating miRNAs can function as ligands for Toll-like receptor (TLR) family receptors in immune cells, triggering a pro-metastatic inflammatory response that may contribute to tumor progression and dissemination. The application of miRNA therapeutics has the potential to intensify these effects, making it more challenging to evaluate their therapeutic efficacy and necessitating careful monitoring. Additionally, the toxicities associated with nanocarriers further complicate the scenario. Besides aggregating serum proteins and disrupting cell membranes, polycationic nanocarriers can also activate the body's innate immune system [162]. Within the realm of combined cancer treatments, it is often the sequential arrangement and timing of distinct medications that ultimately dictate treatment outcomes [163].

6. Future directions and innovations in targeted drug delivery

In the realm of molecular biology, there exists a fervent exploration into the potential applications of nanotechnology-based delivery systems for miRNA drugs. Yet, amidst this pursuit, an array of challenges and enigmas remain uncharted, awaiting resolution in forthcoming research endeavors. A pivotal prerequisite for these nano drugs to grace the pharmaceutical market lies in the meticulous scrutiny of their physicochemical, pharmacokinetic, and pharmacodynamic characteristics, alongside their efficacy and safety profiles. Following administration, nanocarriers disseminate throughout the organism, interfacing with diverse tissues and organs; thus, underscoring the imperative nature of scrutinizing biodistribution patterns to mitigate off-target repercussions and toxicity risks. To safeguard against deleterious health ramifications, it is indispensable that rigorous toxicological evaluations are conducted on these therapies [164].

Recent advancements in the realm of medical imaging have ushered in a new era of tracking nanocarrier systems, with techniques such as positron emission tomography (PET), magnetic resonance imaging (MRI), and fluorescence imaging leading the charge. These cutting-edge methods have proven invaluable in both pre-clinical and clinical settings, offering crucial insights into the biodistribution of nanocarriers. Furthermore, there is a pressing need to refine biocompatible and biodegradable nanocarriers to enhance their efficiency in clearing the body. This refinement not only aims to mitigate potential long-term adverse effects associated with accumulation but also underscores the importance of exploring innovative solutions within this space [165]. The exploration of predictive models through in silico evaluation of miRNA nanomedicines holds promise for anticipating clinical responses before experimental testing. By leveraging these computational tools, researchers can gain valuable insights that may inform subsequent in vitro or in vivo studies. Of equal significance is the consideration of targeting multiple mRNAs with a single miRNA when designing miRNA-based drugs for combating cancer. This multifaceted approach speaks to the complexity inherent in developing effective treatments against cancer and highlights a critical area where further research is warranted [166]. In summary, current investigations into miRNA nanoformulations predominantly focus on well-characterized cancer-associated miRNAs. However, despite numerous reports touting the effectiveness of miRNA drugs, there remains a lingering concern regarding potential unforeseen consequences stemming from their pleiotropic actions. It is worth noting that while cell cultures are commonly utilized in the testing of miRNA-based drugs with nanoparticles, this method may not provide a comprehensive understanding of how these drugs impact gene expression across various cell types. Although the specificity of nanoparticles for cancer cells reduces the likelihood of off-target effects in healthy cells, researchers must expand their investigations to thoroughly assess and mitigate any unintended repercussions associated with miRNAs targeting multiple mRNAs in both targeted and non-cancerous cells [167].

One of the primary obstacles in cancer treatment is the development of resistance by many patients to chemotherapy drugs and radiotherapy. Various miRNAs, including let-7, miR-1, miR-21, miR-34a, miR-122, miR-155, and miR-223, have been identified as key players in modulating chemoresistance [168]. Research has shown that miRNAs can either promote or inhibit radioresistance, with miR-21, miR-124, miR-181a/d, miR-150, miR-155, miR-196a, miR-221, and miR-222 being linked to radioresistance in different cancer types. Conversely, miR-18a, miR-18a-5p, miR-132, miR-133a, and miR-145 have been associated with radiosensitivity. These miRNAs could potentially be targeted in

future studies, with the possibility of using NPs for their administration to improve the efficacy of chemotherapy and/or radiotherapy, as suggested by the reviewed articles [169].

Further research is needed to explore the potential of combining artificial miRNAs with phytochemicals to enhance their therapeutic effects. Kang et al. [170] found that co-delivering curcumin and miR-144-3p using heart-targeted extracellular vesicles improved myocardial infarction outcomes. miR-144-3p was chosen for co-delivery because of its ability to enhance the therapeutic effects of curcumin. While the combination of phytochemicals and miRNAs in cancer miRNA nanomedicine is not well-studied, previous research has shown that phytochemicals can influence the expression of miRNAs involved in cancer development [171]. For example, curcumin [172], galangin [173], and silibinin [174] have been shown to reduce the expression of miR-21 in various cancers, while curcumin [175], oleanolic acid [176], brazilein [177], and isorhapontigenin [178] have been found to regulate tumor suppressor miRNAs like miR-34, miR-122, miR-133a, and miR-145. Therefore, further investigation into nanoparticle-mediated delivery of these combined therapies is warranted.

The introduction of nanoparticles in the manipulation and transport of microRNAs has brought great advancements to the realm of cancer therapy, opening up new possibilities for treatment. Further exploration into this field may reveal the complex molecular mechanisms that drive cancer development and progression through microRNA-mediated genetic regulation. Early studies have shown promising results in using microRNA-based interventions as a key element in combating cancer. The incorporation of nanoplatform delivery systems has improved cell targeting and increased the effectiveness of treatments. However, it is crucial to approach this innovation with caution, ensuring thorough validation of safety, efficacy, pharmacokinetics, pharmacodynamics, biodistribution patterns, and ethical considerations before promoting their widespread use in clinical settings [179].

The precise administration of therapeutic agents to cancerous cells proves to be a formidable task, beset by the intricate and diverse characteristics of tumors. The heterogeneous qualities exhibited by individual cells existing within the tumor microenvironment necessitate the development of nanocarriers equipped with exceptional targeting abilities. Various techniques are being explored, such as surface modifications with specific targeting ligands, designs that react to external stimuli, and the incorporation of tumor-specific antibodies or peptides, all aimed at improving the efficacy of delivering treatments directly to malignant sites. However, further investigation is imperative to perfect and validate these approaches. Recently discovered targeting molecules boasting enhanced specificity and affinity, along with advancements in stimulus-responsive systems, warrant thorough scrutiny to enhance their potential effectiveness. At present, personalized medicine sees a notable surge in innovation through the exploration of miRNA nanoformulations for combating cancer [180]. This burgeoning field is marked by a scarcity of clinical trials registered on ClinicalTrials.gov, with only two such studies presently documented. These include research focusing on a lipid NP-formulated miR-193a-3p mimic, referred to as INT-1B3, devised to target advanced solid tumors (registered under NCT04675996). Additionally, investigations are underway involving EnGeneIC Delivery Vehicles (EDVs) combined with a miR-16 mimic known as TargomiRs for treating malignant pleural mesothelioma and NSCLC (listed under NCT02369198). Such endeavors exemplify the cutting-edge advancements and promising potential within this intriguing domain of scientific inquiry.

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CHAPTER

Peptide-responsive nanocarriers for biomedical applications

16

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1. Introduction

Peptide-responsive nanocarriers, representing one form of revolutionary development in the area of targeted drug delivery and biomedical applications, have further grown from simple peptide-based structures developed in the early 1990s [1], into complex stimuli-responsive nanomaterials. Fundamentally, peptide-responsive nanocarriers depend on the distinctive properties of peptides, biomolecules comprising amino acids joined together through amide bonds, to form dynamic delivery systems capable of offering specific responses toward physiological or external stimuli, including subtle changes in pH, temperature fluctuations, light influencing, the presence of specific enzymes and metals, and the variations in redox species concentrations. More precisely, over the last 10 years, peptide nanocarriers have been successfully combined in a broad range from inorganic nanoparticles and organic polymers to various bioactive molecules, including proteins and nucleic acids [2]. The combinatorial design of peptide-based delivery systems represents a breakthrough in biomedicine, merging diagnostic and therapeutic capabilities into powerful theranostic platforms. Until now, inherent biocompatibility, biodegradability, and the possibility of precise control of their self-assembly behavior have made peptide-based nanocarriers particularly appealing for a wide range of applications, from targeted drug delivery to tissue engineering [3]. Recent advances in peptide design and functionalization have given them wider capabilities by enabling smart delivery systems that can change their morphology due to certain specific biological triggers, consequently enhancing their therapeutic efficacy and specificity [4].

The review chapter focuses on the design principles of peptide-responsive nanocarriers, various stimuli-responsive mechanisms, their synergistic effects in combination with other active materials, and their biomedical applications. Further, in-depth critical examination of the challenges and opportunities opens up prospects toward the future for research and development toward clinical applications.

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2. Peptide-responsive nanocarriers

Structural components, design principles, essential criteria, and mechanisms of response.

2.1 Peptide self-assembly

Insight into the molecular interactions and assembly processes underlying effective peptide-responsive nanocarriers is of paramount importance. In effect, nanocarrier formation and function are precisely controlled from a deep understanding of the basic principles governing peptide organization at the molecular level. These form the basis of the design of sophisticated delivery systems with predictable behavior displaying controlled responses.

Central to this understanding is the process of peptide self-assembly, bridging the gap between isolated molecular components and functional nanostructures. This process is driven mainly by a somewhat complex network of molecular interactions such as hydrogen bonds, hydrophobic interactions, electrostatic interactions, and π-π stacking interactions. Some of the various characteristics on which this process found its base are charge and hydrophobicity of amino acids, size, and polarity. The secondary structures that peptide chains may express are random coil, β-helices, β-sheets, and β-turn; this sort of conformational diversity regulates the final morphology of the assembly [4].

Explanations for such fundamental mechanisms of self-assembly thus set a sound basis for further advances into higher-order peptide-based systems. One important advance in functional nanocarriers was provided by the introduction of stimuli-responsive peptides, which allowed for the development of programmable nanoarchitectures whose structural and functional properties can be precisely controlled.

2.2 Design of stimuli-responsive peptide

Amino acid modifications and functionalized components are used to create stimuli-responsive peptides (SRPs) that react to both internal and external stimuli. Under one or more stimuli, SRPs can assemble into nanostructures with varying sizes and forms. SRPs can self-assemble, deconstruct, or alter in size and shape due to chemical bonding in response to stimuli [5]. In this section, a detailed review will be presented on some basic design principles employed in developing such responsive peptide systems.

2.2.1 Temperature-responsive peptides

Temperature-responsive peptide design represents an intelligent approach in biomolecular engineering, using specific amino acid sequences and structural motifs to obtain controlled conformational transitions. The general design is often based on the α-helix conformation with the heptad repeat motif abcdefg as an important structural element. Strategic amino acid substitutions, especially at position f, are able to make minor refinements in the temperature-dependent behavior of these peptides [6,7].

The design can accommodate various transition mechanisms, including the classical α-helix to β-sheet transformation driven by hydrophilic-hydrophobic interactions, as well as the less common β-sheet to α-helix transition exemplified by DARV16-IV [8]. Perhaps the most ingenious design approach has been that of engineering chiroptical switches, which display temperature-dependent inversions in the helical handedness of peptides [9].

Indeed, nonapeptides composed of Boc-D-amino acids have been reported to undergo reversible transitions from right-handed helices at room temperature to left-handed helices at lower temperatures. The natural peptide trichogen (Tric-ol) and its derivatives have shown that modifications can also be used at the C terminus within the design to produce reversible thermoresponsive changes in conformation, proving in this way the potential of rational design for the creation of peptides with precisely controlled temperature-dependent structural transitions [10].

2.2.2 pH-responsive peptides

While temperature-responsive peptides rely almost entirely on hydrophobic interactions to drive their structure transitions, the development of pH-responsive peptides represents another clear strategy in peptide engineering that takes advantage of the specific properties of ionizable amino acids to drive well-controlled structural transitions. In general, the design strategy relies on the strategic placement of amino acids with ionizable side chains, including glutamic acid, lysine, histidine, and arginine, at precisely defined positions within the peptide sequence.

This architectural principle is realized in a variety of structural frameworks. The most distinctive ones including α-helical systems like homodimeric coiled-coil pHcc and heterodimeric IAAL E3/K3 systems, which position charged residues at distinctive heptad repeat positions that enable pH-dependent coiled-coil formation and disassembly [7,11]. The balance between electrostatic interactions and hydrophobic effects in the design of β-sheet-forming peptides, such as P11-2, enables control over fibril formation and, by extension, gel stability over a range of pH values [12]. More complex designs, such as the peptide A1H1, incorporate both hydrophobic domains driving the aggregation of β-sheets and hydrophilic histidine-rich domains whose charge states vary with pH, yielding materials whose assembly properties can be precisely tuned [13]. Certain peptide designs exhibit exceptional conformational plasticity, such as EAK-12d, which can switch between β-sheet and α-helical conformation by changing the pH value [14].

Through the following rational design, one can identify and precisely position pH-sensitive amino acids that can be employed in constructing peptides with controlled conformational change, self-assembly, or disassembly by targeting particular pH triggers. Hence, this makes them more instrumental in drug delivery applications targeting an acidic pH in pathological tissues such as tumors and sites of inflammation.

2.2.3 Light-responsive peptides

Photochromic molecules developed in the last decades have become overwhelming tools in biological applications since they might change between states due to the influence of light at a certain wavelength without any need of further catalysts [15]. Among different photochromic molecules, azobenzenes (Azos) have proven to be the most successful ones, with the widest range of excitation wavelengths and stability in photoactivated states [16]. The fundamental design mechanism exploits photon-induced E-Z isomerization, wherein light absorption elevates the molecule to an electron-excited state, effectively lowering the energy barrier between different conformational states [17].

These photoswitchable properties can be strategically integrated into peptide structures through modification of cysteine residue thiol groups, where the spacing between modified residues plays a crucial role in determining the resulting structure and photoswitching behavior [18]. For these, the spacing is critical; for example, cysteine pairs at spacings of i, i + 4, or i + 7 along the peptide chain yield α-helical structures that are stabilized by the Z-isomer, whereas i, i + 11 spacings are stabilized

by the E-isomer [17]. Recent advances have resulted in more sophisticated designs incorporating multiple photoswitches through specialized linking molecules, including C2v symmetric linchpins, and have enabled complex photoresponsive bicyclic peptides with improved control over conformational changes, hence extending the range of their possible applications within biological contexts [18].

2.2.4 Metal-responsive peptides

Both natural amino acids and artificial ligands are employed to design metal-responsive peptides for the purpose of attaining controlled self-assembly and functional properties.

The most commonly used natural amino acid ligands are histidine, cysteine, tryptophan, and glutamic acid, all of which coordinate with various metal ions, including Zn^{2+}, Cu^{2+}, Ni^{2+}, and Ag^{+}. These amino acid metal-binding behaviors may induce the conformational transitions and lead to a variety of nanostructures, such as fibers, tubes, and helical ribbons [19]. For example, histidine is an amino acid with an imidazole group possessing a lone pair of electrons on nitrogen and thus can coordinate with metal ions, enabling responsiveness to stimuli. This is evident from the AM1 peptide system, where the addition of Zn^{2+} induces the generation of foam through coordination His-Zn^{2+} [20].

Besides natural amino acids, some artificial ligands like pyridine and imidioacetate have also been introduced into peptide design for the purpose of ensuring structural stability and self-assembly control [19].

This dual approach ensures that the employment of both natural and synthetic metal-binding motifs enables researchers to design biomolecules that can mimic naturally occurring metallopeptides while offering tunable properties toward different applications.

2.2.5 Enzyme-responsive peptides

The enzyme-responsive peptides are designed in a modular approach with sensitive peptide linkers to target enzymes, including but not limited to metalloproteins and cathepsin B [21]. Three main design principles underlie the design: enzyme specificity and compatibility with the nanoparticle system, controlled accessibility of the enzyme-responsive segment, and purposeful morphological changes upon enzymatic cleavage.

More specifically, in the example of Matrix metalloproteinase (MMP)-sensitive systems, PLG/LAG or GPLG/IAGQ linkers may prevail, in which "/" refers to the cleavage site [22]. Subtle tuning can also be done in the design by changing the position of certain amino acids, thus modulating enzyme recognition and response kinetics. A typical example is represented by the peptide linker PX1G/LX2G, whose design is performed by means of three functional regions: a hydrophobic self-assembly domain, the enzyme-responsive sequence, and charged residues affecting enzyme accessibility [21].

The modular nature of the design enables the elaboration of complex drug delivery systems responsive to enzymatic levels indicative of a particular pathological state and providing for a selective, controlled therapeutic response.

2.2.6 Redox-responsive peptides

The design of redox-responsive peptides capitalizes on two different mechanisms: oxidation-responsive elements reacting with reactive oxygen species and reduction-sensitive bonds responding with reducing agents.

In oxidation-responsive designs, peptides contain chalcogen-based bonds (sulfur, selenium, tellurium, etc.) with thioether moieties that undergo oxidation in the presence of ROS species like superoxide anion and hydrogen peroxide [23]. This is exemplified for the PEG-doped poly(methionine) nanomicelles of Hoang et al., wherein the oxidation of methionine residues encourages structural disruption [24].

The reduction-sensitive architectures depend on disulfide (S-S) or diselenide (Se-Se) bonds, which selectively break under reducing conditions in the presence of reductive species like glutathione (GSH). GSH is a tripeptide containing a thiol and γ-amide bond. Its prominent difference in concentration between the extracellular environment and intracellular tumor environment makes it an ideal stimulus for targeted delivery.

Selenium-based systems are especially noteworthy because they can be engineered responsive to either redox condition; however, due care must be taken in designing the system to respond to either oxidizing or reducing conditions because control over cargo release is crucial [23].

2.3 Stimuli-responsive superstructures via peptide self-assembly

Carefully engineered stimuli-responsive peptides serve as building blocks, self-assembling with controlled processes into complex superstructures. The hierarchical assemblies bring together the intrinsic properties of the individual peptide components and emergent collective behaviors, thus enabling sophisticated responsive functionalities in diverse dimensionalities from 0D to 3D morphologies with a view to offering different advantages for a wide range of biomedical applications.

The zero-dimensional structures of peptide nanoparticles (PNPs), nanospheres, and peptide quantum dots (PQDs) have dimensions that are of a very small size with limited specific surface area and are very effective for cellular uptake and intracellular delivery [25]. The 1D morphologies of PNFs, peptide nanowires (PNWs), peptide nanotubes (PNTs), and peptide nanorods (PNRs) offer extended shapes with large numbers of active sites available for a variety of materials. Therefore, these peptide-based nanostructures can be used as connectors to link materials that do not naturally have a binding affinity for each other [26]. Instead, the 2D structure, mainly peptide nanosheets (PNSs) and nanoribbons, utilizes the wide surface and rich functional groups to implement functions in biosensing and photothermal therapies [27]. The 3D hydrogels can provide the macroscopic scaffolds with rich pore structures for drug loading and sustained release at the top level of the structural complexity [28].

By employing the stimulus-responsive properties described earlier, investigators have actually designed sophisticated systems in which dimensional transitions are triggered through environmental cues, thereby increasing the precision and effectiveness of peptide-based therapeutic strategies.

2.4 Responsive peptide-triggered active materials

Peptides can be joined strategically with various materials through different covalent and non-covalent interactions in the hope of functionally tailoring nanomaterials, thus enabling those nanomaterials to achieve enhanced biocompatibility and reduced cytotoxicity [29].

The number of multifunctional strategies so far proposed has embraced multiple material categories. Among these, metals, along with metal oxides/sulfides, wherein representative gold nanoparticles (AuNPs) have been outstanding for their chemical inertness and nontoxicity [30]. For instance, Yang et al. prepared alkaline phosphatase-triggered self-assembling peptide-coated AuNPs

for target therapy against prostate cancer [31], while Chen et al. developed a pH-responsive drug delivery with peptide-functionalized AuNPs bound to silicon nanoparticles (SiNPs) [32]. Besides gold, other metals like silver are also attempted; for example, Li et al. have presented multifunctional peptide-wrapped silver nanoparticles for significantly boosted antimicrobial activity [33]. Applications of iron oxides also show critical uses, such as the work by Yang et al., who used pH/temperature dual-responsive nanogels with Fe_3O_4 in targeted drug delivery and imaging [34].

Another potent strategy involves DNA/RNA/PNA hybridization, where PNAs offer stable RNA target recognition with possibly antitumor effects. Significantly, antisense γPNAs with modifications have been utilized to develop miR-210 inhibition strategies by Gupta and coworkers [35]. Additionally, polysaccharide conjugation has been functional, as by Lu et al., who combined fucoidan with cationic peptides to yield dual stimuli-responsive cancer treatment [36]. Furthermore, the incorporation of photosensitizer achieved specific drug release and improved phototherapy efficacy. Zhang et al. developed polyglutamic acid-based dual-responsive peptide nanoparticles containing tetraphenylporphyrin for photodynamic chemotherapy [37].

The other class of materials involves carbon nanomaterials, which emerged as one of the powerful classes of materials showing enormous potential in biosensor technologies for biomedical applications (Roshani, Mousavizadegan, and Hossei [38] and turned out to be very promising toward the development of flexible sensing systems for wearable health and environmental monitoring [39]. Based on this functionality, Liu et al., for example, have prepared large-size magnetic nanosheets using graphene oxide-targeting proteins and mitochondrial-targeting peptides [40]. Such versatile approaches for functional tailoring have expanded the capabilities of the stimuli-responsive peptide nanomaterials for application in biomedical fields to a great extent.

3. Optimization strategies for peptide-responsive nanocarriers

The strategic development of peptide-sensitive nanocarriers is an important frontier in biomedical engineering, embracing various methodological approaches to achieve maximum therapeutic potential. These complex systems are under development with a number of design elements for improved targeting specificity, enhancement of payload protection, and well-defined release kinetics governed by defined peptide-mediated biological signals.

3.1 Molecular design strategies

Multiple molecular-level strategic alterations have been developed to enhance the stability and functionality of peptide-responsive nanocarriers.

On a simple note, L-amino acids can easily be replaced by protease-resistant D-amino acids. Tugyi et al. showed that the substitution of terminal amino acids with the D-isomers remarkably enhanced peptide stability in human serum [41]. Amino acid substitutions were explored by Chen et al. who introduced a histidine substitution for lysine in cleavage peptides, with their half-life extended to 11 hours in human serum and significantly reduced cytotoxicity against normal cells [42]. Hydrophobic substitutions have similarly been used with success; Han et al. demonstrated a linear relationship between aliphatic chain length and stability; C16 chains extended half-life to 45.4 hours due to increased albumin binding [43].

Taken together, these molecular design strategies illustrate the possibility to tune peptide-responsive nanocarriers for improved stability and targeted delivery.

3.2 Environmental responsiveness enhancement

The transition from single to multistimuli-responsive peptide nanocarriers has overcome the challenges caused by complex physiological microenvironments [44].

Special attention has been drawn to dual stimuli-responsive peptide nanomaterials (DSRPNs) that, in turn, allowed pH/ROS-responsive systems to show their targeted release upon both an acidic tumor microenvironment and high glutathione levels [45]. In this respect, the field has continued to evolve to include many combinations of endogenous stimuli, such as pH/enzyme responsiveness [46], and combinations of internal and external triggers [47]. This, in turn, led to the development of multi-stimuli-responsive platforms that, while responding to cascades of three or more stimuli, enable extremely precise spatiotemporal control over drug delivery [48].

These progresses of multi-responsive systems mark a great leap forward to more efficient and precise therapeutic approaches.

3.3 Surface modification techniques

In order to improve the functioning and stability of peptide-responsive nanocarriers, surface modification is an essential phase in the development process. A proper balance of cellular penetration against systemic stability remains a challenge toward developing an efficient drug delivery system.

The typical cell-penetrating peptides (CPPs) are effective for cellular entry; however, most of the CPPs suffer serious limitations due to their positively charged amino acids that result in rapid clearance and potential enzymatic degradation [49]. This has been successfully overcome by researchers through hydrophilic modification, especially with the conjugation of polyethylene glycol (PEG) to peptides on the nanocarrier surface. For instance, Wu et al. presented that the PEGylation of dual-modified liposomes containing both cell-penetrating peptide R8 and targeting peptide cyclic RGD prolonged their in vivo circulation time to 24 hours: this effect results from the fact that PEG can mask the positive charges of CPPs yet preserve their functional activity [50]. In addition, the presence of targeting peptides may enable cell type-specific delivery, as tumor cells often express receptors like HER-2 and transferrin receptor (TfR) in high levels that can be utilized for targeting selective treatment of the disease [51].

The surface modification optimization will have to consider both the density and spatial distribution of peptides in order to reach an optimal compromise between targeting efficiency and stability for the final goal of nanocarrier therapeutic efficiency.

4. Biomedical applications

With these characteristic stimulus-responsive features and sophisticated controlled-release manners, peptide-responsive nanocarriers find extensive applications in diverse biomedical fields. Table 16.1 presents representative examples of peptide-responsive nanocarriers and their diverse biomedical applications. The following section highlights the major biomedical applications where these smart nanoplatforms have demonstrated remarkable potential:

Table 16.1 Peptide responsive nanocarriers for biomedical applications.

Component name	Stimulus	Conformational changes	Application	References
TPP-RRRKLVFFK-Ce6	Light	structural transformation from nanosphere to nanorod	Drug delivery	[52]
PA2	PH	Self-assemble into nanospheres at pH 7.5 and disassemble at pH 6–6.5	Drug delivery	[53]
BP	Cu2+	ARS/BP complex changes from nanpshperes to nanpfibers	Imaging	[54]
Au-RRVR	Furin Enzyme	Cleavage followed by nanoparticle assembly	theranostic	[55]
CPP- ELP_{BC}	Temperature (37–42 °C)	Unimers self-assemble to form micelles	Drug delivery	[56]
Dendrimer-GEM	Cathepsin B	Cleavage	Drug delivery	[57]
TER-SA (RGDRDDRDD-PLGYLGFFC(Cy))	MMP-2/9 enzymes	Cleavage followed by self-assembles into nanofibers	Imaging	[58]
cationic polyplex core nanoparticle with DMMA-modified PEG-b-polylysine shell	PH	Disassembly	Gene therapy	[59]
albumin-polyplex nanoassembly (BI-SPD)	Dual-stimuli-responsive (NIR/GSH)	Disassembly	Gene therapy	[60]
Alexa-488 labeling of peptide 1 (FFALGLAGKK)	MMP enzyme	Disassembly	Imaging	[61]
Silk-TSP nanosphere	Thrombin enzyme	Cleavage	Antimicrobial	[62]
polypeptide-based FHE hydrogel (F127/OHA-EPL)	Temperature	Gelation	Wound healing	[63]
(HA)-based composite hydrogel, with an antimicrobial peptide [AMP, KK(SLKL) 3 KK]	PH	Cleavage followed by release of AMP	Wound healing	[64]

4.1 Targeted drug delivery

During the last decade, peptide-responsive nanocarriers have set a new frontier in targeted drug delivery. Nanocarriers have demonstrated great versatility in oncology. Advanced systems may utilize unique physicochemical properties from the tumor microenvironment (TME) to achieve precise

spatiotemporal control of therapeutic cargo release, including altered pH, high levels of glutathione, and overexpressed enzymes [65].

For example, Chen et al. fabricated shell-stacked nanoparticles (SNPs) with peptide-based shells, whose size reduces from 145 to 40 nm and charge reverses from −7.4 to 8.2 mV in the acidic tumor environment, which can significantly enhance the tumor penetration and drug efficacy thereof [49]. Complementing this approach, Li et al. prepared dual pH/redox-responsive peptide-calcium phosphate hybrid nanoparticles (CPPT@CaP-CD) for co-delivery of curcumin and doxorubicin, using both the acidic TME and intracellular glutathione for controlled drug release as shown in Fig. 16.1 [66].

Peptide-responsive nanocarriers have the potential for site-specific delivery in gastrointestinal disorders; for example, the nanocarrier based on a zwitterionic peptide scaffold from Xue et al. can mimic albumin to avoid systemic clearance while targeting overexpressed poly(salivary acid) on tumor cells via recognition [67].

To date, peptide-responsive nanocarriers have been applied with promising results regarding immune response modulation in the context of immunotherapy, and some designs are capable of efficiently interrupting PD-1/PD-L1 interactions, hence overcoming limitations in immune checkpoint therapy [68].

These various examples illustrate that peptide-responsive nanocarriers can be adapted for a wide range of therapeutic modalities, from chemotherapy and radiotherapy to targeted delivery in gastrointestinal disorders and even to possible applications in immunotherapy, enabling an unprecedented degree of precision in the treatment of complicated pathologies by specifically tailored drug delivery methods.

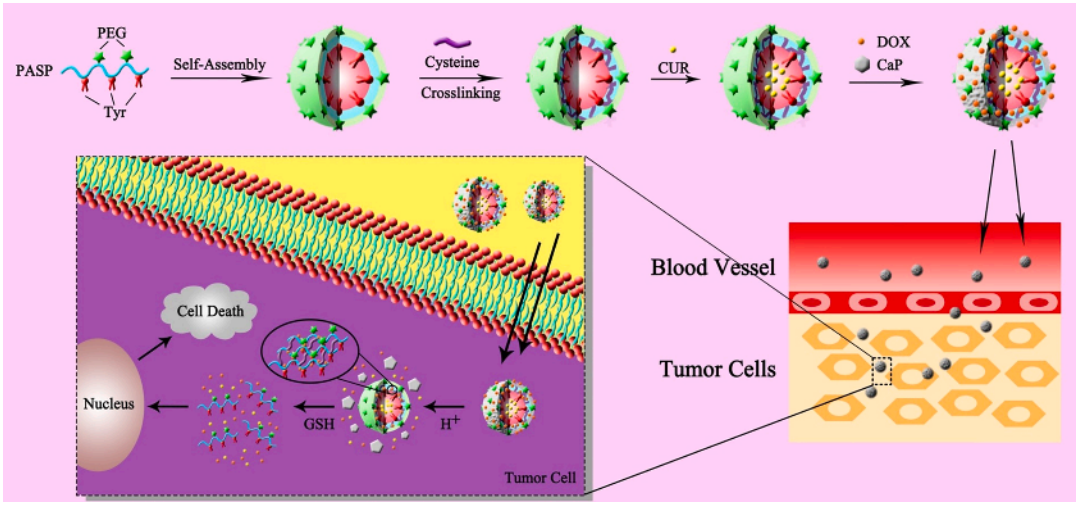


FIGURE 16.1

Schematic representation of preparation process and action principle diagram of CPPT@CaP-CD nanoparticles.

From Q. Li, D. Fu, J. Zhang, H. Yan, H. Wang, B. Niu, biointerfaces dual stimuli-responsive polypeptide-calcium phosphate hybrid nanoparticles for co-delivery of multiple drugs in cancer therapy. Colloid. Surf. B, Biointerf. 200 (October 2020) (2021) 111586. https://doi.org/10.1016/j.colsurfb.2021.111586.

4.2 Antimicrobial applications

Researchers have expanded the idea of peptide-responsive nanocarriers for targeted drug delivery to deal with the growing problem of bacterial infections, especially those caused by strains that are not easily killed with medicine. Peptide-responsive nanocarriers for antimicrobial applications leverage the unique properties of stimulus-responsive peptides and antimicrobial peptides (AMPs) to create smart, stimuli-responsive systems that can effectively combat bacterial pathogens.

This possibility was shown by Chan-Park et al. by preparing a pH-responsive system composed of cationic (CS-PLL) and anionic (CS-PLL-CA) peptidosaccharide copolymers that self-assemble into nanomicelles at physiological levels and have very high biocompatibility. The key to the effectiveness of the system lies in the incorporation of the pH-labile β-carboxylic amide bond within the anionic copolymer. Upon exposure to the pH 5.5 or lower that characterizes the acidic environment at the sites of bacterial infection, hydrolysis of the β-carboxylic amide bond occurs to result in nanomicelle decomposition and release of antimicrobial peptides that effectively target and kill bacteria [69].

More recently, to meet the demand for antimicrobial strategies under alkaline conditions, the authors have designed an original alkaline-triggered antimicrobial hydrogel-entrapped polydiacetylene-peptide system. Upon raising the pH, the hydrogel exhibited a conformational change and colorimetric transition from blue to purple, along with a gel-to-sol phase transition; this was sufficient to provoke selective release of entrapped antimicrobial agents at the site of infection. Thus, the system was a pH sensor for on-demand alkaline-triggered delivery of antibiotics tested on inoculated pig skins [70]. This concept extends the use of peptide-responsive nanocarriers in fighting infections at different pH conditions, offering the possibility to address a wider range of multidrug-resistant pathogens.

4.3 Gene therapy

Gene therapy has emerged as a new treatment strategy against cancer by introducing exogenous normal genes into the target cells using gene transfer technology, which may offer higher efficiencies and selectivities. However, conventional preparation of a gene delivery vector usually faces difficulties, poorly targeted drug delivery, and lower biosafety [71]. Considering these limitations, in recent years rapid development of stimuli-responsive nanocarriers has been carried out that allows highly selective and specific targeted delivery with controlled gene release behavior, which in turn enhances the overall gene therapy efficiencies [72]. Among these, peptide-responsive nanocarriers have emerged as a particularly effective strategy.

Liu et al. constructed one charge-converting biomimetic siRNA delivery nanoplatform with a three-layer core-shell structure for glioblastoma treatment. The nanocomplex Ang-RBCm-CA/siRNA was coated with a vasopeptide-2-modified erythrocyte membrane that enabled blood-brain barrier (BBB) penetration and protected siRNA against immune attack. In the weak acidic TME, the nanocomplex underwent negative-to-positive charge conversion capable of triggering erythrocyte membrane lysis and accelerating siRNA release inside tumor cells [73].

Another noteworthy example is the matrix metalloproteinase 2 (MMP-2)-responsive Micelleplex system shown in Fig. 16.2. Composed of a PEG-poly(ε-caprolactone) block copolymer linked by an MMP-2-sensitive peptide bridge (PLG*LAGr9), it demonstrates the potential to overcome the conflicting requirements of prolonged circulation and efficient cellular uptake. This system utilizes PEG

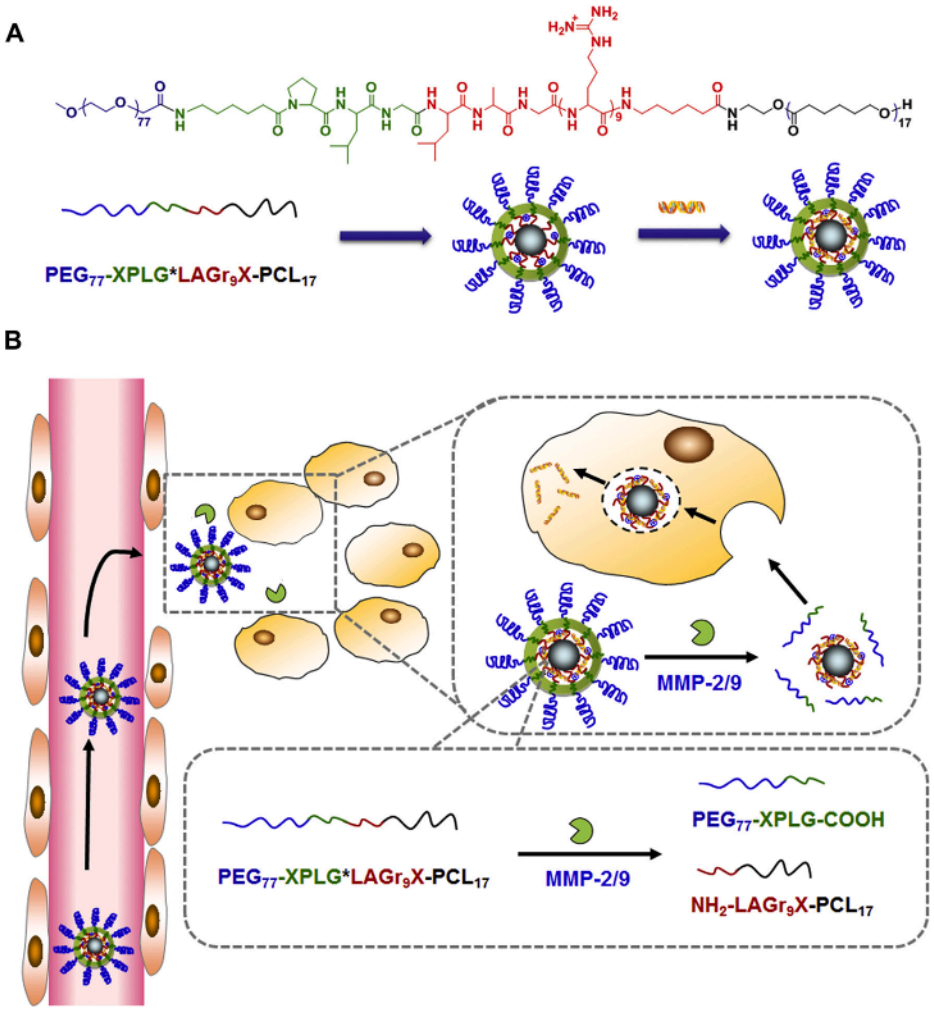


FIGURE 16.2

Matrix metalloproteinase 2-responsive micelle for siRNA delivery.

From H.X. Wang, X.Z. Yang, C.Y. Sun, C.Q. Mao, Y.H. Zhu, J. Wang, Matrix metalloproteinase 2-responsive micelle for SiRNA delivery. Biomaterials 35 (26) (2014) 7622–7634. https://doi.org/10.1016/j.biomaterials.2014.05.050.

for stability in circulation and an MMP-2-cleavable peptide for PEG shedding in the tumor microenvironment, exposing a polyarginine segment for enhanced cellular internalization [74].

4.4 Diagnostic imaging

The birth of peptide-responsive nanocarriers ushered in a new era in precision diagnostic imaging, revolutionizing fundamentally our capability to visualize and understand the processes of disease at a

molecular level. These ingenious smart responsive peptide nanocarriers orchestrate an amazingly sophisticated interplay between molecular recognition and self-assembly to construct powerful diagnostic tools. Such intelligent nanocarriers can enhance the imaging results based on many mechanisms, including responding to tissue or cellular-specific biomarkers for improved selectivity, their accumulation at target sites, improving signal-to-noise ratios relative to free imaging agents, and being engineered to work with aggregation-induced emission (AIE) dyes [65].

For optical imaging, a smart peptide-AIE conjugate probe has been modularly designed by combination of the peptide-tetraphenylethene (TPE) motif with the fibroblast activation protein alpha (FAP-α) responsive elements, as shown in Fig. 16.3. The system exhibits selective fluorescence activation in pancreatic tumor cells, while its Phe-Phe (FF) modification will enable optimal nanofiber assembly onto cancer-associated fibroblasts for enhanced probe retention and imaging capability [75].

For MRI, Wang et al. developed an advanced enzyme-responsive polymer peptide-porphyrin conjugate with a response to bacterial gelatinase. Indeed, this system conferred enhanced T1/T2 dual-mode MRI contrast as well as enabled targeted bacterial infection imaging and treatment monitoring [76].

In the field of photoacoustic imaging, Wang et al. constructed a novel self-assembling nanogel system (SiO2@MCSGs) based on dilysine-iron coordination. This system exhibited both superoxide dismutase and peroxidase activities. When this system was loaded with substrate 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS), it could catalyze the transformation of tumor

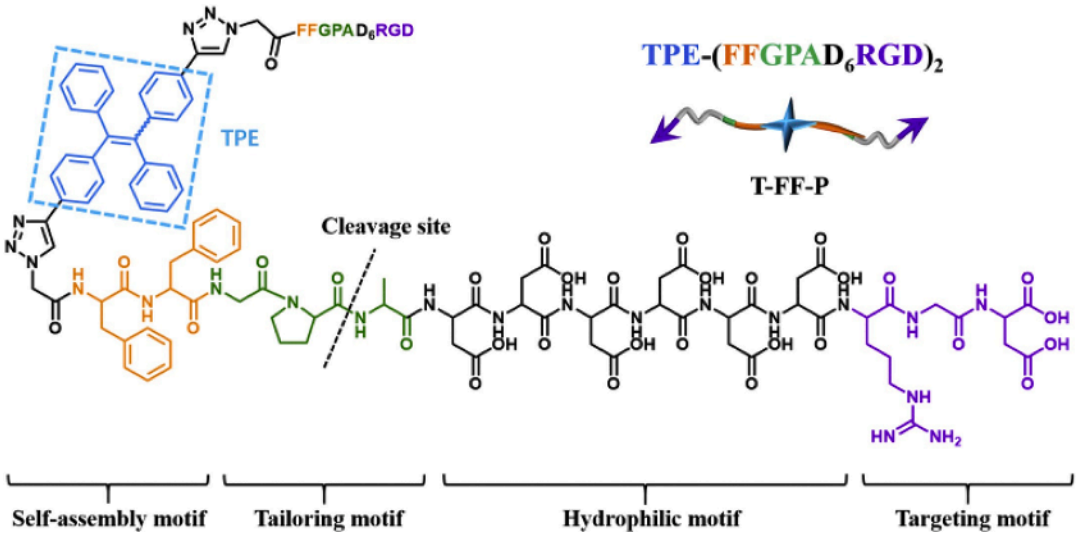


FIGURE 16.3

Chemical structure and modular design of peptide-TPE Chemical structure and modular design of peptide-TPE.

From S. Lu, X. Guo, F. Zhang, X. Li, M. Zou, L.L. Li, Bioactivated in vivo assembly (BIVA) peptide-tetraphenylethylene (TPE) probe with controllable assembled nanostructure for cell imaging, Chin. Chem. Lett. 32 (6) (2021) 1947–1952. https://doi.org/10.1016/j.cclet.2021.01.007.

microenvironment oxygen into an intermediate that strongly absorbs in the near-infrared band and thus act as an ideal photoacoustic imaging contrast agent for tumor detection [77].

4.5 Biosensing

Peptide-responsive nanocarriers have emerged as one of the powerful platforms in biosensing applications by offering a variety of detection mechanisms highly important for modern medical diagnostics.

In hydrogel-based systems, the unique combination of biocompatibility, structural tunability, and retained water properties enables molecular-level detection events to be very precise [78]. These systems operate through a fundamental detection mechanism where the hydrogel structure undergoes specific changes upon interaction with target biomolecules. Indeed, this is done quite brilliantly in the work by Yoshii et al., who developed an advanced multicomponent supramolecular hydrogel system. In this newly designed strategy, a dendritic self-immolative molecule with corresponding enzymes is stably embedded in an H2O2-responsive hydrogel matrix of BPmoc-F3. Upon exposure to various biomarkers such as H2O2, glucose, or uric acid, multiple enzymatic and chemical cascade reactions are initiated within this system, giving rise to controlled degradation of the dendritic molecules and, therefore, a visually detectable gel-to-sol phase transition. This approach provides an improved sensitivity of detection with clear visual confirmation [79].

Besides hydrogels, other responsive-peptide-based fluorescent sensors have also shown excellent performances. A typical example could be the Tetraphenylethylene (TPE)-peptide probe with the GGLHLHLRL sequence, which provides highly selective Zn^{2+} detection based on the mechanism involving TPE aggregation and fluorescence activation upon Zn^{2+} complexation with histidine residues. This sensor represents an unprecedented combination of negligible cytotoxicity and superior cell membrane permeability, making it very valuable for the real-time intracellular monitoring of Zn^{2+} dynamics in living cells [80].

4.6 Tissue engineering and regenerative medicine

Stimuli-responsive peptide nanocarriers have indeed shown great potential in tissue engineering and regenerative medicine owing to their tunable properties combined with biocompatibility. The mechanical strength, response characteristics, and injectability of such systems can be precisely engineered by modifying amino acid sequences with the aim of optimizing these properties toward tissue engineering applications [81].

In vascular regeneration, functionalized self-assembling peptide hydrogels, like, for example, RADA16-I derivatives, were already proven to be effective 3D artificial microenvironments promoting human adipose-derived stem cell (hASC) activity [82]. Temperature-responsive systems containing vascularization peptides, such as QK, have also demonstrated the ability to enhance capillary formation and create well-defined lumina structures in vivo [83]. Generally, the efficacy of such systems depends on the concentration, as demonstrated by hydrogels with the sequential bRGD-CUBE, where the efficiency of blood vessel formation is directly related to the density of bRGD [84].

Such systems effectively biomimic the extracellular matrix. However, long-term in vivo stability and degradation kinetics, which must be systematically investigated, are still required prior to its clinical translation.

4.7 Theranostics

In precision medicine, theranostics represents a paradigm shift wherein diagnostics and targeted therapeutic intervention converge at the molecular level. It involves real-time monitoring of drug delivery, biodistribution, and therapeutic efficacy [85]. The peptide-responsive nanocarriers are some of the advanced platforms that epitomize the development in theranostic systems.

The development of an electrochemical sensing platform based on self-assembling hydrogels of fluorene methoxycarbonyl (Fmoc) group-modified tetrapeptide Phe-Glu-Lys-Phe (FEKF), functionalized with gold nanoparticles and ciprofloxacin, demonstrates dual functionality through its nanofibrous network structure to show both antimicrobial activity and enhanced catalytic properties for neurotransmitter detection [86]. Another striking example is that a hybridized dual-targeting peptide nanoprobe, strategically engineered through the incorporation of RGD homing and KALK mitochondria-targeting motifs into a cyclic heptapeptide sequence Arg-Gly-Asp-Lys-Leu-Ala-Lys, exhibits outstanding theranostic efficacy via Cy5.5-SAPD-(99m)Tc modification, enabling not only dual-modal imaging but also significantly improved therapeutic efficiency against glioblastoma multiforme due to selective receptor binding and mitochondria-mediated apoptosis [87]. In this regard, self-assembling supramolecular nanomedicines through engineering of porphyrin-tyroservatide peptide conjugates represent a significant advance in cancer theranostics that attain simultaneous photoacoustic imaging and chemo-photothermal combination therapy for significantly enhanced histone acetylation, yielding a tumor inhibition rate as high as 70% via single-dose administration, as shown in Fig. 16.4 [88].

These supramolecular systems address precisely how rationally designed nanoplatforms can finally deliver spatiotemporal control of both diagnostic and therapeutic functions with optimized pharmacokinetics and biosafety profiles, important preclinical prerequisites to clinical translation. Their integration within one nanoplatform underlines the strong potential of peptide-responsive nanocarriers toward the development of personalized medicine by active disease management at the molecular level.

5. Advantages and challenges

The peptide-responsive nanocarriers (PRNs), demonstrate improved targeting with active receptor binding, higher retention at target sites, and enhanced cellular uptake in wide therapeutic applications. These engineered platforms give superior pharmacokinetic profiles compared to conventional free drug delivery systems due to reduced systemic toxicity and enabling higher therapeutic indices. This is enabled by the structural versatility of PRNs toward systematic functionalization with diverse bioactive molecules, targeting ligands and diagnostic agents, and establishing their potential in theranostic applications. Compared with the antibody-based delivery system, PRNs exhibit several advantages in terms of simplified synthesis protocols, minimum immunogenic responses, improved physico-chemical stability, and scalable cost-effectiveness [89].

Despite these benefits, PRNs still face critical challenges in translation to clinical applications. Key issues pertain to appropriate sustainable synthesis methods, long-term in vivo stability under multiple parameters, and cytotoxicity caused by their chemical modification. The complexity of physiological environments necessitates highly specific stimuli-responsive mechanisms. One major barrier is increased clearance by the activity of the reticuloendothelial system, which often results in major

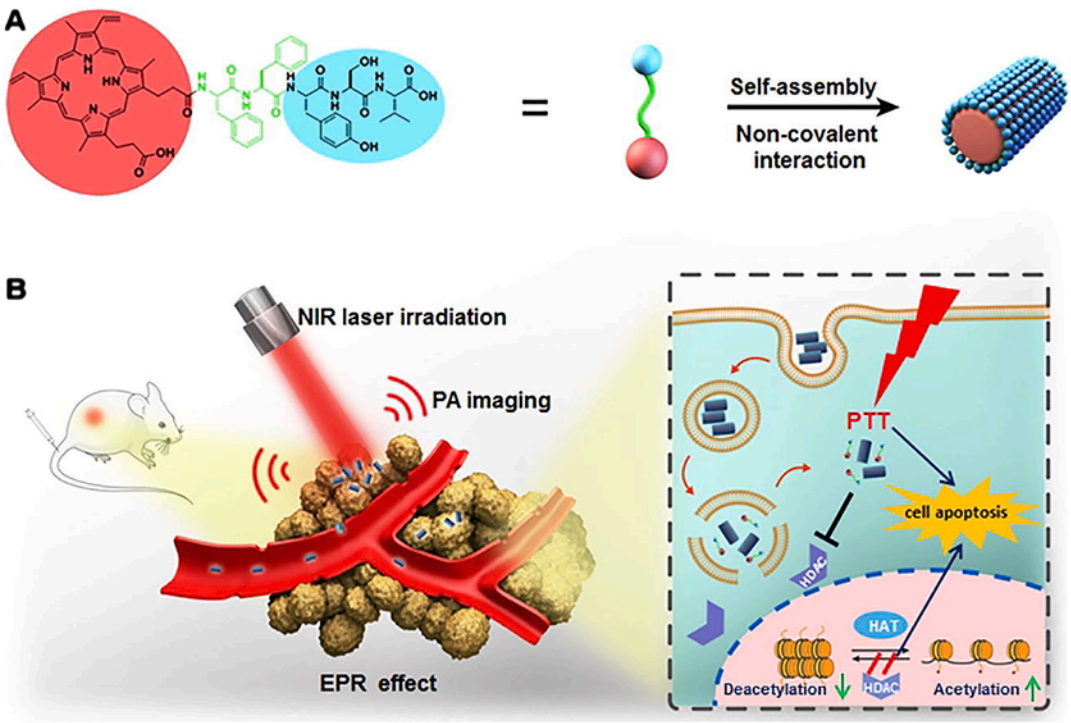


FIGURE 16.4

PpIX-FFYSV self-assembly into functional nanorods as theranostic platforms for imaging and treatment. (A) Molecular structure of PpIX-FFYSV and schematic illustration of the monomers self-assemble into nanorods-like nanoaggregates (PpIX NAs). (B) Schematic illustration of the self-assembled PpIX NAs for in vivo tumor photoacoustic imaging and chemo-photothermal combination therapy.

C. Ren, Z. Wang, X. Zhang, J. Gao, Y. Gao, Y. Zhang, J. Liu, C. Yang, J. Liu, Construction of All-in-One Peptide Nanomedicine with Photoacoustic Imaging Guided Mild Hyperthermia for Enhanced Cancer Chemotherapy. Chem. Eng. J. 405 (September 2020) (2021) 127008. https://doi.org/10.1016/j.cej.2020.127008.

hepatic and splenic deposition. This problem is very much exaggerated by heterogeneous tissue receptor expression. Besides, methodology development enabling real-time monitoring of peptide self-assembly dynamics and therapeutic outcomes in vivo is yet to be made [2]. Overcoming these hurdles in a systematic manner will be important for the further development of PRNs toward clinical applications.

6. Technological evolution and clinical progress

Advanced peptide synthesis technologies have brought a revolution in peptide-responsive nanocarriers. Microfluidic systems transcend fundamental hurdles in synthesis due to enhanced reaction

kinetics, superior safety protocols, and well-controlled parameters that enable one even to generate complex peptide sequences associated with sophisticated nanocarrier architecture [90].

Developments in artificial intelligence and machine learning have taken work in peptide design another step forward. Computer-Aided Peptide Design (CAPD) was used to be able to provide theoretical guidance through data mining and sophisticated algorithms [91], while automated flow peptide synthesis (AFPS) permitted chemists to gain complete control over chemical reactions [92]). Moreover, ionic liquids improved the solubility of peptides while accelerating the coupling reactions, which may potentially promote more efficient processes for nanocarrier production [93].

As the horizon unfolds, converging AI-driven design, automated synthesis, and microfluidic technologies promise to revolutionize the development of peptide-responsive nanocarriers with a possible improvement in more efficient targeted drug delivery systems. This technological progress has translated into significant clinical advancement. Today, over 150 drug candidates based on peptides are under clinical development, and among them peptide-responsive nanocarriers, an important subset holds promise. However, despite FDA approval of several peptide-based therapeutics, there are still some major problems that were discussed previously to be resolved for clinical translation [2].

7. Conclusions and perspectives

In this chapter, we have discussed peptide-responsive nanocarriers, from their basic building blocks of structure to advanced applications in biomedicine. So far, the strategic design of peptides has enabled the construction of nanocarriers capable of responding to a wide range of stimuli through variation in the arrangement of amino acids and the side chain functionalities, from pH to enzymes, redox conditions, and temperature. Recent developments within this area have been largely achieved through surface modification of such systems, embedding of complementary materials, and the design of more intelligent responsive mechanisms to stimuli, affording enhanced targeting and therapeutic effectiveness. Although peptide-responsive nanocarriers represent a very promising class of drug delivery, gene therapy, and bioimaging vectors, challenges such as scalability, potential immunogenicity, and stability in complex physiological environments remain a concern. Ongoing efforts surmount these by artificial intelligence-driven peptide design, development of standardized protocols for scale-up, and optimization of drug loading and release kinetics. Despite all the above-mentioned challenges, the unique advantages of peptide-responsive nanocarriers, in particular, will continue to drive innovation in this field due to biocompatibility and versatility. In the near future, much attention will be paid to further improvement regarding specificity and sensitivity of response mechanisms, in vivo stability, efficacy, and expansion of applications in personalized medicine. Further understanding of peptide self-assembly and stimuli-response mechanisms, combined with advances in synthesis and characterization techniques, is expected to lead to significant progress toward clinical translation in the development of even more effective therapeutic strategies across a wide range of biomedical applications.

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PART

IV

Dual and multimodal-responsive nanocarriers for biomedical application

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CHAPTER

pH-responsive nanocarriers: Smart drug delivery for biomedical applications

17

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1. Introduction to pH-responsive nanocarriers

1.1 Definition and mechanisms

pH-responsive nanocarriers are a newer class of drug delivery systems that are designed to release therapeutic agents in response to the acidic environment of the pathological tissues, especially tumors. The specific drug release mechanism exploits the singular features of the tumor microenvironment (TME), which contains acidity due to the Warburg effect. The phenomenon occurs whenever tumor cells produce lactate and hydrogen ions (H+) as metabolic products of anaerobic glycolysis, which results in lowering extracellular pH than in healthy tissues [1,2]. By using materials that undergo physicochemical changes in an acidic environment, such as protonation, deprotonation, or swelling, these nanocarriers can realize selective drug release at the tumor site with reduced side effects on surrounding healthy tissue [3–5]. More precisely, some polymers bearing ionizable functional groups, like poly(acrylic acid) [6] or chitosan [7], have been found to respond to low pH by a change in their solubility or integrity, thus enabling controlled release. Additionally, nanoparticles with core-shell structures can harbor a positively charged corona under an acidic condition, which enhances cellular uptake and internalization at the tumor site [8]. This pH-sensitive material can also be dissociated or change its surface charge in an acidic TME, thus providing drug release with both precision and efficiency [9–11]. These processes are schematically represented in the following figures: Fig. 17.1 shows how these stimuli, external to the encapsulated molecules in the host material cause release (here, pH changes) for targeted drug delivery, in particular, how drug-loaded pH-responsive nanoparticles release their contents in an acidic tumor microenvironment (pH ~6.5) by mechanisms such as acid-responsive swelling, solubility changes, charge alteration, and cleavage of covalent bonds. Furthermore, pH-responsive nanocarriers can more precisely deliver drugs to the targeted site by selectively responding to pH gradients in tumor tissues, thereby increasing therapeutic efficacy while reducing systemic toxicity [12,13].

Innovative Nanocarriers for Stimulated Drug Delivery. https://doi.org/10.1016/B978-0-443-33641-6.00008-7
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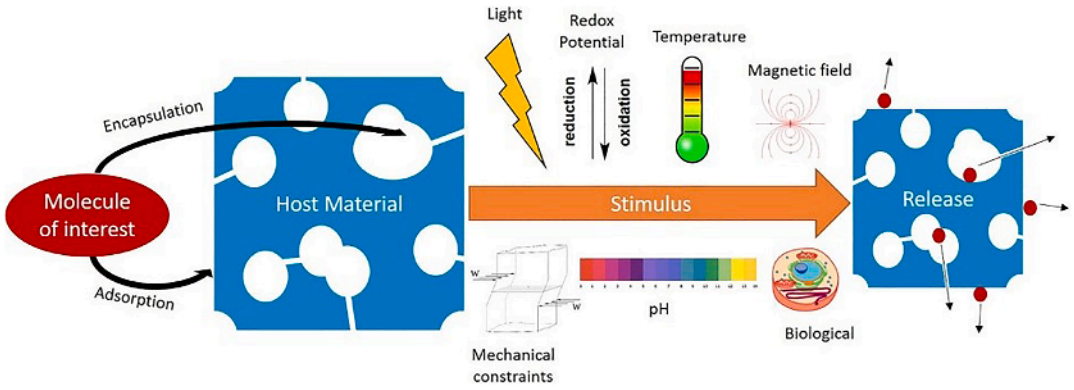


FIGURE 17.1

Stimuli-responsive nanocarrier system: external triggers such as pH, light, temperature, and magnetic fields initiate the release of encapsulated therapeutic agents from host materials for controlled and targeted delivery.

Adapted from R.S. Katiyar, P.K. Jha, Phase behavior of aqueous polyacrylic acid solutions using atomistic molecular dynamics simulations of model oligomers, Polymer 114 (2017) 266–276, https://dx.doi.org/10.1016/j.polymer.2017.03.007.

1.2 Importance in drug delivery

Inflamed tissues, as well as intracellular organelles such as endosomes and lysosomes, have acidic microenvironments that offer strategic targets for the specific delivery of drugs. The design of nanocarriers stable at neutral or physiological pH, which, however, are responsive in an acidic environment, allows the selective release of therapeutic agents in these targeted regions. This methodology enhances the targeting capability of pharmaceutical administration, enabling diseased cells or tissues to be treated more precisely while simultaneously reducing adverse impacts on healthy cells. Consequently, the degradation or activation of nanocarriers in acidic environments enhances the therapeutic effect and, at the same time, reduces systemic toxicity and side effects due to other unforeseen circumstances, thus placing pH-responsive nanocarriers in a potentially valuable position in the field of biomedicine [14,15].

Fig. 17.2 shows different stimuli that could activate responsive nanocarriers for drug delivery systems. It presents how pH, reactive oxygen species (ROS), enzymes, thermal/light stimuli, adenosine triphosphate (ATP), and glutathione (GSH) take part in the triggering role of these subunits. These stimuli communicate with the nanocarriers to cause "charge reversal" or other activation processes that then allow drug release. Of these stimuli, pH represents one of the central triggers using the acidic microenvironment of tumors or inflamed tissues for the initiation of targeted drug release, thereby underlining the flexibility and adaptability of nanocarriers in different biological environments. Exploiting such stimuli allows nanocarriers to achieve a higher degree of drug targeting specificity, which consequently enhances the therapeutic outcomes.

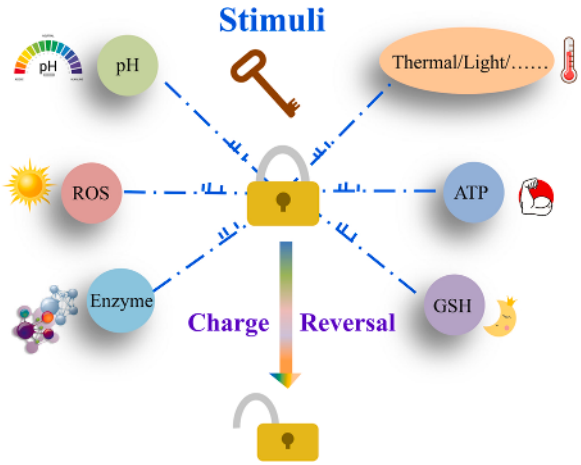


FIGURE 17.2

Schematic representation of stimuli-responsive mechanisms in nanocarriers for targeted drug delivery. Various stimuli, including pH, reactive oxygen species (ROS), enzymes, thermal/light, adenosine triphosphate (ATP), and glutathione (GSH), serve as triggers for activating nanocarriers. These stimuli induce mechanisms such as charge reversal and structural changes, allowing selective drug release in specific microenvironments. pH-sensitive responses, in particular, are essential for targeting acidic regions like tumors and inflamed tissues, enhancing therapeutic specificity and reducing systemic toxicity.

Adapted from H.M. Abdelaziz, M.A. Abdelmoneem, K. Abdelsalam, M.S. Freag, K.A. Elkhodairy, A.O. Elzoghby, Poly(Amino acid) nanoparticles as a promising tool for anticancer therapeutics, Polymeric Nanoparticles as a Promising Tool for Anti-cancer Therapeutics, Elsevier, Egypt, 2019, pp. 167–204, https://dx.doi.org/10.1016/B978-0-12-816963-6.00009-1.

2. Types of pH-responsive nanocarriers

2.1 Polymeric nanocarriers

Polymeric nanocarriers have attracted considerable attention for drug delivery applications by using pH responsiveness to favor the controlled release of therapeutic agents specifically in acidic media, which characterizes tumor tissues, inflamed sites, and intracellular compartments. Among them, polymers such as poly(acrylic acid) (PAA), poly(L-histidine), and chitosan have been widely used for this purpose. Under acidic conditions, PAA is protonated; this change leads to conformational changes that facilitate drug release [16]. Similarly, poly(L-histidine) and chitosan exhibit pH-responsive properties as protonation at lower pH causes swelling, thereby destabilizing the carrier to release drugs effectively [17]. Chitosan is another natural polysaccharide, which also responds to pH changes via its amino groups, which become protonated at lower pH, swell and hence release encapsulated drugs effectively [18,19]. Fig. 17.3 lists various pH-sensitive polymeric nanocarriers, including polymer-drug conjugates, dendrimers, micelles, and nanogels, which are designed to release drugs in response to an acidic environment, and thus enhance the selectivity and efficacy of drug delivery.

Moreover, the potential of pH-responsive nanocarriers has been proven with polydopamine-coated cobalt ferrite nanoparticles (PDA-coated CF-NPs) and MSNs for cancer therapy. PDA-coated CF-NPs

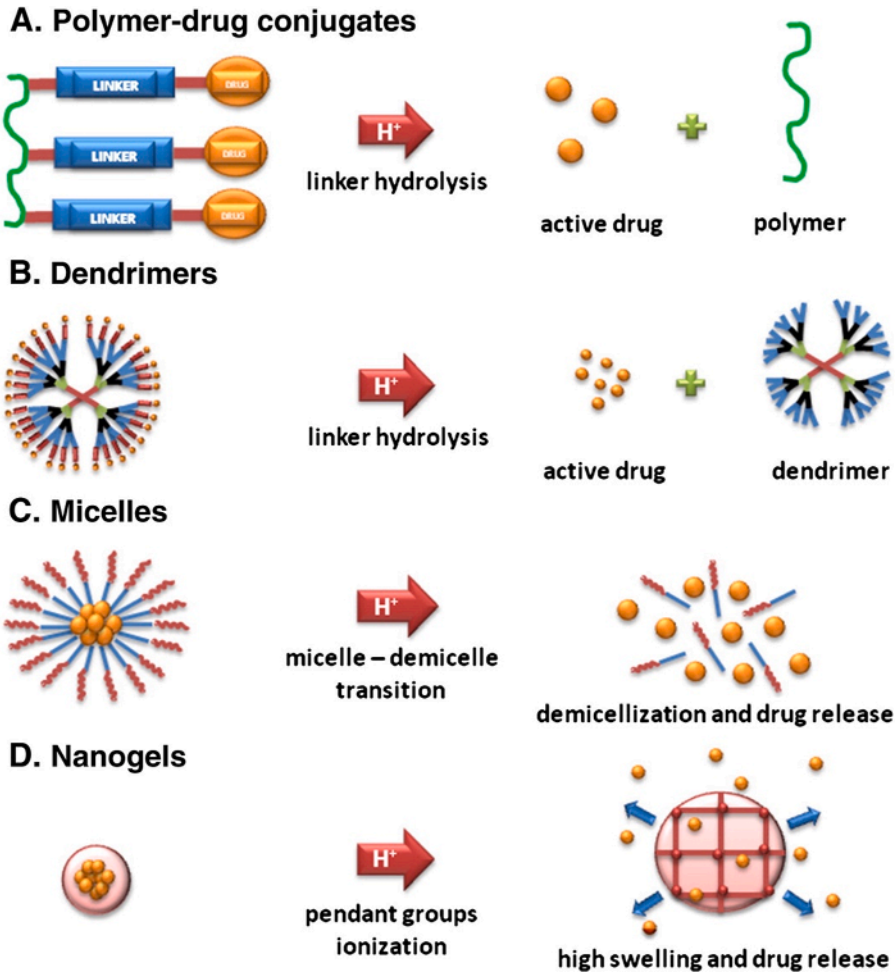


FIGURE 17.3

Different types of pH-responsive polymeric nanocarriers used in drug delivery. (A) Polymer-drug conjugates utilize linker hydrolysis under acidic conditions to release active drugs; (B) Dendrimers similarly release drugs through acid-induced linker hydrolysis; (C) Micelles undergo a micelle-to-demiscelle transition, enabling drug release in acidic environments; (D) Nanogels swell in response to protonation of pendant groups, leading to drug release [15].

were synthesized and evaluated for their drug delivery properties. The nanoparticles showed a spherical shape with increased pore size and enhanced surface area, which indicates improved drug loading and release potential. The drug release of DOX-loaded PDA-coated CF followed Fickian diffusion, indicating a controlled release mechanism. In vitro, these NPs were low toxic to HepG2 liver cancer cells. In vivo studies showed that they could inhibit tumor effectively and thus are promising

candidates for cancer treatment [20]. Similarly, MSNs@P123-DOX nanocarriers, which are made from magnetic iron oxide nanoparticles and silica, also showed pH- and thermo-responsive drug release; DOX could be released more efficiently under an acidic pH (5.4) and at higher temperatures (42°C). The antitumor efficiency of these nanocarriers was further verified in vivo by showing a strong reduction in the size of tumors, hence showing their potential for targeted drug delivery in cancer therapy [21].

2.2 Lipid-based nanocarriers

Lipid-based nanocarriers, such as liposomes and SLNs, have been the focus of intense research in targeted drug delivery, since these carriers exhibit excellent biocompatibility and have shown a great ability to carry different therapeutic agents; moreover, they offer great flexibility in terms of functionalization. Liposomes are highly versatile spherical vesicles made up of one or more bilayers of phospholipids that can encapsulate hydrophilic and lipophilic drugs in the aqueous core and within the lipid membrane, respectively. Their pH-responsive features can be engineered by incorporating pH-sensitive lipids or polymers into their design, allowing them to exhibit targeted release under the acidic conditions found in tumor microenvironments, inflamed tissues, or endosomal compartments of cells [22]. This characteristic of pH-responsiveness is usually attained using lipids that undergo conformational or phase transitions in response to changes in pH and, hence, enable the liposome to destabilize and release its therapeutic payload specifically at the site, minimizing systemic side effects while maximizing the therapeutic effect [23].

Likewise, SLNs, being in a solid lipid matrix at room and body temperatures, provide a stable and controlled release platform for the delivery of drugs. Most of the advantages associated with SLNs are attributed to their potential to protect encapsulated drugs from degradation and their enhanced stability compared to other nanocarrier systems [24]. SLNs can also be designed for drug release in response to changes in pH through either modification of the lipid composition or by incorporation of pH-sensitive materials. This feature is particularly beneficial when the pathological sites have lower pH, as in the case of cancer and diseases related to inflammation. For example, it has been shown that SLNs containing fatty acids or acid-labile linkages can undergo structural changes in an acidic environment, thereby controlling the drug release precisely where it is required [25].

Such advantages of lipid-based nanocarriers may have great potential in enhancing the targeting precision and delivery efficiency in clinical application. pH-sensitive liposomes and SLNs will not only enhance targeting for therapeutic agents but also increase drug stability and bioavailability, reduce toxicity, and allow for dose reduction without affecting the efficacy of the drugs. To be able to engineer nanocarriers that can respond to specific environmental stimuli, such as pH levels, represents a tremendous step in the area of personalized and precision medicine, especially for the treatment of cancers and other diseases where targeted therapy is a must [26].

2.3 Inorganic nanocarriers

Among the inorganic nanocarriers, mesoporous silica nanoparticles (MSNs) and metal-organic frameworks (MOFs) show great advantages for pH-responsive drug delivery because of their good structural integrity, large surface area, and tunable pore size. Fig. 17.4a is a scheme of MSNs,

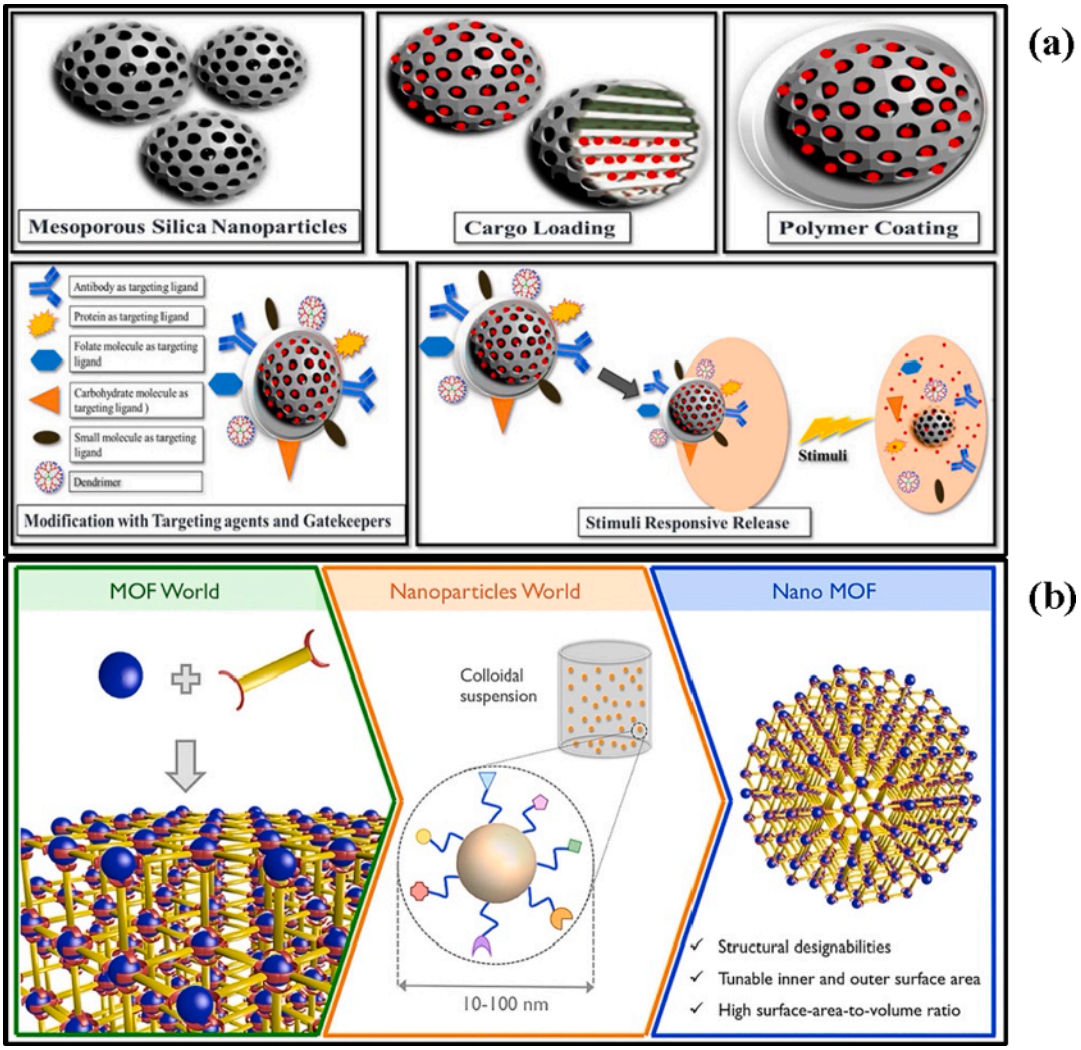


FIGURE 17.4

(a) MSNs as pH-responsive nanocarriers. Illustration of MSN design, including cargo loading, polymer coating, and targeting modifications, allowing for controlled drug release in acidic environments; (b) Metal-organic frameworks (MOFs) as pH-responsive nanocarriers. Depiction of the structural flexibility and high surface-area-to-volume ratio of MOFs, demonstrating their suitability for stimuli-responsive drug delivery, especially in targeted cancer therapy.

Adapted from F. Muhammad, M. Guo, W. Qi, F. Sun, A. Wang, Y. Guo, G. Zhu, PH-triggered controlled drug release from mesoporous silica nanoparticles via intracelluar dissolution of ZnO nanolids, J. Am. Chem. Soc. 133 (23) (2011) 8778–8781, https://dx.doi.org/10.1021/ja200328s; (b) Adapted from L. Liu, K.T. Yong, I. Roy, W.C. Law, L. Ye, J. Liu, J. Liu, R. Kumar, X. Zhang, P.N. Prasad, Bioconjugated pluronic triblock-copolymer micelle-encapsulated quantum dots for targeted imaging of cancer: In vitro and in vivo studies, Theranostics 2 (7) (2012) 705–713, https://dx.doi.org/10.7150/thno.3456China.

illustrating their architectural base, incorporation of cargo, polymer encapsulation, and functionalization with targeting moieties as well as gatekeeping systems. The porous structure of MSNs can be used to encapsulate drugs inside their internal space, protecting therapeutic agents from premature degradation. Combined with pH-sensitive gatekeepers or polymer coatings, MSNs could be designed to release their contents selectively under acidic conditions, such as in tumor tissues or inflamed areas. When exposed to lower pH values, these control structures undergo conformational changes, enabling specific delivery of the drug at the target site [27,28].

In contrast, metal-organic frameworks have high tunability and possess a porous crystalline structure, which is formed by the coordination between metal ions and organic linkers. This indeed allows for extensive tailoring for advanced drug delivery applications. Fig. 17.4b represents the "MOF World," illustrating the design of the structure and flexibility within MOFs. Due to their high surface-area-to-volume ratio and flexible design, MOFs can harbor pH-sensitive linkers, enabling the establishment of a responsive drug release mechanism in acidic microenvironments, such as those found in tumor microenvironments [29]. Due to their high drug loading capacity, MOFs are excellent candidates for the delivery of various therapeutic agents in cancer and other diseases. Such MOFs have been reported to partly dissolve or undergo structural changes in an acidic environment, allowing controlled drug release and enhanced treatment efficiency with reduced systemic toxicity [30] (Fig. 17.5). Taken together, MSNs and MOFs have portrayed the different structural and functional features of MSNs and MOFs in potentially developing targeted and precision drug delivery applications, especially in cancer and inflammatory diseases.

2.4 Hybrid nanocarriers

Hybrid nanocarriers, including Pluronic F127, a triblock copolymer known for its biocompatibility and pH-responsiveness, now offer an advanced platform for targeted drug delivery to acidic environments. More precisely, Pluronic F127, made of polyethylene oxide (PEO) and polypropylene oxide (PPO) blocks, allows the coating of organic materials with characteristics that are sensitive to changes in pH, thus improving the functionality of inorganic cores such as MSNs or MOFs. This copolymer not only stabilizes the nanocarrier but also improves the solubilization and retention of drugs, which is particularly effective in pH-sensitive drug release. Under physiologically relevant conditions (pH ~7.4), drug encapsulation is retained due to the stability of the Pluronic F127-coated carrier matrix. In acidic environments usually found at tumor sites (pH ~5.5–6.5), the Pluronic F127 coating undergoes conformational changes, which, in turn, trigger targeted release of the encapsulated drug precisely where it is most needed [31,32]. More specifically, in Pluronic F127-coated MSN systems, this pH responsive mechanism allows anticancer substances to be retained more in tumor cells, thereby bettering therapy effectiveness while lowering off-target side effects [33]. Moreover, Pluronic F127 is capable of not only improving biocompatibility but also extending in vivo circulation time, which turns out to be a necessary constituent in hybrid nanocarriers for precision oncology [34]. Coupling the stability of inorganic matrices with the pH-sensitivity of Pluronic F127 positions such a hybrid system at one of the most promising approaches for targeted and controlled drug delivery in complex biological environments [35].

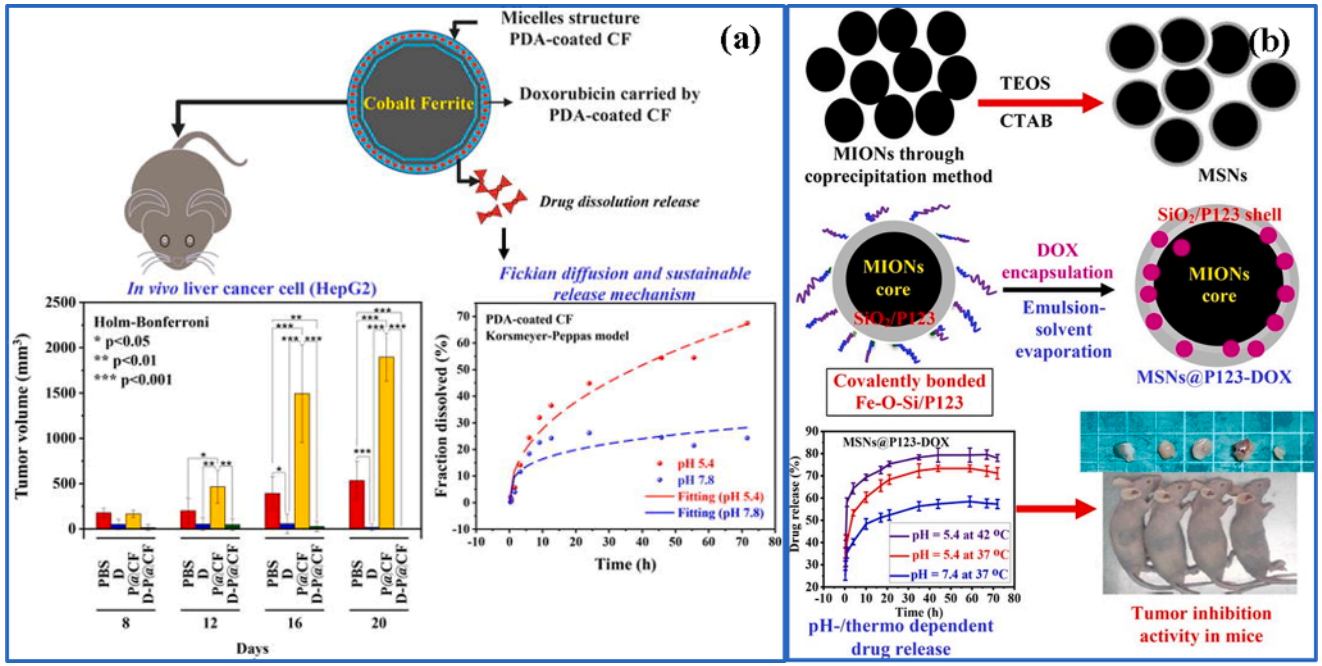


FIGURE 17.5

(a) Evaluation of PDA-coated cobalt ferrite (CF) as a drug delivery system for liver cancer treatment. The top section shows the micellar structure of PDA-coated CF encapsulating Doxorubicin, with drug dissolution release governed by pH conditions [22]; (b) Synthesis and evaluation of dual-responsive MSNs for targeted drug delivery [23].

3. Mechanisms of pH responsiveness

The processes of pH sensitivity in nanocarriers are the key for achieving stimuli-responsive drug delivery, especially for cancer treatment and endosomal drug delivery. These nanocarriers are made small to incorporate certain molecular changes at different pH levels that trigger change in structure to allow for optimal release profiles and improve therapeutics. Fig. 17.6 summarizes these mechanisms involving bond cleavage or formation, change in the electrostatic charges, shift in hydrogen bond interactions, binding and release of molecules, and variations in hydrophobic interactions. All of these mechanisms allow nanocarriers to adapt to change of pH, for example from the tumorous environment with pH at about 6.5 to the normal one with pH at about 7.4. For example, in an acidic environment, functional groups such as amines, carboxyls, protons or deprotons the surface charges and may cause swelling or degradation of the nanocarrier.

These molecular responses contribute to a range of structural alterations in the nanocarrier morphology, which has been presented in Fig. 17.6. Such mechanisms include swelling, cleavage of the linking points, degradation and the physical process of disassembly all of which facilitates releasing of the entrapped drugs. It also gestures responses such as cross-linking and charge conversion which can either increase retention at target sites or increase the selectivity of biomarker sensing. In addition, such mechanisms and structural changes give specific functional results for examples drug delivery, enhancing the sensitivity of the molecular imaging probes and even detecting biomarkers demonstrating the significant role of pH-responsive nanocarriers in the application of precision medicine. The incorporation of these mechanisms into the nanocarrier design means that researchers can develop the respective drug release profiles for the given biological compartment, thereby improving the therapeutic selectivity and minimizing side effects.

3.1 Protonation/deprotonation

The reversible change in the properties of functional groups namely; amines and carboxyl groups by means of protonation and deprotonation leads to pH changes for release of drug from nanocarriers. Where at very acidic pH that are regimes of certain tumors or cellular compartments they undergo protonation breaking the functionalities altering charge and in turn swelling or disruption of nanocarrier enhancing drug release [36]. For example, when the amine groups in polymer coatings are protonated in an acidic environment, the hydrophilicity of the carrier is increased and the swelling occurs, resulting in an increased rate of drug release from the carrier [37]. Likewise, carboxyl groups are capable of depolarization of carboxyl groups at a neutral to basic pH and ensured the stability of entrapment of the drug at a physiological pH (7.4). This dual responsiveness will guarantee that the release of the drugs will be low in health tissue environments, while at the same time being activated in specific acidic site thus enhancing the specificity of the therapeutic mechanisms and therefore reducing side effects [38]. That is, by synthesizing the functional groups within the nanocarrier, researchers can design systems to release at defined low or high pH, and thereby protonation and deprotonation constitutes the basis of pH-sensitive drug delivery system [39].

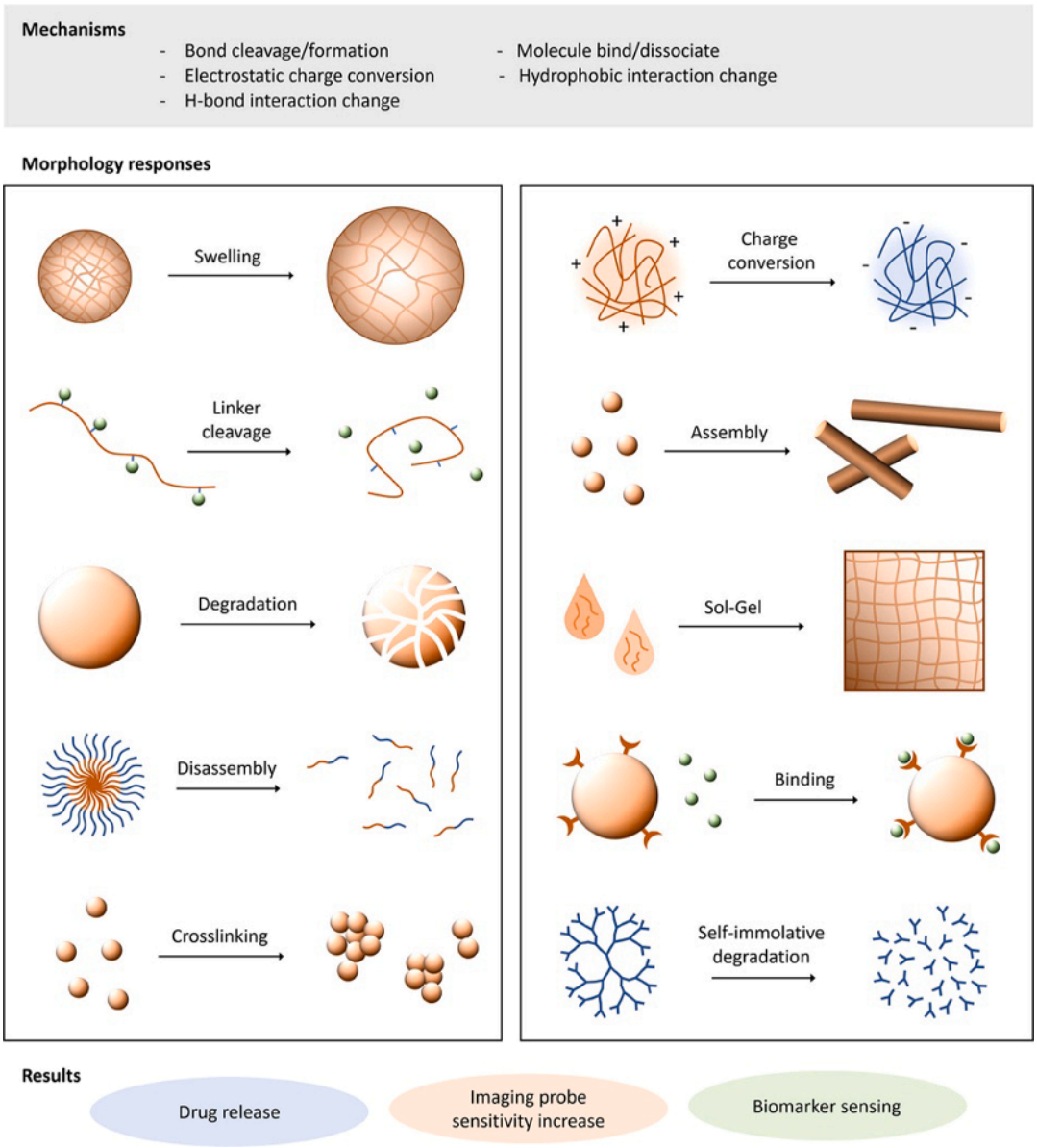


FIGURE 17.6

Schematic representation of the mechanisms and morphological responses in pH-responsive nanocarriers, showing how structural changes are triggered by stimuli to achieve specific outcomes, including drug release, imaging enhancement, and biomarker sensing.

Adapted from M. Al-Shaeli, S. Benkhaya, R.A. Al-Juboori, I. Koyuncu, V. Vatanpour, pH-responsive membranes: mechanisms, fabrications, and applications, Sci. Total Environ. 946 (2024) 173865, https://dx.doi.org/10.1016/j.scitotenv.2024.173865.

4. Applications in biomedical fields

In the "Applications in Biomedical Fields" section, the authors discuss the great prospects of pH-responsive nanocarriers in various branches of biomedicine. These nanocarriers, intended to respond to the pH differences characteristic of biological environments, including tumor regions and particular cellular organelles, allow for both the targeted delivery of therapeutic molecules and improved diagnostic functions at the required time. Through their ability to respond to changes in pH these systems provide localized drug delivery thus raising treatment efficiency and minimizing harm to normal tissues; they are suitable for cancer and similar treatments. Besides drug release, pH-sensitive nanocarriers are also being employed in biomedical imaging and biomarker detection leading to enhanced diagnosis and improving the area of the precision medicine. This section will comprise the versatile use of these smart systems in oncology, regenerative medicine, diagnosis, and biosensing to demonstrate how the ideas of pH-responsive nanocarriers are revolutionizing the concepts of modern healthcare.

4.1 Cancer therapy

pH-sensitive nanocarriers have found successful application in anticancer drug delivery systems since the tumor's environment is generally acidic. The extracellular pH of solid tumors ranges from 6.5 to 7.0 while that of normal tissues is about 7.4 For this reason, pH-sensitive nanocarriers can selectively release anticancer agents at the tumors [40]. For example, the nanoparticles coated by polymers carrying amine or carboxyl groups experience changes in their structure in response to the presence of the acids and the drug is released only within the tumor area without affecting the healthy tissue [41]. Lakkakula et al. [42] have synthesized chitosan nanoparticles (CS NPs), complexed with an anticancer drug tamoxifen and stable in normal condition but, sensitive in the acidic pH environment exist in MCF seven breast cancer cells. The CS NPs also showed a controlled and sustained release of tamoxifen at low pH (4 and 6) than at neutral pH (7) with a loading efficiency of 28% size ranged 100–150 nm. This pH dependent drug-release mechanism could improve the cytotoxicity, and cellular penetration into the tumor cells after causing considerable apoptotic effects without harming the normal cells. An encapsulated pITHA-PEG system in which the pH sensitivity of the system enabled the killing of only cancer cells while sparing normal cells from the toxicity of free tamoxifen concentrations that at high levels triggered apoptotic deaths in normal cells.

In addition, MSNs with pH-sensitive polymers can exhibit similar advantages; the controlled release of drugs only in the acidic tumor microenvironment enhances the anticancer effect and reduces systemic toxicity [43]. Some MOFs also feature pH-sensitivity; here, the protons in a tumor environment cause the disassembly of the MOF cage thereby releasing the encapsulated drug into the tumor cells [44]. Such advancements also put light on the potential use of pH-sensitive nanocarrier in cancer treatment by magnifying the treatment efficiency at the tumor site and reducing the side effect on the other part of the body.

4.2 Inflammatory diseases

pH sensitive effected nanocarrier has proved as one of the most promising systems for the treatment and management of inflammatory diseases since the pH level of the inflamed tissue is slightly acidic

(pH 6.5) while that of normal tissues is near neutral (pH 7.4). Inflammatory sites must have a low pH because of high metabolic activity and production of acidic by products from immune cells. This variation in the pH levels can be harnessed using pH responsive nanocarriers to herald anti-inflammatory drugs at the site of inflammation, hence diminishing the off target impacts and maximizing the therapeutic effect [45]. For example, the polymer–coated nanocarrier from poly (β-amino esters) experiences the protonation in the region of low pH and, hence, structural transformation that liberates the entrapped drug only at the site of inflammation [46].

This pH-sensitive dextran-based methotrexate nanodrug agents were proved to release their payloads in the inflamed tissue as evidenced by the decrease of inflammation markers in a rheumatoid arthritis model [45]. Another strategy entails employing MSNs with poor pH stability coatings that dissolve or swell at the low pH to deliver drugs solely into inflamed tissues. It reduces the toxicity of the treatment to healthy tissues and the rate of delivery, makes the treatment more convenient and efficient for chronic inflammatory diseases [47]. Such developments point to the ability of pH-sensitive nanoparticles to achieve targeted delivery of drugs to sites of inflammation; this forms a promising category within treatment solutions of RA, IBD, and other chronic inflammation conditions.

4.3 Intracellular delivery

Thus, pH-sensitive nanoparticles are of great importance with regard to improving drug delivery across membrane structures that contain intracellular compartments with an acidic environment, like lysosomes with a pH of 4.5–5.0. These carriers are self-assembled to be sensitive to pH differences existing between different intracellular compartments; they can therefore deliver their therapeutic cargoes as accurately as possible at the optimal locations within the cell. Where internalized, nanocarriers can later be internalized through endocytosis and transit through the endosomal-lusosomal transport system where they experience stepwise decrease in pH. Under such conditions, the material used becomes sensitive to pH; covalent functional groups may include amines or carboxyls will become protonated and destabilized or they swell up and burst for the release of the drug [48]. For instance, nanocarriers fabricated from polymeric materials containing pH-sensitive linkers open up lysosomal targeting, which was evidenced by high payload release in lymph nodes and enhanced intracellular drug concentration [49,50]. MSNs [51]and MOFs [52] have also been designed with pH sensitive coatings that dissolve inside the acidic lysosomal environment thus sealing the drug release only in the target tissue while avoiding effects on other tissues.

[53] supported the pH-sensitive MSNs in loading anticancer drugs, the cytotoxicity within cancer cells itself had been enhanced as the drug was selectively released in the lysosomes making the intracellular bioavailability of the therapeutic agent better [53]. Compared to the above types, pH-sensitive MOFs have been reported to show instability at the low pH within lysosomes and hence ensures the release of drugs within the intra cellular environment and a low toxicity at the systemic level [54]. This selective delivery and targeting system can be advantageous especially when drug delivery is required within specific compartments of the cell since most diseases such as cancer and certain infections of the cellular level. Conclusively, pH-sensitive nanocarriers are efficient for intracellular drug delivery since the cellular organelles such as lysosome has acidic pH to enhance therapeutically beneficial results.

5. Advantages and challenges

5.1 Advantages

pH-responsive nanocarriers present several distinct advantages that make them valuable in the field of targeted drug delivery. One major advantage is targeted and controlled drug release. These nanocarriers can be engineered to release their drug payloads in response to the specific pH conditions of diseased sites, such as the acidic environment found in tumors or sites of inflammation. This allows for precise drug delivery to the intended location, maximizing therapeutic effects while minimizing drug wastage and off-target distribution [55]. Such controlled release helps maintain effective drug concentrations at the target site, enhancing treatment efficacy without requiring frequent dosing.

Another significant advantage is reduced side effects. Traditional therapies often lead to systemic drug exposure, affecting healthy tissues and causing adverse side effects. pH-sensitive nanocarriers, by contrast, are designed to remain stable in the neutral pH of healthy tissues, releasing their contents only when they reach the acidic conditions of a target site, such as a tumor or inflamed tissue. This selective release reduces the impact on healthy cells, lowering the likelihood of side effects and providing a safer therapeutic option, particularly in cancer treatments [56].

Lastly, pH-responsive nanocarriers contribute to enhanced drug stability. Encapsulating drugs within these carriers protects them from premature degradation in the bloodstream, where they might be exposed to enzymatic degradation, fluctuating pH levels, or oxidative stress. This stabilization ensures that a higher concentration of the active drug reaches the target site intact, improving its therapeutic effect. For example, encapsulation within MOFs has been shown to enhance the stability and bioavailability of fragile drugs, preserving their potency until release in the desired location [57]. This enhanced stability is especially beneficial for drugs with short half-lives or those that are sensitive to biological conditions.

5.2 Challenges

Despite the promising applications of pH-responsive nanocarriers, several significant challenges must be addressed to fully realize their therapeutic potential. Designing nanocarriers with precise pH sensitivity remains complex, as it requires accurate tuning to ensure that drug release occurs only at specific pH levels associated with target tissues, such as tumors or inflamed areas. This fine-tuning demands a deep understanding of materials chemistry and the interplay between various functional groups that respond predictably to pH changes. Achieving reliable pH sensitivity is challenging, as slight deviations can lead to premature drug release or ineffective targeting [10].

Another critical challenge is ensuring biocompatibility and biodegradability. For clinical use, nanocarriers must be biocompatible to avoid adverse immune reactions and biodegradable to prevent accumulation within the body after drug delivery. Many materials used for pH-sensitive nanocarriers, such as certain polymers or inorganic frameworks, may exhibit limited biodegradability, which can lead to toxicity or long-term biopersistence. Balancing the structural stability of nanocarriers with their ability to safely degrade or clear from the body is a key area of ongoing research [58].

Lastly, overcoming biological barriers for effective delivery is essential for the successful application of nanocarriers. Once administered, nanocarriers encounter various biological barriers, including the mononuclear phagocyte system (MPS), cellular membranes, and endosomal-lysosomal

compartments, all of which can hinder effective drug delivery. For instance, the MPS often recognizes and clears foreign particles, reducing the accumulation of nanocarriers at target sites. Furthermore, for intracellular delivery, nanocarriers must avoid lysosomal degradation to release drugs directly into the cytoplasm. Strategies like surface modification, stealth coatings, and functionalization are being explored to improve nanocarrier stability and navigation through these barriers, yet they add layers of complexity to nanocarrier design and manufacturing [59].

6. Recent advances and case studies

In recent years, there have been significant advances in the development and application of pH-responsive nanocarriers in drug delivery systems. These innovative approaches address the challenges of targeted delivery and controlled release, particularly for cancer therapy and wound healing. Below, three case studies illustrate recent research efforts showcasing the potential of pH-responsive nanocarriers in enhancing therapeutic efficacy. Recent advances in pH-responsive nanocarriers demonstrate their potential across diverse applications, including cancer therapy, wound healing, and sustainable drug delivery solutions. For instance, polymer-grafted cellulose nanocrystals modified with poly(ethyl ethylene phosphate) (PEEP) have shown effective doxorubicin release in tumor environments, displaying biocompatibility and anticancer activity against HeLa cells [60]. Hybrid Liposomes (HLPs) developed via microfluidic technology incorporate PDA for pH-sensitive release, targeting acidic wound environments and promoting healing when loaded with hyaluronic acid or phenytoin [61]. Additionally, MSNs derived from cucumber peels present a sustainable solution for cancer therapy, efficiently delivering doxorubicin to lung adenocarcinoma cells and demonstrating antibacterial activity against infections common in lung cancer patients [62]. Together, these case studies underscore the versatility and potential of pH-responsive nanocarriers in advancing drug delivery systems for targeted therapies and sustainable medical solutions. These recent advances highlight how pH-responsive designs can be tailored for various applications, from cancer treatment to wound healing, while also supporting environmental sustainability through innovative materials sourcing.

6.1 Innovations

Recent advances in pH-responsive nanocarriers have led to the development of multi-stimuli-responsive systems that enhance precision in drug delivery by responding to combinations of pH, enzymes, redox conditions, and other triggers. These innovations allow for controlled, targeted drug release, especially beneficial in tumor environments where specific biochemical conditions prevail. For example, a dual pH- and enzyme-responsive nanocarrier was developed to improve the safety and efficacy of β-lapachone (LAP), an anticancer drug with a limited therapeutic window. In this design, LAP is encapsulated within ferric ion-polyphenol complexes using epigallocatechin gallate (EGCG) and ferric ions. These complex releases LAP in acidic environments, typical of endosomes and lysosomes, whereupon LAP is further activated by tumor-specific enzymes like NAD(P)H: quinone oxidoreductase 1 (NQO1), triggering cell death through multiple pathways, including pyroptosis and ferroptosis. This multi-layered approach not only ensures precise delivery but also enhances therapeutic effects, showcasing how smart, tunable nanocarriers can overcome limitations in conventional drug delivery [63].

7. Future directions

7.1 Emerging trends

The future of pH-responsive nanocarriers lies in the integration with other stimuli-responsive systems, creating multifunctional platforms capable of responding to multiple environmental cues, such as temperature, light, magnetic fields, or specific enzymes. This multi-stimuli approach can significantly enhance targeting specificity and control over drug release, making therapies more effective while minimizing systemic toxicity. For example, combining pH-responsiveness with redox sensitivity enables nanocarriers to release drugs in the acidic and high-glutathione environments typical of tumor cells, thereby achieving dual-selective targeting [64]. Similarly, light-activated and pH-sensitive nanocarriers allow precise control of drug release with external light, suitable for on-demand release during surgical procedures or targeted therapies [65].

Emerging trends in pH-responsive nanocarriers include combining these systems with other stimuli-responsive elements, such as ROS sensitivity and photodynamic therapy (PDT), to enhance therapeutic efficacy. For instance, pH-responsive KPEI nanocarriers facilitate cytosolic delivery of RNase A, an enzyme that degrades intracellular RNA in tumor cells, and are further modified with photosensitizers to enable light-triggered PDT [66]. This dual approach, integrating PDT and RNase A delivery, demonstrates significant anticancer effects in tumor-bearing mice. Additionally, advanced bioorthogonal reactions enable precise delivery of RNase A in targeted tumor cells, as shown in studies using metabolic glycoengineering to enhance cellular uptake of RNase A. These multi-functional nanocarriers highlight the potential of combining pH-responsiveness with other stimuli, pushing the boundaries of targeted cancer therapyThe integration of pH-responsive nanocarriers with such additional responsive mechanisms presents a promising direction for the field, allowing for adaptive, controlled, and patient-specific treatments that address the complexity of diseases like cancer and inflammatory disorders.

7.2 Clinical translation

The clinical translation and commercialization of pH-responsive nanocarriers hold promising potential, particularly in areas such as cancer therapy, targeted drug delivery, and precision medicine. pH-responsive nanocarriers offer the advantage of site-specific drug release, which enhances therapeutic efficacy while minimizing systemic side effects—a critical factor for improving patient outcomes in clinical settings [67]. Despite this promise, translating these technologies from the laboratory to clinical application involves several challenges. For instance, ensuring scalability and reproducibility during manufacturing remains a primary hurdle. Large-scale production requires consistent control over nanocarrier properties, such as particle size, surface chemistry, and pH sensitivity, to maintain functionality and ensure batch-to-batch uniformity [68–71].

In addition to manufacturing challenges, regulatory approval processes for nanomedicine are complex. Nanocarriers interact uniquely with biological systems, and the pharmacokinetics, biodistribution, and toxicity profiles must be thoroughly evaluated to meet safety and efficacy standards. These factors complicate the pathway to clinical approval, as extensive preclinical and clinical studies are needed to address potential immune responses and long-term biocompatibility [69]. However, recent advances in regulatory science, combined with collaboration between industry and research

institutions, are helping to streamline this process. Several companies are investing in good manufacturing practices (GMP) for nanocarrier production and working closely with regulatory agencies to develop standardized safety protocols. With continued progress in these areas, pH-responsive nanocarriers are likely to become a viable option in the pharmaceutical market, paving the way for more targeted and effective therapies [72].

7.3 Regulatory considerations

Bringing pH-responsive nanocarriers to market involves navigating complex regulatory challenges, as these novel delivery systems operate differently from traditional pharmaceuticals. One of the main regulatory considerations is ensuring safety and biocompatibility; nanocarriers must undergo extensive testing to confirm that they do not elicit adverse immune responses or toxicity in vivo. Unlike conventional drugs, nanocarriers interact uniquely with biological systems, meaning they require detailed studies on biodistribution, pharmacokinetics, and potential accumulation in organs [73]. The standardization of manufacturing processes also presents a challenge, as regulatory bodies like the FDA and EMA require consistent quality across large-scale production batches. Achieving batch-to-batch uniformity in properties such as particle size, surface chemistry, and pH sensitivity is essential for ensuring predictable therapeutic outcomes [55].

Additionally, long-term stability and degradability are crucial factors in regulatory approval. pH-responsive nanocarriers need to demonstrate stability during storage and a safe degradation pathway post-administration to avoid accumulation in the body. The lack of standardized guidelines specifically for nanomedicines adds another layer of complexity. Regulatory bodies are still developing frameworks to address the unique aspects of nanocarriers, meaning that companies often face delays and additional scrutiny [74]. Furthermore, demonstrating efficacy through clinical trials is challenging, as nanocarriers require specialized study designs to evaluate targeted release and reduced side effects compared to traditional therapies. To address these regulatory hurdles, industry stakeholders and regulatory agencies are working toward establishing clearer guidelines and collaborative efforts to streamline the approval process for nanomedicines, paving the way for pH-responsive nanocarriers to become viable commercial products [75].

8. Conclusion

The development of pH-responsive nanocarriers offers a transformative approach in targeted drug delivery, providing a means to optimize therapeutic outcomes while reducing systemic toxicity. By exploiting the pH variations inherent in tumor microenvironments, inflamed tissues, and intracellular compartments, these nanocarriers facilitate precise drug release, aligning with the goals of precision medicine. Advances in material science have diversified the types of pH-responsive systems—ranging from polymeric and lipid-based carriers to MSNs, MOFs, and hybrids—each designed to address specific therapeutic challenges. However, translating these technologies from research to clinical application involves overcoming significant hurdles, including achieving consistent manufacturing quality, ensuring safety and biodegradability, and navigating complex regulatory landscapes. Emerging innovations in multi-stimuli responsiveness, manufacturing scalability, and regulatory collaboration are critical to the future success of pH-responsive nanocarriers in clinical settings. As

research progresses, these nanocarriers hold the potential to revolutionize targeted therapies, offering safer, more effective treatment options tailored to individual patient needs and setting new standards in biomedical technology.

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CHAPTER

Biomedical applications of pH and redox dual-responsive nanocarriers

18

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1. Introduction

Numerous novel materials have been developed because of nanotechnology, representing many nanobiomedicine prospects. A technique for medication delivery based on nanomaterials has proven to be a practical approach to control the disadvantages of traditional chemotherapy, including limited drug bioavailability, targeting the nonspecifically unpreferred distribution of biomaterials [1].

Responsiveness of stimuli has been employed to activate the release of prodrugs, including enzyme responsiveness, temperature, pH value, and oxidative/reductive agents. One of the most widely used stimuli is pH responsiveness, using the variation between intracellular and external environments and between pH values in various cells or tissues [2].

The integration of chemotherapy with nanotechnology represents an auspicious therapeutic approach tailored to address the intricate physiological characteristics of the tumor microenvironment (TME) [3,4].

The TME is pivotal in cancer initiation, progression, and metastasis [5].

Thus, extracellular or intracellular markers of TME, such as pH and Redox (glutathione concentration), lead to the generation of intelligent NDDSs that respond to stimuli and release chemotherapeutic medication in reaction to particular biological markers, such as low pH or high glutathione concentration [6].

The differences in pH between the TME and the average environment of the blood induce charge reversal, a key mechanism and one of the most common indicators for TME-specific targeting [7].

An encouraging platform for creating an intelligent platform is charge reversal NDDs (CR_NDDSs) [8].

These competent NPS can transform the surface negative charge to positive, potentially revolutionizing the field of cancer therapy [9].

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Additionally, the variation in the intracellular glutathione levels in tumor tissue is an additional signal for size shrinkage and TME targeting brilliant [10].

NDDSs are usually designed to enhance tumor-targeted delivery. The high glutathione content in the cytosol advances the reduction of disulfide bonds within the polymeric structure of nanoparticles (NPs), facilitating the release of thiols through a redox-driven process [11].

This capability can encourage the NPs to degrade, enabling them to penetrate deeply and reduce their size [12].

Therefore, with the development of charge reversal and size shrinkage strategies, pH and GSH have gained more attention as specific markers to achieve targeted delivery and pH/redox dual-triggered release of therapeutic agents in the tumor cytosol. Incorporating protonation and deprotonation of surface groups and cleavage of disulfide bonds in the structure of our TME-responsive polymeric nanocarriers may provide definitive answers to facilitate the clinical translation of pertinent cancer chemotherapy (Fig. 18.1) [13].

2. Mechanisms of PH and redox responsiveness

2.1 Physiological contexts

PH is a vital physiological signal essential for preserving tissue and cell homeostasis. Mesenchyme in tissues has a pH precisely balanced (7.2–7.4). In eukaryotic cells, the pH of internal sections (such as endocytic vesicles) is also precisely regulated and directly impacts lysosomal degradation, transport across the membrane, cycling of receptors and viral entrance inside cells. At the molecular level, the gradient in pH on the mitochondrial membrane is essential for synthesizing adenosine triphosphate. Besides its typical physiological impacts, pH has a role in many pathogenic mechanisms, including

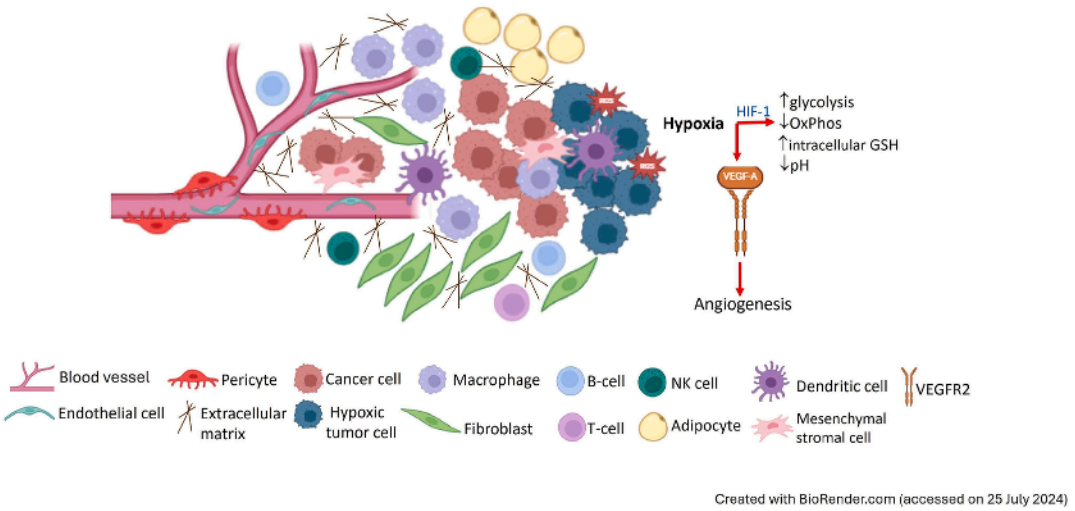


FIGURE 18.1

The attributes of the tumor microenvironment are distinct [14].

infection, inflammation, and cancer. As a result, pH-sensitive nano-transport devices that use pH as a signal for stimulation are crucial for disease imaging and treating analogous conditions [14].

As nanotechnology and nanomaterials have advanced, systems of redox-sensitive delivery have been intensively researched in various essential domains, particularly biomedicine. When in circulation, the redox-sensitive transmission method can be substantially more constant. Furthermore, redox-sensitive carriers can be affected by high glutathione levels inside the cell, and when loaded payloads reach tumor tissue or targeted cells, they are immediately released. Furthermore, redox-sensitive transmission methods are frequently used to raise the focus of drugs in specific cells, improve the efficacy of the therapy, and lessen the adverse consequences of primary medications [15].

2.2 Overview of PH gradients in various body compartments (e.g., tumors, intracellular environments)

Extracellular acidification in tumors has been demonstrated using various measuring methods, including inserting electrodes. Despite being a direct measuring method, probe entry may cause damage to the tissue. Compared to usual tissue, PH inside the cell = 7.2 and outside the cell ≈7.4; several testing methods regularly revealed a reversed pH scale in tumors. The pH of tumors inside their cells is somewhat more fundamental than that of regular tissues, whereas the pH outside the cell is very acidic. The inverted gradient is maintained by transporters such as Na^+/H^+exchangers, H^+/K^+-ATPases, and Na^+/HCO_3^- carriers, along with anhydrases of carbonic (e.g., CAIX and CAXII) and monocarboxylic carriers, which transport (H^+) and compounds of monocarboxylic acids like lactate. It has been proposed that maintaining this inverted gradient benefits cancer cells in two ways: (i) acidification outside the cell prevents the immune response from developing, and (ii) intracellular alkalization promotes increased glycolysis, which in turn promotes cancer cell proliferation, particularly in hypoxia [16].

2.3 Overview of redox gradients in biological systems (e.g., oxidative stress conditions)

To sustain their physiological bodily processes, living things employ a wide range of anabolic and catabolic reactions, including Substrate oxidation and reduction. The goal of the discipline of redox biology is to comprehend how oxidative or reductive stress affects redox homeostasis and which processes are disrupted in various pathological manifestations of diseases [17].

The idea is predicated on the characteristics of the redox (electron exchange) processes that developed alongside the intricacy of biology, enabling synchronization in a system with multiple layers that include the inside of the cells and Interorgan exchange procedures that are necessary for the integration of metabolism of the entire body [18].

The word “oxidative stress” about biological systems was used in the early 1970s. Prof. Sies introduced the notion of oxidative stress in 1985. Oxidative stress was defined as instability between oxidants and antioxidants favoring oxidants, resulting in an imbalance of redox signaling and control and/or molecular damage."

Oxidative stress can be defined as oxidative eustress. Oxidative eustress is defined as “positive stress” and is described by minimum to moderate quantities of oxidants included in regulating numerous biochemical changes, such as hydroxylation and carboxylation or signal transduction

pathway modification. Oxidative distress ("negative stress") describes a state that damages biological molecules like proteins, etc. Nevertheless, oxidative stress is a two-edged sword that may entail a preferred method for cell death, particularly in cancer cells induced by typical reasons or antitumor medications. Free radicals, often known as ROS (oxidants), are species found in every organelle and tissue where they can function. When the notion of oxidative stress was introduced, the detrimental action of ROS was linked to various disease conditions in an organism. It has been hypothesized that by increasing the availability of antioxidants, oxidative stress-associated disorders are "treated". But they provide only minimal health benefits [19].

2.4 Mechanism underlying PH responsiveness in nanocarriers

High temperatures and elevated enzyme expression are characteristics of the unique microenvironment found in tumors; an acidic pH is a redox potential that favors reduction (~6.5). The minimum pH of solid tumors out of the cells is attributable to the tumor of the tissues' predilection for anaerobic respiration. As a result, pH-responsive nanoparticles have been intensively studied for medication delivery to malignancies. These nanoparticles(NPS) launch their cargo in reaction to acidic environments. Two distinct strategies can be used to integrate protonatable groupings or establish acid-convertible bonds.

pH-induced ionization and protonation are commonly utilized to create pH-sensitive NPs. A variety of ionizable groupings are included in the structure of NP. Minimum pH exposure induces reversal of charge or protonation of the functional inserted groupings, disrupting the hydrophilic-hydrophobic balance within the NP, resulting in the disintegration of the encapsulated payload's eventual launch and the structure of the nanocarrier. Amino, carboxyl, sulfonate, and imidazolyl are among the most prevalent ionizable groups. Drug launch from such NPs may occur based on the additional functional group's acid dissociation constant (pKa) through dissociation, aggregation, or precipitation.

pH-sensitive polymers are categorized according to their protonation/ionization as either cationic or anionic. Process and charge at the physiological pH. Cationic polymers transition from non-protonated (hydrophobic/unionized) to deprotonated (hydrophilic/ionized) as pH declines. Anionic polymers transform from hydrophilic to hydrophobic when the pH exceeds their pKa. The polymeric nanocarrier system made of these polymers reforms due to this change in hydrophilicity, ultimately leading to the drug's release [20].

2.5 Mechanism underlying redox responsiveness in nanocarriers

The tripeptide molecule glutathione sulfhydryl (GSH) comprises glutamic acid linked to cysteinylglycine's N-terminus via its side chain. Given that cancer cells have four times the GSH levels of healthy cells, it is employed to generate redox-sensitive nanocarriers (NCs). Specifically, GSH breaks the NCs' disulfide bond in cancer cells, allowing for regulated drug release induced by redox.

Various redox-responsive drug delivery methods have been discovered recently, including liposomes, micelles, nanogels, and prodrugs. When GSH (reducing agents) is present, redox-sensitive liposomes cause the release of medicine via decreasing bisulfite linkers or destabilizing the liposome membrane. For example, a novel Chol–SS–mPEG/HA liposome was developed by Chi et al.; it was joined to PEG by disulfide bonds and demonstrated cytoplasmic drug release triggered by GSH through liposome aggregation and PEG deprotection. In another study, Yin et al. synthesized

conjugated chitooligosaccharides (COS) with cholesterol via a bisulfite linker, with linker reduction leading to COS elimination, liposome instability, and rapid drug release. Micelle-based drug delivery methods are widely employed due to their administrative simplicity, longer circulation duration after PEGylation, high structural balance and conditions. Micelles made with disulfide connections are redox-sensitive, indicating they can cleave when GSH or a reductive environment is present. Redox-sensitive amphiphilic glycan conjugates were produced by Sun et al. by forming disulfide connections between heparosan and deoxycholic acid. The micelles swiftly broke apart and released the drug as soon as they entered the cytoplasm of the cancer cell.

Similarly, Kang et al. used amide bonds to couple gambogic acid with poly(amido amines) to create a redox-responsive system, which formed micelles on its own. Micelle-based drug delivery methods are widely employed due to their administrative simplicity, increased circulation time when PEGylated, high structural stability, excellent hydrophobic drug encapsulation, and capacity to adapt to environmental conditions. Micelles made with bisulfide connections are redox-responsive, meaning they can be cleaved in the presence of GSH (or a reductive environment). Sun et al. created redox-sensitive amphiphilic glycan conjugates by forming bisulfide connections between heparosan and deoxycholic acid. The micelles swiftly broke apart and released the drug as soon as they entered the cytoplasm of the cancer cell. Similarly, Kang et al. used amide bonds to couple gambogic acid with poly (amido amine)s to create a redox-responsive system, which formed micelles on its own. The encapsulated medications disassembled and released quickly due to the existence of bisulfide bonds in a reductive environment.

Although disulfide links are the most crucial component for constructing redox-sensitive NCs, biselenide bonds can create mesoporous silica-based NPs. For example, albumin or myoglobin can control drug release by using biselenide to conjugate them to mesoporous silica-based NPs linkages, resulting in an NC highly responsive to GSH and H2O2. Furthermore, Lei et al. employed iron, zirconium, or aluminum as metal nodes and 4, 4-dithiobisbenzoic acid (4, 4-DTBA) as the neutral ligand to build a unique intrinsic redox-sensitive metal-organic framework transporter [21].

2.6 Types of nanocarriers

There is a growing demand for nanocarriers with various features that target numerous diseases. Nanocarriers outperform conventional medication dosage forms regarding efficacy, side effects, and stability because of their enormous surface area, small size, and potential for targeting. Nanocarriers for drug delivery comprise liposomes, nanotubes, nanocomplexes, niosomes, polymeric, lipidic, inorganic, and gold nanoparticles [22].

2.6.1 Polymer-based nanocarriers

Nanocarriers are made from various polymers; active therapeutic compounds are either physically entangled with the polymeric matrix or covalently bonded to the polymers. The polymeric structures could resemble a capsule, an amphiphile, or a complex branched macromolecule. Nanocarriers are developed using both natural and synthetic polymers [23].

2.6.1.1 Polymeric micelles that respond to pH for theranostics and drug delivery

Each person has a different pH gradient, with the stomach acidic and the intestines alkaline. This pH difference exists in various tissues and areas throughout the body, such as endosomal pH of five to six

and lysosomal pH of 4–5. Tumor tissues have a slightly acidic pH of around 6.5, while irritated tissue has a somewhat lower pH than usual (7.4). This gradient could be utilized to distribute medications in a focused and particular manner. One of the most deliberated drug delivery techniques for customized delivery of drugs is micelles of polymers sensitive to pH. Nanocarriers are pH-responsive polymeric micelles made up of block copolymers having ionizable clusters of pendants connected to the hydrophobic polymer chains in the micelle. These attached groups may be weak acids, such as sulfonic or carboxylic, or basic groups, such as pyridines or amines. When the pH of the surrounding environment changes, the ionizable pendant groups can either be protonated or deprotonated. This causes structural and conformational changes in the polymer chains and hydrodynamic volume changes, allowing the medicine to be released at a precise location.

Common pH-sensitive polymers consist of chitosan, poly[methacrylic acid], poly[glutamic acid], poly[vinyl pyridine], poly[L-histidine], poly[vinyl imidazole], poly[β-amino ester], and poly[glutamic acid]. Polymers can be classified into two categories depending on the polymer chain's pendant group: anionic polymers (which contain sulfonic or carboxyl) and cationic polymers (which include an amine group). Anionic polymers, including polyacids like poly[methacrylic acid], poly[acrylic acid], and poly[glutamic acid], are widely employed in pH-responsive drug delivery. When the pH reaches 7.4 physiologically, the –COOH group ionizes (de protons) and releases its protons. The –COOH group is negatively charged, resulting in a hydrophilic backbone. In acidic pH, the COO− group accepts (H+) protons, causing the backbone to become hydrophobic. This change in backbone structure causes destabilization (bulge/collapse) or a solubility shift, resulting in polymer disintegration and the release of the medicinal component. Poly-acrylates have a pKa of 4.28, making them neutral at pH $<$ 4.28 and negatively charged at pH $>$ 4.28. Sulfonate groups (SO_3H) in poly[sulfonic acids] have pKa values between two and three, allowing them to be ionized across a wide pH range have pKa values between two and three, allowing them to be ionized across a wide pH range. Monomers such as 2-methoxy sulfonic acid make up poly[sulfonic acids], ethylene sulfonic acid, sulfoxy-ethyl methacrylates, and styrene-sulfonic acid. Sant and associates used poly(ethylene glycol)-block poly(alkyl acrylate-co-methacrylic acid) to manufacture self-assembling, pH-sensitive polymeric micelles. The copolymer produced micelles at a pH less than 4.7 and disintegrated at a pH greater than 4.7. The weakly water-soluble medication fenofibrate's oral bioavailability was increased by this method. The micelles stayed clumped together in the stomach's acidic environment, preventing the medicine's launch. At the alkaline pH of the intestine, the entrapped drug's molecularly dispersed form was released when the micelles dissolved, boosting oral bioavailability. Because of their changeable positive charge, pH-sensitive cationic polymers exhibit increased cellular absorption, making them suitable for cancer cell therapy. Polyamines include, poly(4-vinyl pyridine), poly(N,N′-dimethyl aminoethyl methacrylate), poly(β-amino ester), and poly(L-histidine), etc. Most polycations are ionized at low pH levels and contain primary, secondary, and tertiary amine groups. PH alkalinity and protonation at low pH acidity. The medicinal moiety is released when the structure becomes unstable due to this charge shift. Monomers, specifically N′ dimethyl aminomethyl acrylamide, acrylamide, N, and N, N′-diethyl aminoethyl methacrylate, can make pH-sensitive polyamines. The tertiary amine functional groups of poly(N, N′-dimethyl aminoethyl methacrylate) have a pKa of 8 and absorb (H^+) protons from the surrounding medium to positively ionize at pH $<$ pKa. These (H^+) protons are emitted at pH $>$ pKa. Poly (N, N′-diethyl aminoethyl methacrylate) (pKa ~7.3) deprotonates in pH alkalinity. Because of increased hydrophobic contact and decreased ionic amino functional groups, the polymer precipitates

suddenly above pH 7.4. Poly(2-aminoethyl methacrylate), which has a pKa of approximately 7.6, is stable in both acidic and neutral environments, but it degrades slowly in alkaline pH environments because the primary amine group is deprotonated. Ethyleneimine is a linear chain polymer with primary amine group pKa values of 4.5 and secondary amine group pKa values of 6.7. This polymer and its proton buffering capacity are excellent for gene delivery applications. Pyridine, which can be protonated, is a component of the side chains of polypyridines, including poly(4-vinyl pyridine) (pKa 5.39) and poly(2-vinyl pyridine) (pKa 5.9). In acidic conditions, polyimidazoles can become protonated as poly(N-vinyl imidazole) (pKa ~6). Units of D-glucosamine and N-acetyl-D-glucosamine combine to form chitosan, a positively charged polymer. The degree of deacetylation in its structure determines how many amine groups it has, impacting its sensitivity to pH. Poly-histidine is a polycationic peptide with a pKa of 6 due to the imidazole ring on its side chain, which is pH-sensitive. Lipeng, A pH-sensitive micellar system that self-assembles and contains g-poly (L-histidine) and hyaluronic acid, was created by Qui and associates to deliver the anticancer medication doxorubicin to breast cancer cells with MCF-7. The poly (L-histidine) protonation caused the copolymer to disintegrate quickly at lysosomal pH, resulting in efficient doxorubicin administration [24].

2.6.1.2 Redox responsive polymeric micelles for drug delivery and theranostics

Because of the diverse redox states of the body's intracellular and extracellular environments, Redox is among several situations that may be employed as internal triggers for specific delivery. The tripeptide glutathione is made up of γ-L-glutamyl-L-cysteinyl-glycin produced by cells. It possesses the action of antioxidants and is involved in conjugation and reduction events, which provide a mechanism for removing peroxides and xenobiotic substances. Intracellular regions (~2–10 mM) have 100–1000 times greater GSH levels than external areas (~2–10 μM). Additionally, tumorous cancer tissues contain four times as much GSH as ordinary tissues. The redox capacity differential is used for tailored distribution via redox responsiveness. To create such systems, a redox-sensitive unit is inserted into polymers that are amphiphilic blocks, which self-assemble into a micellar structure in a watery substance. Disulfide, ditellurium, and diselenide bonds are redox-sensitive units. The produced polymeric micelles break down at tumor sites but remain stable in systemic circulation [25].

2.6.2 Solid lipid-based nanocarriers

Solid lipid nanocarriers have been utilized as an appropriate vehicle for lipophilic medications since the early 1990s. Solid lipid nanocarriers are formed by dispersing solid lipids that have melted in water and stabilizing them with emulsifiers via High-pressure homogenization or micro-emulsification. Solid lipid nanocarriers are widely made with room-temperature solid lipids, such as mono, di, or triglycerides, steroids, waxes, or free fatty alcohol or acids. Depending on the manufacturing conditions and composition, the drug molecules may be integrated into the solid lipid's core, matrix, or shell. Because of its versatility, this solid lipid nanocarrier may address traditional chemotherapy's drawbacks. The Reticule Endothelial System can easily remove the typical solid lipid nanocarrier, but it also presents a challenge for prolonged drug release when integrating ionic and hydrophilic drug molecules. Recently, solid Lipid nanocarriers have been employed to include ionic and hydrophilic anticancer medicines, as well as lipophilic pharmaceuticals. For example, polymer-lipid hybrid nanocarriers have been investigated as a viable source of oral medication administration. Certain next-generation nanocarriers, such as nanostructured lipid carriers (the mixing of liquid lipid and solid

lipid) and Lipid-drug combinations (transporter molecules that are insoluble in water), have been identified to address the disadvantages of traditional solid lipid nanocarriers. This nanocarrier can be employed for drug delivery by topical, parenteral, and oral routes. Solid lipid nanocarriers can function as a 'perfect tailor-made carrier', delivering any therapeutic molecule to a specific spot. Numerous investigations into solid lipid nanocarriers have been carried out to treat ocular disorders, deliver genes and nucleic acids, release active agents under control, and deliver anticancer drugs in specific locations [25].

2.6.3 Liposomes and micelles with dual responsiveness

Generally, most tumor microenvironments have extracellular pH values between 6.5 and 6.8 and endosome/lysosome pH values between 4.5 and 6.5. These values are both lower than those of normal tissues and blood. Researchers have used this characteristic of tumors to create a range of acidic pH-sensitive nanocarriers; they include micelles, liposomes, pH-sensitive hydrogels, and additional delicate materials. PH-sensitive liposomes have received the most attention among pH-responsive nanocarriers due to their high biocompatibility and adaptability. PH-sensitive liposomes have been widely employed to replace traditional liposomes, improving the transport effectiveness of chemotherapy medicines, siRNA, and poisons.

These pH-sensitive liposomes were primarily designed for the regulated release of medicines under acidic circumstances. The most frequent acid-sensitive technique uses cholesterol hemisuccinate (CHEMS), a mildly amphiphilic acid, and 1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine (DOPE) as liposome constituents. The findings revealed that under acidic conditions in the lysosome, pH-sensitive liposomes changed into the phase of hexagonal crystals, accelerating drug release. Nevertheless, numerous modifications are still required to increase the delivery effectiveness of pH-sensitive liposomes, including tumor targeting, balance, and drug encapsulation efficiency. Nanoparticles that depend on EPR passive targeting impact are more likely to accumulate in the liver and generate increased poisoning, perhaps leading to the failure of a clinical application. Stealthy and actively targeted nanoparticles can decrease the liver's nonspecific absorption while increasing drug delivery efficiency to tumors. Simply adding PEG components creates stealth nanoparticles. Targeting capabilities can also be obtained by integrating target molecules into nanomaterials. Chemicals such as aptamers, antibodies, peptides, and ligands have been converted to pH-responsive liposomes to target tumors better. However, these techniques have limitations because human tumors are heterogeneous [26].

2.6.4 Inorganic nanoparticles for cancer therapy

Nanocarriers are classified as inorganic or organic based on the excipients used in their manufacture. Examples of inorganic nanocarriers include Gold nanoparticles, iron oxide nanoparticles, quantum dots, carbon material carriers, and mesoporous silicon nanocarriers. This type of nanocarrier is usually nontoxic and has high chemical stability, which is helpful for particle processing in severe environments. Inorganic particles' size, porosity, and surface chemistry can be easily controlled. Surface coating with a polymer or lipid shell can improve these particles' biocompatibility and water stability. Furthermore, many inorganic particles have distinct imaging properties that can be exploited to develop theragnostic nanoplatforms [27].

2.6.5 Metal nanoparticles and silica-based carriers exhibit dual responsiveness

2.6.5.1 Gold nanocarriers and their smartness

Because of their unique qualities, such as their easily fabricated nature, high surface-to-volume ratio, thermal ablation of cancer cells, noble optical qualities, and ease of surface functionalization, metallic nanocarriers are desirable. Studies have shown that the size and structure of colloidal nano transporters influence their intercellular absorption. Gold nanoparticles (GNPs) are metallic nanocarriers that can be custom-shaped and sized. GNPs have excellent possibilities as metallic payload delivery candidates. Payloads might be medication molecules or significant macromolecules such as RNA, DNA, and proteins. The surface plasmon resonance (SPR) phenomenon, which enables GNPs to transform light into heat and disperse the heat to destroy cancer cells, is another fascinating feature. GNPs are primarily generated using a variety of ways, such as (1) physical, (2) chemical, and (3) biological methods. Grafting GNPs' surfaces with appropriate ligands could significantly cross the blood-brain barrier (BBB).

Smart nanocarriers should be highly targeted, biocompatible, chemically stable in biological environments, and sensitive to outside or inside stimuli. The RES more easily absorbs unmodified GNPs and is unstable in the blood. To circumvent these constraints, PEGylation is required for gold nanocarriers. Under physiological circumstances, GNPs that have been PEGylated show improved solubility and stability. The surface of GNPs can be changed with various ligands to facilitate targeted drug delivery. For example, transferrin (TF) can be grafted onto the surface of GNPs because many cancers overexpress the TF receptor on their surface. Folic acid may modify the GNP surface, as numerous tumor cells exhibit overexpression of folic acid receptors. The drug can be released from GNPs via (1) external stimuli (laser, ultrasound, X-ray, and light) or (2) internal stimuli (pH, redox state, matrix metalloproteinase). Numerous studies have demonstrated that the cancer-causing gene may be silenced by gene transfection employing GNPs. Using GNPs and fluorescently labeled heparin, cancer spots might be located [28].

2.6.5.2 Silicnanocarriers

2.6.5.2.1 pH-responsive mesoporous silica nanoparticles. Among the various endogenous stimuli-triggered mesoporous nanoparticles under investigation, because pH-sensitive moieties grafted to MSN surfaces are relatively reliable, pH stimuli-responsive MSNs continue to be one of the most often used designs. Through the hydrophobic/hydrophilic transition of the immobilized molecules gating the MSN pores' surface, they also promote the ingestion of cargo. The different pH microenvironments inside the cell (pH ~5.6) are used by the pH-triggered linkers, the stomach (pH 1.5–3.5) and the acidic environment of tumors or inflammatory regions (pH ~6.8). They are usually dormant at physiological pH (7.4) and react when the pH environment is lowered. pH-labile chemical bonds that can be grafted or coated onto the nanoparticle surface are used in most pH-responsive technologies. To selectively cap the porous surface of MSNs, Ionizable polymer/lipid bilayers that depend on electrostatic interaction with surrounding media are used, as are hydrolyzable bonds like amide, imine, acetal, ketal, ester, and hydrazine linkers.

An anion-triggered medication delivery system was the first pH-responsive system that entered an "open" state and released squaraine and vitamin B2 when exposed to high pH values. Using gatekeeping molecules with pKa values near the interstitial tumor pH, such as poly-L-histidine, is another strategy for dealing with pH triggers. Several functional groups are protonated by slight pH variations, altering the gatekeeping molecule's overall solubility. Using layer-by-layer technology, a

polyelectrolyte multilayer (PEM) is applied on MSNs coated with gatekeeping molecules to stop release through electrostatic interactions. Dialdehyde starch, sodium poly (styrene sulfonate), alginate, and chitosan (CHIT) are examples of PEMs. Chitosan is particularly favorable because it is readily available, recyclable, biocompatible, biodegradable, and requires no preparation. CHIT dissolves at an acidic pH for acid-responsive release devices, and the –OH and -NH2 groups can be switched to offer dual functionality. According to current research, a polyelectrolyte-coated MSN demonstrated pH-responsive drug release of 5-fluorouracil (5-FU) and doxorubicin in cancer cell models. The protonation of the polymeric brush-coating of TPP, chitosan, and PEG was transformed, allowing the medications to enter the specific cancer cells through the MSN pores. An MSN system capped with chitosan and folic acid was enhanced by Yan et al. for the pH-responsive release of a photosensitizer (pheophorbide A, PA) and a chemotropic drug (DOX) for targeted and synergistic cancer treatment.

Peng and colleagues synthesized an MSN with a pH-sensitive linker covering a Schiff base copolymer layer that contained the chemotherapeutic medication doxorubicin. The imine-bond linker disintegrated at a minimum pH, releasing more medication than at the physiological pH. Ryplida et al. created a pH-sensitive MSN with a core made of the photothermal dye indocyanine green (ICG) and carbonized zwitterionic PEG-grafted poly[(dimethylamino)ethyl methacrylate-co-sulfobetaine methacrylate] (PEG-g-PDS). As cancer cells absorbed this hybrid complex of carbon dots (CD) and MSNs at an acidic pH, the ICG-complexed CDs generated enough photothermal heat to destroy the cancer cells.

Poor loading and release efficiency are common problems with the pH-responsive gating devices that have been developed, mainly owing to the electrostatic impact induced by the interaction between the MSN's surface and the gatekeeping polymer. Because grafting biopolymers to the surface of the MSN is unpredictable, the distribution and toxicity of gatekeeping polymers vary accordingly. This could have an impact on how well the loaded medicine performs. The loaded bioactive medication is also prone to leaking before reaching its target areas. The translation of evolving gated-response systems is hampered by polymer aggregation and the probable drug movement consequences of complex mechanisms of gates. MSN-gated systems with straightforward designs, including end-capped MSNs that introduce cocondensation of peptides or gating mechanisms into the MSN core, are becoming increasingly popular [29].

2.6.5.2.2 Redox-triggered mesoporous silica nanoparticles. A tripeptide with cysteines, glutathione (GSH), cell redox conditions, and additional cell-cycle processes. It reduces bisulfides of proteins, detoxifies exogenous substances and free radical poisons, and maintains various forms of redox equilibrium inside the cell. GSH levels differ dramatically between the outer (2 μM) and inner (10 mM) of cells. Significant variability of redox conditions is observed inside the tumor microenvironment, with GSH levels measured to be at least four times greater than in regular tissue.

Endogenous intracellular signals observed in the tumor microenvironment are accurately responded to by nanodevice systems designed for redox sensitivity. When GSH enters cancer cells, it can break the disulfide bonds necessary for standard redox-sensitive systems, causing the cargo to be selectively released into the specific cancer cell. Previously, redox-sensitive MSN systems were established by conjugating polyethene glycol (PEG) to MSN surfaces via a disulfide bond. Drug release was initiated by the addition of an atmosphere that is reductive initiated drug release. Polymers, β-CD, polypeptides, and hyaluronic acids are gatekeepers in redox-sensitive systems. Chen et al. produced a paradigm in which the antiCAIX antibody specific to tumors was grafted via disulphide linkages that disintegrated when exposed to GSH at tumor locations, introducing DOX, which was put into breast cancer cells in mice.

Liu et al. stated a redox-responsive system employing a copolymer of PEG-b-PMAFc (PPFc) containing ferrocene to graft β-cyclodextrin (β-CD) as a gatekeeping molecule to the MSN surface. When DOX entered the MSN's core, the cross-linked co-polymer joined with β-CD to form a polymeric coating that trapped the drug until it was subjected to oxidative stress. A redox-sensitive hybrid system was described by Cui et al., consisting of an MSN core doped with fluorescein dye and a disulfide-connected PEG outer layer 10–30 nm thick. Higher GSH levels caused disulide bond cleavage, freeing the drug's release from the mesoporous silica nanoparticles (MSN) matrix and the PEG outer shell.

In a related hybrid formulation, Cystine-dopamine (Cy-DA) pseudotripeptide containing rhodamine B and DOX was coated onto disulfide-linked MSN that is responsive to GSH, enabling it to react to exposure to the pepsin enzyme as well as oxidative stress. This was especially pertinent to treating cancer and stomach-related conditions [29].

3. Biomedical applications

3.1 Drug delivery systems

Modern drug delivery methods and materials make modifying a drug's pharmacological properties possible without sacrificing its intended effect on molecular targets. They can specifically alter a medication's stability, pharmacokinetics, absorption, and exposure to both healthy tissues and tumors. They can also make it easier to administer drug combinations that work well together [30].

3.1.1 Targeted drug delivery strategies leveraging pH and redox responsiveness

In 1906, Ehrlich initially proposed the idea of target drug delivery based on the "magic bullet" concept. The core concept of a targeted DDS was founded on three fundamental elements: identifying the specific disease target, determining the drug that will effectively treat the disease, and choosing suitable target carriers to deliver the drug in a stable form while avoiding other interactions and damaging healthy tissues. A precise dosage of a medicinal material is administered over an extended time to a specified location in the patient's body using targeted medication delivery, an innovative drug delivery system [31].

3.1.1.1 pH and redox dual-responsive drug delivery systems

To initiate drug release, pH-responsive nanocarriers feel pH variations and undergo physicochemical changes in polymeric chains. Depending on the pH of various organs, such as the stomach and intestine. These carriers can distinguish between pH values at specific organelles, tissues, and disease locations. The low pH of tumors may trigger controlled DDSs. The pH stimulation can be coupled with other stimuli, including redox and temperature, to obtain accurate release at specific sites [32].

On the other hand, we use redox-sensitive biomaterials to design intracellular targeted drug delivery systems to treat diseases. The oxidation-reduction potential varies in different tissues and cells, making it helpful in creating TDDSs sensitive to the changes in potential. For instance, the redox system in cancer cells uses nanoparticles responsive to glutathione for targeted drug delivery. The glutathione concentration in cancer cells is 10x higher than in healthy cells, making it a tactical strategy for controlled drug delivery. TDD systems also utilize ROSs, which are more prevalent in inflamed tissue [33].

pH and Redox dual-responsive DDSs were developed; the redox-responsive structure improves cancer cell-targeting and anticancer drug release capabilities while reducing drug discharge in the blood circulation. At pH 6.5, the acidic group is nearly entirely protonated, and further lowering the pH has no impact. Redox-responsive drug release stimulation can be added to improve treatment outcomes and speed up medication release even further. These DDSs have been successfully produced by numerous groups with favorable outcomes [34].

3.1.1.2 Redox and pH dual-responsive drug delivery systems with the layer-by-layer structure

The layer-by-layer (LBL) approaches of polyelectrolyte-based nano- and microcapsules have been extensively researched among today's responsive drug delivery and therapeutic systems. These adaptable capsules can include a variety of functional polyelectrolytes and encapsulate a range of medications to provide targeted and regulated release for synergistic or combination therapeutic effects [35]. Anticancer medications use an LBL structure with spirally wound polymer chains, forming microcapsules with the medication wrapped in the middle. This redox-responsive structure prevents blood breakdown. Examples include the Schiff base and disulfide bond, which have shown promising intracellular delivery and therapeutic effects [34].

3.1.1.3 Redox and pH dual-responsive DDSs with a shell-core structure

It is composed of amphiphilic polymers with a core-shell structure that is particular, with a hydrogen or ester link on the shell and a disulfide linkage in the core. This combination of redox and pH stimulation significantly improves tumor targeting while lowering adverse effects [36]. Drug release is influenced substantially by redox sensitivity, and pH sensitivity may be an expedient factor that facilitates external GSH penetration and disulfide bond exposure in cores. On the other hand, redox and pH dual-responsive drug delivery systems have a core-shell structure, with a redox group on the shell and an acid group in the core. In summary, DDSs' layer-by-layer and core-shell construction can improve tumor targeting, decrease side effects more effectively, and release medications more quickly [34].

3.1.2 Controlled drug release mechanisms for sustained therapeutic effects

To provide therapeutic effects and lower dosing frequency, controlled-release DDSs release active substances over a sustained period in a predictable pattern [37]. A continuous drug-release profile that is in line with zero-order kinetics is what sets controlled-release drug delivery systems apart. In this case, the blood level of the medication remains steady during delivery. Thus, continuous-release drug delivery systems provide some therapeutic benefits, including decreased plasma peak levels and potential adverse effects, exceptional durability and operational length forecasting, and the ability to anticipate in vivo release from in vitro data. Doses taken repeatedly can improve patient compliance and reduce discomfort [31].

The mechanism of drug release from the dosage form determines the classification of controlled drug-released systems: diffusion-controlled, chemically controlled, dissolution-controlled, nanoparticle-based drug delivery, and water penetration-controlled (osmotic pressure-controlled and swelling-controlled) [32].

3.1.2.1 Dissolution controlled drug delivery systems

Drugs in dissolution-controlled release systems are either encased in matrices (monolithic systems) or coated with slowly dissolving polymeric membranes (reservoir systems). Low-solubility polymeric

membranes shield drugs in reservoir systems. Most traditional immediate-release tablets, effervescent tablets, and pills are dissolution-controlled devices, meaning that dissolution is the rate-limiting phase [38].

3.1.2.2 Drug delivery systems controlled by diffusion

Drugs are released through diffusion-controlled devices, which can be categorized into monolithic matrix systems and membrane-control reservoir systems. Fick's diffusion equations govern drug release, with drug diffusion being the rate-limiting stage. Membrane-controlled devices house medication in a core protected by a thin polymeric membrane. The rate of drug release is influenced by drug properties, membrane thickness, and porosity [32].

3.1.2.3 Drug delivery systems controlled by osmosis

Osmotic drug delivery is a method that uses osmotic pressure to regulate the passage of medications through osmogens. This process involves a solvent passing through a semipermeable barrier, separating solutions with varying solute concentrations. It can be used for injection and oral delivery, and polymers like cellulose triacetate, cellulose acetate, and ethyl cellulose are often used. Osmotic-controlled delivery systems offer reduced dose frequency, regulated drug distribution, and enhanced therapeutic efficacy [38].

3.1.3 Enhanced drug efficacy through selective activation at target sites

Poor treatment efficacy is primarily due to low targeting ratios of therapeutics, which can cause side effects on healthy tissues. To overcome these issues, site-specific delivery of medicines to tumor locations is crucial. Nanotechnology formulations have been explored as a solution to high dosage administration, unintended side effects, and undirected biological distribution. Nanoparticles can extend blood circulation duration for improved tumor accumulation and address water solubility issues. This approach offers better delivery, fewer off-target adverse effects, and more therapeutic benefits, making it increasingly popular in individualized treatment plans [39].

Among the stimuli-responsive targeting mechanisms, the TME is characterized by an acidic environment, elevated expression of certain enzymes (MMP-2/cathepsin B), and high levels of GSH and ROS. Therefore, tailored medication delivery and tumor treatment may be made possible by nanoparticles that include TME-responsive components. These endogenous stimuli can change molecular function and dispersion behavior, morphology, and degradation kinetics. This promotes the release of pharmacologically active components and either intracellular internalization or escape from endosome/lysosomal destruction [40,41].

A dual-responsive nanoplatform has been developed for lung cancer treatment, delivering chemotherapy drugs using folic acid as a receptor-mediated targeting chemical. The platform enters tumor cells and exhibits drug-release behavior in the mesenchymal stem, promoting internalization and endosome escape in acidic situations. In mouse models, it shows better cellular uptake, high biocompatibility, and synergistic tumor-killing effectiveness compared to mono-chemotherapy [42].

Nanoparticles are a promising solution to traditional chemotherapy challenges, such as poor drug distribution, toxicity, and high dosage requirements. They target specific cancer cell receptors, delivering drugs more effectively and reducing side effects. Stimuli-responsive nanoparticles release medications responding to environmental cues, enhancing targeted delivery and minimizing damage to healthy tissues. However, nanoparticles can interact with biological systems, disrupt cell membranes,

interfere with protein function, trigger immune responses, and alter their properties in the bloodstream. The physiological environment can also influence their behavior, with carbon nanomaterials and metals generating reactive oxygen species under acidic conditions [39].

3.2 Imaging techniques

Numerous nanoparticles (NPs), including metallic NPs, dendrimers, polymeric NPs, quantum dots, and liposomes, have been functionalized and employed as templates for imaging systems. Biological imaging is a noninvasive technique that records the specimen's three-dimensional structure with the least amount of pain and does not interfere with the many life cycles, such as movement, respiration, etc. In multicellular animals, it helps connect findings of subcellular structure with all tissues. Numerous imaging modalities, such as positron emission tomography (PET), single-photon computed tomography (SPECT), optical imaging, magnetic resonance imaging (MRI), nuclear imaging, ultrasound (US), photoacoustic imaging (PA), and more, have been widely used in clinical applications [43].

3.2.1 Utilization of dual-responsive nanocarriers as contrast agents in medical imaging

Characterizing biological processes at the cellular and subcellular levels in medical imaging requires using contrast agents based on molecules and nanoparticles. Because their surface enables functionalization with targeting molecules and may be adjusted by physical or chemical properties, nanoparticles are perfect for creating targeted contrast agents [44].

Intelligent polymeric capsules that react to tumor tissue microenvironments have improved anticancer efficacy by releasing drugs in response to stimuli. pH-responsive polymers like PAEMA are popular due to their hydrophilic and hydrophobic properties. Redox responses based on disulfide bonds are often used to build intelligent nanocarriers. Dual-responsive or multi-responsive nanoparticles with improved antitumor efficacy have been developed. AIE-labeled polymeric micelles based on mPEG-P copolymer are a strong contender for cancer theranostics due to enhanced antitumor activity in vitro and cellular imaging capabilities (Fig. 18.2) [45].

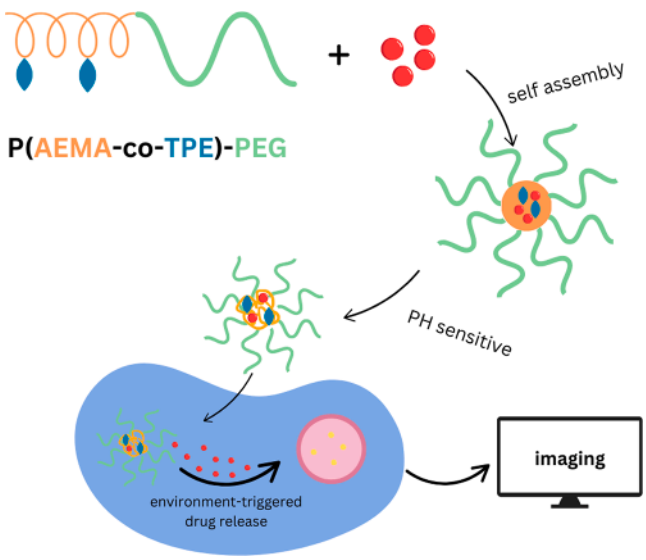


FIGURE 18.2

DOX-loaded polymeric micelles based on mPEG-P (TPE-co-AEMA) for bioimaging, ultrasensitive pH, and redox-triggered drug release [45].

3.3 Theranostics applications combining therapy and diagnostics for personalized medicine

There is a lot of interest in theranostics regarding intelligent drug delivery systems (SDDSs) for cancer treatment. It is still a scientific challenge to create SDDSs with improved drug delivery, effective biodegradability, and early diagnostic capacity [46]. According to Ref. [47], a novel intratumoral pH/redox-dual-responsive nanoplatforms called DOX@MMSN–SS–PEI-cit has been developed to increase therapeutic effectiveness and decrease side effects in cancer treatment. Cancer cells can absorb and release DOX into the intracellular redox environment because the nanoplatforms can collect in tumor tissues and alter their surface charge in weakly acidic situations. With few adverse effects, this dual-responsive trait produces exceptional tumor suppression effectiveness. Comparing DOX@MMSN–SS–PEI-cit's Fe3O4 component to traditional anticancer drug delivery methods, the latter shows promise as a cancer MR imaging probe. The practical application of these multipurpose nanoplatforms with integrated imaging and therapeutic activities will be investigated in future studies.

3.3.1 Targeted responsive theranostic nanoparticles

Theranostic systems have been developed using both passive and active targeting methodologies. Nano drugs can accumulate in solid tumors when passively targeted because of the increased permeability and retention (EPR) effect. Researchers hope to improve localization at the illness site in actively targeted systems by recognizing particular biomarkers or cellular receptors. This targeting can occur by adding ligands like antibodies, peptides, polysaccharides, proteins, aptamers, or tiny compounds like folic acid. The main advantage of targeting techniques, it should be highlighted, is that they improve cellular absorption in tumor settings rather than directly impacting in vivo biodistribution [44].

3.4 Tissue engineering approaches

In the vital life sciences, tissue engineering and regenerative medicine employ biological and engineering principles to restore damaged or sick cells, tissues, and organs. Among the best ingredients for creating tissue engineering materials are biopolymers, including chitosan, collagen, and alginate. Biocompatible and biodegradable materials for tissue/bone engineering and regenerative medicine applications have been successfully developed using stimuli-responsive biopolymer-mediated systems. These materials' mechanical and physical properties can be altered by combining and creating porous assemblies and films with specific architectures. Cell-associated constructions facilitating cellular connection, advancement, propagation, disparity, and displacement are crucial for tissue engineering materials.

Additionally, lipid-based systems have demonstrated promise in developing regenerative medications for treating cancer and other illnesses. Scientists created chitosan-based and stimuli-responsive functional polymerized nanocarriers for tissue engineering and medication administration. Despite being biodegradable and biocompatible, the polymeric nanoparticles (PNPs) disintegrated when both stressors were present [43].

3.4.1 Development of innovative scaffold materials that respond to physiological stimuli

Disease-pathological microenvironments can be used to create intelligent biomaterials for bone regeneration and therapy. These biomaterials can target immunological environments, ionic concentrations, electronegative potentials, mild acidity, and excess reactive oxygen species (ROS). Recent

research has focused on creating multifunctional biomaterials using reactive oxygen species as triggers. Examples include hydrothermal-treated composite scaffolds that selectively inhibit bacterial infection and tumor growth and Fe–CaSiO3 composite scaffolds (30CS) for photothermal and ROS tumor therapy. Acidic environment-responsive techniques have also been developed to address pathological circumstances like chronic inflammation and polluted habitats [48].

3.4.2 Efficient cell delivery systems for regenerative medicine applications

Regenerative medicine uses various techniques to replace or repair damaged tissues or organs, including drug delivery, tissue engineering, and cell therapy. This discipline provides a hopeful remedy for many diseases and injuries that were once thought to be incurable. Regenerative medicine has the potential to completely transform healthcare by overcoming the drawbacks of conventional transplantation [49].

Regenerative medicine involves the delivery of therapeutic agents to damaged tissues, but traditional methods often fail due to rapid diffusion or deactivation. Researchers are exploring drug delivery systems, such as nanofibers, nanogels, and micelles, which can target specific tissues, control release kinetics, and minimize side effects. Cell-based delivery systems are also emerging as a novel approach. Understanding drug properties and biological barriers can lead to innovative strategies for improving therapeutic efficacy and patient outcomes. Nanotechnology offers targeted, controlled-release features like extracellular vesicles, liposomes, and polymeric micelles, offering a fresh medication administration approach [50].

3.4.2.1 pH-responsive materials

Numerous biological processes, including tissue regeneration, depend heavily on pH. For example, the pH values of wounds and tumors differ from those of healthy tissues. Drugs or other therapeutic substances can be released from pH-responsive polymers by taking advantage of this pH differential. Ionizable groups in these polymers react to pH variations by changing their structure and characteristics. This makes it possible to release medications under controlled conditions in particular settings, including acidic tumors. Chitosan, albumin, gelatin, and synthetic polymers are common pH-responsive polymers. These materials' capacity to react to pH changes and release their payload in a regulated manner has made them promising for use in tissue engineering and medication delivery applications [51].

3.4.2.2 Redox-responsive materials

It is possible to design polymers to react to environmental redox changes. This is accomplished by adding labile groups to the polymer structure, including disulfide bonds or acid-sensitive groups. Under reducing circumstances, these groups may cleave, resulting in drug release and polymer breakdown. Furthermore, specific polymers may experience reversible redox reactions that alter their physical characteristics, including swelling and deswelling. Among other uses, this characteristic can be applied to controlled drug release [51].

3.5 Biosensing innovations

Biosensors are advanced instruments capable of identifying and quantifying biological components. They combine biological recognition elements (like antibodies or enzymes) with transducers that

convert biological signals into measurable electrical signals. This technology has revolutionized healthcare by enabling rapid, accurate, and cost-effective diagnosis of diseases. Biosensors can be used for various applications, including disease detection, drug development, and personalized medicine. Biosensors can assist physicians in making well-informed decisions regarding treatment regimens and enhance patient outcomes by providing real-time health monitoring [52].

3.5.1 Advanced detection methods for biomarkers using dual-responsive nanocarriers

Dual-responsive assay techniques can solve this problem since two distinct analytical platforms can concurrently produce two distinct signal outputs to ensure the accuracy and persuasiveness of the detection results. To date, many dual-responsive assay techniques have been developed [53].

Researchers have developed a drug delivery system (DDS) that uses gold nanoclusters modified with an antiEGFR affibody to target cancer cells overexpressing EGFR. The drug, methotrexate (MTX), is encapsulated within the nanoclusters. The system uses targeted delivery and dual-responsive release strategies, releasing MTX based on acidic tumor pH and high intracellular glutathione levels. This method improves therapeutic efficacy and reduces side effects, offering a promising cancer therapy strategy [54].

3.5.2 Point-of-care diagnostic tools for rapid disease detection and monitoring

Nanotechnology revolutionizes traditional disease diagnosis methods by creating rapid, accurate, and affordable diagnostic tools. Nanomaterials, with their unique properties, can create sensitive biosensors for detecting diseases, making them accessible in remote and resource-limited settings. Paper-based sensors are being developed, making them ideal for point-of-care testing in developing nations. Nanomaterials can also enhance the performance of electrochemiluminescence (ECL) sensors, improving sensitivity and specificity. Researchers are working to develop innovative diagnostic tools that can revolutionize healthcare, especially in regions with limited access to advanced medical facilities [55].

Finally, Nanotechnology offers a promising avenue for developing advanced point-of-care diagnostic devices. By incorporating nano-sized sensors into these devices, we can achieve susceptible and accurate detection of various diseases. While significant progress has been made, challenges remain in long-term monitoring, cost-effectiveness, and accessibility. Further research and development are necessary to translate these laboratory-based technologies into practical clinical applications, especially in remote and underserved areas [56].

4. Challenges and future directions

Previous and current studies have confirmed that dual-stimuli responsive nanocarriers are among the most promising methods in biomedical applications, but they are not free of Limitations and challenges.

One of these challenges is the cytotoxicity and stability of nanocarriers; however, some research has already addressed these issues. One way to minimize cytotoxic effects is by encapsulating nanocarriers with chitosan [57,58]. Some nanosystems, such as polyelectrolyte complexes, are unstable under normal physiological pH and the acidic microenvironment. These complexes tend to separate under these conditions due to polymer/polymer ionic interactions [59].

However, one of the solutions for improving stability is to employ nanosystems with distinctive physical properties and sophisticated designs that enhance the efficacy of antitumor drugs. Additionally, DOX-loaded HA-SH/CS, with its properties and functionalization with S–S bonds, will strengthen its deposition in the tumor microenvironment (TME) and increase its stability under biological environments (Fig. 18.3) [57].

The next challenge involves liposomes. Liposomes are a popular type of nanocarrier due to their resemblance to cellular membranes, which makes them highly compatible with biological systems and allows for efficient drug delivery, both for water-soluble and lipid-soluble medications. However, their use is not without difficulties. One major issue is that their similarity to the cell membrane can lead to drug leakage, and the costs associated with their production are still too high for widespread use in commercial and biomedical fields [60].

Immune system interactions add another limitation. One of the limitations is that the immune system may recognize the nanomaterials as nonendogenous to the human body, leading to adverse effects. This causes a buildup in the body due to interactions between the immunoglobulins and the ligands on the surface of the nanomaterials, resulting in the formation of coronas. This, in turn, prevents interaction with the target site, leading to deposition in the vascular system [61].

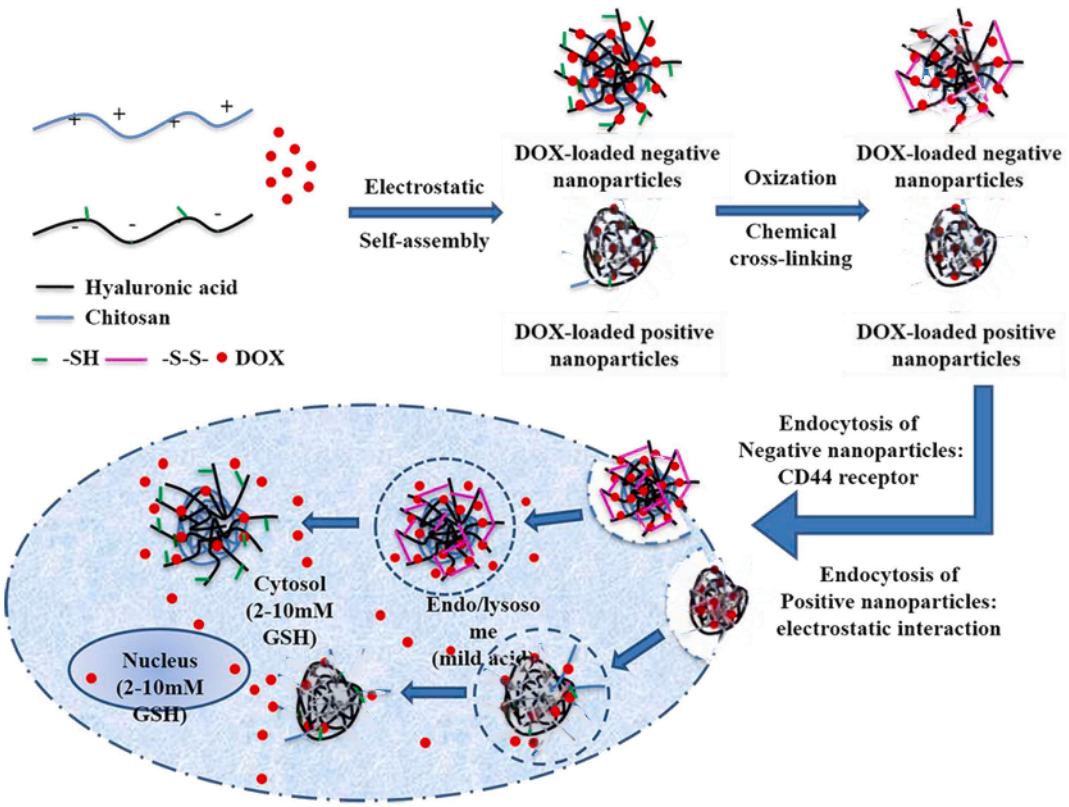


FIGURE 18.3

Diagrammatic representation of HA-SA-CS nanoparticle production, redox, and pH-sensitive drug release [57].

One of the advanced types of nanocarriers is the mesoporous silica nanoparticles (MSN), which has many advantages, including high drug volume and functionalization to improve their efficiency in the target site and their high biocompatibility [62]. However, challenges prevent their current use, primarily biodegradation, due to the presence of Si–O–Si in their framework [63], which complicates the extraction process from the excretory system and results in their accumulation in the body. However, MSNs could be one of the best options for drug delivery if they can resolve this issue, and studies are currently being conducted to find solutions, though there is no resolution yet. Among the proposed solutions is the incorporation of metals such as magnesium to facilitate the degradation of the Si–O–Si framework [64], in addition to adding disulfide bonds to the structure, which undergo degradation in the presence of tumor microenvironment (TME) conditions such as redox potential [65,66], There is also a study on the effectiveness of incorporating calcium and disulfide bonds, which increases their biodegradability [1].

Among the proposed solutions in current research to improve the efficiency of dual-stimuli MSNs are theranostic nanosystems, which combine imaging capabilities with a higher surface area to encapsulate drugs. The choice of inorganic materials is favored due to the advantages previously mentioned [67–69]. CuS@MSN offers real-time monitoring, enhanced targeting effectiveness, and its use in thermal chemotherapy [70]. Current studies have shown that doping with Mn_2^+ within the hollow MSN framework has improved drug delivery efficiency and addressed the issue of biodegradability while also providing real-time monitoring capabilities [71].

Dual-stimuli nanocarriers may suffer from poor stability, leading to aggregation, which reduces effectiveness. However, as a solution to this problem, hydrophilic NPs with narrow size and higher strength can be designed, but this is still a challenge due to the harmful surfactants and stabilizers present in the polymerization process. Although dual-stimuli nanocarriers can improve effective targeting, they may face challenges in avoiding healthy cells. However, in the current study, a novel pH/redox dual-responsive hyaluronic acid (HA) conjugated PMAA (poly(methacrylic acid)/mesoporous organosilica nanoparticles) core-shell structure was designed, characterized by the fact that the core-shell nanoparticles respond to acid pH and redox potential, and the MON shell breaks down in high redox potential. Precipitation polymerization is used to manufacture the PMAA core instead of harmful stabilizers and surfactants, which enhances stability. Incorporation of disulfide bonds increases biodegradability, and conjugation between HA and nanoparticles facilitates more binding to CD44 receptors, increasing targeting because it is overexpressed in cancer cells [72].

Finally, one of the main challenges researchers face is using mouse models for experiments. While the results can be helpful, we cannot be sure they will be the same for humans due to differences between human and mouse tumors, even if they are small, along with differences in how their bodies function. Still, this does not mean they do not help learn about the effects of the systems or drugs being tested [60].

These are some of the challenges faced, along with their ongoing solutions and those that still lack significant resolutions and future directions for resolution.

5. Conclusion

The development of pH and redox dual-responsive nanocarriers has significantly broadened applications such as tissue engineering, cancer treatment, and drug delivery.

Nanocarriers exceed traditional medicine dosage forms in terms of efficacy, side effects, and durability since they are tiny, have a lot of surface area, and can target. Polymeric, lipids, Niosomes, liposomes, nanotubes, nanocomplexes, inorganic nanoparticles, and gold nanoparticles are examples of nanocarriers used for drug delivery. pH-sensitive nanosystems show promise as clever delivery vehicles for anticancer drugs. Among the many forms, redox-responsive nanocarriers are of particular interest. The redox potential difference between average and tumor tissue is viewed as a possible target for tumor treatment, resulting in drug delivery systems responsive to redox (DDS).

Nanotechnology has transformed biomedical applications by providing innovative imaging, tissue engineering, and medicine delivery solutions. By harnessing the unique properties of nanomaterials, scientists are developing targeted drug delivery systems, designing innovative scaffold materials for tissue regeneration, and creating advanced biosensors for early disease detection. These advancements hold the potential to improve patient outcomes and revolutionize healthcare significantly.

Dual-stimuli responsive nanocarriers are promising for biomedical applications but face challenges like cytotoxicity, stability, and immune system interactions. Solutions like encapsulation with chitosan, functionalization with disulfide bonds, and incorporating metals like magnesium and calcium are being explored to address these issues. Advanced designs like theranostic nanosystems and core-shell structures improve targeting and drug delivery. However, challenges like biodegradability and the limitations of animal models remain, requiring further research to ensure safety and effectiveness in humans.

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CHAPTER

Dual-stimuli-responsive nanocarriers for biomedical application

19

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1. Introduction

1.1 Overview of nanocarriers in biomedical applications

Autoimmune diseases are characterized by the immune system attacking healthy parts of the body, which leads to persistent inflammation and lasting harm to essential organs. These illnesses, including conditions like rheumatoid arthritis (RA), multiple sclerosis (MS), lupus, and inflammatory bowel disease (IBD), impact millions of individuals around the globe. Researchers worldwide have made innumerable efforts and proposed numerous ideas for the study of nanoparticles (NPs) and their usage in various nanoscience and nanotechnology-related research. The historical process of synthetic nanoparticles was greatly enriched by the high sophistication of academics from Michael Faraday and Richard Feynman up to the 1980s. Jatzkewitz and Bangham, meanwhile, did their part by developing nanoscale materials such as polymer-drug conjugates and liposomes, which could be finely manipulated at the nanoscale level [1–3]. Eric Drexler's concept of constructing with atoms has changed the industry and opened the doors to its many applications. Nanotechnology focuses on producing, manipulating, and using materials at the nanometer scale, including metal nanoparticles and their oxides. Advancements in modern science have significantly accelerated research in nanomaterials, gaining widespread attention from scientists worldwide. Moreover, there are several types of drug delivery techniques, as depicted in Fig. 19.1, through which these NPs could be delivered.

Nanotechnology offers a way to enhance and modify the key properties of metals in the form of nanoparticles, which have promising applications in diagnostics and medical treatments (nanomedicine) [4,5]. Nanoparticles have special physical, chemical, and biological features, like how they interact with light (surface plasmon resonance), their size variation (polydispersity), how stable they

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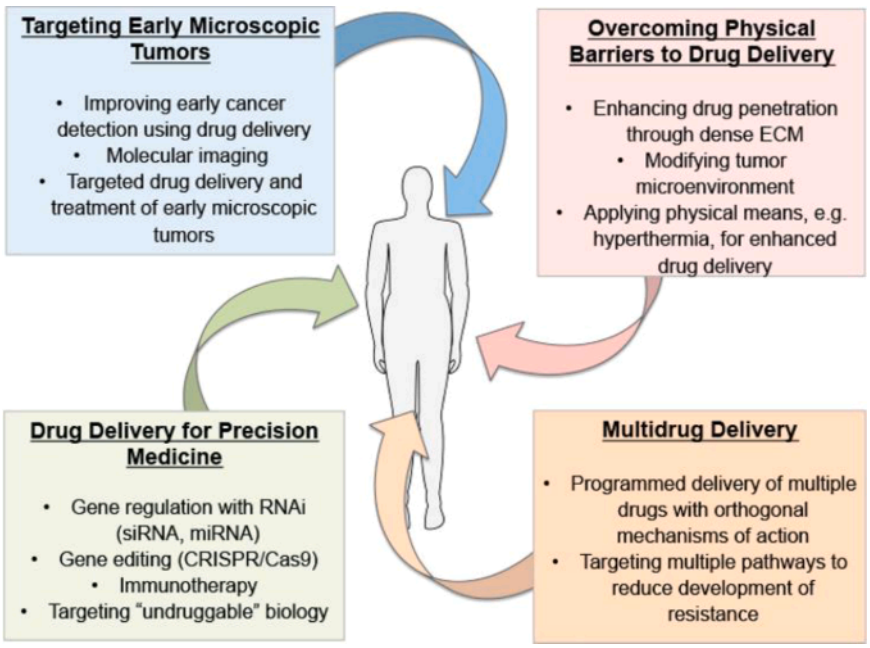


FIGURE 19.1

Types of drug delivery system research for oncological treatment [44], under CC BY license.

are, and how safely they can work with living systems (biocompatibility). These properties make them useful in various medical applications, including cell labeling, biological imaging as contrast agents, antimicrobial treatments, biomarkers for diagnostics, and drug delivery systems [6]. Among the various uses of nanoparticles, their role in drug delivery systems (DDS) to targeted sites using bio-conjugated nanoparticles is one of the most exciting applications in pharmaceutical research. In the past, Tiny vesicles that form naturally at the nanoscale, called exosomes, released by monocytes and macrophages, were utilized to deliver antioxidants for treating Parkinson's disease. However, with advancements in bio-conjugated nanoparticles, such as nano-encapsulated quercetin combined with zinc nanoparticles, it is now possible to improve the absorption of natural plant compounds like flavonoids. This has opened new possibilities for using them as an oral treatment for Alzheimer's disease [7,8]. Although nanotechnology-based drug delivery systems (DDS) offer significant benefits in many areas, some challenges still need to be addressed to fully explore and apply this technology for the benefit of humanity [9]. With advancements in technology, bioengineered molecules can now overcome biological barriers and reach targeted sites in the body, marking significant progress. Drug delivery systems based on nanotechnology can be developed using soft materials (such as organic and polymeric substances) or hard materials (like inorganic ones). Their size and composition can be precisely controlled, allowing them to carry different types of drugs in various forms [10]. Targeted drug delivery systems and controlled release techniques are now being used to treat a variety of medical conditions and ailments, including cancer, neurodegenerative conditions, chronic lung

infections, high blood pressure, tuberculosis, malaria, HIV/AIDS, metabolic conditions, and several others [11,12]. One avenue of research to improve cancer treatment is to increase the concentration of chemotherapeutics in tumors, and drug delivery systems can accomplish this through several approaches, as shown in Fig. 19.1. In the near future, advancements in nanoengineered therapies could lead to nanotechnology-based drugs for almost any disease or health condition. Currently, some nanoparticles providing diverse therapeutic effects are undergoing clinical testing.

In contrast, others have already been approved by the FDA and the regulatory body known as the European Medicines Agency (EMA) to use as potential drugs [13]. This comprehensive literature review explores the progress in developing various nanoparticle systems depending on their source, shape, and core composition. It also discusses its benefits, potential risks, and the future possibilities of drug delivery systems (DDSs).

1.2 Importance of dual-stimuli-responsive nanocarriers

Dual-responsive nanocarriers are getting more attention as they provide more innovative ways to send medicine directly to the right spot in the body. They are engineered in a way that releases therapeutic agents only when triggered by certain conditions in the body, such as variations in pH levels or temperature shifts. This thoughtful response helps the treatment work better and causes fewer unwanted problems, as the drug is released only at the right time and in the specific site where the body requires it most [14]. These tiny carriers are flexible, allowing treatments tailored to each person's body and health needs [15]. This flexibility not only helps to improve treatment results but also allows for advanced therapies that can change and adapt as the disease changes [16]. Progress in the field will change the future of healthcare, bringing treatments that work better and are made to fit each patient's needs [17]. Precision medicine lets patients take a more active role in their treatment because the therapies are designed to match their personal needs. To make this work well, doctors must use modern technologies and innovative data systems when planning and giving care [18]. Artificial intelligence and machine learning in healthcare will help understand patient data, predict health problems, and create treatments for each person. This shift is expected to enhance clinical results and cut costs by reducing trial-and-error practices and ensuring patients receive the right therapy from the beginning [19].

Nanoscale carriers can be engineered to respond to specific biological cues within the body, such as a decrease in interstitial pH, elevated glutathione concentrations, or heightened activity of enzymes like matrix metalloproteinases. Once inside cells, these pH-sensitive systems may either trigger drug release within compartments such as late endosomes or lysosomes or facilitate lysosomal escape, allowing the therapeutic agents to reach the cytoplasm directly [20]. At the tissue level, alterations in the local environment, such as those triggered by cancer, ischemia, inflammation, or infections, can be exploited to achieve targeted drug delivery, with cancer remaining the primary focus in most studies on stimuli-responsive nanocarriers [21]. Drug delivery can also be improved through external physical cues. For instance, magnetic fields can be applied to direct ultrasmall iron oxide nanoparticles, carrying therapeutic molecules, to precise disease sites [22]. Researchers can control exactly when and where a drug is released by designing nanoparticles that react to things like heat, light, or ultrasound. A significant benefit of these systems is that they can be given in many ways, such as by injection, tablets, eye drops, or mucosal routes [23].

2. Fundamentals of stimuli-responsive nanocarriers

2.1 Definition and classification

Stimuli-responsive nanocarriers are tiny drug carriers designed to release medicine only when triggered by changes like pH, temperature, or specific body signals. This way, the treatment goes straight to the right spot, works better, and causes fewer side effects, leading to healthier patient results [16]. Nanocarriers are an important step in personalized medicine because they can be designed to respond to the specific conditions of certain tissues or diseases. This accuracy improves treatments and allows for more targeted ways to treat tough illnesses like cancer and chronic inflammation [24]. Personalized and targeted treatments change healthcare by matching medicines to each patient's needs. This makes it easier to manage diseases and increases the chances of better results. Nanotechnology is expected to drive further innovations, enabling the development of more accurate and adaptable therapies. Turning these advances into everyday medical tools depends on practical cooperation between scientists and doctors, so innovations truly benefit patients and reshape how care is delivered [25,26]. Stimuli-responsive nanocarriers are tiny, smart drug delivery tools that release medicine only when they detect specific triggers in the body, like a change in pH or exposure to heat or light. The idea is to deliver the drug exactly where and when needed, making treatment more accurate and reducing unwanted side effects [27–29]. These nanocarriers are classified based on the type of trigger they respond to.

2.1.1 Internal (biological) stimuli-responsive nanocarriers

2.1.1.1 pH-responsive nanocarriers

pH-responsive materials can help deliver drugs to specific areas in the body by taking advantage of natural pH differences. For example, healthy tissues have a pH of 7.4, the stomach is much more acidic (pH 1.0–3.0), and tumor tissues have a slightly lower pH (6.5–7.0). Nanogels are more stable in the body among different drug carriers, making them a better option. Because they can respond to pH changes, nanogels are ideal for delivering drugs precisely to the correct location [30]. These nanogels release the drug more effectively in acidic conditions while reducing waste in unwanted areas [31]. pH-responsive nanogels can expand or shrink based on their charge, which changes when protons are added or removed. Anionic nanogels with carboxyl groups swell more in high pH environments because they lose protons. On the other hand, cationic nanogels with amine groups gain protons in acidic conditions, causing them to swell more [14,32,33]. The pH-sensitive nature of nanogels can help both store the drug and release it when needed [34]. pH-responsive nanogels can be made using artificial and natural polymers [35].

2.1.1.2 Enzyme-responsive nanocarriers

Enzyme-responsive nanocarriers are tiny drug carriers that release medicine when they come into contact with specific enzymes in the body. Since diseases like cancer, inflammation, and infections often have unusual enzyme activity, these nanocarriers can target sick tissues while avoiding harm to healthy ones. They are made from biodegradable materials that break down when they meet enzymes such as proteases, lipases, or glycosidases. For example, tumors produce certain enzymes in high amounts, which can trigger the nanocarrier to release the drug exactly where it is needed. This makes treatments more effective, reduces side effects, and allows for a controlled drug release over time.

These smart nanocarriers are highly promising for treating cancer, infections, and inflammatory diseases, offering a more precise and efficient way to deliver medicine [36,37].

2.1.1.3 Redox-responsive nanocarriers

Redox-responsive nanocarriers are intelligent delivery systems that release drugs when triggered by alterations in the body's oxidation–reduction balance. Taking advantage of the altered redox potential often seen in diseased microenvironments, these nanocarriers show promise against cancer, inflammation, and oxidative stress-associated illnesses. Compared to healthy tissues, tumor cells often contain significantly higher amounts of antioxidants like glutathione (GSH). Redox-sensitive nanocarriers are designed to exploit this feature, undergoing degradation or modification at these high GSH levels to deliver the drug precisely inside malignant cells. This approach makes therapies more potent and reduces unwanted complications by guiding the drug straight to the site of action [38].

2.1.2 External (physical) stimuli-responsive nanocarriers

2.1.2.1 Temperature-responsive nanocarriers

Temperature-responsive nanocarriers are nanoscale drug delivery systems engineered to release therapeutic agents in response to changes in temperature. Because tumors and inflamed tissues are typically warmer than healthy tissues, temperature-responsive nanocarriers can be directed to these sites more precisely. These systems are often made with materials like poly(N-isopropylacrylamide) (PNIPAM), which change their structure at a specific temperature to release the drug. This method makes drug delivery more precise and controlled, helping treatments work better while causing fewer side effects. Current research is looking at their use in treating cancer, hyperthermia, and other conditions where temperature changes can help control when drugs are released [39,40]. Based on the necessity and the microenvironment surrounding the tumor region, various external stimuli could be employed to target a carcinoma; these varieties of stimuli have been illustrated in Fig. 19.2.

2.1.2.2 Light-responsive nanocarriers

Light-responsive nanocarriers are tiny drug carriers that release medicine when exposed to certain types of light, such as UV, visible, or near-infrared light. These carriers are often made from light-sensitive materials, such as gold nanoparticles, which change their structure when exposed to light. This ensures the drug is released only at the right spot, giving more precise treatment and reducing harm to healthy tissues. Among the different light types, NIR light is beneficial because it can go deeper into body tissues. This makes NIR-responsive nanocarriers very promising for treating cancers inside the body. They are also being studied for photothermal therapy, where light creates heat to kill diseased cells. This method is efficient because it does not require surgery. Doctors can decide exactly when and where the medicine is released inside the body. This improves the treatment and makes patients more comfortable, making light-based drug carriers an important step forward in personalized healthcare [41,42].

2.1.2.3 Magnetic-responsive nanocarriers

Magnetic nanocarriers are smart drug carriers that make use of magnets from outside the body to guide medicines exactly to the right spot. This targeted method helps the treatment work better and lowers unwanted side effects. It also lets doctors decide when and where the medicine is released, so treatment can be tailored to each patient's needs [43]. These nanocarriers can deliver medicines more accurately

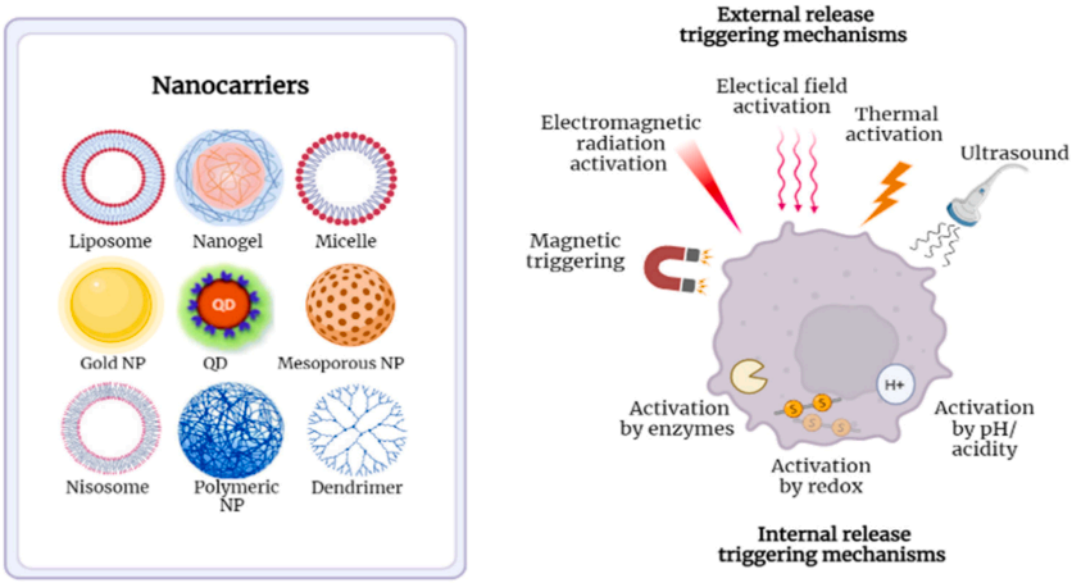


FIGURE 19.2

Dual stimuli-responsive nanocarriers that release drugs in response to combined triggers like temperature, ultrasound, magnetic and light for targeted delivery [40], under CC BY license.

and effectively by adding control, especially for hard-to-treat diseases like cancer and brain disorders [44,45]. Researchers are now studying how these nanocarriers can pass through the body's natural barriers to release medicine exactly where needed. This could improve treatments, cause fewer side effects, and improve patients' health and quality of life [46]. Scientists are working to make nanocarriers more powerful, so they can be used for cancer treatments and targeted gene therapies. Applications involving nanocarriers with responsiveness toward a magnetic field have been provided in Fig. 19.3.

2.1.2.4 Ultrasound-responsive nanocarriers

Ultrasound-responsive nanocarriers are intelligent systems that release medicines when triggered by ultrasound waves. By tuning them to specific sound frequencies, doctors can treat exact spots in the body. This makes the therapy more accurate, causes fewer side effects, and gives doctors better control over when and how the medicine is given [47]. This method delivers medicine directly to tumors, so smaller doses can be used while protecting healthy tissues from damage. It is primarily for tough diseases like cancer and infections since the treatment targets only the affected area instead of the whole body. Ultrasound-responsive nanocarriers can carry and release several drugs simultaneously, making treatments work better and helping to overcome drug resistance often seen in cancers. As this technology keeps improving, it brings us closer to truly personalized medicine, where treatments are designed specifically for each patient's needs. This is a big step toward care that focuses more on the patient, offering better results and quality of life for people with long-term illnesses [48,49].

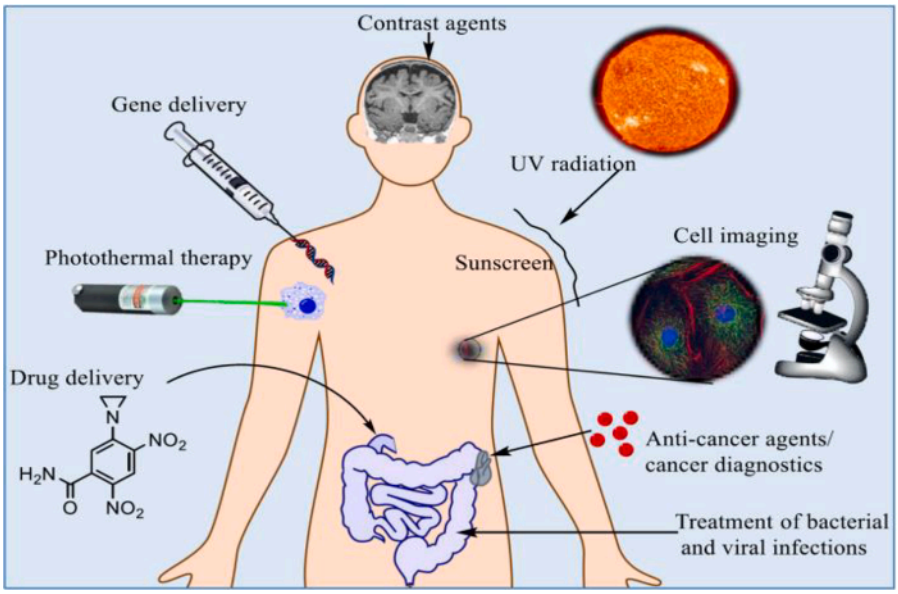


FIGURE 19.3

Example applications for magnetic responsive nanocarriers (MNCs) in Biomedicine [44], under CC BY license.

3. Dual-stimuli-responsive nanocarriers: Mechanisms and design strategies

3.1 Concept and working principles

The emergence of stimuli-responsive nanocarriers represents a key advancement in drug delivery, turning these systems from simple transporters into dynamic contributors to therapy. Often referred to as "smart" systems, these nanocarriers can adjust their structure in response to pathological cues, ensuring controlled and localized drug release. Single-stimulus responsive platforms, for instance, those tuned to changes in pH, temperature, or redox state, have yielded encouraging findings, yet they lack complete specificity. Because such triggers often occur beyond the diseased tissue, they may cause early release and unwanted side effects. Researchers have developed dual stimulus-responsive nanocarriers to overcome these limitations and provide greater precision in drug delivery. The core idea of these advanced systems is that they are designed to respond to two or more distinct triggers, which may come from within the body or external sources. This combined strategy acts like a logical "AND" gate, ensuring that drug release occurs only when multiple, well-defined conditions are present simultaneously or occur in a specific sequence. Incorporating dual triggers ensures greater accuracy in controlling when, where, and how drugs are administered, ultimately boosting treatment effectiveness and limiting adverse impacts on healthy areas. For instance, a nanocarrier might be designed to remain stable in circulation and only release its payload upon encountering both the acidic pH of the tumor

microenvironment (TME) and an externally applied trigger like localized hyperthermia, ensuring release is confined to the intended site [1–3,44].

The working principle of dual-stimuli-responsive nanocarriers is predicated on the rational design of materials that undergo a profound structural or chemical transformation upon encountering the requisite stimuli. These transformations are the primary mechanism for drug release. They can include the protonation of ionizable polymer groups, the cleavage of stimulus-sensitive covalent bonds such as acid-labile hydrazones or redox-labile disulfide bonds, or (supra)molecular conformational changes that alter the nanocarrier's integrity. These triggered events lead to the destabilization or disassembly of the nanocarrier's architecture, such as the collapse of a micelle or the swelling and disruption of a nanoparticle, facilitating the controlled release of the encapsulated therapeutic payload directly at the pathological site [2,44].

The stimuli exploited for this purpose are broadly classified as endogenous or exogenous. Endogenous, or internal, stimuli are derived from the target tissue's unique physiological or pathological conditions. Among the most widely exploited is the aberrant pH of diseased sites. Solid tumors often exhibit an acidic extracellular microenvironment (pH $\approx$ 6.5–7.2) due to increased glycolysis, which contrasts with the physiological pH of blood ($\approx$7.4). Upon cellular internalization, nanocarriers encounter even lower pH values within endosomes (pH $\approx$ 5.0–6.5) and lysosomes (pH $\approx$ 4.5–5.0), providing distinct triggers for intracellular drug release. Another critical internal stimulus is the redox potential, which leverages the significantly higher concentration of reducing agents, particularly glutathione (GSH), in the cytosol ($\approx$2–10 mM) compared to the oxidizing extracellular environment ($\approx$2–10 μM). This sharp gradient is ideal for triggering the cleavage of disulfide bonds within the nanocarrier structure. Additionally, the overexpression of certain enzymes, such as matrix metalloproteinases (MMPs), proteases, and lipases, in tumor and inflammatory tissues serves as a highly specific trigger for nanocarriers functionalized with corresponding enzyme-cleavable substrates [3,44].

In contrast, exogenous, or external, stimuli are physical triggers applied from outside the body to achieve precise spatiotemporal control over drug release. Local hyperthermia, where the target tissue is heated to approximately 40–42°C, is a well-explored strategy to activate thermosensitive materials. Light, particularly in the near-infrared (NIR) region, offering deeper tissue penetration, can induce photochemical reactions or photothermal effects, destabilizing the nanocarrier. Magnetic fields are another versatile external tool; an alternating magnetic field (AMF) can generate localized heat from embedded magnetic nanoparticles, while a static field can be used for magnetic guidance of the carrier to the target. Finally, ultrasound can be focused on a specific site to generate thermal and mechanical effects, such as cavitation, which can disrupt the nanocarrier structure and enhance tissue permeability. By ingeniously combining these endogenous and exogenous triggers, dual-stimuli-responsive nanocarriers represent a sophisticated and highly promising strategy to overcome the barriers of conventional drug delivery and advance the development of precision nanomedicine [1,3].

3.2 Types of dual-stimuli responses

The strategic advantage of dual-stimuli-responsive nanocarriers lies in the rational combination of triggers to enhance therapeutic precision. These combinations can be broadly classified into two principal categories based on the origin of the stimuli: those that respond to two distinct endogenous cues (internal-internal) and those that integrate an endogenous signal with an externally applied

physical trigger (internal-external). Each category represents a unique strategic approach to achieving on-demand drug release, with the choice of combination depending on the specific pathological target and desired therapeutic outcome [4].

Nanocarriers designed with an internal-internal dual response leverage multiple, coexisting pathological signals within the tumor microenvironment or intracellular compartments to achieve a highly specific and autonomous release profile, independent of external intervention. The most extensively investigated combination in this class is the dual sensitivity to pH and redox potential. These systems are engineered to remain stable under physiological conditions but disassemble upon sequential exposure to the acidic environment of endosomes/lysosomes (pH 5.0–6.5) and the high glutathione (GSH) concentration of the cytosol (up to 10 mM). This sequential triggering is highly advantageous; the initial pH-induced change can facilitate endosomal escape, positioning the nanocarrier in the cytosol, where the subsequent redox-triggered cleavage of disulfide bonds ensures payload release directly at its intracellular site of action. This can result in sophisticated release profiles, such as a two-phase kinetic pattern that mimics clinical dosing by providing an initial high loading dose followed by a sustained maintenance dose. Another powerful internal-internal combination is pH and enzyme responsiveness. For example, nano micelles have been designed with pH-sensitive ketal groups and an enzyme-labile dipeptide linker. Such systems demonstrate a synergistic effect, where the combination of an acidic environment and an overexpressed enzyme like trypsin results in a significantly accelerated and more complete drug release compared to the response from a single stimulus alone [5,6].

The internal-external combination represents a second primary strategy, which pairs a biological trigger with a physical, externally applied stimulus to afford an exceptional degree of "on-demand" spatiotemporal control. In this paradigm, the nanocarrier first uses the internal stimulus, typically the acidic TME, to accumulate and prime itself at the target site. The therapeutic release is initiated with high precision only when and where the external energy source is applied. A prominent example is the development of magnetic and pH-responsive nanocarriers. These systems often have a superparamagnetic iron oxide core coated with a pH-sensitive polymer shell. The magnetic core allows for MRI-based tracking or magnetic guidance to the tumor. At the same time, the acidic pH of the endosome triggers the final release of the cargo, effectively combining diagnosis, targeting, and therapy. Similarly, nanocarriers have been designed to respond to pH and ultrasound, where focused ultrasound disrupts the nanocarrier structure only after being localized within the acidic tumor tissue. Other approaches combine pH sensitivity with light, using gold nanostructures that respond to near-infrared (NIR) irradiation for photothermal therapy, or even create triple-responsive systems sensitive to pH, redox potential, and NIR light to achieve multifaceted control over the therapeutic action. This coupling of a biological prerequisite with an external command allows for precise control over the initiation of therapy, minimizing off-target effects and maximizing efficacy at the intended site [7–9].

3.3 Types of nanocarriers

A diverse array of nanocarrier platforms can be engineered to exhibit dual-stimuli-responsive behavior, offering distinct advantages for drug encapsulation and delivery. The selection of a specific nanocarrier architecture is dictated by numerous factors, including the physicochemical properties of the therapeutic payload (e.g., hydrophobicity or hydrophilicity), the desired release kinetics, and the specific combination of biological and physical triggers to be exploited. The most prominent systems

developed for dual-stimuli response are predominantly organic-based structures such as polymeric micelles, liposomes, and nanogels. However, inorganic-organic hybrid systems are gaining significant traction for their unique theragnostic capabilities [10].

Polymeric micelles are versatile nanocarriers formed by the self-assembly of amphiphilic block copolymers in an aqueous medium, resulting in a core-shell structure. The hydrophobic core serves as an effective reservoir for poorly water-soluble anticancer drugs. At the same time, the hydrophilic shell, typically composed of poly(ethylene glycol) (PEG), provides colloidal stability and "stealth" properties to prolong circulation time. Dual-stimuli sensitivity is integrated by synthesizing block copolymers with specifically designed responsive moieties. For a combined pH and redox response, polymers can incorporate acid-labile linkages, such as hydrazones, and redox-cleavable disulfide bonds, which can be strategically placed at the block junction, within the polymer backbone, or as cross-linkers in the core. Similarly, enzyme and pH dual-responsive micelles have been developed from linear-dendritic block copolymers that feature an enzyme-cleavable peptide at the junction point and pH-labile ketal groups on the periphery, enabling sequential disassembly in the tumor microenvironment [11,12].

Liposomes, which are vesicular structures composed of one or more phospholipid bilayers enclosing an aqueous core, are another major class of nanocarriers. Their unique structure, as illustrated in Fig. 19.4, allows for the simultaneous encapsulation of hydrophilic drugs in the aqueous interior and hydrophobic drugs within the lipid bilayer. Dual responsiveness is achieved by incorporating functional lipids and modifying the liposomal surface. For an internal pH and redox response, liposomes can be formulated with ionizable lipids that become charged in acidic endosomes, alongside lipid like materials containing both pH-sensitive amines and redox-cleavable disulfide bonds. To create systems responsive to both internal and external triggers, liposomes can be modified with pH-sensitive fusogenic peptides like GALA to promote endosomal escape, while also featuring a detachable PEG shield linked via an enzyme-sensitive peptide that is cleaved in the tumor microenvironment.

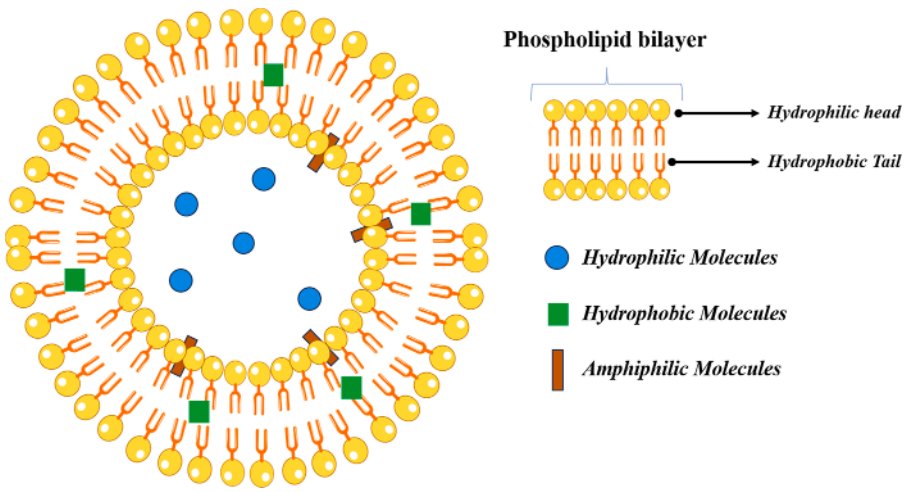


FIGURE 19.4

A generalized illustration of a liposome.

Furthermore, combining thermosensitive lipid compositions with external triggers like high-intensity focused ultrasound (HIFU) or conjugating liposomes to microbubbles has enabled advanced, externally controlled release mechanisms [13,14].

Nanogels and hydrogels are three-dimensional, crosslinked polymer networks capable of absorbing large amounts of water. Their porous structure is ideal for high-capacity drug loading, and the swelling, deswelling, or degradation of the polymer matrix typically governs release. Dual-stimuli functionality can be programmed directly into the polymer network. For instance, hydrogels crosslinked with pH-sensitive acyl hydrazone and imine bonds can exhibit controlled release profiles in response to multiple internal cues. For an internal-external response, magnetic nanoparticles can be embedded within a thermosensitive hydrogel matrix to form a "ferrogel," which allows an external alternating magnetic field to induce localized heating, causing the gel network to shrink and expel its therapeutic payload on demand. Inorganic and composite nanomaterials offer distinct advantages, particularly for therapies involving external physical stimuli, due to their inherent magnetic or optical properties. These systems are typically hybrid structures with an inorganic core coated with a responsive polymer shell. A prime example is a nanocarrier with a superparamagnetic iron oxide (Fe_3O_4) core and a multilayer polymer shell containing a pH-responsive poly(methacrylic acid) (PMAA) layer. The iron core enables magnetic guidance and MRI tracking, while the PMAA layer triggers drug release in the acidic conditions of endosomes. Other inorganic platforms, such as gold nanoparticles (nanorods or nano shells), are excellent photothermal agents and are often integrated into composite systems that respond to both NIR light and a second stimulus, like pH or enzymes. Mesoporous silica nanoparticles (MSNs) are another versatile inorganic platform, whose pores can be capped with "gatekeepers" made of dual-responsive polymers or molecular machines, allowing for highly controlled, triggered release in response to multiple specific signals [15,16].

4. Biomedical applications of dual-stimuli-responsive nanocarriers

In the last 20 years, researchers have focused a lot on nanoscale carriers because of their potential to deliver drugs directly to target cells while reducing unnecessary exposure to healthy tissues [50–52]. Their small size and high surface-area-to-volume ratio grant them unique capabilities, enabling them to efficiently transport various drugs, therapeutics, and imaging agents with enhanced loading capacity [53–55]. A wide range of nanocarriers, including dendrimers, liposomes, nanostructured lipids, micelles, polymeric nanoparticles, nanoemulsions, Janus nanoparticles, organic-based nanocarriers, as well as mesoporous silica nanoparticles, carbon nanotubes, quantum dots, magnetic nanoparticles, and inorganic nanocarriers, have been investigated for targeted therapeutic delivery. The first-generation nanocarriers, designed primarily for drug transport, helped overcome bioavailability, stability, and controlled release challenges. The majority of stimuli-responsive nanomedicines are based on a single type of trigger, which often results in less-than-ideal drug release behavior during systemic circulation and can cause unintended release in healthy tissues, owing to the inherent constraints of individual endogenous or exogenous stimuli sources [56,57]. For instance, nanomedicines responsive to external physical stimuli offer greater control and adaptability in regulating drug release. However, their clinical utility is often restricted to surface-level tumors due to the limited penetration depth of such stimuli [58–60]. However, the next-generation nanocarriers were developed with multifunctionality,

offering enhanced targeting precision and simultaneous tissue imaging, effectively addressing the complexities of various diseases [61].

A significant breakthrough in nanocarrier research involves the development of stimuli-responsive nanocarriers, which can alter specific properties in response to endogenous or external triggers. These advanced systems have found diverse biomedical applications, particularly in the controlled and localized release of therapeutics, thereby reducing adverse effects on healthy tissues [62]. Stimuli-responsive nanocarriers generally consist of a hydrophobic core enclosed by a hydrophilic or amphiphilic outer shell, which is often composed of stimulus-responsive amphiphilic polymers that react to internal or external stimuli. The amphiphilic component may include natural lipids, lipid like materials, or surfactants that respond to pH changes, redox conditions, temperature variations, light exposure, enzyme activity, or magnetic fields. In certain nanocarrier designs, the hydrophobic region of these amphiphilic polymers is functionalized with cationic groups, facilitating the binding of anionic agents, such as nucleic acids, within the hydrophobic core [63].

4.1 Targeted drug delivery

Precise and efficient delivery of drugs to targeted diseased tissues is essential for effectively treating various conditions, including cancer, cardiovascular diseases, and inflammatory disorders [64–66]. Conventionally, most therapeutic agents are formulated without tissue specificity, requiring higher doses to achieve the desired effect. Such nonspecific administration often leads to reduced therapeutic efficacy and increased risk of adverse effects on healthy tissues. Several molecular prodrugs have been recently developed to overcome these limitations and increase selectivity toward diseased cells and tissues, thereby enhancing therapeutic effectiveness. Another promising approach involves the use of drug delivery systems employing nanoscale carriers [7–9]. Nanocarrier-based delivery platforms provide multiple benefits in disease treatment, including reduced degradation of therapeutic agents, lower required dosages, prolonged systemic circulation, improved therapeutic outcomes, and minimized side effects on healthy tissues [1,2]. Among various external triggers, light has emerged as a particularly effective and noninvasive stimulus, offering precise spatial control for localized diagnosis, drug release, and therapeutic intervention. This is achieved by modulating key light parameters such as wavelength, polarization, intensity, and exposure time [28]. In light-induced phototherapy, drug release is typically confined to the targeted area, enabling what is referred to as "dual selectivity." [29] Drug-loaded nanocarriers can penetrate diseased tissues via passive diffusion or active targeting mechanisms. Once accumulated at the target site, light exposure can be selectively applied—both spatially and temporally—ensuring that only the affected region is irradiated while minimizing impact on healthy surrounding tissues.

4.2 Cancer therapy

Cancer, being one of the deadliest diseases with over 10 million fatalities in 2020 [67], necessitates the development of novel drug delivery techniques to overcome the hindrance. As we are well aware, in contrast to normal tissues, the tumor microenvironment is characterized by a lower pH [68,69], elevated levels of reactive oxygen species (ROS) [41–43], and increased glutathione (GSH) concentration [70], largely due to the abnormal metabolic activity associated with rapid tumor cell proliferation. To exploit these unique features, prodrug-based nanomedicines are often designed with

chemotherapeutic agents linked via cleavable chemical bonds that can be selectively triggered by tumor-specific stimuli, thereby enhancing drug release at the target site while minimizing premature leakage during systemic circulation [71]. Notably, dual-responsive nanocarrier systems have demonstrated superior drug delivery efficiency and reduced systemic toxicity compared to those that respond to a single stimulus. Zhang's team developed an innovative polymeric nanomicelle system that responds to both acidic pH and elevated ROS levels, aiming to improve the effectiveness of cancer chemotherapy [72]. In this design, doxorubicin (DOX) was loaded into micelles formed from a polymer containing methionine-modified dimethyl maleic anhydride (DMA) and a PEG-based copolymer linked with α-tocopheryl succinate. Within the acidic tumor environment, DMA triggers a charge reversal on the micelles, enhancing their uptake by cancer cells. When α-tocopheryl succinate interacts with mitochondria, it produces reactive oxygen species (ROS). These ROS then oxidize the thioether groups in the polymer, turning them into more water-loving sulfoxides. This chemical change causes the micelles to break apart, allowing for an amplified and targeted release of DOX at the tumor site [73]. Li and coworkers developed a multifunctional nanoparticle system using a hydrogen peroxide-sensitive chiral mesoporous silica nanorod (PCMSR) as the core platform [74]. This pH/H_2O_2 dual-responsive nanocarrier was designed to deliver doxorubicin (DOX) specifically for cancer chemotherapy Fig. 19.5.

Intelligent nanocarriers containing ROS-sensitive components—such as thioketal and oxalate ester linkages—can undergo structural changes in environments rich in reactive oxygen species, enabling targeted drug delivery [75–77]. Similarly, nanomedicines designed with disulfide bonds are responsive to high glutathione (GSH) levels, allowing for controlled drug release triggered by GSH [78]. Since tumors typically exhibit elevated concentrations of both ROS and GSH, designing nanocarriers that respond to both stimuli presents a promising approach for cancer treatment. In this context, Pang and colleagues developed a dual-responsive nanoparticle, sensitive to ROS and GSH, to achieve precise docetaxel (DTX) delivery for cancer chemotherapy [79].

Although disulfide bonds are widely recognized for their strong responsiveness to reductive conditions, they can also undergo oxidation—similar to thioether linkages—when positioned appropriately within the carbon chain of prodrugs, making them susceptible to oxidative environments as well [80]. Building on this dual reactivity, Sun and colleagues developed a prodrug nanoassembly incorporating disulfide bonds, enabling redox-responsive drug release for targeted cancer therapy [81]. In the Paclitaxel (PTX)-citronellol conjugate, the disulfide bond contains two sulfur atoms that can be cleaved by glutathione (GSH) to form thiol groups, or oxidized by reactive oxygen species (ROS) to yield polar sulfoxide or sulfone groups. These transformations enhance the hydrolysis of nearby ester linkages, thereby promoting the release of PTX. This redox dual-responsive prodrug nanoassembly demonstrated vigorous chemotherapeutic activity against KB tumors. Compared to the redox transformation of disulfide bonds, diselenide bonds undergo oxidation to seleninic acid or reduction to selenol more readily [82], making them attractive candidates for use as stimuli-responsive linkages in drug delivery systems [83]. For instance, Leong's group developed diselenide-bridged mesoporous silica nanoparticles that enable the targeted release of ribonuclease A (RNase A) at tumor sites in response to both oxidative and reductive conditions [84].

Hypoxia is a defining characteristic of the tumor microenvironment. Hypoxia-activated prodrugs offer the potential for tumor-specific cytotoxicity by being selectively reduced in low-oxygen conditions, making them highly promising for the targeted induction of cancer cell apoptosis [85]. However, the uneven oxygen distribution within solid tumors often hampers efficient prodrug

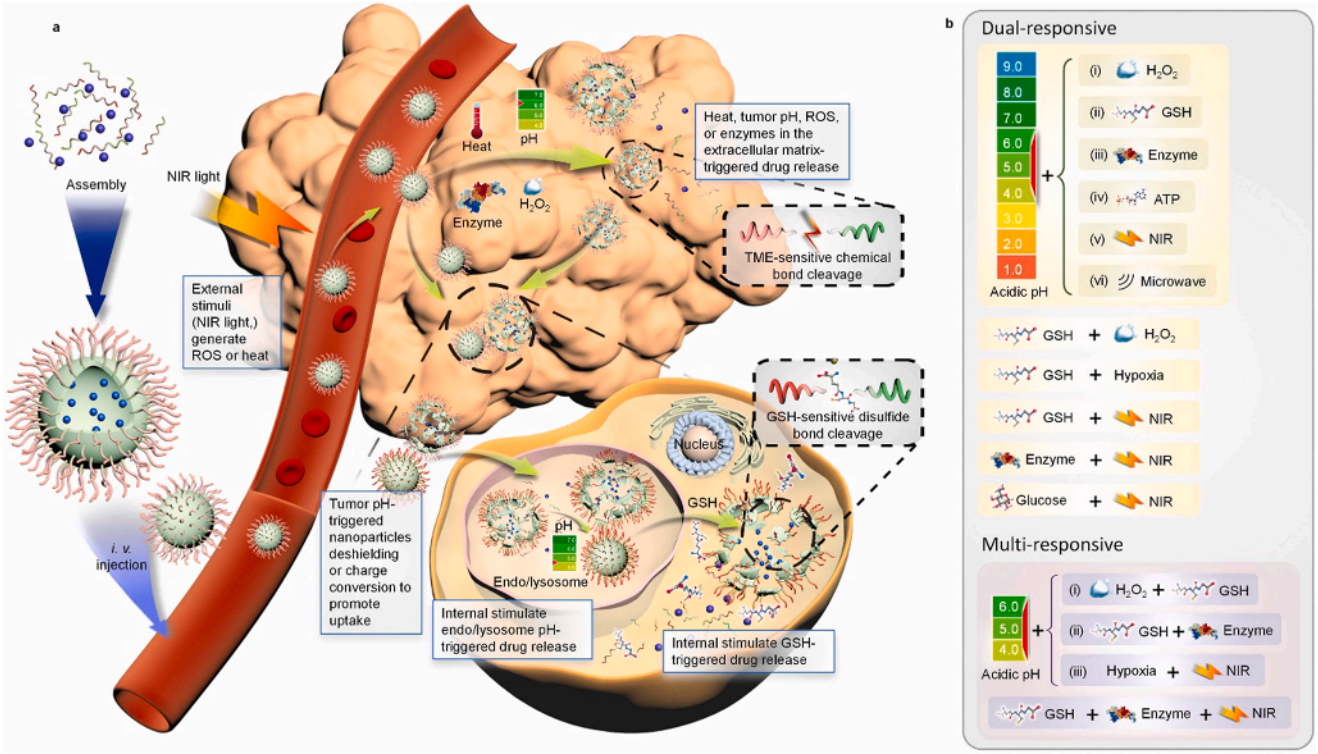


FIGURE 19.5

Illustration of precision cancer therapy enabled by dual- and multi-stimuli responsive nanomedicines, highlighting enhanced therapeutic efficacy and reduced adverse effects, reused with permission from Ref. [76], copyright @ Elsevier, 2022.

activation. To overcome this limitation, Yu's group developed a dual-responsive organosilica nanoparticle system that responds to both hypoxia and glutathione (GSH), enabling enhanced antitumor therapy [86].

4.3 Gene therapy

Genetic alterations are associated with numerous human diseases [87]. Gene therapy has emerged as a transformative strategy for managing such conditions, offering new avenues for treating disorders that remain unresponsive to conventional therapies. Nonviral vectors have shown significant promise among the various gene delivery approaches explored due to their lower production costs and reduced risk of adverse immune responses [88]. One particularly well-studied class of these vectors includes stimulus-responsive nanoparticles, which enable nucleic acid delivery by responding to specific internal or environmental cues.

The primary objective of gene therapy is to promote the expression of therapeutic nucleic acids—such as DNA, RNA, or oligonucleotides—within target cells, thereby addressing disease symptoms stemming from faulty gene expression. For instance, pH- and temperature-responsive nanocarriers have been developed using sulfonamide-functionalized poly (N-isopropylacrylamide)-based micelles [89]. When loaded with a model antiproliferative agent, both sulfadimethoxine- and sulfamethazine-functionalized micelles exhibited improved cellular uptake in mildly acidic environments (pH 6.8) at temperatures just above their lower critical solution temperature, demonstrating their utility for intracellular gene or drug delivery. Recently, considerable focus has been directed toward designing dual-responsive nanocarriers for gene delivery, particularly those sensitive to pH and redox conditions. These systems facilitate the endosomal escape of nucleic acids through acid responsiveness while enhancing cytoplasmic release efficiency via redox sensitivity. A notable example is a dual pH- and redox-responsive polyplex engineered to codeliver MDR-1 siRNA and doxorubicin to drug-resistant cancer cells [90]. This delivery vehicle, composed of methoxy-PEG–polylactide–polyhistidine–ss–oligoethylenimine (mPEG-b-PLA-PHis-ssOEI), showed enhanced endosomal/lysosomal escape. Another innovative system includes a pH- and magnetic field-responsive nanocarrier, developed using a vitamin A-conjugated, pH-sensitive copolymer [VA–polyethylene glycol–polyethyleneimine–poly (N-(N′, N′-diisopropylamino ethyl)-co-benzyl amino) aspartamide (T-PBP)]. This polymer formed cationic micelles decorated with superparamagnetic iron oxide nanoparticles for targeted miRNA delivery, highlighting the potential of multifunctional nanocarriers in gene therapy [91].

4.4 Antimicrobial and anti-inflammatory applications

In recent years, the growing resistance of bacteria to traditional antibiotics has made infectious diseases a significant global health concern. A notable example is the rise of methicillin-resistant Staphylococcus aureus (MRSA) in both the United States and Europe, which is a leading cause of skin and soft tissue infections [92]. Antimicrobial peptides (AMPs) have emerged as a promising class of therapeutic agents with potent antibacterial activity. Antimicrobial peptides (AMPs) are naturally found in a wide range of organisms, including mammals, plants, fungi, and bacteria. Their unique features—such as strong therapeutic potential, tunable toxicity, availability in both broad- and narrow-spectrum forms, and suitability for bioengineering—make them attractive candidates for incorporation

into drug delivery systems. For example, nisin, a cationic antimicrobial peptide, exhibits potent activity against Grampositive bacteria but shows limited effectiveness against Gramnegative strains. However, the therapeutic application of AMPs faces certain limitations, including their short half-life and rapid degradation by enzymes. In general, we employ nanogels, nanocomposites, quantum dots, and many others in order to transport these AMPs [93,94]. Nanogels are considered an effective platform for the delivery of antimicrobial peptides (AMPs) due to their high drug loading capacity, biodegradability [8], tunable physicochemical properties [28], and ability to provide controlled drug release [33]. Among various nanogel types, stimuli-responsive nanogels have attracted significant interest for their potential in advanced drug delivery applications. For instance, Pikabea et al. developed a pH-responsive nanogel for the controlled release of an anticancer agent [95], while Xu et al. investigated a multi-responsive nanogel designed for intracellular delivery of doxorubicin, achieving precise control over the drug's release profile [96]. In the field of thermosensitive nanogels, poly(N-isopropylacrylamide) (PNIPAAM) is one of the most widely used polymers. However, its clinical translation is limited by a key drawback—its lack of biodegradability.

Inflammation is a fundamental biological response that serves as a protective mechanism against both external and internal insults, such as pathogens (e.g., microbial or viral infections), irritants (e.g., allergens, radiation, or harmful chemicals), and apoptotic cells resulting from injuries, autoimmune conditions, or chronic diseases [97]. Persistent or chronic inflammation is a key feature of numerous diseases, including autoimmune disorders (e.g., inflammatory bowel disease [IBD]), inflammatory joint diseases (e.g., rheumatoid arthritis and osteoarthritis), neurodegenerative conditions (e.g., Alzheimer's disease and multiple sclerosis), and cardiovascular diseases (CVDs) [98]. Standard treatments for chronic inflammation typically involve nonsteroidal antiinflammatory drugs (NSAIDs) or glucocorticoids, either as primary therapies or adjuncts [99]. However, these conventional approaches are associated with several drawbacks, including systemic side effects, insufficient drug accumulation at the target site, limited tissue penetration, gastrointestinal toxicity (especially with oral administration), and poor stability in the bloodstream. Reformulating these agents into nanomedicine-based delivery systems offers a promising alternative to overcome these therapeutic challenges [100]. Lee et al. [101] A dual-responsive nanoparticle tailored for high specificity toward inflammatory microenvironments was designed by combining an ROS-sensitive dextran–naproxen conjugate with a pH-responsive acetylated dextran polymer. In this system, the antiinflammatory drug naproxen was chemically linked via a phenylboronic acid (PBA) moiety, which is reactive to reactive oxygen species (ROS), and incorporated into an acid-sensitive acetylated dextran matrix. The resulting nanocarrier enhanced drug release under inflammatory conditions, effectively scavenged ROS, and significantly suppressed proinflammatory cytokines, including IL-6 and TNF-α [102]. Leveraging the characteristic low pH and high ROS levels of inflamed tissues, Xu et al. developed a dual-responsive nanocarrier (PBAE-SA-PAPE) by conjugating 4-(hydroxymethyl) phenylboronic acid pinacol ester (PAPE), which responds to ROS, onto a pH-sensitive PBAE side chain using a succinic anhydride (SA) linker. Curcumin was encapsulated within these nanoparticles and further incorporated into a chitosan/alginate hydrogel matrix to target macrophages. This formulation enabled accelerated drug release under acidic and oxidative conditions, leading to marked antiinflammatory effects in a murine model of ulcerative colitis by modulating the TLR4-MAPK/NF-κB signaling pathway.

5. Conclusion

The development of dual stimuli-responsive nanocarriers represents a significant advancement in the field of biomedical nanotechnology, addressing many limitations inherent in conventional and even single-responsive drug delivery systems. Dual-responsive nanocarriers are designed to take advantage of two different physiological or external triggers, allowing for a more precise and adaptive drug release in complex disease environments. Unlike single-trigger systems, they provide tighter spatial and temporal control, ensuring drug release occurs only when both conditions are present. This selective behavior helps to limit systemic exposure, reduce side effects, and improve therapeutic efficiency. Many pathological sites, especially tumors, present unique microenvironmental features such as acidic pH, high levels of reducing agents like glutathione, abnormal enzyme activity, hypoxic conditions, or elevated temperature. By combining responses to factors such as pH and redox state, enzyme activity and oxidative stress, or pH and temperature, researchers have achieved more targeted delivery within tumor tissues. This dual-trigger strategy significantly reduces the risk of premature drug leakage and enhances localized treatment. The concept has also shown relevance in areas beyond cancer. In infectious and inflammatory diseases, for example, nanocarriers that respond to changes in pH and enzyme levels at affected sites have demonstrated improved delivery of antimicrobial and antiinflammatory agents, providing better control over treatment at the disease site. Similarly, dual-responsive hydrogels and scaffolds in tissue engineering can modulate the local release of growth factors or bioactive molecules in response to environmental signals, aiding in controlled regeneration and healing processes.

A wide range of nanomaterials has been adapted for dual-stimuli responsiveness. These include polymeric micelles, liposomes, dendrimers, mesoporous silica nanoparticles, metallic and magnetic nanoparticles, nanogels, and core–shell hybrid nanostructures. These materials are engineered to retain stability during systemic circulation and undergo rapid transformation or disassembly in response to target-specific triggers through chemical conjugation, labile linkers, and responsive crosslinking. Incorporating surface ligands or targeting moieties (e.g., folic acid, peptides, antibodies) further improves target specificity, cellular uptake, and intracellular trafficking of the nanocarriers. Despite the rapid progress, several challenges continue to impede clinical translation. The complexity in synthesis, batch-to-batch variability, and difficulty scaling up production while maintaining functional integrity are critical hurdles.

Furthermore, these advanced systems' long-term toxicity, immune responses, and pharmacokinetics remain underexplored in human settings. Regulatory pathways are not yet fully equipped to evaluate such multifaceted therapeutic platforms, making standardization and safety evaluation a pressing need. The future of dual stimuli-responsive systems is likely to be shaped by advancements in multidisciplinary integration. The convergence of nanotechnology, materials science, biomedical engineering, and artificial intelligence is expected to lead to next-generation smart nanocarriers that are self-regulated, feedback-responsive, and even capable of real-time monitoring and adaptive response. AI-assisted design can further optimize material combinations, predict responsiveness, and tailor systems based on patient-specific molecular profiles, paving the way for personalized nanomedicine. Another promising direction is the incorporation of multi-modal functionalities, where dual-responsive systems are therapeutic and diagnostic (theranostic), enabling simultaneous imaging,

monitoring, and treatment. Such integration can enhance treatment accuracy and reduce intervention times in critical conditions like metastatic cancers, chronic infections, or neurodegenerative diseases.

In summary, dual stimuli-responsive nanocarriers represent a powerful and versatile platform in the biomedical arena. Their ability to integrate environmental sensitivity, precision targeting, and controlled release aligns perfectly with the needs of modern therapeutic paradigms. As innovation continues and translational bottlenecks are addressed, these systems can redefine how drugs are delivered, diseases are treated, and patient care is personalized in the years ahead.

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CHAPTER

Stimuli-responsive charge-reversal nanocarriers for biomedical application

20

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1. Introduction

Research aimed at improving the bioactivity, bioavailability, stability, solubility, and tissue targeting of drugs has significantly progressed through the design and development of nanocarrier systems in the 21st century with the boom of nanotechnology in biomedicine. Nanocarriers are nanoscale drug delivery vehicles that transport therapeutic agents to specific tissues within a living organism. Pharmaceutical drugs or other substances may be included in the nanocarrier matrix or encapsulated within the nanocarrier for delivery. Commonly used nanomaterials in nanocarrier systems include polymers, liposomes, micelles, nanotubes, nanocomplexes, and inorganic nanoparticles. Due to their configurable nanoscale structure, tunability, and multifunctional physicochemical characteristics, these transport modules enhance biodistribution, enable controlled release, improve solubility, and facilitate targeted drug administration, all while reducing side effects within the body. For these advantages, researchers have considered nanocarriers more useful over several traditional drug therapies for effective drug targeting and delivery [1,2]. Despite the numerous advantages of nanocarriers in biomedicine, various nanoparticles demonstrate adverse effects on the body, including nanotoxicity, cytotoxicity, and genotoxicity. Furthermore, enhancements in drug absorption capacity, bio-environmental stability, regulated drug release, and interactions with biological barriers are also significant areas of concern that must be addressed before the commercialization of nanotechnology-based delivery systems [3,4]. Nevertheless, these limitations enable researchers to thoroughly study, investigate, and manipulate various physiochemical aspects of a nanomaterial to create more varied and optimized nanocarriers. In this chapter, we focused into a novel and advanced nanocarrier system emphasizing how the surface charge of nanomaterials influences delivery efficacy and biostability in the human body. This emerging paradigm of nanodelivery systems is referred to as 'stimuli-responsive charge-reversal nanocarrier' [5].

Charge-reversal nanocarriers show neutral or negative charges under normal physiological conditions and may shift their surface charge to a positive state when activated by particular environmental stimuli, including pH, temperature, redox potential, reactive oxygen species (ROS), enzymes, or light. This dynamic charge-switching capability enables precise delivery of therapeutic agents in vivo. The positive charge promotes interaction with negatively charged cell membranes, boosting cellular uptake

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through endocytosis. Furthermore, this characteristic allows these nanostructures to remain in the bloodstream longer by avoiding rapid clearance and undesirable interactions within the body [5]. Additionally, charge reversal nanodrug delivery systems demonstrate significant versatility in drug delivery through the effective encapsulation and transport of drugs, genes, and vaccines. These systems can address multi-drug resistance through mechanisms such as improved intracellular drug retention and decreased efflux pump activity. Charge reversal nanocarriers represent an innovative and effective alternative to traditional nanodelivery systems, as they enhance cellular uptake, improve tumor targeting, reduce rapid clearance, and facilitate stimuli-responsive drug release. These benefits position them as viable options for sophisticated drug delivery, particularly in the context of cancer treatment. By using the distinctive physiological traits of tumors, including acidic pH, specific enzyme activity, and redox potential, these systems exhibit increased accumulation and penetration at tumor areas, leading to enhanced therapeutic effectiveness and diagnostic precision [6]. Besides being an effective tool for the delivery of anticancer agents, these innovative nanosystems have widespread applications in other areas of biomedicine namely gene delivery, immunotherapy, tissue engineering, wound healing, antimicrobial therapy, biosensing, and other diagnostic applications. Notable examples include the use of charge reversibility in nanocarriers to improve antigen uptake by antigen-presenting cells (APCs), resulting in a stronger immune response. The positively charged carriers function as immune stimulators independent of any adjuvants [7]. The stimulus-responsive nature of charge reversal nanosystems improves targeted imaging of tissues and organs in the diagnosis of diseases. Moreover, its charge-switching capability can add to the binding affinity of biosensors for disease indicators. Charge-reversible nanostructures offer a promising approach to overcome biofilm resistance and enhance targeted antibiotic therapy by using pH and enzyme-responsive charge reversal mechanisms, together with efficient biofilm penetration [8]. This system also finds application in areas of regenerative medicine through enhanced cellular adhesion, facilitating tissue generation and controlled release of growth factors, stem cells, and bioactives for wound healing and tissue repair [9].

This chapter explores the groundbreaking charge-reversible nanocarrier systems and their importance in transforming contemporary healthcare and nanomedicine. By discussing the charge reversible mechanisms influenced by diverse stimuli, along with their design and synthesis techniques, readers will acquire an in-depth understanding of the performance of these systems and their beneficial implications in the administration of diverse therapeutic substances for disease treatment. Furthermore, different drug delivery patterns and their role in biomedicine as well as their current limitations, are explored, along with insights into future aspects of this innovative system in revolutionizing disease diagnosis and treatment.

2. Mechanisms of charge-reversal in nanocarriers

Charge-reverse nanodelivery vehicles alter their surface charge from negative to positive in response to specific stimuli, enhancing the delivery of encapsulated therapeutic compounds. Specific factors typically initiate this transformation. Depending on the triggers or stimuli, charge-reversal nanocarriers can be classified into pH, enzyme, redox potential, temperature, and light charge reversal systems (outlined in Table 20.1). The following sections provide a more detailed discussion of these systems.

Table 20.1 Types of stimuli-responsive charge-reversal nanocarriers.

SL No.	Stimulus type	Mechanism of charge reversal	Common materials used	Biomedical applications	References
01	pH-responsive	In low pH levels or acidic conditions, acid-labile bonds hydrolyze, changing neutral or negative charge into positive	Polyethylene glycol (PEG), poly (acrylamidoglycolic acid), chitosan, polyacrylic acid (PAA), gelatin, liposome, and acid-labile linkages (amide, hydrazone, imine, ortho ester)	Tumor-targeted drug delivery gene delivery, protein delivery	[5,10]
02	Enzyme-responsive	Enzymatic cleavage of functional groups reveals cationic groups (e.g., lysine, arginine) altering the surface charge from negative to positive.	Enzyme-sensitive polymers such as matrix metalloproteinase (MMP)-responsive polypeptides, lipid and mesoporous silica nanoparticles with enzyme-responsive moieties	Enzyme-specific drug delivery in cancer therapy and gene therapy	[11]
03	Redox-responsive	In high-glutathione (GSH) environments, disulfide bond cleavage causes the surface charge to shift from neutral to positive.	GSH-sensitive lipid and polymeric linkages	Intracellular drug delivery, gene therapy, and cancer therapy	[5,12]
04	Light-responsive	Upon exposure to light energy, photosensitive bonds are cleaved causing charge conversion from neutral to positive	Azobenzene-modified and spiropyran (SP)-functionalized polymers	Tumor-targeted drug delivery, photodynamic therapy (PDT), molecular diagnostics	[13]
05	Temperature-responsive	Thermo-sensitive moieties undergo physiochemical changes upon exposure to varying levels of heat leading to charge reversal	Thermo-sensitive polymers (e.g., PNIPAM, PEG)	Controlled and targeted multi-drug delivery in cancer therapy and gene therapy, Wound healing	[14]

2.1 pH-responsive charge reversal

pH-responsive charge reversal is the process of modifying a nanoparticle's surface charge in response to variations in the body's pH levels. Most responsive charge-reversal systems frequently used in gene therapy and drug delivery applications are pH-sensitive systems. Its charge conversion methods can be explained via the protonation and deprotonation mechanisms and pH-sensitive functional groups [6]. Fig. 20.1illustrates the method of achieving positive charge reversal in acid responsive systems.

2.1.1 Protonation/deprotonation mechanisms

The nanoparticle surface comprises functional groups (carboxyl, amine, tertiary amine, imidazole, and sulfonamide) that are capable of undergoing protonation (gain H+) and deprotonation (lose H+) in response to pH fluctuations in the biological environment. At a particular pH threshold, the material transitions from a positive charge to a negative charge, or vice versa. This charge reversal can initiate drug release and cellular uptake This mechanism alters the surface zeta potential and does not entail the breaking of chemical bonds. It provides a more rapid response to pH fluctuations than systems with acid-unstable bonds, which often need longer periods, particularly making them useful in tumor and anti-microbial therapy [6].

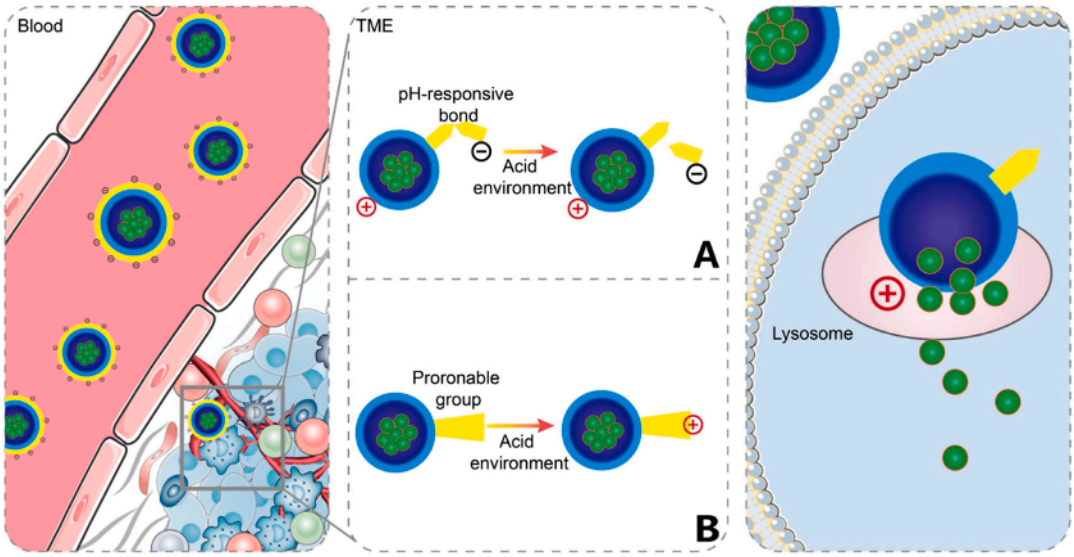


FIGURE 20.1

Sub-images A and B illustrate two methods for achieving a positive charge reversal in acid. -responsive systems for charge reversal: Protonation or the breaking of pH-sensitive bonds.

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2.1.2 pH-sensitive functional groups

The carboxyl group is the predominant functional group that responds to pH changes in the body. Polymers containing carboxyl groups, such as polyhistidine (PHis), polyacrylic acid (PAA), and polymethacrylic acid (PEAA), are commonly used to develop pH-sensitive charge-reversal nanocarriers for targeted tumor therapy in cancer treatment. At alkaline or higher pH, carboxyl groups are deprotonated by losing electrons, resulting in a negatively charged surface on the nanocarrier. Conversely, at low or acidic pH values, these groups gain electrons and become protonated, which creates a positive charge on the surface of the nanocarrier, facilitating biofilm penetration, particularly necessary for the treatment of infectious diseases [15].

The incorporation of amine groups, particularly tertiary amines, as pH-responsive functional entities, presents significant potential for the advancement of nanocarriers aimed at targeted drug delivery to tumor cells. In acidic pH they protonate, while in alkaline pH they deprotonate, releasing drugs and bioactive compounds. Typical instances include PDEAEM (Poly (diethylaminoethyl methacrylate), which features tertiary amine groups within its side chains. Nonetheless, their application in tumor treatment remains limited because of insufficient sensitivity. Conversely, PBAE, or poly (β-amino esters), is recommended for targeted administration since it uses either primary or secondary amines with diacrylate. In anticancer treatment, the charge became positive upon reaching the tumor microenvironment due to the protonation of the tertiary amine on the PBAE under mildly acidic or low pH conditions. For example, the experimental work by Shi et al. on PBAE-based nanoplatforms shows a shift from -2.8 mV to $+15.4$ mV at pH 6.5, enhancing tumor targeting [6,16]. Research also investigates groups such as sulfonamide and imidazole due to their charge-switching capability for targeted administration of drugs. For instance, the Histidine-based PEG system (PEG-His@BPC) utilizes protonation to tear the PEG shell, thereby revealing a positively charged nanoparticle that facilitates tumor penetration upon encountering the tumor microenvironment under weakly acidic conditions [17].

In addition to these, pH-responsive charge reversal through acid-labile bond cleavage is also investigated. Drug delivery systems (DDSs) rely on these chemical bonds, which are stable in neutral environments but undergo charge reversal when exposed to acidic ones. These bonds can be found in the main polymer structure, in side chains, or between charged layers. In cases of tumors or infections, the positively charged core can be exposed when the outer shell is hydrolyzed, allowing for cellular uptake. Several critical acid-sensitive bonds demonstrating pH sensitivity include amide bonds, imine and benzoic-imine bonds, Schiff bases (C═N bonds), orthoester linkages, and borate ester bonds. For example, the MTGZ@PPD nanocarrier, which has a C═N link, alters its charge after 2 h at pH 6.5, thereby enhancing drug internalization. Borate ester linkages are beneficial in the treatment of pneumonia and enhance mitochondrial targeting. Similarly, these interactions increase drug accumulation at tumor sites, facilitate tumor penetration, and promote rapid release of therapeutic cargo, all while diminishing the potential for systemic toxicity in vivo [5,6]. Figs. 20.2 and 20.3 illustrates tumor therapy using pH responsive charge reversal nanocarriers.

2.2 Enzyme-triggered charge reversal

Enzymes are crucial elements that govern cellular function and various physiological processes. These biomolecules serve as triggers for initiating the dynamic charge-switching in the development of charge-reversal nanocarrier systems. The nanoparticles are synthesized with functional groups or

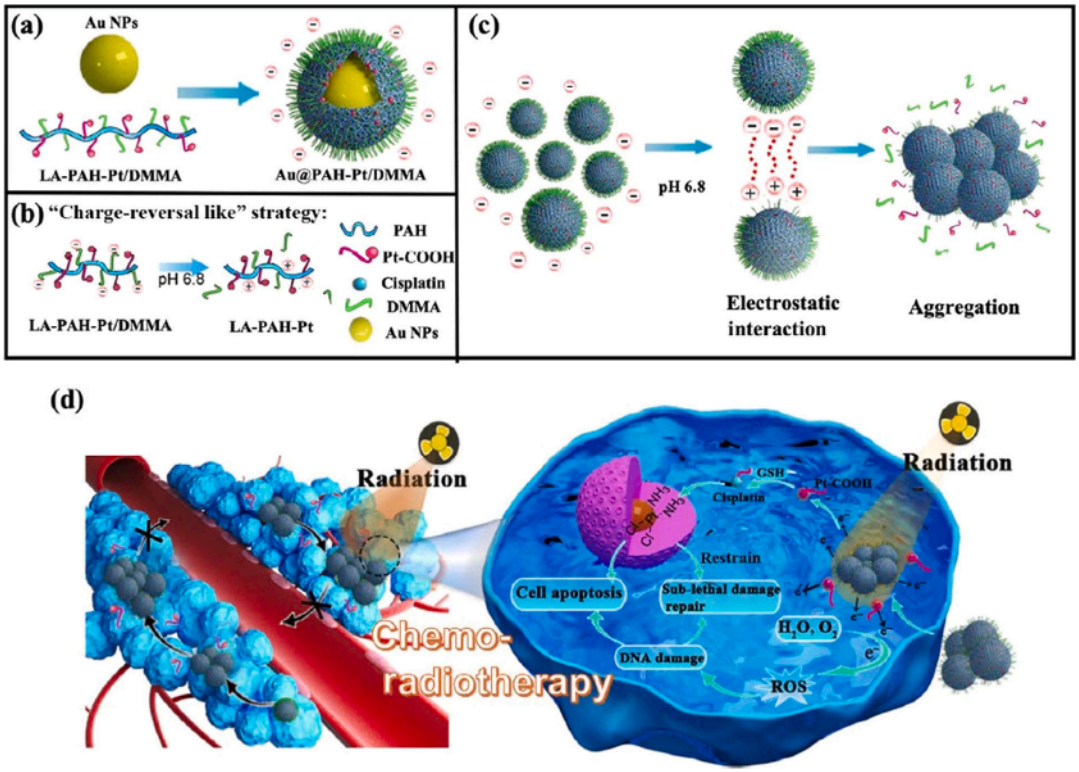


FIGURE 20.2

pH-responsive charge-reversal nanoparticles (Au@PAH-Pt/DMMA) to improve the effectiveness of tumor treatment: (a) Au@PAH-Pt/DMMA composites; (b) Au@PAH-Pt/DMMA's charge conversion mechanism; (c) electrostatic aggregation; (d) the in vivo functions of Au@PAH-Pt/DMMA combined chemotherapy and radiation.

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enzyme substrates that can be cleaved by specifically overexpressed enzymes both intracellularly and extracellularly, effectively reversing the molecule's overall charge and behavior. This mechanism facilitates targeted drug delivery for tumor treatment and gene therapy. Notable enzymes involved in this process include matrix metalloproteinases (MMPs), aminopeptidases (APNs), hyaluronidases (HAases), γ-glutamyltransferases (GGTs), esterases, and proteases [12].

2.2.1 Enzyme-specific cleavage of functional groups

In enzyme-triggered charge reversal nanocarriers, specific enzymes found in inflamed areas, tumors, and infections can identify functional groups on nanocarriers and cleave them, allowing for increased cellular absorption and targeted drug administration. These groups preserve a neutral or negative

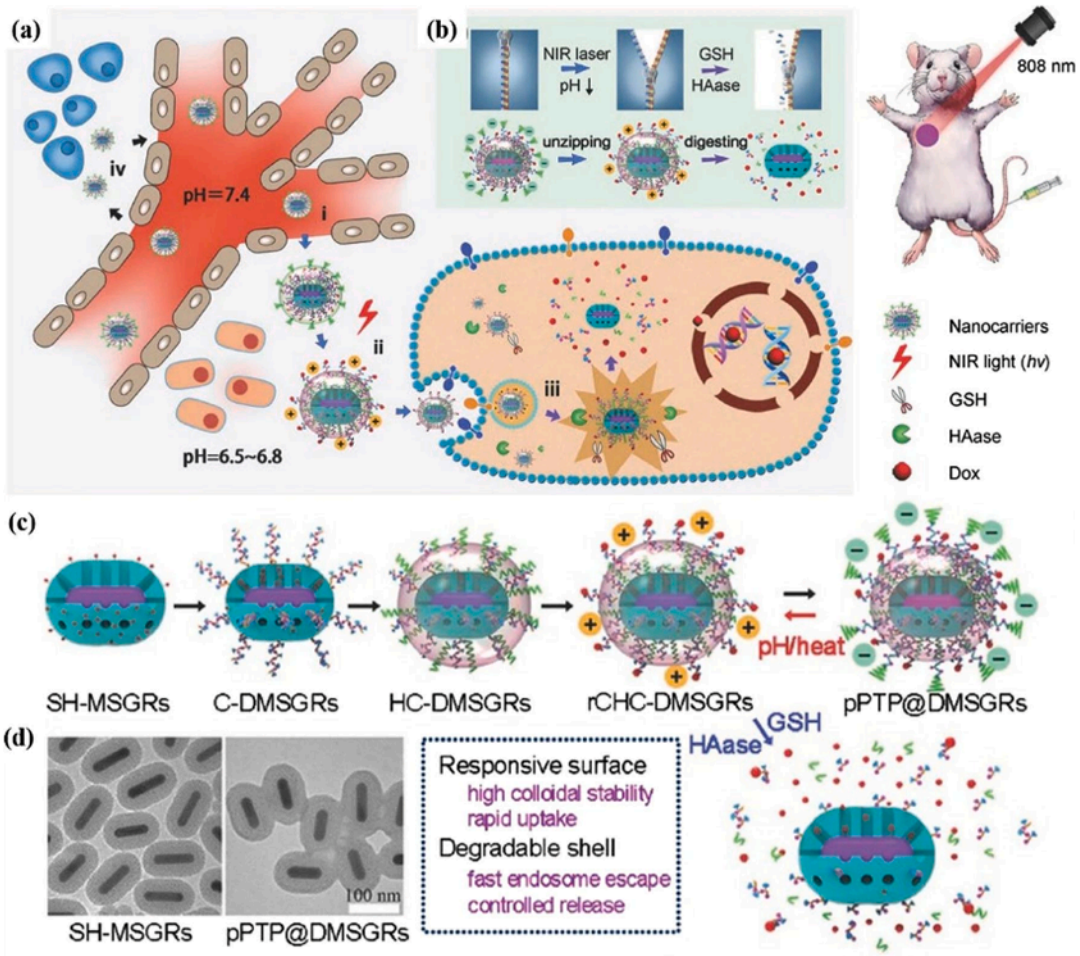


FIGURE 20.3

A drug delivery system for tumor therapy that is responsive to pH, thermal conditions, and glutathione, facilitating charge reversal. (a) Charge reversal and drug release process: (i) Accumulation through the enhanced permeability and retention (EPR) effect; (ii) near-infrared (NIR) and pH-activated surface modification for cellular uptake; (iii) endosomal escape and controlled release in response to glutathione (GSH) and hyaluronidase (HAase). (b) Surface charge transformation and degradation of the polymer shell. (c) Preparation and the process of surface charge conversion triggered by pH and thermal conditions. (d) TEM images.

Reprinted from P. Zhang, D. Chen, Lin L., K. Sun, Charge reversal nano-systems for tumor therapy, J. Nanobiotechnol. 20 (1) (2022), doi: 10.1186/s12951-021-01221-8, Copyright, Springer 2022. This open access article is distributed under the terms of creative commons license [CC BY 4.0].

charge of the nanocarrier under normal physiological conditions. Enzymatic cleavage alters these moieties, revealing positively charged groups and augmenting interactions with negatively charged cell membranes in the body.

Matrix metalloproteinases (MMPs) are universally expressed across all cancers, playing a crucial role in tumor progression. MMPs cleave enzyme-sensitive peptide linkers attached to the polymer structure or integrated into the nanocarrier surface, thereby exposing positively charged residues. Lang et al. developed MMP9-responsive micelles (pDM) that release docetaxel (DTX) by PEG hydrolysis in tumor locations, hence enhancing drug concentration, particularly within cancer cells [18].

Alkaline phosphatase (ALP) is an enzyme that targets and removes phosphate groups from peptide chains through dephosphorylation, thus converting anionic groups to cationic forms. In tumor drug delivery, enzymatic action can be used for enzyme-triggered charge reversal, wherein APN cleaves a neutral or negative functional group from a nanocarrier, thereby exposing a positively charged moiety to improve cancer cell uptake [19]. Aminopeptidases (APNs), an enzyme involved in diverse cellular functions similarly hydrolyze neutral amino acids at the N-terminal of peptide chains, revealing a positively charged amine ($-NH_3^+$) that facilitates cellular absorption in malignancies [20]. GGT, an enzyme participating in glutathione metabolism, hydrolyzes γ-glutamyl amide bonds, yielding a primary amine. GGT, which is overexpressed in certain cancers, is used for enzyme-mediated charge reversal in drug delivery systems. Dai et al. developed a GGT-responsive peptide that maintains a negative charge in normal tissues while shifting to a positive charge in tumors, enhancing drug penetration and therapeutic efficacy [21].

Hyaluronidases (HAase) play a key role in the degradation of glycosidic bonds in hyaluronic acid (HA). CD44, the receptor for HA, is often overexpressed in multiple cancers and correlates with tumor progression. HAase-based charge-reversal nanocarriers have been developed for targeted drug delivery. Yang et al. developed Must-nano, a nanocarrier that is multi-responsive and loaded with CRISPR/Cas9, featuring a core sensitive to redox conditions and a shell that can be cleaved by HAase. This system allows for charge reversal in tumor environments, promoting targeted gene editing and therapeutic interventions [6,22].

2.2.2 Role of proteases and hydrolases

Proteases, including peptidases and proteins, are essential in catalyzing the cleavage of anionic peptide bonds on the nanocarrier surface. When these peptides are degraded, the underlying cationic charges are revealed, hence improving cellular uptake by electrostatic attraction. A prevalent example is nanocarriers engineered with matrix metalloproteinases (MMP)-cleavable peptides that experience charge reversal following MMP exposure, resulting in increased tumor cell uptake and therefore improving drug delivery efficacy. This reversible charge mechanism, facilitated by proteases, is particularly advantageous in cancer treatment. Additionally, proteases effectively cleave the enzyme cathepsin B, which is used in the development of polymeric nanoparticles for intracellular drug delivery. Given that specific proteases are overexpressed in tumor microenvironments, the development of nanocarriers featuring protease-cleavable sites facilitates targeted and regulated drug delivery to cancer cells [12,23].

Hydrolases are enzymes that facilitate the hydrolysis of chemical bonds, including esters, phosphates, amides, and glycosides. They facilitate the charge reversal mechanism by eliminating surface functional groups, resulting in the shift of surface charge from a neutral or negatively charged state to a positively charged state. This method enables the nanovehicle to deliver the drug precisely to target

cells, enhancing cellular absorption through electrostatic interactions with cell membranes bearing a negative charge. Furthermore, by breaking down the carrier material, hydrolases such as esterase, phosphatase, lipase, glycosidase, and amylase enable localized drug release while reducing adverse effects. Hydrolases are important biomarkers that have prompted the development of highly sensitive nanoparticle-based sensors for their detection. They are also being studied extensively to develop enzyme-responsive charge-reversal nanosystems for ELISA, biosensing via FRET, synergistic drug delivery, and prostate cancer diagnosis [24].

2.3 Redox-responsive charge reversal

The cells in our body maintain redox homeostasis by balancing oxidants (ROS) and antioxidants within the cell. Glutathione concentrations are directly correlated with cellular redox status, serving as a principal regulator of redox equilibrium by easily transitioning between its reduced and oxidized states. Redox-sensitive charge reversal is an innovative drug delivery strategy for designing nanocarriers, wherein nanosystems transporting therapeutic agents undergo charge transformations in response to local redox conditions within the body. The charge reversibility of this specific type uses intracellular glutathione (GSH) and reactive oxygen species (ROS) levels to facilitate drug release and enhance cellular uptake while minimizing toxicity risks. This approach is especially useful in cancer therapy and gene delivery, positioning it as a promising method for the emergence of next-generation smart drug carriers [5].

2.3.1 Glutathione-responsive mechanisms

Glutathione-responsive delivery methods are the most efficacious in tumor therapy. The tumor microenvironment shows increased oxidation, resulting from higher production of reactive oxygen species (ROS) and the overexpression of antioxidant systems, which together sustain a delicate equilibrium that promotes tumor growth [25]. The differing levels of GSH result in redox variations that facilitate the selective degradation of GSH-sensitive linkages in nanocarriers. Disulfide (S–S) and diselenide (Se–Se) bonds are stable in circulation but are cleaved in tumor microenvironments, making them commonly utilized in these systems. Research has shown their efficacy in enabling targeted drug delivery, promoting endosomal escape, and achieving controlled drug release for cancer treatment. For instance, Zhang et al. developed a core-shell nano-complex that used anionic hyaluronic acid (HA)-disulfide bonds alongside a cationic PAMAM@DOX core, leading to improved cellular uptake and regulated doxorubicin (DOX) release via charge reversal. He et al. developed diselenide-containing PTXD nanoparticles targeting triple-negative breast cancer (TNBC), demonstrating effective tumor accumulation and improved in vivo anti-tumor efficacy. In addition, ditelluride (Te–Te) bonds show lower bond energy, which increases their sensitivity to tumor redox environments. The nanoparticles composed of PEGylated folic acid-TeTe DOX exhibited improved tumor suppression in vivo [5,26].

2.3.2 ROS-responsive mechanisms

Tumor patients often experience redox imbalances that lead to excessive ROS buildup, prompting the design of a ROS-responsive nanocarrier system. A few common types of reactive oxygen species are hydroxyl radicals (OH·), hydrogen peroxides (H_2O_2), nitric oxide (NO·), and superoxides ($O_2-\cdot$). Researchers have developed a variety of ROS-sensitive nanocarriers, such as chitosan–drug conjugates with ROS-sensitive thioacetal linkers that release therapeutic agents following ROS-triggered cleavage and

Fe_3O_4-based nanoparticles that deconstruct in response to H_2O_2 in the tumor microenvironment. Furthermore, selenium-containing polymers and ROS-unstable polymers like poly (propylene sulfide) have been investigated for targeted tumor therapy in cancer management. Because ROS levels are high in cancer cells, ROS-responsive charge-reversal nanocarriers can aid in enhancing drug delivery efficacy. When oxidized, a variety of ROS-sensitive linkages, including thioketal, peroxalate esters, and boronic acid, allow charge conversion, which facilitates the release of drugs or genes. These systems have been used to treat glioblastoma with ROS-sensitive polymers, treat Alzheimer's disease, and transfect genes. However, to improve therapeutic effects, combinational techniques such as pH/ROS dual-sensitivity systems may be necessary because intracellular ROS levels may not always cause rapid bond-breaking [5,6].

2.4 Light and temperature-induced charge reversal

Charge reversal in nanosystems generated by light and temperature uses photosensitive and thermo-sensitive molecules for regulated drug delivery and targeted therapy. These stimuli-responsive devices use environmental changes to adjust surface charge, hence improving cellular uptake, tissue penetration, and drug release efficacy in the treatment and diagnosis of many diseases.

2.4.1 Photoresponsive groups

Light-responsive nanocarriers typically trigger cargo release upon exposure to a light stimulus. The core principle of their mechanism originates from the concept of altering the nanoparticle with various photosensitive moieties that respond to light energy. This action results in changes to the electronic structure, bond cleavage, and molecular interactions facilitating either reversible or irreversible charge conversion of the nanocarrier. This process allows for targeted cellular interaction and drug release. Due to their great biodegradability and biocompatibility, light-induced charge reversal nanosystems serve as exceptional nanocarriers for targeted drug delivery in cancer and photodynamic therapy (PDT) and have been included as a feasible component in diagnostic tools. Key mechanisms involved in light-responsive charge reversal are photoisomerization, photocleavage, and photocross-linking-decross-linking. Azobenzenes are among the most extensively used photosensitive compounds in the design of light-sensitive charge reversal nanosystems. When exposed to UV light, azobenzenes change from a thermodynamically stable trans form to a bending cis form, making them useful for photo-triggered isomerization. Their π-π transitions enable this reversible change, and their low photo-bleaching, quick photoisomerization, and high quantum yields make them perfect candidates for clinical applications. Under UV light, spiropyran (SP), another well-known photochromic switch, reversibly changes into its hydrophilic merocyanine (MC) form by breaking carbon-oxygen bonds. SP shows a light-driven fluorescence shift, characterized by a colorless ring-closed form and a colored ring-open MC form that responds to UV and visible light. SP-based charge reversal systems are used in photo-switchable sensing devices in various diagnostic applications. Light-responsive polymers experience irreversible modifications via photocleavage processes. The o-nitrobenzyl (ONB) group serves as a frequently used photolabile moiety in biomedical applications, undergoing photolysis to liberate functional groups such as carboxylic acids, amines, or alcohols. There are four distinct categories of photocleavage-based polymer systems namely self-immolate polymers (SIPs), light-degradable polymers featuring photocleavable side chains such as amines, alcohols, and thiols, hydrophobic-to-hydrophilic transition polymers, and SIPs that incorporate multi photocleavable

linkers. Light-induced reversible dimerization entails the covalent connection of two free molecules, which can be reversed upon irradiation, and photocross-linking-decross-linking is another way to accomplish light-induced charge reversal in nanocarriers. This feature is shown by compounds such as cinnamylidene acetate, and anthracene; the most prevalent of these are coumarin and cinnamic acid derivatives. Higher energy photons with shorter wavelengths initiate the decross-linking process. Nevertheless, UV-based systems are limited in their use due to the light's shallow tissue penetration depth and the possibility of cellular genetic harm in both laboratory and biological settings [13].

2.4.2 Thermosensitive polymers and materials

Thermosensitive charge reversal nanocarriers have emerged as intriguing and exciting possibilities for drug delivery due to their ability to selectively target predetermined sites in response to temperature variations. Various thermo-sensitive polymers that are incorporated or conjugated onto the surface of the nanoparticle are triggered via heat changes. This leads to physical and chemical changes, ultimately transforming the surface charge of the nanocarrier. PNIPAM, known as poly(N-isopropylacrylamide), is a thermosensitive polymer that is widely used in the creation of thermoresponsive charge reversal nanocarriers for drug delivery. The polymer maintains its hydrophilicity at temperatures below the lower critical solution temperature (LCST), but it changes phases and becomes hydrophobic at temperatures above the LCST. The change in hydrophobicity caused by temperature has the potential to reverse the nanocarrier's surface charge. Furthermore, LCST polymers are frequently combined with PEG and its derivatives to increase biocompatibility and temperature responsiveness. Thermoresponsive hydrogels are also used in the fabrication of thermosensitive nanocarriers that can undergo sol-to-gel transition. Nanocarriers with this feature are commonly used to make injectable or implanted drugs that stay stable at room temperature but transform into a gel-like solid when heated. To control the change in surface charge of the nanocarrier and enhance the release capacity of the therapeutic payload, several lipids and polypeptides are used in addition to the thermosensitive groups in the structure of the nanocarrier system. This new class of nanocarriers is currently under extensive research for their potential in sustained release of the chemotherapeutic agent paclitaxel in cervical cancer, controlled multidrug release, encapsulation, targeted delivery of genes and antibodies, and applications in wound healing [14,27].

Fig. 20.4 illustrates different mechanisms of activated charge reversal.

3. Design and synthesis of charge-reversal nanocarriers

Driven by substantial advancements in materials science and drug delivery, charge-reversal nanocarriers are emerging as effective tools for the administration of drugs, genes, vaccines, and other bioactive agents for disease diagnosis and therapy. Due to their charge-reversal ability in response to particular stimuli, they can improve cellular absorption, extend circulation duration, and facilitate targeted drug release at disease locations, such as tumors. Their capacity to overcome biological obstacles enhances treatment efficacy while reducing adverse effects, making them advantageous for precision medicine applications.

The subsequent sections explore diverse materials and fabrication techniques used in the development of smart nanocarriers, as well as the distinct properties and surface functionalization strategies used to enhance their functions.

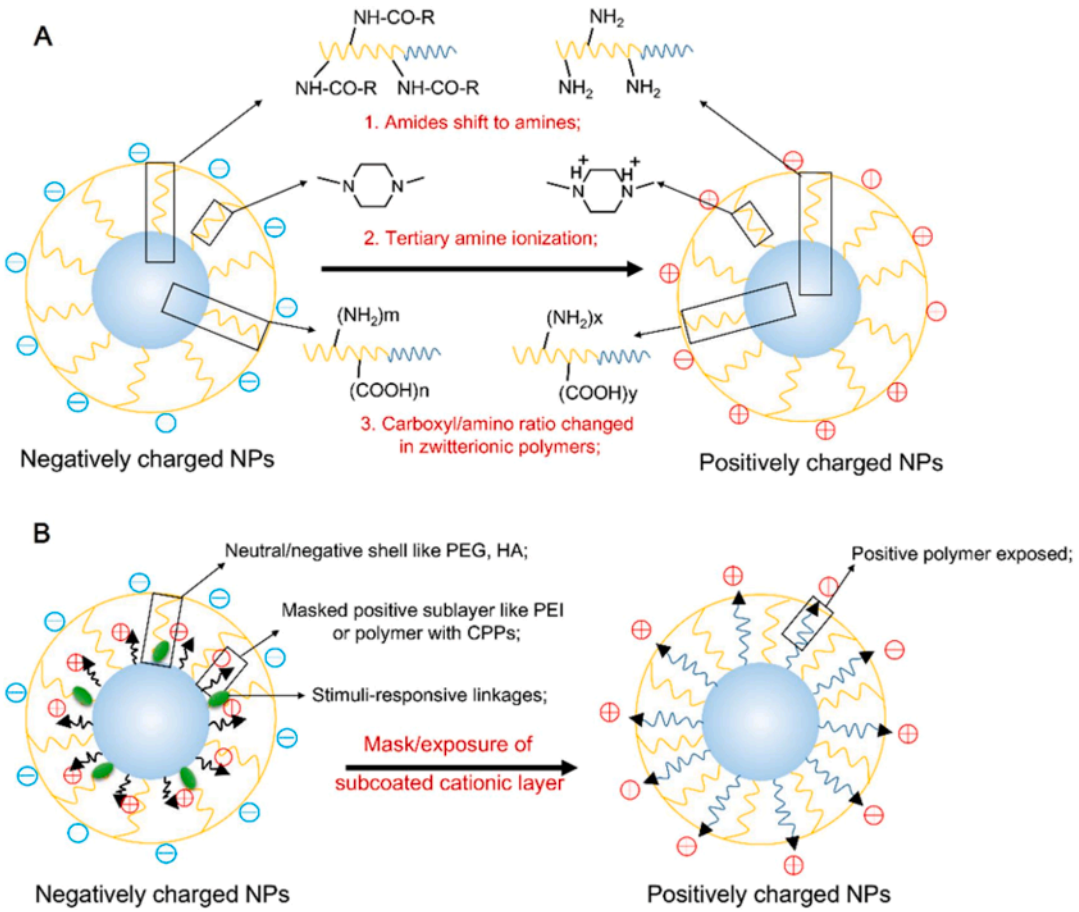


FIGURE 20.4

Mechanisms of activated charge reversal through the (A) protonation and deprotonation of polymer and (B) the masking and exposure of the subcoated cationic nano-layer.

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3.1 Materials used for charge-reversal nanocarriers

3.1.1 Polymers and polyelectrolytes

In recent decades, polymers have been a popular choice for developing charge-reversal nanocarriers for targeted and controlled medication delivery. Polymers offer enhanced biostability and biocompatibility in physiological conditions, improving the circulation time of therapeutic agents. Additionally, they can be designed or altered to facilitate charge conversion in reaction to specific stimuli, including pH, enzymes, or redox conditions because of their surface tunability and responsiveness to

environmental changes. This facilitates controlled drug release, rendering them suitable for targeted drug delivery. Diverse polymeric systems have been engineered to attain pH-responsive charge inversion. Poly (β-amino ester) (PBAE) serves as an example of a cationic polymer characterized by tertiary amines, which undergo protonation under acidic conditions. Micelles composed of amphiphilic polymers with PBAE-grafted hydrophilic segments, such as polyethylene glycol (PEG), demonstrate drug release driven by acidity. Another instance involves the formulation of charge-reversal poly (L-glutamic acid-co-L-lysine) nano-aggregates that encapsulate the chemotherapeutic drug cisplatin, showing a regulated transition from negative to positive charge within the tumor microenvironment. Moreover, neutral polymers such as PEG, poly (ethylene oxide), and poly (acrylic acid) can protect anticancer agents from in vivo clearance, leading to prolonged circulation time and improved drug accumulation at tumor sites via the EPR effect. Furthermore, certain studies have emphasized the function of polymer-based charge reversal nanocarriers in enhancing the cellular absorption of pharmaceutical drugs. PCL-b-P(AEP-g-TMA/DMA) nanoparticles, containing tertiary amine and carboxyl groups inside pH-sensitive amides, demonstrated charge conversion resulting in increased uptake by MDA-MB-231 breast cancer cells and improved tumor accumulation in vivo. Furthermore, enzyme-sensitive polymer-based charge reversal nanosystems are increasingly recognized for their capacity to facilitate targeted drug delivery by modulating enzyme activity at specific sites. Researchers have developed a PEG-MMP2 substrate peptide-quantum dot (QD) system in which the enzyme MMP cleaves the enzyme-responsive substrate, facilitating charge conversion and the release of therapeutic cargo. Numerous polymer-based charge reversal nanoparticles are currently under investigation, responding to redox changes, UV light, and heat for drug delivery. These nanosystems are being evaluated in various phases of preclinical and clinical trials. Moreover, Furthermore, charge-switchable nanocarriers can target cancer stem cells (CSCs), which demonstrate considerable resistance to conventional therapies and contribute to tumor recurrence. These systems enhance drug penetration in heterogeneous tumors and environments rich in cancer stem cells, presenting a promising strategy for improved anticancer efficacy [27,28].

In charge reversal nanomaterials, polyelectrolytes serve as essential components that modify the surface charge of nanoparticles through adsorption driven by electrostatic interactions. The addition of a polyelectrolyte with a charge opposite to that of the nanoparticle results in a strong binding to the nanoparticle surface. This process effectively neutralizes the initial charge and can induce a net opposite charge, resulting in a shift in surface charge. They maintain the characteristics of both polymers and electrolytes and have been used in the design and fabrication of charge-reversal nanosystems. For example, scientists have developed pH-sensitive polyelectrolyte-coated mesoporous silica NPs with the polymer combination P(DMA-co-TPAMA)/PAH for carrying and releasing cisplatin to tumor tissue upon changes in intracellular pH levels [29,30].

3.1.2 Lipid-based nanocarriers

Lipid-based nanoparticles represent a highly promising category of colloidal carriers for bioactive organic compounds due to their superior temporal and thermal stability, exceptional biocompatibility, great loading capacity, low toxicity risk, and favorable scalability for industrial manufacturing. Recently, researchers used the efficient pH-responsive behavior to construct lipid-based charge reversal nanocarriers that release drugs in response to environmental triggers such as pH. These nanoparticles demonstrate a dynamic charge-switching capability in acidic conditions, coupled with improved cellular uptake and endosomal escape, rendering them particularly effective for tumor-targeted drug administration. Among

lipid-based nanocarriers, liposomes are the most extensively researched particles. The integration of acid-sensitive moieties, such as carboxyl groups and tertiary amines, which become protonated at higher pH levels, results in a transition to a positive charge, hence augmenting electrostatic interactions with negatively charged cell membranes. Research has been continuously conducted on the advancement of various lipid-based nanocarriers for medication delivery. Researchers have developed a positively charged lipid, (2,3-dioleoyloxy-propyl)-trimethylammonium-chloride (DOTAP) liposomal vesicle, and included 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) as an additional or supplementary lipid component. DOTAP can maintain a neutral charge in blood circulation but transitions to a cationic charge in acidic conditions, facilitating endosomal escape. DOPE's fusogenic properties are active at low pH, enhancing intracellular delivery. These carriers are capable of encapsulating both hydrophilic and hydrophobic drugs. The biostability of the nanocarrier formulation is enhanced, thereby preventing premature clearance of the nanosystem. Furthermore, parameters such as temperature, pH, and enzyme activity can be adjusted to facilitate the controlled release of an anticancer drug through the application of an external trigger in the design and development of charge-reversal nanocarriers utilizing liposomes. A liposome conjugated with the thermo-sensitive polymer poly (NIPMAM-co-NIPAM) has been developed to encapsulate docetaxel, improving this anticancer drug's stability. Additionally, an MMP2-responsive PEG layer has been utilized to create a liposomal vehicle for therapeutic drug delivery [31–33].

Solid lipid nanoparticles (SLNs), composed of physiological lipids, indicate a novel approach in the realm of colloidal drug delivery systems. They can be stabilized by integrating polymers or surfactants and can encapsulate a wide range of drugs. These nanoparticles combine the benefits of polymeric and liposome nanoparticles, including low toxicity, the ability to release hydrophilic and lipophilic therapeutic agents, enhanced biocompatibility protection of labile compounds, low cost, and ease of manufacture thus they are promising candidates for synthesizing lipid-based charge reversal nanocarriers. Thus, SLNs can enhance endosomal escape, improve intracellular drug delivery, and boost therapeutic efficacy by implementing charge-reversal techniques, particularly for gene therapy, protein delivery, and cancer treatment. SLNs can demonstrate charge reversal capability to facilitate drug release in response to different environmental stimuli. SLN incorporating stearic acid and glyceryl monostearate (GMS) with doxorubicin has been developed to switch charge from negative to positive in acidic or low pH conditions typical of the tumor microenvironment, facilitating the controlled delivery of DOX. Additionally, MMP-sensitive peptide-lipid conjugates are applied in solid lipid nanoparticles (SLNs) to facilitate the transport of paclitaxel through charge reversal resulting from enzymatic cleavage [31,34].

Additionally, nanostructured lipid nanocarriers are gaining popularity in biomedical research due to their size, enhanced drug-loading capacity, and targeted therapeutics delivery.

3.2 Fabrication techniques

3.2.1 Self-assembly

The self-assembly process, in which building units (atoms, molecules) spontaneously arrange themselves to form ordered patterns or structures, is a fundamental strategy for developing nanostructures. The fabrication of advanced nanomaterials through self-assembly requires the interplay of specific supramolecular forces among amphiphilic polymers, polyelectrolytes, or lipids possessing functional groups that react to particular stimuli. These forces are primarily non-covalent interactions, including hydrogen bonding, electrostatic forces, hydrophobic interactions, π-π stacking interactions, and van

der Waals forces. The spontaneous creation of controlled ordered structures marks the end of the self-assembly processes. The process is characterized by a transition from an initial, disordered configuration (such as a dispersion of macromolecules or aggregates) to a final, more favorable, ordered, self-assembled nanostructure (such as a condensed state or crystal). Self-assembly enables precise regulation of the dimensions, morphology, and surface characteristics of nanocarriers, allowing them to have dynamic charge reversibility. Furthermore, the self-assembled nanostructures provide improved stability, biocompatibility, drug encapsulation, and multifunctionality. Due to these characteristics, self-assembly techniques serve as successful tools for creating charge-reversible nanocarriers, rendering them very efficient for drug delivery and biomedical applications [35].

3.2.2 Surface functionalization

Nanocarriers frequently demonstrate inefficiencies in their ability to precisely target specific diseased sites and extend their circulation duration in the bloodstream. This necessitates the emergence of the concept of surface functionalization. To enhance the physicochemical properties of nanoparticles, thereby enhancing their in vivo drug delivery capacity and making the nanosystem more adaptable to biological environments, this strategy entails the modification of their surfaces with ligands. The charge reversal in stimulus-responsive charge reversal nanosystems is achieved by incorporating functional groups on the nanoparticle surface that can undergo conformational changes in response to a triggering stimulus. This process leads to a change in the overall surface charge, facilitating the release of the encapsulated drug substance. Physical adsorption, covalent binding, and encapsulation are the three mechanisms involved in these nanocarriers' modification. Physical adsorption involves the noncovalent surface binding of molecules to the nanocarrier. This can be achieved through electrostatic, hydrophobic, or van der Waals intermolecular forces of attraction. A covalent bond, on the other hand, is a chemical bond between the nanocarrier's surface and the ligand's changed functional group. Polymer coatings are the most frequently applied to nanoparticle surfaces to modify their surface properties due to their superior properties of enhanced biostability, low toxicity, tunability, and prevention of premature drug clearance. Various polymeric materials, including biodegradable and low-toxic poly (lactic acid) (PLA), poly (ethylene glycol) (PEG), chitosan, polycaprolactone, poly (L-cystine), polyelectrolytes, etc., are used in conjunction with pH-sensitive moieties such as amides or tertiary amines for targeted and controlled release of drugs. Enzyme-sensitive linkers have been used with polymers to create enzyme-responsive charge reversal nanostructures for drug and gene delivery. MMP2-responsive PEG is one of the most prevalent types of polymeric coatings used in the design of such carrier vehicles. Furthermore, UV-sensitive, thermoresponsive, and redox-responsive groups are also used with polymeric materials to design various types of nanocarriers. In addition to polymeric coating, the surface of the nanocarrier can be altered with ligands including proteins, polysaccharides, antibodies, aptamers, small molecules, cell-penetrating peptides (CPP), growth factors, and ionizing functional groups. Ligand conjugation enhances the targeting capabilities of the nanocarrier, allowing for precise drug delivery to specific cells, thereby increasing the efficacy of the administered therapy. Furthermore, these surface-modifying molecules are also used in nanocarrier surfaces made of lipid, metal, carbon, dendrimers, or even nanocomposites [27,36,37].

3.3 Characterization of charge-reversal nanocarriers

3.3.1 Zeta potential analysis

The surface charge of the nanocarrier system is quantifiable through zeta potential, indicating the electrical potential difference between the nanocarrier's surface and the surrounding liquid medium. Zeta potential analysis serves as an essential method for evaluating the surface charge and charge-

reversal properties of nanocarriers. This measurement provides insights into the stability, interactions, and reactivity of nanocarriers under diverse environmental conditions, while also confirming charge reception behavior under various physiological conditions and aiding in the optimization of targeted drug delivery efficiency by monitoring charge fluctuations at different points. In charge reversal nanosystems, the zeta potential varies in reaction to external stimuli such as pH, enzymes, redox potential, light, and heat, hence validating their charge-switching characteristics. To assess the zeta potential, the nanocarriers are initially disseminated in an appropriate medium. The nanocarriers are subjected to various stimuli to investigate their charge reversibility. Subsequently, a zeta potential analyzer is used to detect zeta potential, commonly using procedures such as electrophoretic light scattering. A high absolute value of zeta potential (positive or negative), indicates enhanced stability resulting from robust electrostatic repulsion among particles. ΔZP assesses the efficiency of charge reversal by measuring the difference in zeta potential before and after exposure to stimuli. Ideally, the charge reversal should be rapid and responsive to stimuli, whereas the nanocarrier must maintain stability post-charge switching [5,38].

3.3.2 Size, morphology, and stability assessment

To analyze and evaluate the physicochemical properties, charge-switching behavior, and functional efficacy of charge-reversal nanocarriers, it is necessary to measure the size, morphology, and stability of the engineered smart nanosystems. Nanocarrier systems must have an optimal size range that will help them to traverse various biological barriers inside the body, influencing the circulation time, biodistribution, cellular uptake, and potential for nanotoxicity of the encapsulated therapeutic molecules. Biophysical techniques including transmission electron microscopy (TEM), dynamic light scattering (DLS), atomic force microscopy (AFM), nanoparticle tracking analysis (NTA), and small-angle X-ray scattering (SAXS) are used to measure the size of the nanostructure. Currently, there is a significant focus on the necessity for expert management, standardized approaches, and transparency in the reporting of nanoparticle size to improve the methodologies for characterizing nanoparticle dimensions [39]. Similar to nanoparticle size, the structure and shape of nanocarriers are crucial factors in evaluating their interactions with cells and their drug-release characteristics. This subsequently influences the charge-switching behavior of the stimuli-responsive nanocarrier systems. Nanocarriers can be fabricated in various shapes, including nanospheres, nanocubes, nanorods, and nanotubes. Microstructural characterization provides insights into their shape, size, morphology of different phases, number of phases, surface chemistry, distribution, and surface properties. Charge-reversal nanocarriers depend on alterations in surface charge to improve cellular internalization, thus necessitating an analysis of whether the morphology facilitates this process. Irregular or highly porous structures can show uneven charge distribution, which impacts charge-switching efficacy Maintaining uniform morphology facilitates predictable and reproducible drug release profiles. Morphological evaluations are most commonly performed using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) [40]. Size and morphological characterization, together with assessments of integrity during storage and circulation, drug clearance and release, and charge loss, are critical parameters determining the overall stability of the charge reversal nanocarrier system. Methods such as transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential analysis, centrifugation, and X-ray diffraction are commonly used to assess and monitor the stability of a nanofabricated delivery system. These approaches provide valuable insights into storage stability, colloidal stability, particle aggregation,

degradation rate, light scattering patterns, electrostatic stability, morphology, and size distribution of the nanostructure. Furthermore, charge reversal systems are subjected to specific stimuli such as pH, temperature, and light and are applied just to evaluate their functionality and stability in adverse environmental conditions [41].

4. Stimuli-responsive drug delivery using charge-reversal nanocarriers

The following parts and Table 20.2 explore the various charge reversal methods of stimuli-induced nanocarriers for targeted drug delivery in biomedical applications, their in vitro and in vivo performance as well as the potential of new hybrid stimuli-responsive charge reversal nanosystems to revolutionize modern drug delivery in biomedicine.

4.1 pH-sensitive drug release in tumor microenvironment

4.1.1 Targeting tumor acidity

During tumor development, cell proliferation and angiogenesis become uncontrolled and dysfunctional, resulting in a complex tumor microenvironment characterized by severe hypoxia, a slightly acidic pH, and elevated levels of reactive oxygen species (ROS). Because the environment around tumor cells is very low in oxygen, glycolysis produces a lot of acidic byproducts. This results in a mildly acidic environment surrounding the tumor tissues, with a pH value lower than that of blood and healthy tissues (pH 7.4). Researchers exploit this complex tumor microenvironment to get specific responses. pH-responsive charge reversal nanocarriers demonstrate relative stability in blood and normal tissues; however, they are unstable in the tumor microenvironment characterized by acidic pH, leading to rapid fluctuations in the ionization of pH-responsive groups which leads to protonation/deprotonation of the materials and cleavage of pH-sensitive groups through degradation and hydrolysis, eventually resulting in charge reversal to a positively charged state. This phenomenon enables the release of anti-tumor drugs, thereby achieving the goal of targeted tumor therapy. Scientists incorporate pH-sensitive groups that primarily include hydrazone, imine, oxime, amide, acetal/ketal, ester/orthoester, and β-thiopropionate in the nanostructure to enable their surface charge switching in acidic environments, resulting in the disintegration of the nanoparticle membrane to release the therapeutic agent to the targeted tumor site. A common example is the use of the polymer poly (acrylic acid) for the encapsulation of the chemotherapeutic agents doxorubicin and paclitaxel. This anionic polymer shows enhanced hydrophilicity at lower pH due to the protonation of its carboxylic acid group, which aids in the targeted release of the antitumor drug at cancer tissues [10].

4.1.2 Drug release kinetics

Effective drug release from the nanocarrier shell is crucial for attaining optimal therapeutic effectiveness. In the tumor microenvironment (TME), pH-sensitive nanocarriers show a drug release kinetic profile with a significantly faster release rate in acidic conditions common in tumors compared to normal tissues, which have a neutral pH. This phenomenon leads to a rapid release of the drug when exposed to low pH, thereby enhancing drug concentration specifically at the tumor site. This is mainly achieved by engineering the nanocarrier using pH-responsive polymers that experience structural alterations, such as swelling or dissociation, at acidic pH, facilitating drug release from the nanocarrier system. Moreover, depending on the polymer configuration, a controlled release phase may occur

Table 20.2 In vitro and In vivo performance of charge-reversal nanocarriers.

SL No.	Nanocarrier type	Model system used	Stimulus sensitivity	Drug release kinetics	Cellular uptake efficiency	In vivo outcome	References
01	pH-sensitive polymeric micelles	HeLa cell line	pH-sensitive	Continuous drug release in acidic environments (low pH) occurs after a transition from a negative to a positive charge state, facilitated by micelle hydrolysis or dissociation upon cellular uptake through the endocytic pathway.	Enhanced cellular uptake	Enhanced targeting of specific tumor cells, increased cellular internalization, accelerated drug release, and mitigated multidrug resistance contribute to tumor suppression.	[42]
02	pH-sensitive liposomes	HepG2 cells	pH-sensitive	Burst release of drug molecules upon encountering endosomal pH levels	Significantly higher cellular uptake than non-responsive liposomes	Increased tumor penetration, low systemic toxicity	[5]
03	Enzyme-responsive micelles	U87MG cells	Enzyme (MMP-2)-sensitive	Enzyme-cleavage-dependent release upon exposure to MMP enzyme in the acidic TME	Enhanced cellular internalization through enzyme-triggered charge shift	Higher tumor selectivity reduced off-target side effects	[8]

04	Redox-sensitive polymeric nanoparticles	Prostate cancer cell line	Redox (GSH)-sensitive	Rapid and controlled drug release via disulfide bond cleavage and charge reversal due to exposure to elevated levels of GSH	Increased cellular uptake	Substantial inhibition of tumor growth observed in murine models.	[43]
05	Temperature-responsive nanogels	Lung cancer cell line	Heat-sensitive	Enhanced release of drugs at increased temperatures	Improved cellular uptake under hyperthermic conditions	Decreased tumor volume in animal models subjected to heat treatment	[44]

following the initial burst, allowing for prolonged drug exposure within the tumor. Factors including the pKa of the polymer or molecule used in the nanostructure's design, the structure itself, drug loading efficiency, and degradation rate affect the kinetics of drug release. Improving these kinetics facilitates controlled drug release, reduces the risk of premature leakage and clearance, and enhances therapeutic efficacy [45,46].

4.2 Enzyme-responsive nanocarriers for targeted therapy

4.2.1 Enzyme overexpression in disease states

Enzymes possess remarkable catalytic powers and biorecognition capabilities, making them a vital part of the bio-nanotechnology toolkit. Abnormal enzyme expression in cancer, resulting from metabolic and signaling changes that promote uncontrolled cell proliferation, survival, and invasion, presents numerous opportunities for the design of nanocarriers with enzyme-labile linkages. Research indicates the overexpression of various enzymes, such as matrix metalloproteinases (MMPs), hydrolases (including proteases, phospholipases, and glycosidases), cathepsin B, transferases, oxidoreductases, and hyaluronidase, in several cancers, including breast, lung, pancreatic, glioma, liver, prostate, and oral carcinoma. Enzyme-responsive charge reversal nanocarriers are engineered to exploit the tumor microenvironment (TME) by incorporating an enzyme-sensitive moiety. This linkage is cleaved upon recognition and interaction with a specific enzyme at the tumor site, resulting in a change in the nanocarrier's charge and facilitating the release of the therapeutic molecule. The selection of enzymes is dependent upon the target tissue, given that distinct enzymes exhibit overexpression in different cancer types [47].

4.2.2 Drug release and selectivity

Enzyme-responsive nanocarriers are nanoparticles engineered to release therapeutic drugs selectively upon encountering specific enzymes at target sites in targeted tumor therapy. On-demand drug release is attainable via alterations to the physical or chemical characteristics of nanocarriers or through the enzymatic cleavage of the drug. This enables targeted and selective delivery exclusively at the tumor site as incorporating enzyme-cleavable linkages into the nanocarrier design the encapsulated drugs are released solely in the presence of the targeted enzyme in the diseased area, thereby maximizing therapeutic efficacy at the intended site and preventing unwanted release in healthy tissues. The overexpression of matrix metalloproteinases (MMPs) has been documented in various tumor types, correlating with cancer pathophysiology. Matrix metalloproteinases-2 (MMP-2) and matrix metalloproteinases-9 (MMP-9) are predominantly studied for enzyme-mediated drug delivery applications. In a study, paclitaxel (PTX) was coupled with a peptide linker that functions as a substrate for MMP-2. The drug delivery systems are engineered to possess a positive charge initially, facilitating cellular uptake and accumulation at specific tumor sites characterized by overexpression of MMP-2. Subsequently, they transition to a negative charge when exposed to MMPs in the tumor microenvironment. Due to this charge switching, the attached enzyme experiences cleavage, releasing PTX and inhibiting cancer cell proliferation. Additionally, glycylphenylalanylleucylglycine tetra-peptide (Gly-Phe-Leu-Gly, GFLG) is frequently used as a linker in the design of enzyme-responsive nanocarriers. Exposure to the enzyme Cathepsin B results in the hydrolysis of the peptide bond, facilitating drug release following endocytosis in many cases of breast and pancreatic cancer. Additionally, various enzymes are used to develop these "smart" nanocarriers with enzyme-sensitive substrates aimed at

targeting specific cancer cells associated with tumor growth, proliferation, angiogenesis, invasion, metastasis, mutagenesis, and inflammation, thereby promoting effective cancer management and treatment [47–49].

4.3 Redox-responsive nanocarriers for cancer therapy

4.3.1 Intracellular redox environments

Cancer cells frequently show higher oxidative stress as a result of enhanced metabolism, mitochondrial dysfunction, and oncogenic signaling pathways. This results in redox imbalances that may cause oxidative damage if not adequately regulated. Therefore, cancer cells increase their survival by developing antioxidant defense mechanisms, leading to the overexpression of molecules such as glutathione (GSH), cysteine, and thioredoxin. That is, cancer cells exploit altered redox homeostasis to enhance proliferation, tumor progression, survival, and resistance to therapy within the organism. Manipulating redox homeostasis in the tumor microenvironment facilitates the targeted generation of oxidative stress in cancer cells through the development of redox-responsive nanocarrier systems, subsequently improving treatment efficacy and overcoming resistance mechanisms. The concentration of GSH in tumor cytosol was slightly elevated compared to normal cells, as it is often upregulated to counteract oxidative stress and prevent ROS-induced apoptosis. Redox-based therapies use GSH-sensitive linkages, typically disulfide bonds, integrated into the nanocarrier system to covalently link chemotherapeutic drugs with the structural components of the nanocarriers. Disulfide bonds, characterized by their reduction sensitivity, show significant redox responsiveness. In the reducing environment of the tumor microenvironment (TME), these bonds are cleaved by elevated levels of GSH, resulting in a charge reversal and the release of the encapsulated anticancer agent within cancer tissues [50].

4.3.2 Drug release in cancer cells

The design and production of GSH-responsive nanoparticles may serve as an option for on-demand intracellular drug delivery. In reaction to the elevated levels of GSH in tumor cells, disulfide bonds have been extensively used as the "gold standard" for the construction of redox-responsive charge-reversal nanocarriers. Disulfide bonds have a significant role in cancer, as their dysregulation leads to protein misfolding and abnormal cellular behavior contributing to cancer development. These bonds are commonly used as hydrophilic and hydrophobic polymers, as connectors between polymers and pharmacological agents, or as cross-linking agents in polymer drug carriers, and can be reduced to the hydrophilic thiol group by glutathione (GSH). When the redox-nanocarrier with disulfide linkage interacts with cancer cells, a thiol-disulfide exchange reaction takes place between glutathione (GSH) and the disulfide-containing nanocarrier, causing the nanocarrier to shift from a neutral/negative state to a positively charged state. This results in the targeted release of the medication in GSH-rich environments, such as cancer cells, following particle collapse. For instance, scientists have synthesized polyethylene glycol-disulfide bond-polyphosphate (PEG–SS–PEEP) and polyethylene glycol-disulfide bond-polycaprolactone (PEG–SS–PCL) incorporating the anticancer agent doxorubicin (DOX). These polymer micelles infiltrate the tumor cells, where the elevated levels of GSH cleave the disulfide link, therefore dismantling the nanocarrier shell and liberating DOX. Furthermore, diverse inorganic and lipid-based nanocarriers are constructed with redox-sensitive coatings for intracellular drug delivery. Disulfide linkages in the main chain confer biodegradability and bio-reducibility to the nanocarriers and hence more research is focusing on developing redox-based therapies targeting

disulfide bonds. However, the large-scale production and clinical application of redox-responsive nanosystems continues to pose a significant barrier [50,51].

4.4 Combination of multiple stimuli for enhanced efficacy

4.4.1 Dual & multi stimuli-responsive systems

Research has progressed beyond single stimulus-responsive charge reversal systems to discover nanocarrier systems that respond to more than one stimulus in the extracellular and intracellular environments of diseased cells, such as in cancer. These advanced and hybrid systems are referred to as 'dual stimuli-responsive systems.' The system depends on two triggers that sequentially alter the surface charge of the nanocarrier. Typically, the nanocarrier is predominantly negatively or neutrally charged to prevent premature clearance and reduce interactions with healthy cells. The initial stimuli induce a partial charge reversal, resulting in the carrier being slightly positive which is then followed by a second stimulus induces a total charge shifting, disrupting the nanocarrier and resulting in drug release into the diseased cells. Common examples include pH/GSH, ROS/Near-Infrared Light, pH/Thermal/GSH pH/ROS, ROS/Near-Infrared Light, etc. Due to their improved antitumor efficacy and decreased systemic toxicity, dual stimuli-responsive charge reversal nanostructures are attracting a lot of attention in anticancer therapy research. However, the intricacy of these delivery systems' designs poses serious difficulties. Sometimes it is challenging to precisely manage the distribution of therapeutic medicines because the multiple stimuli may not respond individually or concurrently as expected. Researchers are actively working to address these barriers to clinical translation [5,52].

4.4.2 Synergistic drug release mechanisms

Multi-stimuli-responsive systems have the capacity to improve treatment efficacy for diseases like cancer by using a synergistic approach that integrates several environmental stimuli. The integrated responses take place either simultaneously at the same site or sequentially across distinct compartments within the cell. For example, pH changes and enzymatic cleavage of anionic substructures can concurrently facilitate charge conversion. Upon exposure to the initial trigger or stimulus, the acidic tumor microenvironment protonates the polymer, shifting its charge from negative to positive. This improves the cellular uptake of the nanocarrier. Upon entry, the secondary stimulation initiates drug release via multiple processes. This sequential release mechanism leads to increased drug absorption, controlled release, improved tumor cell eradication, and enhanced combination therapy to counteract resistance. Examples include PCDMA micelles that utilize pH and reactive oxygen species (ROS) responsiveness for the delivery of podophyllotoxin and cucurbitacin B. This approach reverses charge in tumors to enhance uptake, while ROS further accelerates drug release to address multidrug resistance (MDR). A pH/thermal/GSH multi-responsive system uses disulfide-based polymers containing guanidinium and phosphate groups to achieve charge reversal in response to tumor acidity and heat. This design facilitates prolonged circulation, regulated drug release, and enhanced tumor suppression [53,54].

5. Biomedical applications of charge-reversal nanocarriers

Due to their remarkable capacity to alter their surface charge in reaction to specific environmental stimuli, charge-reversal nanocarriers have emerged as a transformative approach in biomedical science (outlined in Table 20.3). This characteristic improves their controlled and site-specific drug delivery, which makes

Table 20.3 Biomedical applications of stimuli-responsive charge-reversal nanocarriers.

SL No.	Application area	Stimulus type	Nanocarrier composition	Delivered therapeutic agent	Clinical/ Preclinical stage	References
01	Cancer therapy	pH-responsive, enzyme-responsive, redox-responsive	pH-sensitive functional groups linked lipid-based nanoparticles, polymeric micelles, dendrimers, hydrogels	Doxorubicin, Paclitaxle	Preclinical	[6]
02	Gene therapy	pH-sensitive, redox-responsive	Disulfide-linked polymeric nanoparticles and liposomes	siRNA, mRNA, DNA	Preclinical	[6]
03	Photodynamic therapy (PDT)	Light-responsive	Photosensitizer-conjugated lipid-based nanoparticles	Photosensitizing agents	Preclinical	[55]
04	Infectious disease	Enzyme-responsive	Enzyme-sensitive substrate-linked polymers, micelles, liposomes	Antibiotics	Preclinical	[1]
05	Immunotherapy	pH-responsive	pH-sensitive functional groups linked lipid-based nanoparticles and polymeric micelles	Cancer vaccines	Preclinical	[56]

them valuable in a variety of applications in medicine. Charge-reversal nanocarriers have shown great promise in the following important areas.

5.1 Cancer therapy

5.1.1 Effect of surface charge on nanocarrier drug delivery to tumors

The effectiveness of nano drug delivery systems (NDDSs) in cancer therapy relies on the absorption of therapeutics by tumor cells. The cellular uptake profile indicates delivery efficiency and the bioavailability of nanocarriers [57–59]. Surface charge is a critical physicochemical property of NDDSs that significantly affects biological responses to delivery systems. Studies demonstrate that electrostatic interactions between charged nanocarriers and the cytomembrane play a crucial role in cellular uptake. Positively charged nanocarriers generally demonstrate greater internalization and improved cellular uptake efficiency relative to negatively charged ones; however, this positive surface charge also results in the swift clearance of nanocarrier drug delivery systems from the bloodstream. The principal determinant for the clearance of cationic nanocarriers is the electrostatic interaction between negatively charged albumin and positively charged nanoparticles [60,61]. The normal blood pH range is 7.35–7.45, whereas the isoelectric point of most blood proteins is lower than 7, suggesting that these proteins predominantly possess a negative charge [62,63]. Human serum albumin (HSA) has a negative charge during blood circulation due to its isoelectric point of 4.7. As a result, through electrostatic attraction, positively charged nanocarriers strongly associate with negatively charged proteins, affecting how nanoparticles interact with cells and how nanodrug delivery systems distribute within the biological system. Neutral or slightly negatively charged nanoparticles show prolonged resistance to protein adsorption [64]. Once nanoparticles interact with biological environments, they undergo modifications due to the adsorption of biomolecules on their surface, a phenomenon referred to as the protein corona. The development of a protein corona modifies various physicochemical characteristics of nanoparticles, such as surface charge, dimensions, and aggregation state. These alterations can directly or indirectly affect biological processes, including pharmacokinetics, therapeutic efficacy, and the mechanisms of endocytosis for the cellular uptake of nanomaterials. Although it has been suggested that positively charged nanoparticles typically attract negatively charged proteins, empirical evidence demonstrates that biological environments primarily support negatively charged, corona-covered nanoparticles [65]. The negatively charged cytomembrane, attributed to surface anionic chemical entities, facilitates high adhesion of positively charged nanocarriers. This association improves cellular uptake owing to the robust electrostatic attraction between charged nanoparticles and cell membranes. Cationic nanocarriers may enhance clathrin-mediated and caveolae-mediated endocytosis, which are the principal mechanisms for the cellular uptake of nanodrug delivery systems [66]. The impact of the surface charge of positive particles on cellular uptake is still a topic of debate. Positively charged magnetic nanoparticles demonstrated enhanced internalization in human breast cancer cells relative to negatively charged nanoparticles. However, no notable internalization effect was detected in human umbilical vein endothelial cells [67]. The protein corona may explain this outcome. A positive surface charge can result in considerable negative consequences, as it may lead to strong interactions with serum components in blood and non-specific adhesion to normal cells, potentially causing hemolytic reactions and tissue or cell toxicity [5,68,69].

5.1.2 Gene therapy for cancer using charge reversal nanocarriers

Gene therapy plays a significant role in tumor treatment, using viruses as gene delivery vehicles. Viral vectors present challenges including immunogenicity, tumorigenicity, and various other adverse effects [70]. Consequently, the development of gene vectors with high transfection efficiency and low cytotoxicity is crucial for effective gene therapy. Charge-reversal nanosystems have become increasingly relevant in gene delivery applications in recent years [71,72]. Researchers have designed a nanodelivery system where the coding plasmid (pDNA) was encapsulated within lipid nanoparticles (LNPs), which were altered with fish sperm protein conjugated with palmitic acid on the surface and then coated with sodium tripolyphosphate (TPP) as the outermost layer. The transfection efficiency was significantly increased compared to pDNA alone, and human alveolar epithelial cells (A549) displayed remarkable tolerance [73]. Zhang et al. fabricated a polymeric nanocarrier (BTIL) comprising B-PDEAEA/DNA polyplex cores and liposome coatings infused with IR780. Low-intensity ultrasonic irradiation of IR780 generated increased amounts of reactive oxygen species (ROS), leading to a charge reversal of the ROS-responsive B-PDEAEA polymer, which promoted intracellular gene release and improved gene transfection efficiency. BTIL displays extended stability in blood circulation and shows significant efficacy in gene transfer while reducing negative effects [74]. This study introduces novel concepts for non-viral gene delivery vectors [6].

5.1.3 Delivering contrasting agents for tumor imaging

For the screening, diagnosis, and assessment of tumors, imaging methods like computed tomography (CT) and magnetic resonance imaging (MRI) are essential. Contrast agents enhance diagnostic accuracy by augmenting contrast levels [75,76]. There is a pressing necessity to create nanocarriers capable of selectively delivering contrast agents to tumor tissue. Researcher Feng et al. created a tumor microenvironment-responsive nanocarrier (MB@MSP) utilizing mesoporous silica nanoparticles (MSNs) and Fe3O4 as the core material, with the photosensitizer methylene blue (MB) enclosed in the mesopores of the MSNs. The MMP2-degradable peptide PDPPA-1 was conjugated to the core via disulfide bonds. In the tumor matrix, increased levels of MMP2 cleave PDPPA-1, whereas elevated concentrations of GSH promote disulfide bond cleavage. This process results in size reduction and charge reversal, enhancing the ability of nanocarriers to penetrate tumors. MB@MSP enables in vivo MR and μCT imaging and enhances the apoptosis of immunogenic malignant cells [77]. He et al. co-encapsulated gadolinium oxide (Gd2O3) and doxorubicin (DOX) within mesoporous silica nanoparticles (MSNs) to manufacture hybrid nanoparticles (Gd2O3@MSNs), which were further coated with pH-responsive polyelectrolytes. Charge reversal took place in acidic conditions, enabling the simultaneous administration of the contrast agent (Gd2O3) and the therapeutic agent [6,78].

5.1.4 Chemoimmunotherapy with charge reversal nanocarriers

Chemotherapy may have cytotoxic effects on tumor cells in malignant tumors, although immune escape and suppression hinder its actual effectiveness [79]. Immunotherapy in conjunction with chemotherapy has emerged as a novel approach for cancer treatment. Drug transport efficiency is improved and biological obstacles are successfully addressed by the development of size- and charge-variable nanocarriers. Cancer treatment often uses immune checkpoint blockade (ICB) therapy, which inhibits T cells from activating, sends signals that weaken the immune system, and helps tumor cells avoid the immune system by working with PD-1 and PD-L1. Thus, obstructing the PD-1/PD-L1 axis has become a viable approach for augmenting T cell-mediated anti-tumor immune responses [80]. Liu

et al. created a yolk-shell liposome co-loaded with JQ1 and DOX, exhibiting fast changes in zeta potential from −4.90 to +2.35 under low pH or acidic conditions, hence enhancing cellular internalization. The blockade of the PD-L1 pathway and DOX-induced immunogenic cell death (ICD) through JQ1 facilitated the effective implementation of chemoimmunotherapy [6,81].

5.1.5 Co-delivery system with charge reversal nanocarriers

The study revealed that a single pharmacological treatment often fails to achieve the desired therapeutic effect and is also likely to cause difficulties, including drug resistance in cancer cells. Combination therapy for cancers uses two or more distinct therapeutic agents that impede tumor growth via diverse mechanisms, yielding a therapeutic effect that exceeds that of a singular drug and mitigating tumor resistance. CRNDDS-based co-delivery systems, including dual-gene, dual-drug, and co-loading systems of drug and gene, are expected to be used as a holistic approach for the treatment of malignancies. Researcher Zhang et al. developed a dual-responsive drug delivery system (DMA-ss-NPs) that is pH and redox sensitive for the co-delivery of all-trans-retinoic acid (ATRA) and paclitaxel (PTX) in cancer treatment [82]. The findings indicated that the co-carrier delivery method outperformed the single drug delivery approach. In comparison to control groups, the DMA-ss-NPs effectively inhibited tumors. Numerous more pharmaceuticals for tumor-targeted CRNDDS functioned as multiple drug co-delivery systems, including triptolide and doxorubicin [43], as well as paclitaxel and disulfiram. An improved method for the delivery of plasmid DNA (p53) and Killer Red, activated by tumor acidosis, was developed [83]. At a pH of 6.8, the acidic environment led to the protonation of amino groups in the DNA complex, resulting in a reversal of charge that enhanced cellular absorption and transfection. The carriers were easily accessible and effective in targeting the tumor tissue microenvironment. The drug-gene co-delivery system has gained more extensive research attention compared to dual-drug and dual-gene co-carrier delivery systems. A unique charge-reversal PLGA nanobubble was produced. The result of the study indicated that these nanobubbles were able to co-deliver DOX and P-gp shRNA into tumor cells, effectively suppressing P-gp expression and enhancing the overall therapeutic effects. Zhang et al. designed a pH- and redox-responsive nanoparticles utilizing a charge-reversal pullulan derivative (CAPL) and disulfide-containing poly(β-amino ester) (ssPBAE) for the concurrent delivery of gene therapy and the anitneoplastic agent methotrexate (MTX) [84]. The findings indicated that these nanoparticles could markedly hamper cellular proliferation. Additionally, Wang et al. created pH-responsive core-shell nanoparticles that can effectively deliver both the chemotherapeutic drug DOX and plasmid DNA (pDNA) which was further validated by a laser confocal microscope [85]. Guan et al. devised a pH-sensitive charge-reversal technique for the co-delivery of the anticancer agent cis-aconityl doxorubicin and DNA [86]. Furthermore, Yu et al. developed a triple-layered pH-responsive micellular system for the targeted treatment of metastatic breast cancer [87]. The study's findings suggested that the micelleplex co-loaded with siRNA-p65 and alkylated cisplatin prodrug could inhibit tumor growth and reduce distant metastasis of cancer cells [88].

5.1.6 Protein delivery using charge reversal nanocarriers

Over the past decade, advancements in biotechnological methods and genetic engineering have led to the development of pharmaceutically active peptides and proteins. Nonetheless, the application of these novel therapeutic biomolecules is limited by their considerable size, short plasma half-life, high elimination rate (vulnerability to enzymatic and body fluid destruction), difficulty to penetrate cell

membranes, and poor oral bioavailability. This leads to the consistent administration of drugs over a prolonged treatment period when these biomolecules are used in clinical environments [12]. Protein drugs are typically delivered through oral and parenteral routes. Deteriorating factors, including water and enzymes, limit the effectiveness of these methods.

The encapsulation of these drugs within a designated carrier offers a feasible approach to mitigate these challenges. The use of nanoparticles for protein delivery to biological compartments is a promising method to improve protein bioavailability. Polylactic acid (PLA) and its copolymer with glycolic acid (PLGA) have received approval from regulatory authorities for parenteral administration in the form of implants (e.g., Zoladex) and microparticles (e.g., Decapeptyl, Parlodel LA, and Enantone Depot) [89]. The design of a novel protein nanoparticle enhances protein targeting and activity in vivo. This approach may (1) enhance treatment outcomes while minimizing adverse effects; (2) prevent drug resistance, as excessive dosing and imprecise targeting can induce resistance in pathological conditions; and (3) address drug resistance mechanisms through sustained local drug accumulation [90,91].

Charge-reversal polymeric carriers have been recognized as progressively efficient protein carriers. Compared to conventional protein carriers, these demonstrate superior characteristics, such as increased stability, modified site specificity, enhanced blood circulation stability, and stimuli-responsive release [90,91]. Akiyoshi et al. devised an effective system for intracellular protein delivery through the use of self-assembled cationic nanogels. This study examined the interaction between proteins and self-assembled nanogels derived from cationic cholesteryl group-bearing pullulans (CHPNH2). The cationic nanogels demonstrated significant interactions with bovine serum albumin (BSA), leading to the creation of monodispersed nanoparticles under 50 nm in size. The complex was successfully internalized into HeLa cells. Kissel et al. [92] developed negatively charged nano-carriers using polymer blends of PLGA and poly (styrene-co-4-styrene-sulfonate) (PSSS) for the delivery of positively charged proteins, including lysozyme. The nanoparticle demonstrated a significant density of negative charge, hence augmenting the protein loading capability. Moreover, Raichur et al. [93] constructed stable hollow microcapsules composed of sodium carboxymethyl cellulose (CMC) and PAH using layer-by-layer procedures, enabling the adsorption of polyelectrolytes onto CaCO3 microparticles. Bovine serum albumin (BSA), a positively charged protein, was spontaneously encapsulated below its isoelectric point within hollow microcapsules. Within the acidic pH range, the fluorescent probe FITC-dextran exhibited effective loading, signifying the permeability of the shell. An elevation in pH led to a reduction in permeability. FITC-dextran encapsulation did not transpire at pH values over 7.0, indicating that the capsule walls demonstrated impermeability. At acidic pH, the amino groups of PAH become protonated, leading to a localized concentration of positive charges. The electrostatic repulsion of these charges resulted in a shift from a continuous morphology to a nanoporous structure within the walls of microcapsules. Elevating the pH led to a reversal of the microcapsule wall charge, reducing electrostatic repulsion and consequently resulting in layer compaction and pore occlusion [12,94].

5.2 Gene therapy

5.2.1 Using enzyme-responsive charge reversal polymeric nanocarriers

Researcher Qiu et al. introduced an esterase-responsive cationic polymer, PQDEA, designed for intraperitoneal gene therapy as an alternative to chemotherapy agents. PQDEA undergoes degradation

by intracellular esterases, leading to the production of negatively charged poly (acrylic acid). Thus, in the cytosol, polyplexes can efficiently dissociate and release free DNA for transcription. The lipidic-PQDEA/DNA polyplexes, LPQDEA/DNA, demonstrate resistance to serum and various bodily fluids, thereby enhancing gene delivery efficiency, particularly in intraperitoneal tumors with ascites. The vector delivers a suicide gene, pTRAIL, and effectively induces apoptosis in tumor cells. In the HeLa i.p. tumor model, LPQDEA/pTRAIL therapy significantly inhibits tumor growth, showing low tumor recurrence and substantially extended survival during the treatment-free interval compared to PEI 25K/pTRAIL and two first-line anticancer agents, CPT11 and PTX. Moreover, the gene treatment shows much diminished unfavorable effects. This study demonstrates that, given the present gene-delivery efficiency, nonviral gene vectors may have a specific role in certain localized gene therapy applications [11].

5.2.2 siRNA Silencing by charge reversal polyelectrolyte gold nanoparticles

Scientist Guo et al. demonstrated the application of charge-reversal polyelectrolyte-deposited gold nanoparticles as carriers for gene delivery. Innovative gold nanoparticles enhance gene delivery efficiency and modulate gene expression in RNA interference. This suggests that charge reversion in acidic environments facilitates the release of gold nanoparticle/nucleic acid complexes from endosomes and lysosomes, leading to the subsequent release of functional nucleic acids into the cytoplasm. The layer-by-layer method effectively produces multifunctional gold nanoparticles for therapeutic applications. The application of a polyelectrolyte layer containing PEG and ligand moieties on the surface of PEI/PAH-Cit/PEI/AuNPs increases their blood circulation time and enhances drug bioavailability. These nanoparticles possess considerable potential for in vivo delivery of nucleic acids in clinical settings [95].

5.3 Attacking infectious agents using charge reversal nanocarriers

Antibiotics are among the greatest medicinal advances. As microorganisms become more resistant to antimicrobials and humanity may face a post-antibiotic era, new pathogenic germ-fighting tactics are needed. Up to 80% of infections caused by bacteria involve biofilms. Due to delayed diffusion, antibiotic inactivation or metabolic dormancy, and persistent cells, this biofilm makes bacteria more antibiotic-tolerant [96,97]. As protection, microorganism-infected tissues and biofilms have a pH below seven to create anionic and acidic barriers. Bacterial metabolism and the human immune system cause localized infection area acidification, which reduces antibiotic efficacy. As acidic conditions reduce antibiotic activity, charge-reversible NPs that can encapsulate these drugs are being developed. Cationic nanoparticles show significant potential; however, their antibiotic efficacy is limited by adsorption to extracellular compounds, neutralization by charged ions, degradation by extracellular enzymes, and inactivation due to environmental factors such as pH or redox conditions [98]. Charge-reversible NPs for antibiotic delivery may solve this problem [99]. At physiological pH 7.4, pH-responsive charge-reversible NPs mask nonspecific interactions and bind bacteria at pH 6.0, according to Zhu et al. The antibiotic, vancomycin when encapsulated in such NPs worked better at lower pH levels than as a free drug [100]. Hu et al. created charge-reversible pH-responsive NPs to improve biofilm penetration and cellular uptake. NPs were created by combining α-CD-CD-NO and α-CD-Ce6 into a pH-sensitive polypeptide copolymer (PEG-KLAKLAK)2-DA, resulting in α-CD-Ce6-NO-DA NPs. The enhanced penetration of these NPs into biofilms is attributed to their surface

charge transition from negative at physiological pH to positive at acidic biofilm pH [101]. Additionally, Burcu et al. studied charge-reversible enzyme-responsive NPs. Antifungal protein (PAF) and polyphosphate (PP) were used to create phosphate-degradable NPs. A zeta potential change from −12 mV to virtually neutral indicated PP breakage by alkaline phosphatase in Candida albicans biofilm, enhancing antifungal effectiveness [102]. Da Costa et al. created rifampicin-encapsulated PLA NPs. Biofilm penetration was higher than positively charged NPs [103]. Photodynamic treatment is a new way to study microbial biofilm in illnesses. High-activity cationic photosensitizers are harmful but useful for antibacterial action. Wu et al. created pH-sensitive photodynamic NPs from Rose Bengal (RB) PDA coated with polymyxin B (PMB) and gluconic acid (GA). At physiological pH, these NPs are negatively charged, but biofilms' acidic pH makes them positively charged, allowing them to cling to bacteria and promote photoinactivation [104]. Later research by Wang et al. examined guanidine-caged biofilm-responsive nanoparticles. Covalently attaching guanidine to acid-cleavable moieties to hide the positive charge made CGNs negatively charged at neutral pH. Upon reaching the biofilm, the acidic pH cleaved the negative functional groups, leading to positive surface charges on the CGN, which facilitated deep biofilm penetration, bacterial attachment, and elucidation [105]. Silver nanoparticles (AgNPs) are extensively used as antibacterial agents owing to their significant antimicrobial efficacy and limited resistance. Mammalian cell cytotoxicity presents significant challenges. Qiao et al. developed a zwitterionic system by functionalizing AgNPs with carboxyl betaine groups (AgNPs-LA-OB). Acidic conditions altered the charges of these nanoparticles. AgNPs-LA-OB exhibited antibacterial activity, and biofilm eradication without harming any healthy cells [106]. Wu et al. investigated pH-responsive charge-reversible silver nanoparticles. Poly (ethylene glycol)-poly (aminopropyl imidazole-aspartate)-polyalanine served as a ligand. As pH decreased, imidazole underwent ionization. The nanoparticles enhance antibiofilm activity by altering their zeta potential from −3.85 to +1.00 mV under physiological and acidic conditions [107]. Gao et al. synthesized azithromycin-conjugated clustered (AZM-DA) nanoparticles for antibiofilm evaluation in a separate study. The electrostatic complexation of AZM, amino-ended poly(amidoamine) dendrimer (PAMAM), and 2,3-dimethyl maleic anhydride (DA) with poly(ethylene glycol)-block-polylysine resulted in the formation of nanoparticles (NPs). At acidic pH, negatively charged AZM-DA nanoparticles disassembled, resulting in the formation of positively charged secondary PAMAM-AZM nanoparticles, which enhanced penetration and antibiofilm efficacy [108,109].

6. Clinical translation potential and challenges

The transition from the development of stimuli-responsive drug-delivery systems from laboratory research to applying in clinical settings is challenging. This complexity arises from their typically sophisticated designs, which complicate potential drug development, particularly regarding manufacturing processes, reproducibility, and quality control. Furthermore, the translation of each stimulus from preclinical research models to routine clinical practice frequently necessitates substantial optimizations and improvements. Endogenous triggers are challenging to regulate due to their variability among patients, exemplified by factors such as tumor pH and the presence of reducing agents in the bloodstream. While systems that respond to external stimuli show greater potential, significant advancements are required to enhance tissue penetration depth and focus to prevent damage to healthy tissues [110].

The biocompatibility and toxicity of nanocarriers used for drug delivery in the human body must be meticulously monitored and regulated, especially for inorganic materials such as carbon nanotubes and mesoporous silica nanoparticles (MSNs) in the development of charge-reversal nano-drug delivery systems (CRNDDSs). Studies demonstrate that the nature and concentration of metal impurities, the dimensions and varieties of carbon nanotubes, the inclusion of solubilizers, and the functionalization of carbon nanotubes can affect their toxicity [111]. It is essential to carefully assess these factors when utilizing carbon nanotubes for the development of CRNDDSs. Additionally, carbon nanotubes present challenges such as limited solubility, low biodegradability, and inadequate dispersibility, indicating an urgent requirement for optimization or replacement of this material [112]. The toxicity of MSNs is influenced by factors such as particle shape, size, pore size, and the method of synthesis. Research demonstrates that MSNs with a particle size of 55 nm show lower cytotoxicity in comparison to other particle sizes [113]. Researchers using MSN as a carrier must evaluate its surface modification to determine biological distribution, alongside the MSN's properties to reduce cell toxicity. The size of dendritic polymers primarily determines their toxicity. Poly(amidoamine) dendrimers (PAMAM) are classified into various generations. The dimensions of PAMAM from Generation 0 to 5 (G0–G5) remain below 5.7 nm, are predominantly metabolized by the kidneys, and demonstrate low toxicity. PAMAM from Generation six to 9 (G6–G9) exceeds 7.2 nm in size, is metabolized in lymph nodes or the liver, and shows high toxicity [114].

There exist concerns about the factors that initiate charge reversal. The tumor microenvironment (TME) shows heterogeneity, characterized by variations in pH values and specific enzyme concentrations among different tumor types. These differences may influence the charge reversal of pH-responsive and enzyme-responsive charge-reversal nanodrug delivery systems (CRNDDSs) within the TME [115]. Most photoresponsive CRNDDSs necessitate stimulation by near-infrared (NIR) light. NIR light shows greater penetration depth compared to visible and ultraviolet light; however, its effectiveness may be limited in thicker skin and muscle tissues [116]. Currently, the majority of research involves mice with subcutaneously implanted tumors, and the effectiveness of treatments for human tumors remains uncertain [117]. Consequently, tumors appropriate for the application of photoresponsive CRNDDSs must be identified to facilitate the effective penetration of light.

The tumor microenvironment (TME) shows considerable heterogeneity, which has attracted substantial interest in recent years because of its essential influence on treatment efficacy and the emergence of drug resistance. Different tumors display distinct tumor immune microenvironments, necessitating targeted drug development to deliver specific treatments for these varied immune microenvironments. CRNDDSs should not be confined to the presently recognized stimulating factors; further specific triggering conditions require investigation [6].

The potential of charge-reversal nanocarriers in cancer nanomedicine has gained significant interest; however, the biosafety concerns related to charge reversal in biological or physiological environments remain poorly understood and warrant thorough and systematic investigation. Nanocarriers with a positive surface charge are recognized for their ability to enhance cellular uptake; however, they also provoke unwanted interactions with anionic serum components. This leads to enhanced absorption by the reticuloendothelial system (RES) in the liver and spleen, resulting in a reduction of the injected dose, decreased circulation time, and a notable immune response. The dilemma may be addressed by implementing a charge-reversal strategy. The integration of a charge-reversal moiety (i.e., negative-to-positive conversion) into the nanocarrier facilitates the temporary masking of its positive surface charge. This modification confers anionic or neutral properties during

blood circulation, resulting in extended circulation time and diminished immune response. Currently, most research has concentrated exclusively on the systemic toxicity or immune response resulting from the systemic administration of charge-reversal nanocarriers in murine models. Prior to negative-to-positive conversion, smart nanocarriers are generally neutral or anionic, exhibiting high biocompatibility as a result of the strategic design of biocompatible nanocarriers. Upon the negative-to-positive conversion at the target site, specifically the tumor microenvironment, nanocarriers with a cationic surface charge are recovered. A modification in surface charge may induce charge disturbances within the tumor microenvironment, potentially adversely affecting the anionic cell membrane [118,119], physiological polyanions including enzymes and cell receptors [120], and possibly the physiological functions of organs. Following charge reversal, the regenerated positively charged nanocarriers are expected to demonstrate a toxic effect akin to that of traditional cationic nanocarriers. At present, there is a lack of research on the potential toxic effects or immune responses associated with charge-reversal nanocarriers transitioning from a negative to a positive charge. Due to the significant relevance of these biosafety issues, they require comprehensive investigation in future research [9].

7. Conclusion

Stimuli-responsive charge-reversal nanocarriers present a groundbreaking method in drug delivery, providing distinct advantages like enhanced targeting, controlled release, and increased cellular uptake. By altering the surface charge dynamically, these nanocarriers enhance the delivery of therapeutic agents in a controlled way, improving drug bioavailability and reducing systemic side effects. This chapter emphasizes the essential influence of environmental triggers, such as pH, enzymes, and redox conditions, on the charge-reversal process. These triggers facilitate targeted delivery of drugs directly to diseased areas, especially within acidic tumor microenvironments or inflamed tissues, thereby enhancing therapeutic effectiveness. The ability to alter the surface chemistry of these carriers significantly boosts their adaptability, allowing for customization across various biomedical applications, including cancer therapy and gene delivery. Even with these encouraging benefits, challenges remain in advancing these technologies from the lab to clinical settings. Challenges like large-scale synthesis, biocompatibility, and long-term stability need additional research. Nevertheless, the swift progress in nanotechnology and materials science offers a hopeful perspective for addressing these challenges, bringing us nearer to personalized, precision medicine. Stimuli-responsive charge-reversal nanocarriers present considerable potential for advancing targeted drug delivery in the future. Ongoing research and innovation in this field are expected to produce more efficient, adaptable, and personalized therapeutic strategies, ultimately leading to more effective treatments for complex diseases.

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CHAPTER

Stimuli-responsive nanocarriers for image-guided therapy 21

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1. Introduction

With the interdisciplinary development of nanotechnology, material sciences, and pharmaceutical sciences, the research of nanotherapeautics have achieved great progress [1,2]. Some novel nanomaterials with good biocompatibility and unique properties, such as liposomes, polymeric micelles, and various nanoparticles (NPs), have been applied as nanocarriers to construct nano drug delivery systems (DDS). Comparing with traditional drugs (e.g., small molecule drugs, nucleic acid drugs, and protein drugs), nanocarrier-based nanotherapeautics exhibit many advantages. First, several different therapy modalities can be integrated into one nanoplatform, and these modalities may have synergistic effect, which can improve the outcome and reduce side effects. Second, external or endogenous stimuli-responsive moieties can be designed into the DDS, therefore the loaded drugs can be released on demand at the designated disease sites. Third, apart from the therapy modules, different imaging modules can also be integrated into the NPs and realize image-guided therapy [3–5].

In comparison with the free small-molecule drugs, NPs with a size of approximately 100–300 nm could accumulate passively in tumor location via the enhanced permeability and retention (EPR) effect [6]. They exhibit the abilities to efficiently carry the drug to the tumor site, increase drug uptake, reduce drug toxicity, and prevent early drug release. To utilize the pathological cues for precise treatment, intelligent nanomaterials capable of degradation or conformation changes have been devised with specific responses to external stimuli or endogenous stimuli for targeted delivery and on-demand drug release at disease sites, which could significantly enhance therapy efficacy and reduce off-target and adverse side effects. Stimuli-responsive controllable DDS appear to be particularly important in alleviating the poor efficacy and cytotoxicity caused by premature drug release [7–9].

Commonly, the stimuli applied in responsive DDS can be divided into two main categories: external stimuli and endogenous stimuli. External stimuli mainly include light irradiation, ultrasound (US), magnetic field, and X-ray radiation. These stimuli can be applied directly at the disease sites, and the parameters as well as duration of action can be set according to the requirement of therapy [10–12].

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Once subjected to these stimuli, the stimuli-responsive nanocarriers would release the loaded cargoes and exert imaging functions at the same time. Endogenous stimuli commonly refer to the specific physiological environment at disease sites that are quite different from healthy tissues. For example, tumor microenvironment (TME) is characterized by acidic microenvironment, high concentrations of glutathione (GSH) and reactive oxygen species (ROS), and the relatively anoxic environment [13,14]. The differences in the physiological microenvironment between TME and normal tissue provide an attractive strategy to achieve selective accumulation of NPs at the tumor site. Therefore, TME-responsive nanotheranostic agents have been extensively explored for precise cancer therapy and imaging.

The integration of therapeutic functions and imaging functions into one single nanoplatform allows precise treatment of disease, real-time monitoring of drug release, and evaluation of treatment efficacy, which can in turn guide the process of therapy. At the moment, the mainstream imaging modalities that can be integrated into nanotherapeutics mainly include fluorescence imaging (FLI), US imaging, near infrared thermal imaging, photoacoustic imaging (PAI), computed tomography (CT), and nuclear magnetic resonance imaging (MRI) [15,16]. Among them, CT is one of the most commonly used non-invasive clinical imaging modalities because of its wide availability, high spatial resolution, unlimited depth, and accurate anatomical information with reconstructed three dimensional (3D) imaging. MRI does not involve ionizing radiation and achieves 3D anatomical coverage with flexible imaging orientations even for tissues deep in the body. PAI is a novel imaging modality that has been widely used in recent years. It relies on the photothermal effect of nanomaterials to generate acoustic waves, which can be detected and converted into imaging signals [17–19].

In this chapter, we center on stimuli-responsive nanocarriers, and divide the nanocarriers into three main categories: external stimuli-responsive nanocarriers, endogenous stimuli-responsive nanocarriers, as well as external and endogenous stimuli dual-responsive nanocarriers. In each part, we introduce how these smart DDS are constructed, and highlight how the stimuli trigger the drug release from the nanocarriers, and how the imaging properties guide the therapy of diseases.

2. External stimuli-responsive nanocarriers

2.1 Light irradiation-responsive nanocarriers

Photo-based therapeutic approaches such as photothermal therapy (PTT) and photodynamic therapy (PDT) have gained extensive attention owing to their noninvasiveness, controllability, high spatiotemporal precision, as well as negligible drug resistance [20–22]. External light sources that optimally penetrate the target tissues can cover solid tumors more efficiently, and the penetration depth of the laser increases with increasing wavelength. Light in the second near-infrared window (NIR-II, 1000–1700 nm) allows for the deeper tissue penetration due to its reduced tissue scattering and decreased interference by proteins compared to the light in the first near-infrared window (NIR-I, 700–1000 nm) [23]. Among the lasers of different wavelengths, 808 nm laser is especially attractive due to the minimum absorption of water in this region, which can significantly overcome the heating effect and mitigate the damage to surrounding healthy tissues [24].

The clinical application of light-responsive drug delivery systems is severely restricted by uncontrollable drug loading, release, and adverse effects caused by the introduction of other contrast agents. To solve this problem, Wang and coworkers designed light-responsive dual prodrug polymer

NPs (DPP NPs) for precise synergistic chemotherapy under the guidance CT imaging without introducing any additional imaging agent [25]. DPP NPs enabled visible light (430 nm)-triggered prodrug polymer backbone cleavage and bioactive Pt(II) release at tumor site. In addition, the light-cleaved polymer fragments were further hydrolyzed into dimethyl cantharidin. Due to the high content of Pt in the polymer chain, the spatial/temporal biodistribution and metabolism of DPP NPs in vivo could be monitored by CT imaging. In vivo synergistic anti-cancer efficacy of DPP NPs was evaluated in U14 xenograft tumor-bearing KM mice. Comparing with other groups, DPP NPs with light irradiation exhibited superior tumor growth inhibition effect. The tumor volume was smaller than 100 mm^3 and 75% of mice in the DPP NPs group were fully cured after CT imaging-guided light irradiation.

Deng et al. developed CuS@Cu_2S@Au nanohybrid which can not only realize a photoswitchable targeting effect but also act as a PAI/NIR thermal imaging agent, providing a versatile platform for image-guided and NIR laser-responsive targeted cancer therapy [26]. The hollow-structured CuS (HCuS) was prepared by one-pot sacrificial template method, and then gold NPs were deposited on the surface of CuS. After the surface reduction of CuS to Cu_2S, the functional polymers of azobenzene-RGD-contained SH-PEG and p(OEOMA-co-MEMA) were modified on the surface of HCuS@Cu_2S@Au in sequence by Au-S bond. The cis-conformation RGD molecules were embedded in the long-chain p(OEOMA-co-MEMA) to form the nonadhesive surface. Under 808 nm laser irradiation, the thermally sensitive polymer and *cis-to-trans* isomerism of azobenzene units would shrink and make the RGD molecules expose and then realize the photoswitchable targeting effect. HCuS@Cu_2S@Au solution (Cu: 100 ppm) under 808 nm laser irradiation (0.8 W cm^{-2}, 7 min) induced a 63°C temperature increase in vitro and temperature at the tumor site increased to 47.8°C. Meanwhile, the elevated temperature could be applied in NIR thermal imaging. In addition, the prepared CuS@Cu_2S@Au nanohybrid was also ideal PAI agent. PA signal can be obviously detected only after 30 min of HCuS@Cu_2S@Au-P-RGD injection with the NIR pretreatment. In vivo tumor-inhibitory effect of CuS@Cu_2S@Au nanodrug was evaluated on U87MG tumor-bearing nude mice. The combination of PTT and chemotherapy induced a maximum tumor recession in all the mice. In addition, the treatment did not cause obvious body weight change of the mice, demonstrating the prepared nanodrug could reduce off-target toxicity and nonspecific absorption.

Zhang et al. synthesized fluorinated aza-boron-dipyrromethene (NBF) with high NIR absorption property to load DOX and liquid PFCs (PDNBF NPs) for imaging-guided therapy of cancer [27]. Four amino groups were introduced to aza-BODIPY and a significant redshift from 659 to 818 nm in the absorption compared to the parent 1,3,5,7-tetraphenylaza-BODIPY was achieved, which made it an ideal agent for PTT. PDNBF NPs were stable at physiological temperature. While under 808 nm laser irradiation, the hyperthermia generated by NBF resulted in liquid-gas phase transition of PFC droplets, which not only triggered the burst release of DOX, but also enhanced echo signals for ultrasound (US) imaging. PAI results showed that after intravenously injection, PDNBF NPs exhibited high efficiency tumor accumulation. Together with the burst release of DOX triggered by 808 nm laser irradiation, the remarkable synergistic effect of PTT and chemotherapy was achieved to inhibit tumor growth.

The ubiquitous hypoxia in solid tumors and inadequate content of endogenous H_2O_2 extremely confine the potency of stimuli-responsive nanotherapeautics. To increase the PDT efficacy of cancer, Ding et al. chose CuS NPs as nanocarrier, which has excellent photothermal properties, to engineer peroxymonosulfate (PMS)-loaded hollow mesoporous CuS NPs for 1064 nm laser-responsive tumor therapy in an oxygen-independent manner (Fig. 21.1A) [28]. The prepared CuS@PMS NPs were embedded into thermosensitive hydrogel (CuS@PMS-Gel) to ensure affluent drug accumulation in the

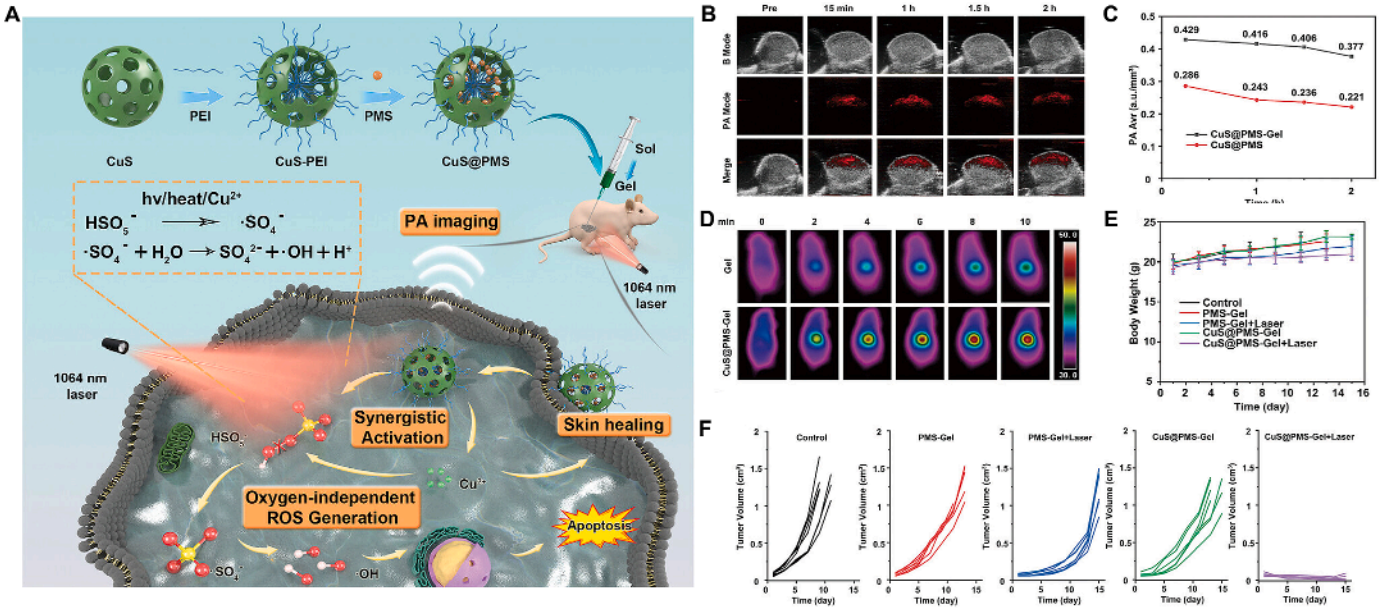


FIGURE 21.1

(A) Schematic illustration of the fabrication and theranostic functions of CuS@PMS-Gel for PAI-guided PDT of cancer. (B) PA images in tumor sites before and after intratumoral injection of CuS@PMS-Gel. (C) The average value of PA signal at tumor sites at different time points. (D) The infrared thermal images of B16F10 tumor-bearing nude mice with intratumoral injection of Gel or CuS@PMS-Gel after 1064 nm laser irradiation (1 W cm^{-2}) at different time points, (E) Body weight curves of mice after different treatments. (F) Individual tumor growth curves of mice after different treatments. Adv. Sci. 2022, 9, 2200974.

tumor region. After intratumoral injection of CuS@PMS-Gel, its PAI capability in NIR-II region was evaluated at different time intervals. Comparing with CuS@PMS solution, CuS@PMS-Gel presented distinct and persistent PA signal (Fig. 21.1B and C), demonstrating CuS@PMS-Gel can serve as PAI agent to guide tumor therapy. Photothermal imaging was performed after intratumor injection of 50 μL of Gel and 50 μL of CuS@PMS-Gel. It was found that the temperature of the tumor site injected with CuS@PMS-Gel increased to 47.8°C under 1064 nm laser irradiation, while the temperature of the tumor injected with Gel only reached 40.1°C after same irradiation (Fig. 21.1D). This result demonstrated that CuS@PMS-Gel could respond to 1064 nm laser irradiation and induce photothermal ablation of tumor tissues. In addition, the generated heat could activate the production of sulfate radical, which was able to further improve the therapeutic efficacy. In vivo antitumor property of the nanodrug was evaluated on B16F10 melanoma tumor-bearing nude mice. As shown in Fig. 21.1F, the tumor volume of the Control group and PMS-Gel group increased rapidly. The PMS-Gel with NIR-II laser irradiation group and the CuS@PMS-Gel group presented a certain degree of tumor growth inhibition at the initial stage, while tumor volume was still increasing promptly at the later stage. It is worth noting that the tumor volume in CuS@PMS-Gel with NIR-II laser irradiation group remained at a very low level during the entire monitoring period, demonstrating its excellent anti-tumor effect. Furthermore, no significant body weight change was observed among all groups (Fig. 21.1E), confirming the good biosafety of prepared nanodrug.

2.2 Ultrasound-responsive nanocarriers

Ultrasounds (US) is a unique method for achieving spatiotemporal control of drug release at the desired site. Additionally, US are attractive due to its non-invasiveness, the absence of ionizing radiations, and the easy regulation of tissue penetration depth by tuning the frequency, cycles and exposure time [29,30]. In recent years, various US-responsive nanocarriers have been developed for drug delivery, and many of them can be applied as US imaging agents at the same time [31–33].

Sonodynamic therapy (SDT) can inhibit plaque angiogenesis via macrophage apoptosis-induced endothelial cell apoptosis without causing obvious side effects. However, its clinical application is limited by the lack of a technique for realtime monitoring of the distribution of sonosensitizers in vivo. To optimize and monitor the efficacy of SDT in plaque neovascularization therapy, Yao et al. fabricated a nanoplatform consists of manganese ferrite ($MnFe_2O_4$), hematopor-phyrin monomethyl ether (HMME), and perfluoropentane (PFP) stabilized by PLGA shell and conjugated with anti-VEGFR-2 antibody. Upon low-intensity focused ultrasound (LIFU) irradiation, MRI/PA/US multimodal imaging in plaque-bearing rabbits could be conducted to realize realtime observation of the nanoplatform biodistribution and guide the therapy (Fig. 21.2A). In vivo contrast-enhanced MRI performance was assessed in plaque-bearing rabbits. After intravenous injection of PFP-HMME@PLGA/$MnFe_2O_4$-Ram NPs, obvious bright effect was observed in the plaque region, and the signal peaked at 90 min postinjection. However, the plaque-enhanced signal of PFP-HMME@PLGA/$MnFe_2O_4$ did not change (Fig. 21.2B and C). Notably, after injection of Magnevist (Gd-DTPA), the enhanced signal in the plaque area was significantly lower than that in both targeted and nontargeted groups and it decreased with time. Furthermore, the PA and US imaging property of the nanoplatform was also verified in plaque-bearing rabbit models. Finally, the efficacy of PFP-HMME@PLGA/$MnFe_2O_4$-Ram NPs to suppress neovascularization was assessed. It was found that on day 3, PHPMR NP-mediated SDT induced apoptosis in neovessel endothelial cells and improved hypoxia in the rabbit advanced plaque.

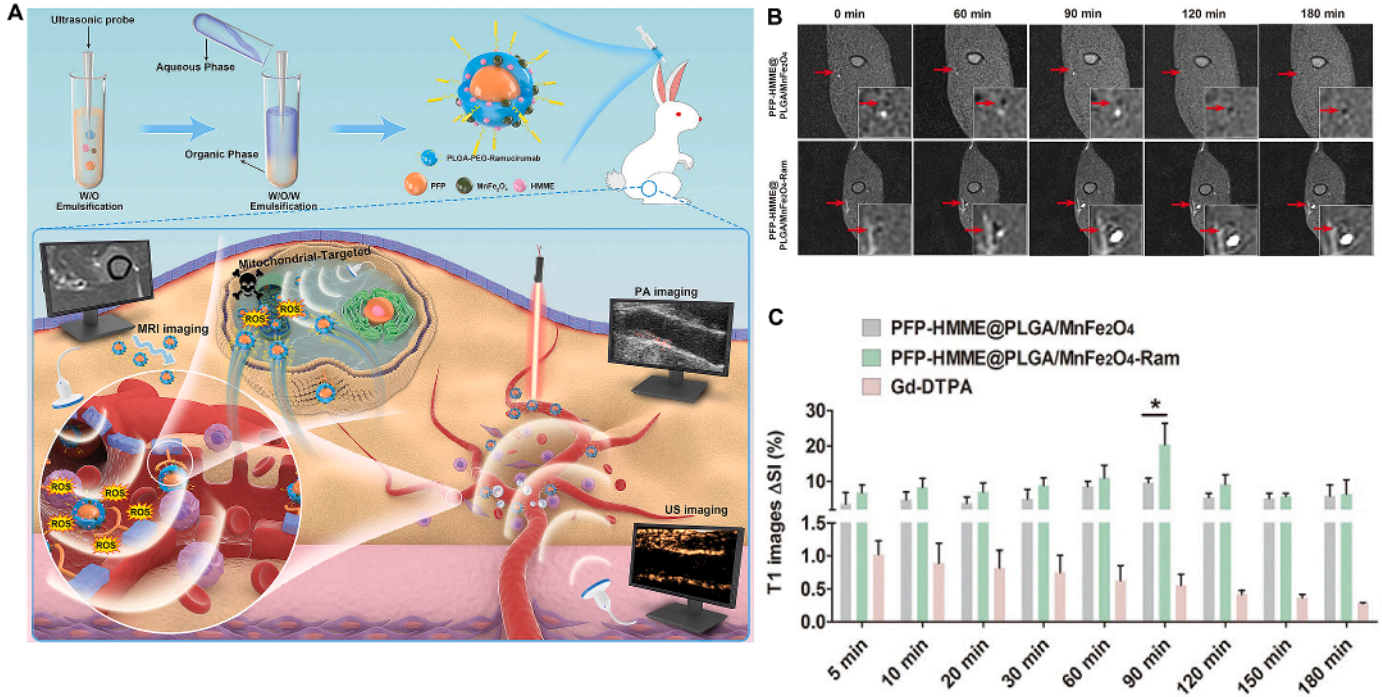


FIGURE 21.2

A. Schematic illustration of the synthetic process for PFP-HMME@PLGA/$MnFe_2O_4$-Ram nanoplatform and the MRI/PA/US multimodal imaging-guided SDT of plaque neovascularization. B. In vivo MRI T_1 images of rabbit femoral plaque after injection of PFP-HMME@PLGA/Mn Fe_2O_4 and PFP-HMME@PLGA/$MnFe_2O_4$-Ram NPs. C. Changes in MRI T_1 signal intensities within plaque regions at the corresponding time points in the indicated groups.

On day 28, PHPMR NP-mediated SDT reduced the density of neovessels, subsequently inhibiting intraplaque hemorrhage and inflammation, and eventually stabilized the plaque.

MRI is a very essential imaging modality for clinical diagnosis, and MRI-guided high-intensity focused ultrasound (MRgHIFU) is a powerful technology for targeted therapy. The applications of MRgHIFU primarily use hyperthermia and ablation to treat cancer, while the thermal damage caused to healthy tissues is undesirable. Cheng et al. developed a biofriendly MRgHIFU-responsive mesoporous silica nanoparticle (MSN) platform, which is stimulated within a physiological safe temperature range, to deliver cargos in a spatial- and temporal-controlled manner monitored in real time by MRI [34]. This nanoplatform consists of three main parts: MSN as the carrier, gadolinium-diethylenetriamine pentaacetic acid $(Gd(DTPA))^{2-}$ as the cargo, and ultrasound-responsive PEG as the cap material. The conjugated PEG seals the pores of MSNs and exposes them under the stimulation of HIFU. The released $Gd(DTPA)^{2-}$ causes T_1-weighted MRI contrast changes, therefore the amount of cargos release induced by HIFU can be monitored in realtime. It is worth noting that, the released amount of cargos can be controlled by adjusting HIFU parameters, such as power level, stimulation duration, and number of stimulation cycles. In addition, only minimal to mild heating is required to release the cargos and it is beneficial for treating diseases in regions where it is impractical to heat.

Gadolinium (Gd) is the most widely used contrast agent in MRI, while free ions of Gd (Gd^{+3}) are toxic. The chelate ligand complexes Gd-DOTA are used instead which can reduce the metal ions toxicity. Maghsoudinia et al. prepared ultrasound-responsive Gd-DOTA/DOX-loaded nanodroplets (NDs) for MRI-guided therapy of melanoma cancer [35]. The NDs were prepared by nano-emulsion method, and the obtained NDs were spherical with a mean size of 25.5 ± 4.1 nm. Drug releasing profiles were investigated with or without ultrasound induction. It was found that only 2.3% of DOX was released during 10 min in the absence of ultrasound, and the releasing percentage increased to 88.7% under ultrasound. This result demonstrated that ultrasound can effectively promote the release of DOX from the NDs. Gd-DOTA/DOX NDs significantly increased MRI signal intensity ($r1 = 6.34\ mM^{-1}S^{-1}$). In addition, ultrasound imaging confirmed that NDs can turn into microbubbles by vapourization phenomenon.

2.3 Magnetic field-responsive nanocarriers

Magnetic field-triggered drug release involves the application of an alternating high or low frequency magnetic field. It can realize both the monitor of magnetic materials through MRI using a static magnetic field and controlled drug release in combination with Alternating Magnetic Fields (AMF). The use of AMF to trigger drug release is based on the property of magnetic materials to convert electromagnetic energy into heat, and then cause structure change of temperature-sensitive nanocarriers [36–38].

Liu et al. prepared magnetic field-responsive anethole dithiolethione (ADT)-loaded magnetic nanoliposomes (AMLs) for US imaging and MRI-guided accurate cancer therapy [39].As shown in Fig. 21.3A and B, AMLs were prepared by hydration and a membrane-extrusive method, and the hydrated particle size was 211.1 ± 4.64 nm. In vivo tumor internalization of the nanoliposome was monitored with US/MRI dual mode imaging. ADT-encapsulated liposomes (ALs) and blankliposomes were set as control. MRI (7T) images were collected at equivalent time intervals (0, 2, 4, 6, 8, 12, and 24 h) pre- and postinjection of the three different kinds of liposomes (Fig. 21.3C). It was found that the blank liposome and AL solution groups did not give any tumor T_2^* signal decrease after injection,

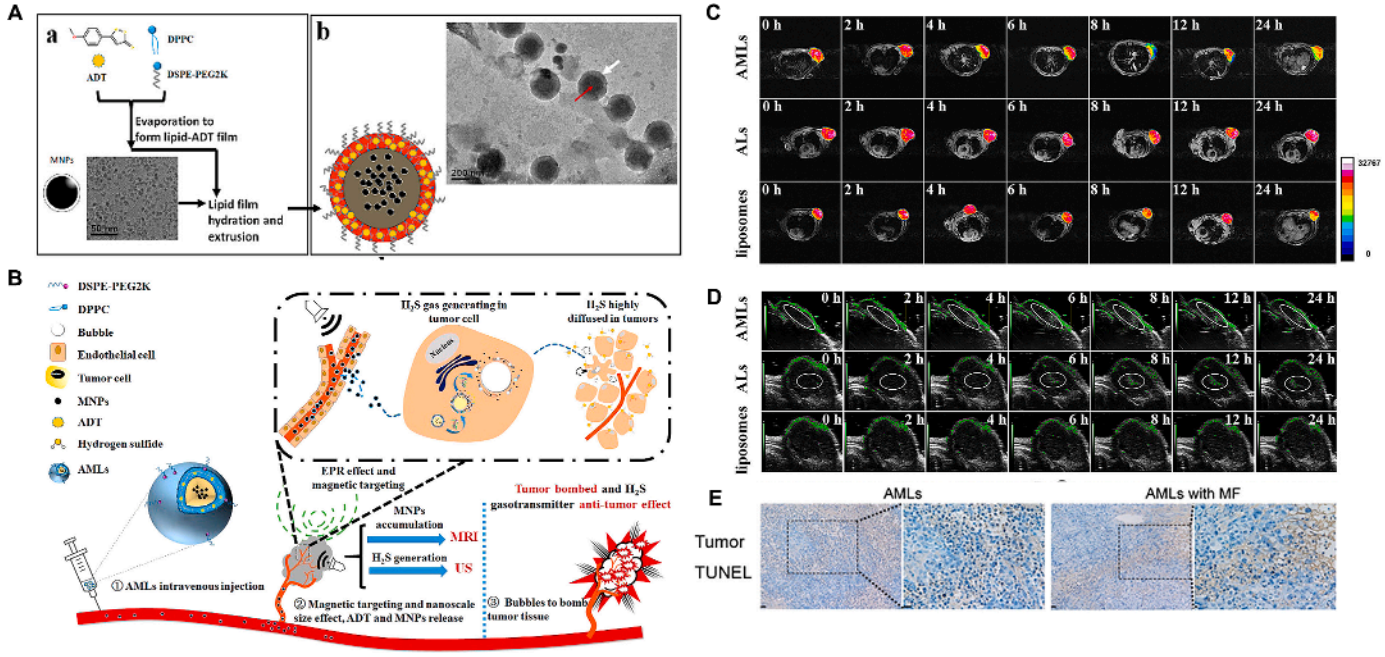


FIGURE 21.3

A. (a) Diagram of AMLs fabrication method. (b) Representative TEM images of AMLs. B. Schematic illustration of AMLs and the nano to micro conversion for US/MR dual modal imaging and the spatiotemporal-bombed combination tumor accurate therapy. C. In vivo T_2 MR images before and after AML, AL, and liposome injection. D. In vivo US images of mouse tumor before and after AML, AL, and liposome injection. E. TUNEL assay for tumors treated by AMLs with and without magnetic field guidance.

while the mean gray value of tumor area in AML group decreased about 22% at 6 h after injection. In addition, MR images showed that the injected AMLs diffused to the whole tumor regions at 6h after injection. The US imaging property of AMLs in vivo was further explored after intravenous injection. As shown in Fig. 21.3D, the enhanced US imaging can be observed in the inner region of tumor. In addition, the echo intensity value of US in AMLs group reached the peak 4 h after injection, which was 3.4 times higher than that in the AL group. When exposed to an external magnetic field, AMLs could pass through the blood vessels under the guidance of magnetic force, causing AMLs to passively target the tumor site. The therapeutic efficiency of AMLs was evaluated in the tumor-bearing nude mouse model. HE staining results indicated enhanced antitumor effect after magnetic field treatment. Besides, the TUNEL assay results showed that the tumors exhibited extensive cavitation regions and high populations of apoptotic cells (brown cell) for AMLs in the magnetic field group (Fig. 21.3E), which could be attributed to potentially higher amount of H_2S bubbles generated from AMLs compared to ALs because of magnetic guidance.

Lee group developed magnetic field-inducible drug-eluting NPs (MIDENs) for MRI-guided chemo-hyperthermia treatment [40]. MIDENs were prepared by incorporating super-paramagnetic iron oxide nanoparticles (SPIONs) and doxorubicin (DOX) into temperature-responsive PLGA matrix. MIDENs can generate heat in response to an AMF and subsequently release DOX to realize the combination of thermal therapy and chemotherapy. The amount of DOX release for treatment is controlled by AMF and thus non-specific toxicity can be reduced. The hyperthermia properties of MIDENs were evaluated using an AMF at 4.4 kW ($H = 2$ kA/m and $f = 205$ kHz). The results showed that the medium temperature raised by 5.2°C (from 37 to 42.2°C) after 10 min of AMF application, demonstrating that MIDENs are suitable for thermal therapy. In addition, it was found that DOX release rate was temperature-dependent, likely due to the glassy-to-rubbery transition of the PLGA nanomatrix leading to the release of the loaded DOX. In vivo therapeutic efficacy of MIDENs combining with AMF was assessed in CT26 tumor-bearing mice. Thermal images revealed that the temperature at tumor site reached 44–46°C during AMF treatment, indicating that MIDENs could induce hyperthermic tumor apoptosis. Comparing with PBS-treated group, MIDENs plus AMF group significantly inhibited the tumor growth ($V/V_{initial} = 0.68$ on day 15), suggesting that both AMF-induced thermal energy and DOX contributed to tumor regression.

The applications of magnetic hyperthermia therapy (MHT) have been largely hindered by low magnetic-to-thermal conversion efficiency of MHT agents. To handle this issue, Zhang and coworkers developed genetically engineered magnetic nanocages for magneto-catalytic therapy of cancer [41]. The prepared encapsulin-produced magnetic iron oxide nanocomposites (eMIONs) have excellent magnetic saturation and remnant magnetization properties with an ultrahigh specific absorption rate of 2390 W/g. In addition, eMIONs can act as a nanozyme and have enhanced catalase-like activity in the presence of an AMF, leading to tumor angiogenesis inhibition with a sharp decrease in the expression of HIF-1α. PAI and US imaging were applied to evaluate the catalytic efficiency after i.v. injection of eMIONs. It was found that tumor O_2 level of eMIONs-treated group was about 13 times higher than that of PBS-treated group. The excellent anticancer efficacy was confirmed in A549 tumor-bearing mice.

2.4 X-ray-responsive nanocarriers

X-ray radiation is a promising external trigger because of its high penetration depth in solid tumors [42]. Among the stimuli-responsive nanocarriers, NPs that can be applied in deep tissue imaging, such

as CT imaging, are more appealing for clinical applications. X-ray-responsive NPs are particularly popular due to their high spatial resolution in 3D reconstruction of interested tissues [43–45].

PDT is a safe and minimally invasive treatment modality of cancer using reactive oxygen species (ROS) produced by photo-sensitizers (PSs) under light excitation. It is widely believed that the penetration depth of the excitation light determines the reachable depth of PDT. However, most of clinically approved PSs are excited by UV/vis light with relatively short wavelength. X-ray is a kind of ionizing radiation source with unlimited penetration depth in the human body, which has been widely applied in CT imaging and radiotherapy. Zhong et al. developed cerium (Ce)-doped Na-CeF_4:Gd, Tb scintillating NPs (ScNP) for X-ray-responsive and multimodal imaging-guided therapy of cancer (Fig. 21.4A) [46]. In this system, Ce^{3+} ions acted not only as sensitizers to improve the fluorescence emission of terbium ions under X-ray excitation, but also as photocatalysts to induce the generation of $\bullet O^{2-}$ and hydroxyl radical •OH. Due to the existence of heavy atoms (Ce, Gd, and Tb), a significant enhancement of local radiation dose deposition was achieved. After exposing to X-ray, 29.7% of cells were damaged at the dose of 6 Gy, and about 80% of the cells were damaged when simultaneously received ScNP-PEG treatment and X-ray irradiation of 6 Gy, demonstrating a synergistic enhancement on radiotherapy. It was found that T_1-weighted MRI exhibited a concentration-dependent brightening effect (Fig. 21.4B), and T_2-weighted MRI exhibited a concentration-dependent dimming effect (Fig. 21.4C). In vivo T_1-and T_2-weighted MRI was conducted. A slightly brighten effect appeared within the tumor site from T_1-MRI and remarkable dimming effect from T_2-MRI, respectively (Fig. 21.4D). In addition, a remarkable brighten effect was also achieved by intratumor injection of

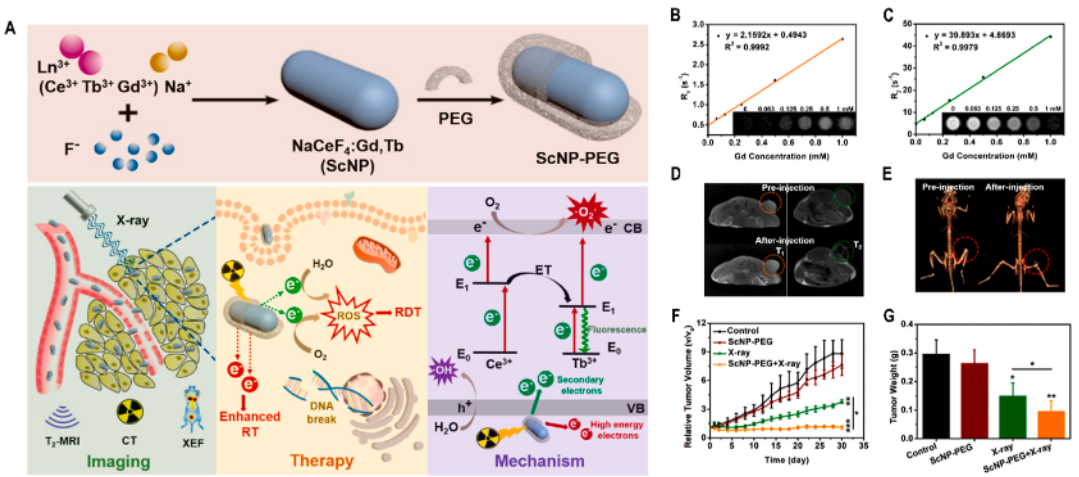


FIGURE 21.4

A. Schematic illustration of X-ray activated NaCeF4:Gd,Tb scintillator for XEF/CT/T_2-MRI-guided synchronous RT and RDT of cancer. B and C. In vitro T_1/T_2-MRI of different concentrations of ScNP-PEG, respectively. D. In vivo T_1/T_2- MRI by intratumor injecting ScNP-PEG in A549 tumor-bearing nude mice. E. In vivo CT imaging of different concentrations of ScNP-PEG and in vivo CT imaging by intratumor injecting ScNP-PEG in A549 tumor-bearing nude mice. F. Tumor growth curves of different groups of mice after various treatment groups. G. Average weights of tumors collected from the mice at the end of different treatments.

ScNP-PEG (Fig. 21.4E). In vivo therapeutic efficacy of ScNP-PEG was investigated in A549 tumor-bearing BALB/c nude mice. Tumor volume of each mouse were monitored every 2 days during the whole course of treatment (Fig. 21.4F),and average weights of tumors collected from the mice were recorded at the end of different treatments (Fig. 21.4G). The results showed that tumor growth inhibition value of X-ray irradiation was 49.45%, and the synergistic treatment with ScNP-PEG increased the final antitumor rat to 63.67%.

Zhou et al. rationally designed an X-ray activatable drug delivery system for synchronous chemoradiation [47]. This nanoplatform was fabricated by self-assembly of ROS-responsive PPS-PEG amphiphilic polymers and Au NPs tethered with X-ray labile linoleic acid hydroperoxide (LAHP) molecules. Chemotherapy drug DOX was loaded in the inner core of the nanoplatform. PPS-PEG polymers were stable at the low level of ROS. Under X-ray irradiation, the generated hydroxyl radical (•OH) would cause efficient degradation of the nanoplatform and the release of DOX. In this manner, drug release is synchronously confined when radiation therapy is applied, which may mitigate possible development of resistance to monotherapy and potentially improve the synergistic therapeutic effect.

Zheng and coworkers reported the application of X-ray activated Au/Ag core/shell nanorods (NRs) to enhance tumor radioimmunotherapy (RIT) and monitor the therapeutic response using PAI in NIR-II region [48]. Au/Ag NRs can be etched by X-ray irradiation to release silver ions (Ag^+), which can promote dendritic cell (DC) maturation and enhance the capability of T lymphocytes to kill cancer cells. In addition, the released Ag^+ can trigger a red shift of the surface plasmon absorption peak, and this property enabled monitoring of the therapeutic response by NIR-II PAI in vivo. The Au/Ag NRs achieved good antitumor efficacy in primary and metastatic tumor-bearing mice, with the survival of metastatic mice extended by 16 days compared with control mice.

3. Endogenous stimuli-responsive nanocarriers

3.1 pH-responsive nanocarriers

Commonly, the extracellular region of solid tumors is acidic (pH 6.0–6.8) owing to excessive glycolysis and poor perfusion, which is different from that of normal tissues (pH 7.2–7.4). This mildly acidic environment can be applied as an endogenous stimulus to change the properties of nanodrugs, and thus realize smart drug delivery [49,50]. Moreover, once internalized into cells, the NPs will undergo fast acidification, beginning from endosomes (pH 5.2–6) to lysosomes (pH 4.5–5.2) during the endosome maturation. Therefore, pH-sensitive nanocarriers have been one of the most frequently used materials for targeted tumor therapy [51–53].

In recent years, drug-eluting beads (DEB) have been developed for transcatheter intra-arterial (IA) delivery to hepatocellular carcinoma (HCC). However, it is difficult to control drug loading/release and to monitor selective delivery to the targeted sites. To address this issue, Park et al. designed pH-triggered drug-eluting nanocomposite microspheres (pH-DENs) for MRI-guided transcatheter drug delivery [54]. Poly(lactide-co-glycolide) (PLGA) and iron oxide nanocubes (IONCs) were used as base materials to prepare this nanocomposites (NCs). pH-responsive additive (pH-ADT) and anti-angiogenic drug sorafenib were incorporated into the NCs. pH-ADT was in a stable solid state at a neutral pH, and it would change into water-soluble form in an acidic environment ($pH < 7.4$) due to ionization of imidazole groups. Therefore, the solubility change of pH-ADT would trigger drug release

from pH-sensitive NCs (Fig. 21.5A–C). In vivo imaging properties and therapeutic effects of NCs were evaluated in orthotopic HCC rat model. The tumor sites were visualized as hyperintense regions relative to the normal liver tissues in T_2-weighted images (Fig. 21.5D, left panels). Signal reductions were observed at the tumor sites immediately after transcatheter infusion of NCs (Fig. 21.5D, right panels), indicating efficient accumulation of NCs exhibiting high R_2 relaxivity. The deposition of NCs within the liver was confirmed by histological staining with Prussian blue. As shown in Fig. 21.5E, blue signal was observed not only at the peripheral rim of the tumor but also at the intratumor vessel. These results demonstrated that sorafenib-pH-DENs were effectively localized to the targeted tumor sites.

Zhao et al. designed and synthesized polyacrylic acid (PAA)-capped and Ln^{3+}-doped porous BiF_3 nanostructures for pH-dependent DOX loading and release under the guidance of CT imaging for cancer theranostics [55]. Due to the porous structure and PAA chains, the BiF_3 spheres were ideal DOX loading and delivery vehicles for cancer therapy. It was found that drug loading efficiency increased with the increase of pH in PBS buffer. Inversely, the carboxyl groups in PAA are protonated in acidic environment, which weakens the binding of DOX with PAA and thereby achieved pH-responsive release of DOX. Because of favorable X-ray attenuation of Bi, the prepared nanodrug presented good CT imaging performance. The CT signals were gradually enhanced with the increase of BiF_3:Yb,Er concentrations. In vivo antitumor efficacy of BiF_3:Yb,Er@DOX was evaluated on A549 tumor-bearing mice. Though pure DOX exhibited slightly superior efficiency than BiF_3: Yb,Er@DOX, the latter presented milder side effects, which could be ascribed to the acidic pH-responsive drug release property.

Due to relatively low accumulation of nanotherapeutics at disease sites, the therapy efficiency is often limited. To solve this problem, Zhu et al. developed a novel theranostic platform with pH-responsive charge conversion property to improve cellular uptake toward cancer cells for enhanced SPECT/CT dual mode imaging-guided radiotherapy of cancer [56]. PEI was first linked with PEG, and then alkoxyphenyl acylsulfonamide (APAS) was modified on PEG. The functionalized PEI was used as carrier to entrap Au NPs and label with radioactive ^{131}I. Zeta potentials of APAS-Au PNPs and Au PNPs were measured under different pH, respectively. It was found that these two nanostructures approached to neutral in physiological microenvironment (pH 7.0–7.5), and only APAS-Au PNPs turned to be positively charged in acidic microenvironment (pH 5.0–6.5). Due to APAS modification, the charge conversion property of APAS-Au PNPs made it possible to achieve improved cellular internalization in cancer cells via electrostatic interaction. SPECT and CT imaging properties of ^{131}I-APAS-Au PNPs were verified both in vitro an in vivo. Radiotherapeutic efficacy of ^{131}I-APAS-Au PNPs was assessed in tumor-bearing mice. The results showed that ^{131}I-APAS-Au PNPs treatment presented lower tumor growth rate than ^{131}I-Au PNPs, confirming that APAS-mediated enhanced cellular internalization increased the therapy efficacy. In addition, ^{131}I-APAS-Au PNPs effectively prolonged the lifetime of mice, which verified the improved radiotherapeutic efficacy indirectly.

3.2 GSH-responsive nanocarriers

GSH is a kind of strong reducing agent present in most of the living organisms. Many researches have revealed that the intracellular concentration of GSH in tumors can be 100 times higher than that in healthy tissues [57,58]. Therefore, GSH-responsive nanocarriers take advantage of the fact that certain bonds, such as disulfides (S-S) and Se-Se, are cleaved in the presence of high concentration of GSH

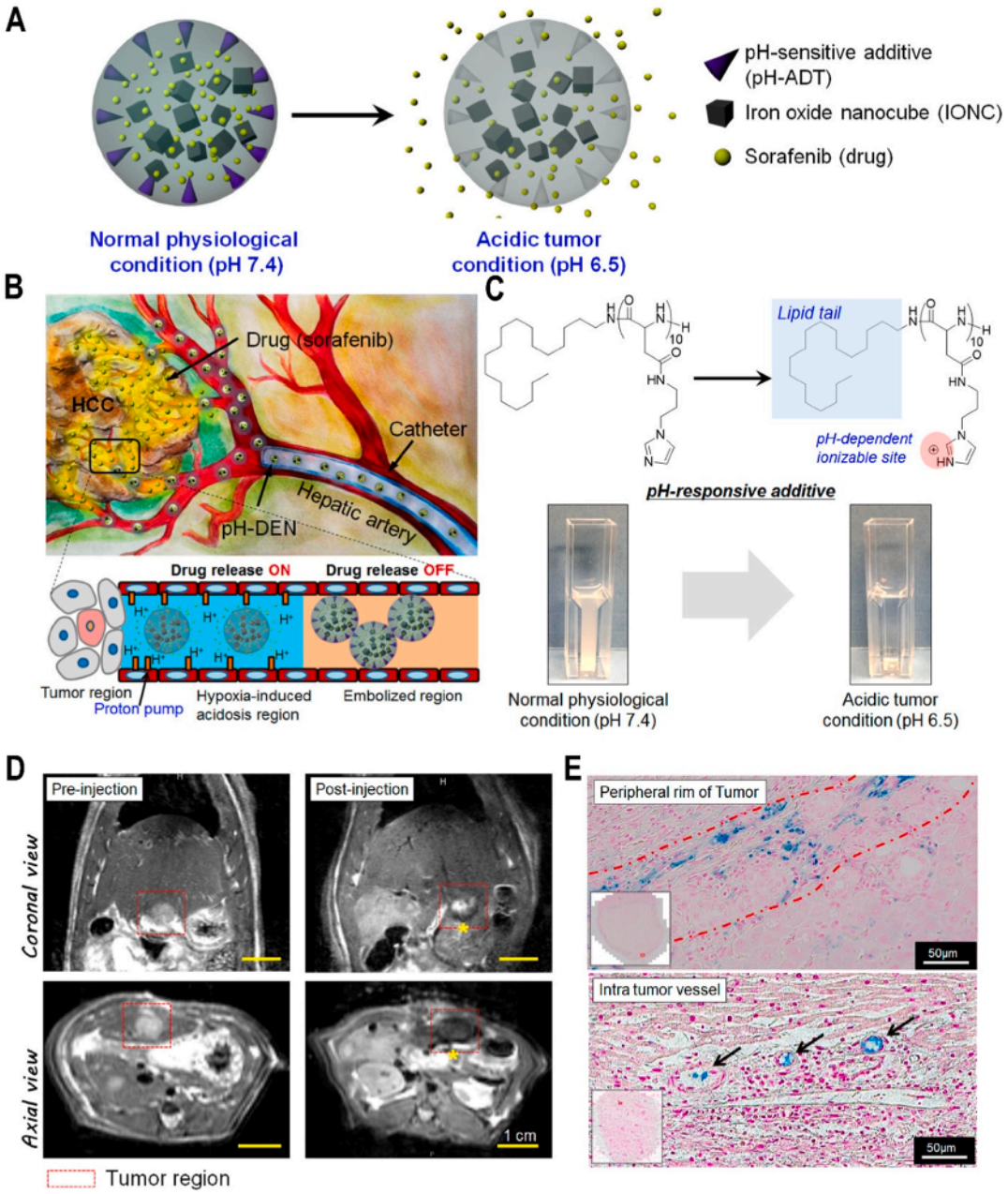


FIGURE 21.5

A. Schematic illustrations of acidic pH-triggered drug release behavior. B. Transcatheter hepatic intra-arterial delivery of pH-DEN. C. Chemical structure of pH-responsive additive (pH-ADT) at different pH values and their pH-dependent phase transition. D. In vivo T_2-weighted MR images acquired before and after transcatheter infusion of pH-DENs. E. Prussian blue staining of treated tumor-bearing liver tissues 24 h post-IA injection.

and then release the drugs in tumor sites. The linkages can be used to directly bind the drugs of interest or to cross-link a gating or capping molecule on the nanocarrier surface [59,60].

Monitoring the drug release from NPs in vivo has been a long-standing challenge. To this end, Wang et al. developed GSH-responsive polymeric magnetosome (PolyMag), which not only integrated the merits of nanotechnology, such as tumor selectivity, TME-responsiveness, but also realized non-invasively monitor of in vivo drug release by MRI [61]. As shown in Fig. 21.6A, the responsive PolyMags were constructed by concurrent encapsulation of T_2-contrast agents (SPIO) and DOX into a disulfide-cross-linked micelle (DCM). Upon interaction with the abundant GSH in TME, the payloads in PolyMags could be responsively released, accompanied by T_2-MRI contrast and R_2 increase as well as the release of DOX. The GSH-responsive DOX release of PolyMags@DOX was monitored by the addition of GSH. It was found that the drug release rate could be extensively accelerated by the addition of GSH. The higher GSH concentration, the faster drug release rate was reached. In vivo drug release behavior was monitored with MRI. As shown in Fig. 21.6B, T_2-weighted images, T_2 signal-to-noise ratio (SNR), T_2 and T_2^* maps, and R_2 and R_2^* values were recorded at different time points after injection of PolyMags. The T_2-MRI signal recovered due to the increased drug release at tumor site. Therefore, it was able to assess and monitor the drug release by combining T_2-and T_2^*-MR sequences. In addition, PolyMags@DOX exhibited better photothermal effect than both PolyMags and DCM (Fig. 21.6C), demonstrating its potential as PTT agents. In vivo therapeutic efficacy of PolyMags@DOX was evaluated in MCF-7 breast cancer bearing animal models. Experimental results showed that PolyMags@DOX-mediated photothermal and chemotherapeutic treatment was more efficacious than either PTT or chemotherapy alone.

PTT is a safe and promising cancer treatment modality. However, the expression of the heat shock protein (Hsp) is commonly upregulated by hyperthermia, which decreases the tolerance of heat stress to cancer cell. To address this issue, Wu et al. constructed GSHresponsive nanotheranostic agent to deliver Hsp 90 inhibitor, 17AAG, for CT and PAI-guided PDT and PTT of cancer [61]. In this system, hollow mesoporous organosilica NPs (HMONs) with disulfide bonds served as nanocarriers to load 17AAG. BSAIrO_2 complexes with thiol group modification were attached onto the surface of HMONs to prevent leakage of 17AAG during blood circulation. Then, polyethylene glycol (PEG) was modified on HMONs, and the nano platform 17AAG@HMONs-BSA-IrO_2-PEG (AHBIP) was prepared. After AHBIP NPs are ingested by cancer cells, the BSA-IrO_2 gatekeepers are opened due to the breakage of disulfide bond by GSH, and subsequently the released 17AAG down-regulates the level of Hsp90. In vitro release performance of 17AAG triggered by GSH was investigated. Negligible 17AAG released from AHBIP NPs at pH 7.4 and 5.0, indicating good gating effect of BSA-IrO_2. However, the release of 17AAG over 48 h increased to around 45.2% and 76.8% at pH 7.4 and 5.0, respectively. This can be attributed to the GSH-induced cleavage of disulfide bonds and thus leading to the abscission of BSA-IrO_2 on pore outlets. In addition, the feasibility of AHBIP as in vivo CT and PA imaging agents was verified in MDA-MB-231 tumor-bearing mice. Finally, the synergistic PTT/PDT therapy effect was evaluated. It was found that the tumors were completely destroyed without recurrence in the synergistic group, demonstrating the high efficacy of PTT/PDT treatment.

Zheng et al. designed and constructed GSH-responsive nanoplatform (AB-DS@BSA-N_3 NYs) for NIR-II fluorescence/photoacoustic imaging-guided PTT and gas therapy (GT) of cancer [62]. The embedded H_2S donor DATS can specifically release H_2S gas by the overexpressed GSH in TME. Subsequently, $–N_3(-)$ can be reduced to $–NH_3(+)$ by the elevated H_2S for prolonged tumor retention. H_2S production performance was measured in PBS with or without GSH (2 mM). It was found that in

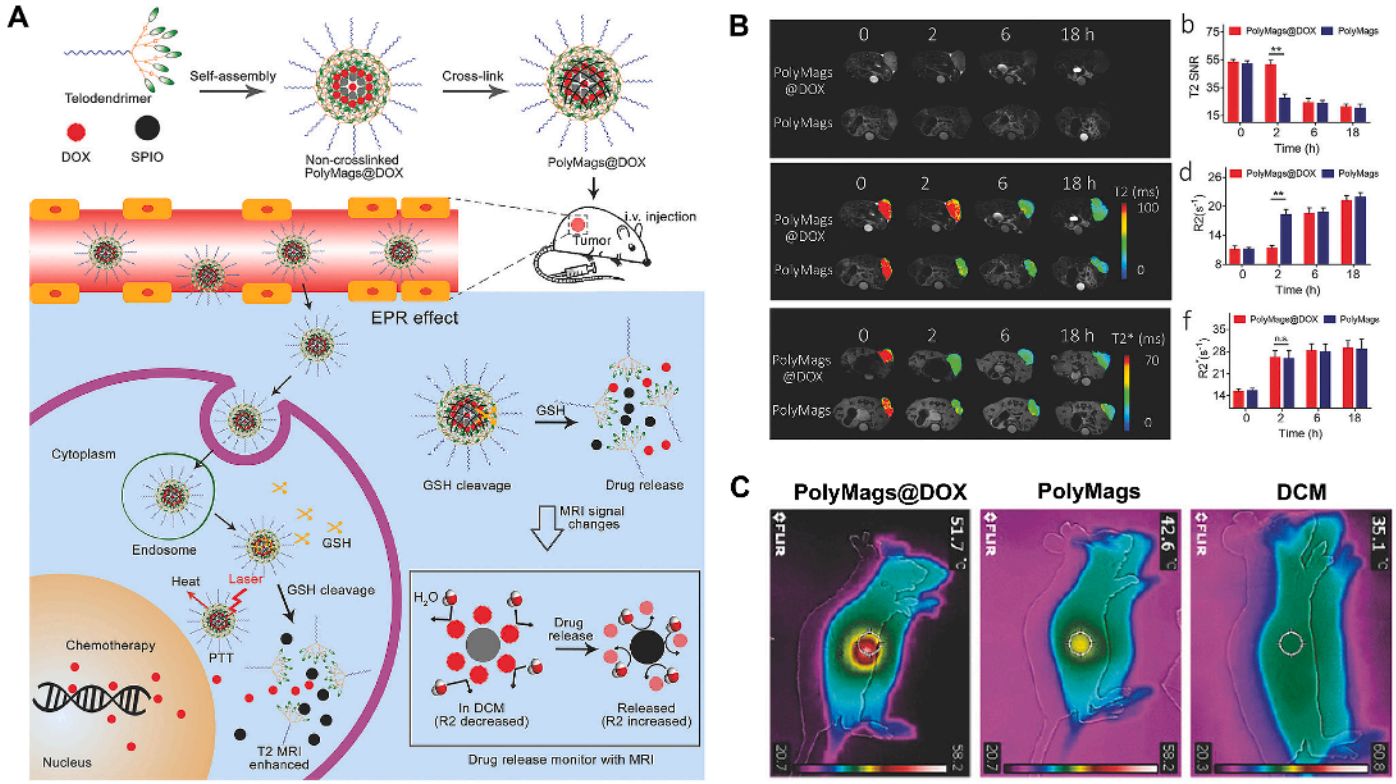


FIGURE 21.6

A. Schematic illustrations of the design of MRI-based theranostic polymeric magnetosomes. B. T_2-weighted images, T_2 SNR, T_2 map, R_2 values, T_2^* map, R_2^* values of the tumors of the nude mice bearing breast cancer at pre-injection, and 2, 6, and 18h postinjection of the PolyMags@DOX (2 mg mL^{-1}) and empty PolyMags. C. Representative thermal images of tumors after light irradiation at 12 h postinjection of empty PolyMags, PolyMags@DOX, and DCM.

the presence of GSH, H_2S concentration reached the maximum value (11.9 μM) within 5 h, which was significantly higher than in PBS without GSH. This result indicated that GSH could effectively promote H_2S generation. In vivo NIR-II fluorescence and PA imaging properties of AB-DS@BSA-N_3 wereinvestigated in Hep2 tumor-bearing mice. At 2 h after intravenous injection, the NIR-II fluorescence and PA signals at the tumor site began to appear and reached maximum values at 6 h postinjection, and the signals increased in a concentration-dependent manner. Anti-cancer therapy results showed that AB-DS@BSA-N_3 combining with laser irradiation efficiently suppressed tumor growth and realized near-complete tumor regression after 20 days of treatment. Moreover, the body weight of the mice was not affected significantly after treatment, indicating minimal systemic side effects during therapy process.

3.3 Hypoxia-responsive nanocarriers

Hypoxia has been well established as a prominent feature of solid tumor microenvironment, and it significantly influences tumor behavior and treatment response [63–65]. For example, the resistance to radiotherapy is a well-known phenomenon related with tumor hypoxia. Besides, many researches have confirmed that hypoxic cells are responsible for decreased sensitivity to certain chemotherapeutic drugs [66,67].

NPs with size of around 100–300 nm can accumulate in tumor location via EPR effect, while they are inferior in tumor penetration. On the contrary, NPs with size below 20 nm have better distribution within tumors but they more likely subjected to metabolization and clearance. Therefore, idea nanocarriers should have large size to enhance blood circulation time and release small therapeutic agents upon arrival of the tumor site under TME stimuli [68,69]. To this end, Zhang et al. constructed a hypoxia-responsive and size-switchable MOF nanosystem for synergistic chemodynamic therapy and sonodynamic therapy (CDT/SDT) of cancer [70]. As shown in Fig. 21.7A, the nanosystem consists of copper clusters bridged by organic ligands loaded with sono-sensitizers chlorin e6 (Ce6). The MOF NPs with large size were effectively accumulated in the tumor region via EPR effect, and then the hypoxia in TME triggered the degradation of MOF to release Cu^{2+} and Ce6 and realized deep tumor penetration. The redox between Cu^{2+} and intracellular high-level GSH resulted in GSH depletion and reducing Cu^{2+} to Cu^{+}. The data of Calcein-AM/PI double staining assay in Fig. 21.7B showed that Cu-MOF/Ce6 was hypoxia-responsive, which killed cancer cells with high specificity. The size-switchable property endowed Cu-MOF/Ce6 with long blood circulation time, and this was confirmed by PAI in Fig. 21.7C. Compared to the control group, much stronger PA signal was observed in the tumor region after tail vein injection of Cu-MOF/Ce6 into mice for 12 h. In vivo anticancer performance of the nanosystem was assessed on MCF-7 cancer-bearing nude mice. Cu-MOF/Ce6 plus US irradiation presented the best tumor growth suppression ability (Fig. 21.7D) without causing noticeable body weight change (Fig. 21.7E).

Azobenzene derivatives with hypoxia-sensitive property have been applied as hypoxia-responsive fluorescent probes to detect hypoxia levels and for the delivery of therapeutic drugs to hypoxic cancer cells. However, azobenzene-based NPs with hypoxia-responsive dissociation capacity to enhance tumor penetration and therapeutic efficiency have not been well investigated. Yang and coworkers reported a hypoxia-responsive human serum albumin (HSA)-based nanoplatform for enhanced tumor penetration and therapeutic efficacy [71]. Ce6 and oxaliplatin prodrug (Oxa(IV)) were separately

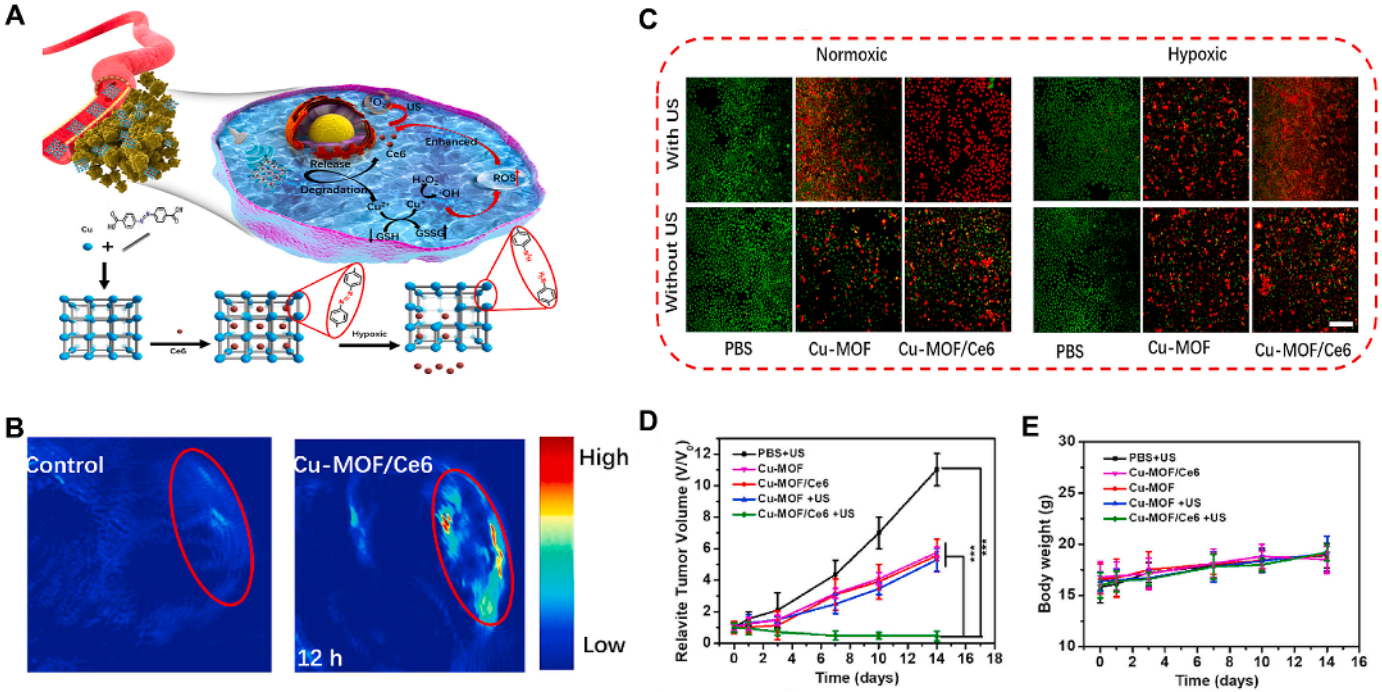


FIGURE 21.7

A. Schematic illustration of the synthetic procedure and hypoxia-responsive copper metal-organic frameworks nanosystem for improved cancer therapy. B. CLSM images of MCF-7 cells in normoxic or hypoxia conditions treated with PBS (pH 7.4, 10 mM), Cu-MOF, and Cu-MOF/Ce6 (100 μg/mL) with or without US irradiation for 10 min. C. In vivo PAI with (right) and without (left) injection of Cu-MOF/Ce6. D and E. Relative tumor volumes and body weight of mice received different treatments.

conjugated with HAS to produce ultrasmall HC and HO NPs with the diameter of less than 10 nm. Then, hypoxia-sensitive linker was used to crosslink HC and HO to yield HCHOA assemblies. Under hypoxic conditions in TME, the azobenzene moieties in the HCHOA would be reduced and cleaved, causing the dissociation of the nanoplatform into HC and HO therapeutic agents with enhanced penetration into deep tumor tissues for better anti-cancer efficacy. In vivo accumulation of HCHOA was verified by Maestro EX optical imaging system. It was found that HCHOA NPs presented higher tumor accumulation than either Ce6 or HCHOH NPs, which can be ascribed to the recovery of Ce6 fluorescence at hypoxic TME after hypoxia-responsive dissociation into ultrasmall NPs. The results of cancer therapy experiments revealed that the HCHOA NPs with 660 nm light irradiation presented the best antitumor efficiency among all the treatments, demonstrating the effectiveness of this therapy strategy.

3.4 ROS-responsive nanocarriers

Due to the overexpression of superoxide dismutase, cancers tend to produce high levels of intracellular ROS. In addition, elevated concentration of ROS is also accompanied with the inflammatory process [72–74]. Therefore, ROS offers an ideal target for accurate drug delivery in the treatment of cancers and inflammatory diseases.

Mesenchymal stem cell (MSC) therapy has been proven to be an effective approach for idiopathic pulmonary fibrosis (IPF) therapy. However, the poor curative effect and insufficient comprehension of in vivo condition of the transplanted MSCs has limited this strategy. To address these issues, Li and coworkers prepared Au-based Janus NPs loaded with Pirfenidone (PFD) for ROS-responsive drug release and prolonged CT imaging tracing of post-transplanted MSCs in the treatment of idiopathic IPF [75]. As shown in Fig. 21.8A, the Janus NPs consist of a Au core and mesoporous silica shell loaded with drug PFD. Then, the NPs were asymmetrically decorated with two targeting moieties: one is ROS-sensitive thioketal grafted methoxy poly(ethylene glycol) (mPEG-TK), and the other is DSPE. The NPs can anchor on cell membranes via DSPE modification on the hemispherical surfaces and inhibit the cellular endocytosis by PEG modification. In vitro drug release performance was investigated in PBS with or without H_2O_2. Around 40% of PFD released from Janus-PFD NPs within 46 h in the absence of H_2O_2, and almost 91% of PFD released in the presence of 0.8 mM H_2O_2 (Fig. 21.8B). It can be ascribed to the fracture of thioketal linker by H_2O_2, resulting in PFD release caused by the shedding of PEG layer from the surface of the Janus NPs. The possibility of Janus-PFD NPs for long-term tracing of MSCs in the treatment of IPF was assessed in IPF murine model. One day after transplantation of the labeled MSCs, enhanced CT imaging signals (red circle) from the labeled MSCs were observed in both the axial section (Fig. 21.8C, the first row) and coronal section (Fig. 21.8C, the second row). In vivo therapeutic effect of Janus-PFD NPs-labeled MSCs was assessed by CT imaging. No significant changes in lung parenchyma were observed in the Janus-PFD-treated group, which was much better than Janus NPs treatment.

Ma et al. reported a ROS-responsive theranostic nanoplatform for two-photon aggregation-induced emission (AIE) imaging and therapy of inflammation [76]. In this system, anti-inflammatory drug prednisolone (Pred) was bridged to a two-photon fluorophore (TP) via a ROS sensitive bond, and then loaded by amphipathic polymer PMPC-PMEMA (PMM) to form core-shell structured micelles (TPP@PMM). The micellar structure was first interrupted by the ROS triggered hydrophobic-to-hydrophilic conversion of PMEMA, which allowed the release of TPP. Then the ROS -responsive bond in

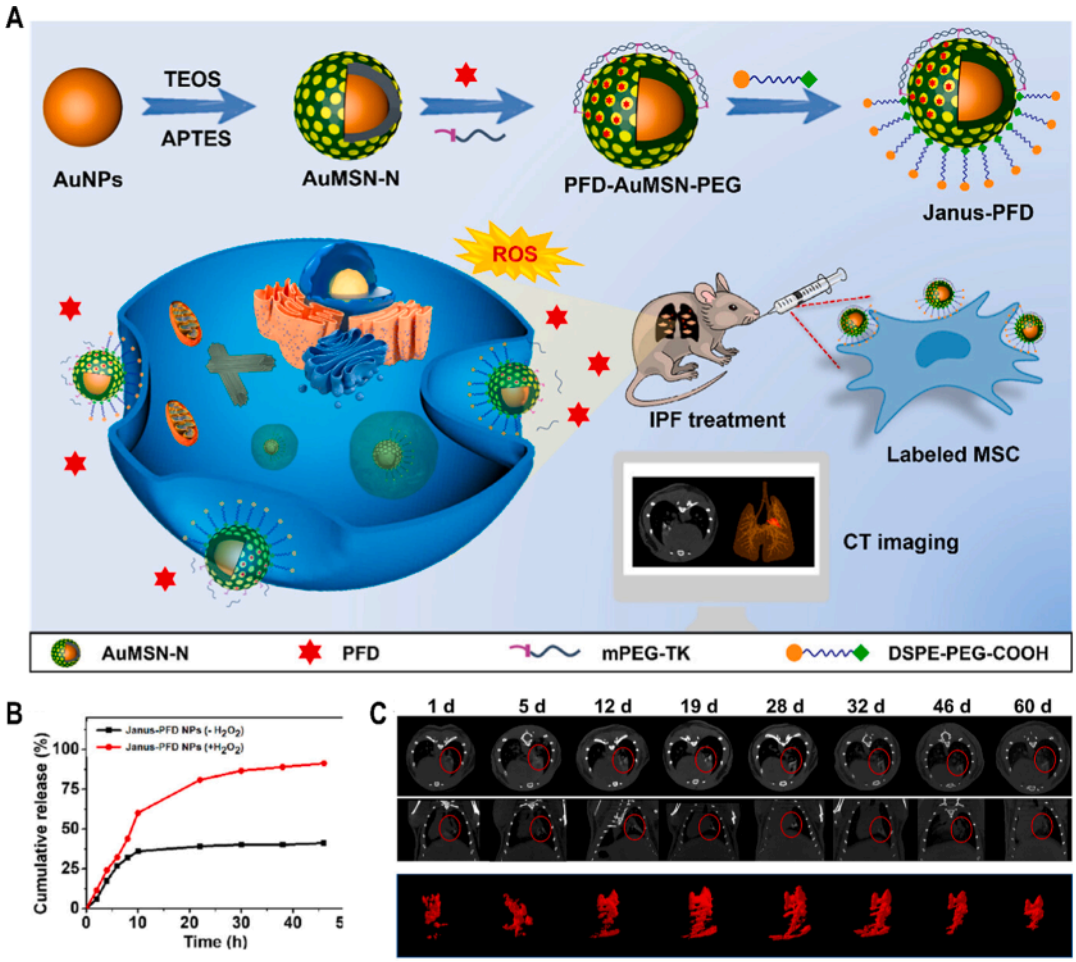


FIGURE 21.8

A. Schematic illustration of the preparation of Janus-PFD NPs and CT imaging tracing of the post-transplanted MSCs combined with ROS-responsive drug release for IPF therapy. B. Cumulative release of PFD from the Janus-PFD NPs in PBS with or without H_2O_2. C. In vivo micro-CT scans of the Janus-PFD labeled MSCs after transplantation at different time intervals. Axial section (top row) and corresponding coronal section (bottom row) were captured at various time points.

TPP was broken, resulting in the release of Pred and therapy of inflammation. Furthermore, TPP@PMM can be traced in vivo with a distinct two-photon imaging due to the AIE active fluorophore TP. The anti-inflammation efficacy and AIE imaging property of TPP@PMM were successfully verified in animal models of acute lung injury, arthritis, and atherosclerosis.

Shen et al. developed ROS-responsive bimodal probe Fe_3O_4@SiO_2-CDs (FC) for atherosclerosis diagnosis and therapy [77]. Polyglycidyl methacrylate (PGMA) and PEG were linked by oxalyl chloride to synthesize ROS-responsive diblock polymer (PGMA-PEG), and cardiovascular drug

simvastatin (Sim) was then modified on PGMA via oxalyl chloride to achieve ROS-responsive drug release. The target agent ISO-1 was modified on PGMA for targeted drug delivery to plaques, and carbon fluorescent quantum dots (CDs) were encapsulated in nanomicelles to monitor the therapy process. In atherosclerotic plaques, the dicarbonyl group in oxalyl chloride can be cleaved in response to high concentrations of ROS, thus releasing Sim and the bimodal probe, and finally realizing therapy and imaging at the same time. The drug release rate was measured by UV standard working curve. It was found that Sim release was related to the concentration of H_2O_2. The maximum release rate of Sim was $12.5 \pm 3.0\%$ when the concentration of H_2O_2 was 0 mM, while when the concentration of H_2O_2 was increased to 1 mM, the maximum drug release rate reached $21.5 \pm 2.6\%$. In vivo fluorescence imaging and MRI imaging properties of FC were also evaluated. The fluorescence intensity increased with time, demonstrating that the ROS-responsive release of FC still existed in vivo. In addition, the MRI signal became stronger with the increase of FC NP concentration, confirming that FC can be used as T_2 contrast agents for MRI.

4. External and endogenous stimuli dual-responsive nanocarriers

Though nanocarriers responsive to single external or endogenous stimulus can increase the accuracy and intelligence of drug delivery, there are still some issues to be addressed before truly realizing precision medicine. For example, the temporal and spatial nature of nanotherapeautics cannot be well controlled in many cases. Thus, multistimuli-responsive nanoplatforms with programmable functions to combat multiple biological barriers have been developed to enhance spatiotemporal controllability for activated targets and overcome the drawbacks of single stimulus-responsive nanocarriers [78–80].

As an alternative to traditional cancer therapy modalities, CDT induces cancer cell death without damaging normal tissues. It uses metal ions (e.g., Fe, Mn, Ag, and Cu) to convert abundant H_2O_2 into highly toxic ·OH to kill cancer cells [81]. However, most of the inorganic CDT agents cannot self-degrade in the body and thus will cause long-term toxicity. In addition, high level of GSH in tumor region can reduce hydroxyl free radicals and lessen the efficacy of CDT. To solve these problems, Chen et al. developed TME and 808 nm laser dual-responsive and biodegradable Fe-doped MoO_X (FMO) nanowires (NWs) for MRI-guided CDT of cancer [82]. As shown in Fig. 21.9A, Fe^{2+} and Mo^{5+} in FMO act as Fenton reagents to convert H_2O_2 into poisonous ·OH. Moreover, FMO has excellent photothermal effect under 808 nm laser, and the photothermal conversion efficiency can reach 48.5%. FMO nanowires achieve good contrast enhancement in vivo and can be used as contrast agent for T_1-weighted MRI. FMO NWs are stable at acidic pH (5.4) and quickly degrade under the physiological pH (7.4). This property endows FMO NWs with better biocompatibility than non-degradable inorganic nanoagents. GSH consumption caused by FMO in cells was measured by GSH assay kit. It was found that the concentration of GSH decreased rapidly with the addition of FMO ($1000\ \mu g\ mL^{-1}$), demonstrating that FMO can regulate TME. The potential of FMO as MRI and infrared thermal imaging agent was evaluated in HeLa tumor-bearing mice (Fig. 21.9B and C). The therapeutic efficacy of FMO was also assessed. As shown in Fig. 21.9D, the tumor growth was inhibited only after FMO treatment compared to control group, and FMO plus laser irradiation completely eradicated the tumor after 15 days of treatment.

PDT and PTT are novel strategies for cancer treatment because of minimal invasiveness and spatiotemporal controllability. However, hypoxia can reduce the efficiency of PDT by limiting the generation of ROS, and the antioxidant glutathione GSH can reduce cytotoxic ROS and cause PDT

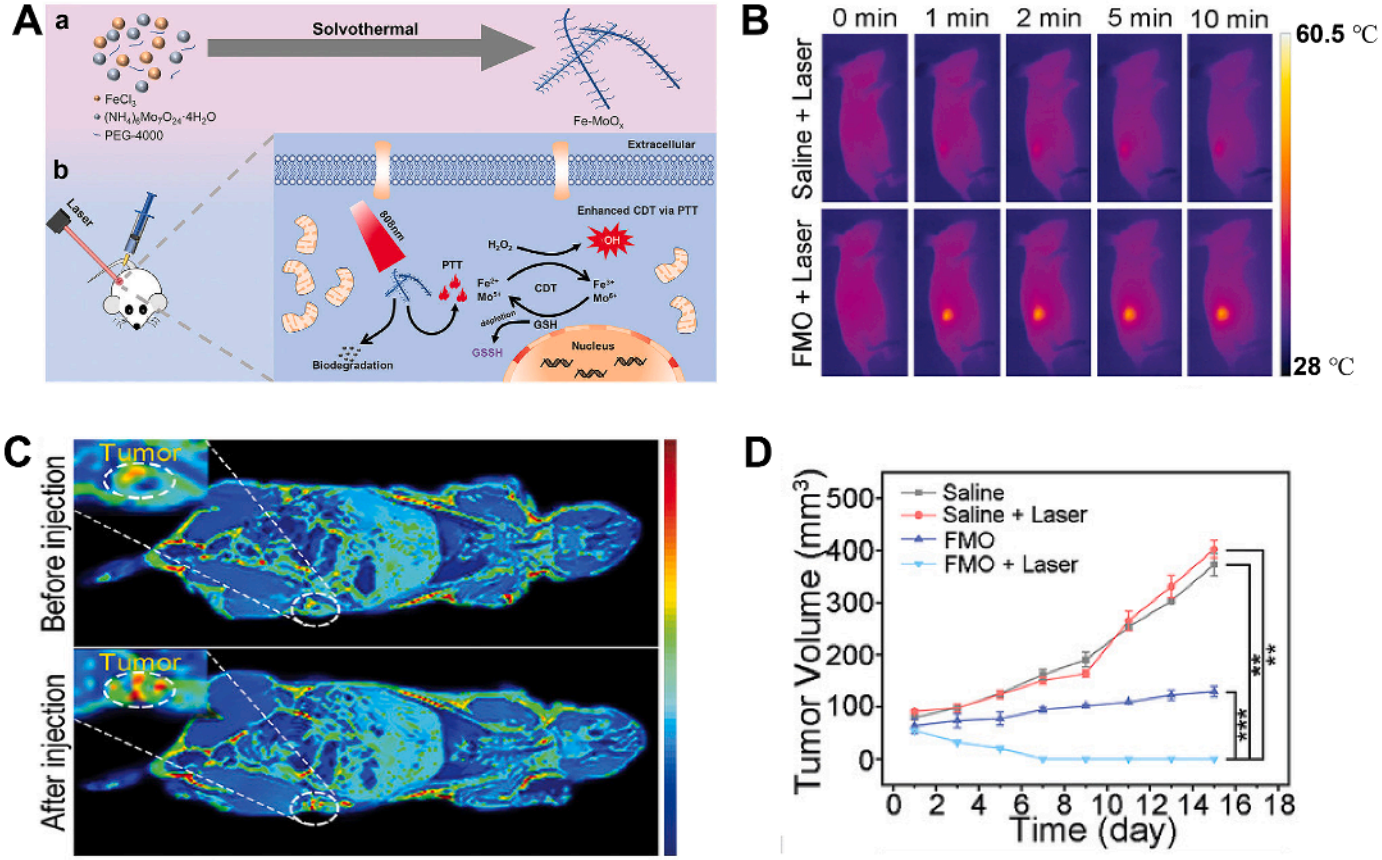


FIGURE 21.9

A. (a) Synthesis process of FMO nanowires. (b) Schematic illustration of FMO for photothermal enhanced CDT and GSH-depleted amplified CDT. B. Infrared thermal images at different time intervals of HeLa tumor-bearing mice in which saline and the FMO suspension were injected into the tumor upon 808 nm laser irradiation. The laser power density was 0.7 W cm^{-2}. C. T_1-weighted MRI before and after intratumor injection. D. Tumor volume of mice from different groups.

resistance. Nanoplatforms combining strategies to overcome hypoxia and scavenge GSH are very promising for improving PDT efficacy. Ma et al. developed a NIR and acidic TME dual-responsive nanoplatform named $PMIC^{Apt}$/ICG for synergistic PTT and PDT of cancer [83]. PTT and PDT can be activated simultaneous by 808 nm laser irradiation. Acid-degradable $CaCO_3$ promoted effective tumor accumulation and TME-responsive payload release in situ. The imaging capability of the nanosystem was assessed in 4T1 tumor-bearing mice. Photothermal images showed that 24 h post-intravenous administration of $PMIC^{Apt}$/ICG, the photothermal imaging signal was stronger than at other time points, and the temperature at the irradiated tumor region reached around 60°C. In vivo MRI images showed that T_2-weighted signal 24 h post-intravenous administration of $PMIC^{Apt}$/ICG was significantly stronger than that at 0 h, demonstrating the high accumulation of the nanodrug at the tumor site. Furthermore, $PMIC^{Apt}$/ICG showed good synergistic anticancer efficacy of PTT and PDT in 4T1 tumor-bearing mice, with some of the tumors were even totally eliminated after 2 weeks of therapy.

Extracellular vesicles (EVs), man-sized cell-derived membrane vesicles, have emerged as versatile nanocarriers for drug loading and delivery in recent years due to the good biocompatibility, prolonged blood circulation, as well as minimal immunogenicity [84,85]. Cao et al. developed EVs-based ultrasound and pH dual-responsive sonosensitizers for PAI-guided chemo-SDT of cancer [86]. The sonosensitizer and PAI agent indocyanine green (ICG), anticancer drug paclitaxel (PTX), and $NaHCO_3$ (sodium bicarbonate, SBC) were co-loaded into EVs. In acidic TME, SBC decomposes spontaneously and generate CO_2 bubbles to cause EV membranes burst and release of payloads. US irradiation activates ICG to generate ROS in cancer cells, which subsequently results in synergistic anticancer effects with PTX. In vivo PAI of ICG-loaded EVs and free ICG was performed using MCF-7 tumor-bearing mice. Strong PA signals were observed in the tumor region after the mice were injected with ICG-EVs, while no strong PA signals were identified in free ICG group, demonstrating good accumulation of the nanosystem at the tumor site. The evaluation results of cancer therapy efficacy showed that SBC-EV (ICG/PTX) exhibited better tumor growth suppression than both EV(ICG/PTX) and free ICG/PTX.

Shi et al. developed US and H_2O_2 dual-responsive nano-riceball (NGR@DDP) for enhanced sono-chemotherapy of cancer [87]. This nanosystem possesses a core-shell structure. The inner core contains bottlebrush-like unimolecular dextran-$POEGMA_9$-b-$PMTEMA_{22}$ (DOS) with co-loaded DOX and Purpurin 18, and the shell is NGR (Asn-Gly-Arg)-modified RBC-membrane. Transmission electron microscope images showed that NGR@DDP micelles were degraded under stimuli of US and H_2O_2 treatment. In addition, the loaded DOX was released rapidly under ultrasound (0.56 W cm^{-2} for 5 min) due to the fast structural collapse, followed by continuous release over time in the presence of H_2O_2. PAI data confirmed the gradual accumulation of NGR@DDP in the tumor region, and it reached the peak at 24 h post injection. In vivo antitumor efficacy of NGR@DDP was evaluated in nude mice bearing orthotopic Luc-HepG2 tumors. After 15 days of treatment, NGR@DDP plus US irradiation group presented great antitumor efficacy with complete tumor elimination.

5. Summary and perspectives

The past few years have witnessed the rapid development of stimuli-responsive nanotherapeutics for image-guided therapy of various diseases, especially for cancer treatment. These nanoplatforms integrate therapy and imaging modules into one system, and release the loaded drugs or exert the therapy functions at designated disease sites in response to stimuli, therefore increasing the therapeutic efficacy and reducing side effects. In addition, the embedded imaging modules enable monitoring of

the drug distribution and metabolism, which can be utilized to guide the therapy. In this chapter, we divide the stimuli-responsive nanocarriers into three categories: external stimuli-responsive nanocarriers, endogenous stimuli-responsive nanocarriers, as well as external and endogenous stimuli dual-responsive nanocarriers. In each part, the working mechanisms and typical application examples of these nanoplatforms were introduced in detail.

The basic research of nanocarrier-based stimuli-responsive drug delivery systems has gained extraordinary progress, while some bottlenecks still hamper the clinical translation.

(i) Precise control of the stimuli. Although theoretically the designed stimuli-responsive nanomedicine can release the loaded drugs and exert therapy effect in response to stimulations, in some conditions the stimuli cannot be controlled as we expect, especially to the endogenous stimuli. In addition, the parameters of external stimuli should be standardized.

(ii) Large-scale fabrication of the nano drug delivery systems. Due to the complexity of some nanoplatforms, which integrate both therapy and imaging modules into one system as introduced above, it is difficult to prepare NPs with uniform quality on a large scale. Therefore, simplification should be taken into consideration in the design of stimuli-responsive nanomaterials.

(iii) Uncertain fate of the nanocarriers in the body. The nanomaterials, especially inorganic nanomaterials, cannot be degraded completely in the body. Therefore, through safety evaluation of the nanocarriers, including immunological and cytotoxic characteristics, in vivo biodistribution and metabolic process, as well as short-term and long-term effect to the body should be thoroughly investigated before clinic translation.

We expect that with the development of science of various disciplines, the issues pointed above will be gradually addressed, and more intelligent stimuli-responsive nanomedicine will be developed to cure human diseases and improve life quality.

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Conflict of interest

The authors declare no conflict of interest.

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PART

V

Therapeutic aspects of stimuli-responsive nanocarriers

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CHAPTER

Stimuli-responsive metal nanocarriers in the treatment of various cancers

22

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1. Plasmonic materials

Plasmonic nanoparticle-based drug carrier systems are a class of inorganic materials represented over the past 2 decades as the quintessential accomplishment of nanotechnology in smart drug-delivery systems with the absolute potential to break the pharmaceutical market. The series of fascinating features, such as biocompatibility, low toxicity, minimum immune response, etc., facilitates targeted therapy and widens their application range besides drug-delivery, like bioimaging, sensing, enhanced spectroscopy, and photothermal therapy (PTT). In light of these facts, over the next parts, we will have a comprehensive but concise look at the ruling principles and underlying mechanism in this domain, as it is of great importance to help readers have a deeper understanding and those who seek to prosper in this field by either devising new structures or ameliorating the existing ones [1].

1.1 LSPR effect

Upon studying plasmonics, the first concept to encounter is "plasmon", which has led the field to be called plasmonics. A plasmon is a quasiparticle and the quantum of the collective oscillation of the electron sea in a metal when it's illuminated by a beam of light, but to gain a deeper perspective on this phenomenon, understanding the light-matter interaction at the nanoscale and electronic structure of the metals is crucial. The electronic structure of the metals is characterized by two distinct bands: the valence band and the conduction band. Electrons in the conduction band of metals are assumed to make a "sea" or "gas" of electrons, and this sea is not bound to specific atoms; on the other hand, it can move collectively through the whole metal lattice. When the dimensions of materials are reduced to nanometers, usually unpredicted optical responses are observed, such as the appearance of gold colloids with different colors relative to their size. When the thickness of a metal layer reaches a few nanometers, under the specific conditions called resonant conditions, the energy of the incident light matches the energy of the plasmons at the surface, causing them to oscillate and propagate along the surface to only a few nanometers. These propagating waves at the interference of metal and dielectric are named "surface plasmon polaritons" shortly known as surface plasmons, and the corresponding phenomenon is named "surface plasmon resonance" (SPR). Nanoparticles are smaller in all three

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dimensions compared to the wavelength of visible light; thus, the electric field component of the electromagnetic wave can uniformly oscillate the plasmons (resembling a harmonic oscillator) to the nonpropagating collective oscillation, generally known as "localized surface plasmons" (LSPs). LSPs decay rapidly from the material's surface, reaching only a few nanometers in the surrounding dielectric, and are widely used for bioimaging, biosensing, drug delivery, and other similar applications. Same as SPR, but only here surface plasmons are localized to a limited area; therefore, it is called localized surface plasmon resonance (LSPR) [2,3].

1.2 Physiochemical principles

LSPs exhibit important properties: first, they are sensitive to the material composition, size, shape, and surrounding dielectric, meaning that the resonance frequency is not set at a certain unchangeable frequency; rather, it shifts or changes in the shapes of the peaks in the spectrum through altering these parameters. The ability to tune the plasmon peak enables the customization of nanoparticles for imaging applications (enhancing contrast) and improved drug delivery systems by using specific wavelengths for activation. For instance, the plasmon peak of gold nanorods has two distinct peaks, one corresponding to the longitudinal plasmon mode and the other to the transverse mode. Second, LSPs can create enhanced electromagnetic fields in the neighborhood of nanoparticles, which is referred to as "hot-spot". This enhanced field can dramatically boost various optical phenomena, such as scattering and absorption, especially in biosensing applications. Third, these oscillations result in strong scattering and absorption cross-sections that are calculated by Mie theory. The Mie theory explains the absorption and scattering cross-sections of light by spherical particles and the final extinction cross-section, which is the sum of both ($C_{ext} = C_{abs} + C_{sca}$). This parameter is crucial for understanding how nanoparticles behave in different media and conditions. Both SPR and LSPR produce evanescent waves that decay rapidly from the surface due to the dissipation of the energy under various mechanisms, which will not be discussed here, but these evanescent waves are means to detect small concentrations of analytes and even atoms with good precision and accuracy. To observe SPR and LSPR phenomena, plasmonic materials are required to have a high density of electrons and satisfying conductivity, thus mostly gold (Au), silver (Ag), platinum (Pt), and palladium (Pd) are widely used, but due to their rare amount and costly expenses, other abundant elements such as aluminum (Al), copper (Cu) and indium tin oxide (ITO) have been considered as substitute in some cases. However, for medical purposes, gold still remains the first top candidate due to its lower toxicity, relatively simple surface functionalization, biocompatibility, and stability, especially over a wide range of pH and ionic strength [4–6].

1.3 Development

The field of plasmonics has progressed a long way and evolved rapidly over the mid-20th century. The trajectory starts with the prediction of the existence of surface plasmons by David Pines (1956) [7]. The discovery of SPR in the 1970s laid the groundwork for biosensing applications in the following years and accelerated the research field. The advent of nanotechnology in the late 20th century catalyzed the transition from bulk plasmonic materials to nanoplasmonics. The invention of different nanofabrication techniques facilitated the exploration of the LSPR effect, and in the subsequent 2 decades, integration of plasmonic materials in photonics and electronics became easier. The

confluence of plasmonics with therapy and medicine dates back to the late 20th and early 21st century when initial explorations on the biocompatibility of AuNPs started. The major breakthroughs that happened during that decade were not attributable to a single scientist or paper but rather to a growing body of work from many research groups. The main focus was mostly concentrated on the biocompatibility, cytotoxicity, colloidal stability, and optical properties of AuNPs, plus some exploratory studies investigating the potential of AuNPs in biomedical applications, such as their functionalization with various biomolecules. The subsequent decade witnessed a convergence of several disciplines, which extended the application range of plasmonics [8]. Over these years, PTT, especially through using gold nanoshells and rods, nanoparticle-based assays capable of simultaneous detection of multiple biomarkers, utilization of AuNPs for computed tomography (CT), photoacoustic imaging, and optical coherence tomography (OCT), have occurred. The result was targeted PTT and drug delivery systems for cancer treatment, better diagnosis, and monitoring of disease due to enhanced imaging techniques and also SERS-based sensors. Since 2010 up until now, progress has been incremental and multifaceted, with many research groups contributing to advancements across several areas which makes it challenging to pinpoint the superlative ones, but the most impressive ones will suffice to show their application includes: dendron-coated AuNPs that are capable of encapsulating drugs within their dendritic structures as the external stimuli triggers the drug release in a controlled way. Another example is hollow gold nanocages with several potencies to be used in targeted drug delivery, PTT, biosensing, and most importantly as contrast agents in imaging techniques such as computed tomography (CT) and photoacoustic imaging [9].

1.4 Advances

The ongoing research on plasmonics has pushed the boundaries and turned it into an interdisciplinary field, fostering hybrid technologies like plasmonic circuits, metamaterials, and optoelectronic devices. However, in the current landscape of smart drug-delivery systems, plasmonics stands out owing to the successful achievements in fabrication techniques and designing strategies during the last couple of years. The design and optimization procedure of plasmonic devices is notoriously complex, requiring intricate simulations such as finite-difference time-domain (FDTD) and extensive experimental validation [10]. After the introduction of artificial intelligence, all the disciplines have been revolutionized more or less, and simulations have not remained untouched. By training algorithms on large datasets of simulated or experimental results, the creation of predictive models capable of rapidly evaluating the performance of different plasmonic designs without the need for computationally intensive simulations has become possible [11].

Meanwhile, the targeted drug delivery has evolved from passive targeting (works based on the enhanced permeability and retention (EPR) effect) to active targeting via combining sophisticated targeting ligands (e.g., aptamers, antibodies, peptides) with gold NPs. In Active targeting, the attached ligands to the surface of NPs bind to the special receptors or markers on the surface of tumor cells, which in turn facilitates the accumulation of AuNPs at the desired site and reduction of the off-target effects. Stimuli-responsive release mechanisms (e.g., pH-sensitive, enzyme-sensitive, light-activated), have been an idea behind the design of this set of nanocarriers that release their drug payload in response to specific stimuli present at the target site. Interestingly, the simultaneous capability of

plasmonics in diagnosis and therapy manifested itself in "theranostic" which has been a considerable step in this dynamic field. These multimodal platforms enable real-time monitoring of drug delivery, distribution, and therapeutic efficacy through imaging techniques, coupled with the simultaneous administration of therapeutic agents. The pursuit of personalized medicine promises to unlock new therapeutic potentials, enhancing precision, efficacy, and safety in medical applications, heralding a new era in nanomedicine [12,13].

1.5 Classification

The systematic study of plasmonics requires classifying them based on different parameters such as composition, dimension, application, drug delivery mechanism, etc., into distinct categories. As discussed earlier, substances that support SPs are not excluded Au and Ag, but rather a diverse class of materials, comprising metals from different groups of the periodic table (Aluminum (Al), Copper (Cu), Cobalt (Co), Platinum (Pt), Palladium (Pd), Ruthenium (Ru)), metal alloys (gold-silver, gold-copper), transition metal oxides (indium tin oxide (ITO), titanium dioxide (TiO)), Graphene, Titanium Nitride (TiN) and core-shell nanostructures (e.g., silica core with plasmonic shell). Although there are many numbers, only a few of them are considered suitable for medical purposes and have passed the clinical test or are still in trial or are in progress to be validated [14,15].

By controlling the size, shape, and surface modification of Au NPs, a variety of structures with exclusive characteristics, each for distinct biomedical requirements, are prepared. So, herein, we believe categorizing only the Plasmonic gold nanoparticles based on their structure should suffice.

The most common structures applied for different medical purposes are [16,17]

- Gold nanospheres: Drug Delivery, Imaging, Contrast Agents, Surface-Enhanced Raman Scattering (SERS)
- Gold nanorods: PTT, Photoacoustic Imaging, Drug Delivery, Contrast Agents
- Gold hallow nanorods: Drug delivery, PTT, computed tomography (CT), photoacoustic imaging, biosensing, Photodynamic Therapy (PDT), fluorescence imaging
- Small gold nanorods: Photoacoustic imaging, PTT, SERS
- Gold nanostars: PTT, Surface-Enhanced Raman Scattering (SERS), Imaging
- Gold nanocages: Drug Delivery, Imaging, PTT
- Gold nanodimers: SERS, PTT, Drug delivery, Fluorescence Enhancement
- Gold core-satellites: SERS, imaging, drug delivery
- Gold nanoshells: Photoacoustic Imaging, Drug Delivery, Contrast Agents
- Gold nanobelts: Sensing, Imaging, SERS, surface Enhanced Fluorescence (SEF)
- Gold nanodisks and nanoplates: Imaging, Sensing
- Gold nanoclusters: Imaging, Fluorescent Markers
- Gold nanoechinus: Photoacoustic imaging, biosensing, PTT
- Gold nanodumbbells: Drug delivery, imaging, Biosensing and Diagnostics
- Gold nanocorals: Optical Imaging and Tomography, Antimicrobial Applications, PTT
- Gold nanochains: SERS, PTT, drug delivery
- Gold nanoframeworks: PTT, imaging, photo-chemotrophy
- Gold nanovesicles: Drug delivery, PTT, Antimicrobial Agents

2. Nanoscale drug-carriers based on plasmonic species

An important area of interest has been created for NPs as drug-delivery vehicles due to their nanometer size, extensive surface-area-to-volume ratios, and better adsorption capabilities. Among these, special attention has been attracted to plasmonic nanomaterials due to their LSPR effect, as elaborated earlier. Nanoparticles fall under two wide categories of organic and inorganic, and the two nanoparticle classes reflect the diverse functionalities and applications of nanoparticles in advancing medical science and engineering. Liposomes and polymeric micelles form the organic NPs and are routinely used for their biocompatibility and targeted drug delivery ability. Metal nanoparticles like Au NPs, mesoporous silica nanoparticles, and carbon-based nanoparticles form the inorganic NPs [18].

2.1 Inorganic nanoscale drug carriers

Three characteristics that inorganic nanoparticles possess are hydrophilicity, nontoxicity, and biocompatibility. Inorganic nanoparticles are more stable than organic nanoparticles. Gold (Au), platinum (Pt), and silver (Ag) metal nanoparticles exhibit intense absorption and light-scattering properties. Plasmonic optical properties of metal nanoparticles can be tuned by controlling the shape, size, and composition of nanoparticles from the visible to the near-infrared portion of the EM spectrum. Further, the color of plasmonic nanoparticles dispersed in a fluid or in a film can be altered by shifting the absorption and scattering [19].

2.1.1 Gold nanoparticles

A crucial reason why Au nanoparticles are important in plasmonics is that they can be used for imaging deep tissues because they absorb light better than other compounds in the near infrared (NIR) range (1350–650 nm). This is because tissues and blood components don't absorb as much light. What is specific to AuNPs is that their LSPR is composed of coherent localized oscillations of free conduction band electrons. Au and Ag nanoparticles exhibit unique spectral responses due to their specific wavelengths of light, which can force the conduction electrons to oscillate in unison. These plasmonic nanoparticles exhibit up to 40% higher absorption and scattering intensities than nonplasmonic particles at their resonances. Their brightness and tunability make them extremely helpful for detection, imaging, and tracking molecules. The creation of plasmonic nanostructures is primarily based on two dominant methods [20]:

In the last 20 years, gold nanoparticles (AuNPs) have been widely studied due to their remarkable optical, electronic, and chemical characteristics stemming from surface plasmon resonance. Strong light absorption and scattering characteristics cause AuNPs to exhibit intense colors upon reduction to the nanoscale [19–21]. This is a result of an electromagnetic field causing a collective oscillation of electrons in the conduction band. This is known as localized surface plasmon resonance (LSPR). Au nanoparticles are inorganic nanoscale drug delivery vehicles. Other than AuNPs, Other gold contrast agent morphologies, such as gold nanorods (AuNRs), gold nanoprisms (AuNPrs), gold nanocages (AuNCs), gold nanostars (AuNSts), gold-based nanoplates, and gold nanocrystalline assemblies, have also been used in NIR-I PA imaging. These gold-based contrast agents can be used in the PA imaging technology to allow deep tissue optical imaging with high spatial resolution [21]. Gold nanoparticles are effective in photoacoustic imaging, chemotherapy, surface-enhanced resonance spectroscopy, gene therapy, and PTT. Enzymes, carbohydrates, fluorophores, peptides, proteins, and DNA can conjugate

with gold nanoparticles that surpass cell barriers to deliver chemicals. Gold nanocarriers are utilized to image tumor cells effectively. The nanoshell and optical coherence tomography agents can provide three-dimensional tissue images. Positron emission tomography, single photon emission computed tomography, and CT analysis make use of gold nanocarriers [22].

2.1.2 Magnetic nanocarriers

Magneto-plasmonic nanoparticles exhibit physico-chemical properties which are applied in drug storage or other molecules of interest, and whose magnetic characterization reveals their potential use as magnetic resonance imaging (MRI) contrast agents. Secondly, their plasmonic activity in the NIR renders them efficient in photothermal application whereby they establish a high temperature gradient after brief irradiation time. Inmaculada Urries et al. synthesized nanoparticles using a model compound, Rhodamine B isothiocyanate. The nanoparticles that were synthesized can be employed as theranostic nanoparticles with imaging capability and a dual therapeutic application, drug delivery and hyperthermia [23].

2.1.3 Mesoporous silica

Mesoporous silica possesses high pore volume with large surface area, porous structure, biocompatibility, high drug loading capacity and thermochemical stability that enable the encapsulation of hydrophobic and hydrophilic drugs that can be conjugated to a ligand molecule to target the drug.

2.2 Organic nanoparticles

Organic nanoparticles represent a cutting-edge class of nanocarriers engineered from biocompatible and biodegradable organic materials, such as lipids, polymers, and proteins, designed to revolutionize therapeutic delivery and diagnostic precision. These nanoparticles, including liposomes, polymeric micelles, and dendrimers, are celebrated for their tunable physicochemical properties, high encapsulation efficiency, and sustained release kinetics, making them versatile platforms for targeted drug delivery, gene therapy, and imaging applications.

2.2.1 Polymeric micelles

Over the past few decades, hydrogels have captured the interest of scientists across numerous disciplines. These materials have a wide range of uses due to their adaptability; some examples are medication delivery, tissue engineering, biosensing [24], actuator manufacture, wastewater treatment, and many more.

Hydrogels are defined by the three-dimensional network that forms from the crosslinking of polymers containing hydrophilic groups. The capacity to hold significant quantities of water is what makes these materials special. Supramolecular hydrogels, and especially low-molecular-weight peptide hydrogels, show a lot of promise as drug delivery methods because they can change how drugs dissolve, target, break down, and cause side effects. The release of drugs from magneto-plasmonic liposomes is improved by thermal stimulation, such as magnetic and optical hyperthermia. These liposomes can be programmed remotely by applying an external magnetic field. Because the two materials are mixed, magnetoplasmonic lipogels can do many things, such as heat up and deliver drugs

in a controlled way at specific times and places [25]. Seungki Lee synthesized spatiotemporally controlled drug delivery via photothermally driven conformational change of self-integrated plasmonic hybrid nanogels. We develop self-integrated plasmonic hybrid nanogels (PHNs) to control drug delivery spatiotemporally through light-driven conformational change and photothermally boosted endosomal escape. The simultaneous integration of gold nanoparticles (GNPs), thermo-responsive poly(N-isopropyl acrylamide), and linker molecules during polymerization easily synthesizes PHNs. Wave-optic simulations show that the density of the integrated GNPs and the size of the PHNs are two very important factors that affect photothermal conversion [26]. Different linkers with different molecular weights are added to find the best PHNs. The alginate-linked PHN (A-PHN) converts heat more than twice as well as the others. Light-mediated conformational changes occur transiently, allowing for spatiotemporally controlled drug delivery. Also, light-activated heat production from A-PHNs inside cells lets them deliver specific molecules to the cytosol by breaking open endosomes. Finally, multicellular spheroids validate the deeper penetration and enhanced delivery efficiency by A-PHNs [27]. Hakan Erdoğan et al. investigated plasmonic PTT-based synergistic drug release application through gold nanoparticles coated and embedded polymer Poly (D-lactic-co-glycolic acid) (PLGA) [28].

2.3 Recent instances in cancer therapy

Valéria Gomes et al. developed a magnetoplasmonic lipogel based on a peptide hydrogel for use in combined cancer therapy. In this case, they made a new peptide hydrogel and added magneto-plasmonic liposomes to it to make it work better. After carefully studying the magneto-plasmonic lipogel's rheological, spectroscopic, and magnetic properties of each part, they tested it to see how well it could deliver drugs under controlled conditions. They achieved this by measuring the magneto-plasmonic lipogel's reaction to near-infrared light after loading it with 5[1]-carboxyfluorescein. Based on the findings, the system provides an ideal vehicle for hydrophilic drug delivery and opens the possibility of photo-responsive medication administration [29]. A new age in cancer and other illness therapy may be on the horizon, thanks to these facts plus the produced composite gel's magnetic navigation and hyperthermia capabilities. Jacopo Cardellini et al. made magnetic plasmonic nanoscale liposomes that can change their optical and magnetic properties. These can be used for imaging in multiple modes and delivering drugs. The study looks at the structure and movement of magnetic plasmonic liposomes created by Au–Fe_3O_4NPs and synthetic liposomes that put themselves together on their own, based on the kind and number of lipid vesicles that are present. We used a mix of cryogenic electron microscopy, ultraviolet-visible spectroscopy, and dynamic light scattering to show that the lipid membrane's bending rigidity and fluidity affect how well the NPs stick to it and how stable the hybrids are as colloids. The tests show that it is very important to have 10 nm Fe_3O_4 magnetic seeds and nm Au– Fe_3O_4 NPs together in order to make lipid-NP magnetic–plasmonic hybrids. This thermodynamic control allows for fine adjustments to the hybrid's size and composition, thereby enhancing and tuning the magnetic response. Overall, these results make it possible to create multifunctional nanomaterials with controlled magnetic and plasmonic properties. These can be made through spontaneous self-assembly and can be used as combined nanoprobes and nanovectors for imaging in multiple modes and delivering drugs [30].

3. Design, preparation and characterization of plasmonic drug-carriers

The design of plasmonic nanoparticles is a long, multistep process, comprised of tailoring their physical, chemical, and optical properties, which is not carried solely, but rather requires a multidisciplinary approach that integrates nanomaterials science, pharmacology, and biomedical engineering to gain efficient therapeutic outcomes.

3.1 Synthesis

The whole synthesis methods are divided into two main groups based on their use approach including bottom-up and top-down techniques, along with hybrid techniques. While techniques in the bottom-up group rely on the assembly of atoms or molecules to construct nanoparticles, top-down techniques are focused on the fragmentation of bulk materials into nanoscale particles, yielding high-purity products but often with limited control over size and morphology. A range of innovative synthetic methodologies have been developed to fabricate AuNPs with dimensions below 10 nm, including:

1. **Chemical Reduction Method:** This method, as a versatile method for synthesizing gold nanoparticles, uses reducing compounds such as sodium borohydride ($NaBH_4$), citrate or (H_2O_2) to reduce gold salts (such as $AuCl_4^-$ or $HAuCl_4$) to AuNPs [31].
2. **Sol-Gel Method:** In this method, a gold precursor solution is used to create AuNPs, such as Au (III), and then it is converted into a gel. This method can be used to synthesize gold nanoparticles with controlled size, which is very suitable for drug delivery [32].
3. **Laser Ablation Method:** A physical method, employing the energy of laser radiation for evaporation, breaking the surface or destroying a target material. Usually performed under vacuum or even wet conditions like water, ethanol, or even other liquids [33].
4. **Green Synthesis Method:** The natural materials of plant extracts, fungi, bacteria, or any other natural compounds act as potential substitutes instead of chemical reducing agents into nanoparticles through the reduction of gold salts in a controlled and precise manner, achieving nanoparticle sizes of less than 10 nm.

Synthesizing gold nanoparticles by reduction reaction, it is probably one of the most common as well as effective methods to produce gold nanoparticles, where the salt that suits to prepare gold ions (the most common salts being gold chloride ($HAuCl_4$) or ($AuCl_4^-$)) is chosen, and the gold ions are reduced into AuNPs by suitable reducing agents. The selection of a reducing agent is usually based upon properties such as the size of the nanoparticles, reaction rate, and stability of the final nanoparticles. Citrate is one such reducing agent that also acts as a stabilizer preventing the aggregation of the nanoparticles. Sodium borohydride ($NaBH_4$), a potent reducing agent, has been playing a significant role in the synthesis of gold nanoparticles (AuNPs) below 10 nm in dimension with a uniform morphology. Here, this chemical agent induces the reduction of gold from its oxidized (Au^{3+}) state to metallic (Au^0) in a molten-alloyed, nanoscale-sized particle dispersed into solution. Parameters such as type of reducing agent, solution pH, temperature and reaction time have a great effect on the size and characteristics of nanoparticles. After the formation of nanoparticles, it is required to use stabilizing agents such as polymers to prevent the aggregation of nanoparticles, which counts as an undesired reaction. They help to maintain the colloidal stability of AuNPs in various physiological and synthetic environments by providing electrostatic, steric, or electrosteric stabilization [34–37].

The second method discussed here is the sol-gel process, which is a famous and popular technique for the synthesis of porous and metallic nanoparticles, specifically for the fabrication of silica-gold nanoshells. This technique enables scientists to control the size and morphology with exceptional precision and tailored optical properties. The principal steps of this process revolve around the formation of a sol, gelation, drying, and calcination to produce the final nanoparticles. In the first step, which is the production of the sol, metal salts or oxide precursors such as $AuCl_3$, $AgNO_3$ or other metal compounds such as sulfates are dissolved in an appropriate solvent (e.g., aqueous or organic solvents) to form a homogeneous sol solution. This sol serves as the foundation for gel formation. Upon the application of specific conditions, such as heating or chemical reduction, the precursors undergo transformation into metal nanoparticles. This transformation is usually conducted through using different crosslinkers like organic acids, amines, or alkalis, which slow down the evaporation rate of the solvent and create crosslinks between the molecules creating a gel with a three-dimensional network. This gelation process can span from several hours to days, depending on the system parameters. For strengthening the chemical networks and fully form the NPs, the obtained gel from the previous step must be subjected to a calcination process to thoroughly dry. Via this stage of the process, the strength of the chemical bonds that are present in the gel is multiplied by raising the temperature to higher degrees (typically between 300 and 600°C), and the nanoparticles in their solid, crystalline state are stabilized. With this method, the size and structure of NPs can be controlled by changing conditions such as temperature, synthesis time, concentration of precursors and type of crosslinking agent. NPs in this method are usually produced in nanometer sizes (between 1 and 100 nm) with different shapes such as spherical, star-shaped, prismatic or needle-shaped. Uniform size distribution and surface properties using specific precursors and production of NPs with different compositions, as well as the possibility of creating special structures with high stability, are other advantages of this method. However, limitations of this technique remain as a challenge for scientists, like multiple steps for drying and calcination, which can be time-consuming and resource-intensive, plus achieving complete control over the surface properties of the NPs is quite difficult and limits their application in certain contexts. Despite these obstacles, this method remains a powerful tool for fabricating NPs for applications in drug delivery systems and other biomedical fields [38–41].

Laser ablation is a physical method in which the energy of a laser is applied to evaporate, break or destroy the surface of the target material. This method is usually performed in a vacuum or in wet environments (e.g., water, ethanol, or other liquids). If it is carried out in aquas condition, the process of synthesizing nanoparticles occurs due to the rapid contraction and expansion of the liquid, and it can produce nanoparticles with specific sizes and properties. The associated advantages of this method can be called controlled size, variety of materials and ease of synthesis process. Among the various applications of this method, it can be said that the synthesis of nanoparticles with LSPR properties that are suitable for use in drug delivery, medical imaging, and industrial applications is possible through this technique [42].

The last method to look into is the green synthesis methods, which are recognized as the revolutionary, highly modern and biocompatible techniques because of utilizing natural, nontoxic and environmentally friendly substances. In stark contrast to conventional chemical methods that employ harmful reagents, this innovative technique harnesses the potential of plant extracts, microorganisms (such as bacteria and fungi), and other biological resources to synthesize AuNPs in a safe and eco-conscious manner. These days, this method has attracted much attention of researches due to its elimination of toxic byproducts and its aligned principles with green chemistry. In the plant-mediated

synthesis, plant extracts or plant tissues, as natural, sustainable resources, are introduced into a metal precursor solution, where they act as natural reducing agents and facilitate the transformation of metal ions into nanoparticles. Various plants are rich in bioactive compounds that can act as good reducing agents or as stabilizing coatings during the formation of NPs. The leaf or seed extract of Moringa oleifera (Moringa) [43] is known to synthesize gold and silver nanoparticles, acting as a natural reducing agent due to its extensive phytochemicals. Other examples include turmeric, wherein the active compound curcumin serves as a potent reducing agent for the synthesis of gold NPs with a size less than 10 nm. This makes the plant a champion of nano synthesis and provides credence for green nanotechnology. Some microorganisms, like bacteria (*Bacillus subtilis* and *Pseudomonas aeruginosa*), fungi [44–46], (*Fusarium oxysporum* and *Aspergillus niger*), and yeast (*Saccharomyces cerevisiae*) [47–49], are considered as natural nanofactories to synthesize nanoparticles through biosynthetic mechanisms for their unique ability to adsorb metal ions from their environment and convert them to nanoparticles biogenically, [50–52].

3.2 Characterization

The NPs synthesized by the above methodologies need to be characterized to assess their physical, chemical and optical properties, and this has been accomplished by various advanced microscopic and spectral techniques. Scanning Electron Microscopy can provide images with high-resolution of surface morphology and size of NPs, while Transmission Electron Microscopy (TEM) is used when detailed insights into the internal structure, size distribution and shape of NPs at the atomic level are needed. The LSPR peak, as the most characteristic feature of plasmonic materials, is studied by UV-visible spectroscopy. Functional groups along with surface chemicals of nanoparticles are screened by Fourier Transform Infrared Spectroscopy (FTIR). In addition to spectroscopic and microscopic techniques, measuring the average hydrodynamic size and size distribution of nanoparticles in suspension is possible through using Dynamic Light Scattering (DLS). The LSPR absorption peak is often observed as a distinctive feature in the UV-Vis spectrum, appearing only at specific wavelengths—typically around 520–550 nm for gold NPs and 400–500 nm for silver NPs [53,54]. The position of the peak shifts directly by the relative size of the NP; larger NPs exhibit a red shift to longer wavelengths, while smaller ones display peaks around 500 nm or lower. By analyzing the peak position, intensity, and shape in the UV-Vis spectrum, researchers can infer the size, concentration, and synthesis quality of NPs. If they are distributed in a uniform size and optimal surface area, sharper and more intense LSPR peaks are likely to be observed. Conversely, the presence of impurities or polydispersity can lead to a reduction in peak intensity and a broad spectrum. In the context of green synthesis, plant extracts often exhibit absorption spectra in the UV-Vis region, which may be caused by the presence of compounds such as flavonoids, alkaloids, phenolic acids and other antioxidant compounds that produce absorptions in the UV-Vis spectrum. Plant materials usually reduce the size of nanoparticles and may also cause changes in their shape (e.g., becoming nanoplatelets, nanoblocks or nanoneedles). In green synthesis and biological synthesis of NPs, changes in the UV-Vis spectrum can indicate the progress of the synthesis reaction. An increase in the intensity and clarity of the LSPR peak usually indicates the growth of NPs (approximately 250–300 nm), which can influence the optical properties of the synthesized NPs [55].

4. Principal approaches in treatments with plasmonic drug-carriers

The application of plasmonic drug carriers is rooted in their optical behavior which provides a toolbox for smart therapeutic approaches. Although targeted drug delivery by conjugating targeting ligands (e.g., antibodies, peptides, aptamers) to their surface is the primary approach, PTT, photodynamic therapy, and stimuli-responsive drug-release are the developed applications for enhanced treatment. Other therapeutic uses of plasmonics encompass applications like wound healing, imaging purposes (i.e., photoacoustic imaging, optical coherence tomography imaging, etc.), gene therapy, and treatment of inflammatory diseases. The specific approach employed depends heavily on the disease, the desired therapeutic effect, and the design of the nanocarrier. Here, the three common and promising approaches of plasmonic drug-carriers besides the targeted therapy are explained in detail.

4.1 Photothermal applications (heat-generating strategies)

"Hyperthermia" is known as an almost noninvasive and complementary treatment technique to other methods, operating based on the deliberate temperature rise of a specific body part or the entire body above normal (>40°C/104°F) to achieve various medical benefits. This elevation is either local or total and is usually applied to help cancer treatment through protein denaturation, metabolic rate acceleration, and cell membrane disruption of the cancer cells. Cancer cells are more susceptible to this technique for their higher metabolism compared to normal cells, and thus, heat generation is counted as an effective approach for curing the diseased tissue or tumor cells. PTT, is a specific type of localized hyperthermia that is deeply focused and works in a more sophisticated way by utilizing plasmonic nanoparticles and their accumulation in targeted cells or tissues. Looking profoundly into the mechanism, upon irradiation of noble metal nanoparticles with light, electrons are excited above the Fermi energy—the highest occupied energy level at absolute zero temperature and produce hot electron-hole pairs. Then the energy of these nonthermally distributed hot electrons is redistributed among them via electron-electron scattering in a timescale of (100fs to 1ps) and subsequently transferred to the nanoparticle's lattice (the metal atoms themselves) through electron-phonon collisions. The lattice absorbs this energy, causing a temperature increase in the nanoparticle, which is proportional to the sphere's volume (R^3) and lasts for about 100 ps to 1ns. This process ends with the diffusion of heat from the nanoparticle to the surroundings and is controlled by considering factors like the materials' properties (heat capacity, density, conductivity) and diminishes in the surrounding water as the distance (r) increases, (proportional to R/r). Photophysical aspects of this phenomenon are frequently studied using ultrafast laser spectroscopy to monitor the dynamics of these processes on very short timescales (femtoseconds to nanoseconds) [56,57].

Biological tissues (e.g., blood, muscle, skin) and components (hemoglobin, water, and melanin) in the human body are capable of absorbing and scattering a minor fraction of the NIR-I light (650–950 nm), which lowers the penetration depth and effective intensity. Light scattering is a major obstacle in deep tissue penetration, whether by nanoparticles or biological substances, it is crucial to engineer particles with a high absorption-to-scattering ratio for PTT purposes. Even though the size reduction of nanoparticles improves the mentioned ratio, the blue shift of the LSPR into the visible spectrum is the resulting disadvantage, plus, as the nanoparticles get smaller, they grow to lose efficiency in converting light into heat. Therefore, although smaller particles absorb light more effectively, this absorbance is not located in the NIR range, which is necessary for deep-tissue penetration.

Sufficient light penetration requires higher irradiation intensities, but exceeding the American National Standards Institute (ANSI) safety standards for skin exposure can lead to harm and sideeffects. Besides, the maximum permissible exposure (MPE) for NIR-I (0.33 W/cm^2) is rather poor, especially when it's compared to NIR-II radiation (1 W/cm^2). To overcome these challenges, the NIR second window (NIR-II, 1000–1700 nm) has been suggested as an alternative. The dominant scattering mechanism in biological tissues is Rayleigh scattering which is inversely proportional to the fourth power of the wavelength (λ^{-4}) and it implies the fact that longer wavelengths (NIR-II) experience significantly less scattering than shorter wavelengths (NIR-I) and therefore, lower irradiation power is needed to reach the same therapeutic effect, besides, sufficient depth of penetration is guaranteed. The outcome advantage is the minimized risk of tissue damage by staying below ANSI/MPE limits [58].

So far, various plasmonic materials have been employed for PTT, such as silver, platinum, palladium, copper, and gold, because of their large absorption cross-sections in the visible and NIR regions. While Ag and Cu suffer from less stability in different media (the formation of an oxide layer on the surface of the nanoparticle results in damping of the LSPR), Pt and Pd are less tunable in the NIR region, which leaves gold as the only option for its higher biocompatibility and tunable plasmon peak via different morphologies.

The outcome advantages of this technique over traditional hyperthermia are the noninvasive treatment, for its precision in targeting the selected area and minimized damage to surrounding healthy cells, along with the Real-time monitoring capabilities. Beyond PTT, photothermal strategies utilize heat generation for controlled drug release from nanocarriers, enhancing drug efficacy and minimizing side effects. In recent years, smart vehicles covered by polymers, lipid layers, etc., for targeted drug/gene delivery were fabricated in which the induced thermal by laser corrupts the outer cover and results in the better release of the drug into the cytosol [59–61].

4.2 Photodynamic applications (electronic strategies)

Photodynamic therapy (PDT) represents a minimally invasive treatment, predicated on the synergistic interaction of light, a photosensitizing agent (PS), and molecular oxygen to generate cytotoxic reactive oxygen species (ROS), predominantly singlet oxygen ($^1O_2$), for the selective ablation of neoplastic cells, bacterial pathogens, and other deleterious microorganisms. The photophysical and photochemical processes involved in PDT are significantly amplified by integrating plasmonics into the PDT process. The underlying mechanism of Photodynamic therapy describes the photon absorption of the PS at a specific wavelength, which elevates it from the ground singlet state (S_0) to its first excited singlet state (S_1). A subsequent intersystem crossing (ISC) process facilitates a transition from the short-lived S_1 state to the metastable triplet excited state (T_1), which is pivotal due to its prolonged lifetime. This version of PS is highly reactive and is capable of interacting with existing molecular oxygen (O_2) to form singlet oxygen ($^1O_2$) via transferring the energy. This radical is a potent oxidizing agent (singlet oxygen) and causes oxidative damage to cellular components such as lipids, proteins, and DNA to finally terminate the cell (apoptosis, necrosis, or autophagy) [62]. This process is highly localized at the site of photoactivation, wherein plasmonic nanoparticles play their key role. By dramatically multiplying the rate of light absorption by photosensitizers located near the nanostructure, stronger excitation of the photosensitizer molecules becomes possible, followed by the multiplication of the triplet-state population and greater singlet oxygen production.

Among the various PSs, the selection of the most suitable PS for plasmon-enhanced PDT is often controlled by factors such as absorption spectrum, biodistribution, and cellular uptake, and it is critically dependent on the desired application and the type of plasmonic nanostructure used. Plasmon-enhanced photodynamic therapy commonly uses the following photosensitizers: chlorins, phthalocyanines, indocyanine green (ICG), and porphyrin derivatives such as hematoporphyrin derivative (HpD) and photofrin (a purified mixture of porphyrins). PSs can be conjugated, coated, or encapsulated on/inside plasmonic nanoparticles (e.g., Au nanorods or nanoshells) and applied for PDT + PTT treatment by enhancing the efficacy of both modalities. The current trend is toward designing and synthesizing novel advanced hybrid composites that offer synergistic advantages, leading to superior tumor cell killing than either modality alone. The heat produced by plasmonics also enhances the permeability of cell membranes, making them more susceptible to ROS attack, and diffusion of oxygen within the tumor, alleviating hypoxia, a common problem in many tumors that limits the effectiveness of traditional PDT [63].

4.3 Stimuli-responsive drug release

The third approach that we will investigate in this section is the stimuli-responsive drug release, a strategy based on employing advanced materials to modulate the liberation of therapeutic agents in a spatiotemporally controlled manner. This approach deviates from conventional drug delivery methods, which often exhibit uncontrolled and often diffuse release profiles. Upon inducing exogenous or endogenous stimuli, a conformational change in the structure of the drug carrier begins, which is responsible for effectuating the release of the therapeutic payload. These stimuli are assorted into three groups: physical (e.g., changes in temperature, pH, light intensity, or magnetic fields), chemical (e.g., enzymatic activity or redox potential), or biological (e.g., ligand-receptor interactions). The design of such systems requires careful consideration of physicochemical parameters to optimize drug encapsulation efficiency, release kinetics, and biocompatibility. The unique optical and thermal properties of plasmonic nanoparticles help to control the release of therapeutic agents in a targeted and controlled manner. In the stimuli-responsive plasmonic drug assemblies, external stimulus interacts with the plasmonic nanoparticles and starts the structural changes to the carrier's structure or properties, which in turn leads to the start of the controlled release of the drug [64,65].

The main popular systems based on the kind of stimulus are:

1. **Light-responsive systems:** in this group, the drug leaches out of the carrier when specific wavelengths of light are irradiated in order to trigger the light-responsive materials. For instance, reversible [2 + 2] cycloaddition of coumarin under long-wave (365 nm) UV irradiation, and reversed reaction with a shorter wavelength (254 nm).
2. **Temperature-responsive systems:** within this class, thermoregulated release of the drug to the surrounding medium occurs when the surface-modified molecules or encapsulating component expands or contracts under temperature gradients. A regular example of the thermosensitive material is poly(N-isopropylacrylamide) (PNIPAM) with a lower critical solution temperature (LCST) of 32°C, employed for encapsulating drug carriers in numerous reports.
3. **pH-responsive systems:** protonation and deprotonation of molecules and polymers in certain pH ranges results to the conformational changes of them **(e.g., poly (acrylic acid)).** This strategy is especially useful in the case of tumor cells with slightly lower pH-levels (6.5–7.2) than normal

tissues (~7.4), to ensure the targeted-drug release and minimized off-target effects on normal cells [66].

5. Clinical challenges

Tremendous research has focused on the multifunctional plasmonic systems, though, only a small group of them are considered promising to reach clinical trials. This probably stems from the lack of attention to the fact that whether a nanocarrier can fulfill the safety and biocompatibility expectations rather than spotlighting complexing them to polyfunctional structures. Almost all the nano drug-carriers share challenges in common, including biocompatibility and toxicological aspects, pharmacokinetic complexity and clearance, plus limitations in manufacturing and reproducibility, which will be discussed in the next part. Notice that all the plasmonic drug carriers are generally counted as drug entities in this context.

5.1 Prerequisites

According to FDA regulations, preclinical research, as the second stage of the *drug development process,* involves a number of prerequisites for a typical nanoformulation carrying the pharmaceutics. The summation of them includes the following:

Preclinical Evaluation: Before clinical testing, detailed cell culture assays evaluating cytotoxicity, oxidative potential, and immune activation pathways must be tested in both in vitro and in vivo conditions. For instance, the oxidative behavior of AuNPs is a paradoxical issue that requires extra investigation since their uncontrolled usage can lead to significant oxidative damage. Gold NPs are observed to *catalyze the* ROS generation, specifically hydroxyl radicals (·OH) and superoxide anions (O_2^-) under certain conditions, like X-ray or UV irradiation. However, carefully controlled circumstances can bias the reaction to prevent the activation of signaling pathways that lead to ROS overproduction. Moreover, the toxicity profiles of affecting parameters comprising size, shape, zeta potential, and surface functional groups need to be elaborated. The density and type of surface ligands, along with the average charge of NP, are considered to be contributing factors in the penetration of biological barriers such as the blood-brain barrier (BBB) or tumor vasculature. The enlargement of NPs by the formation of a dense layer on the surface of NPs consisting of proteins known as "protein corona", can become a further obstacle in their entrance to the cells, plus increasing their risk of aggregation [67].

Reproducible and Scalable Synthesis: Synthesis protocols should be amenable to industrial-scale production and in compliance with good manufacturing practice (GMP) guidelines. All the researchers are obligated to apply good laboratory practices (GLP) for this set of experiments to find information on dosing and toxicity levels [68,69].

5.2 Clinical phases

After a vaccine, medical treatment, drug, or therapeutic nanocarrier passes preclinical studies to decide (e.g., in vivo and in vitro tests) whether it has the potential to be studied on human beings, it enters the next stage of clinical trials. Clinical trials are a series of experiments conducted by scientists or drug developers on volunteer groups of people in multiple steps, named phases. Before starting the first

clinical phase, the Investigational New Drug (IND) application must be sent to the Food and Drug Administration (FDA) on behalf of developers, which comprises a detailed explanation of the protocols and analysis of conducted research so far.

The first phase begins with a small group of patients, usually less than 100 individuals, to search for the safe dosage of the drug, its metabolism, and how it's being excreted from the body. After the safe dosage is discovered, it proceeds to the second phase, which includes more people (up to hundreds of people) and lasts for a longer time compared to the previous phase. Through this phase, scientists study the efficacy and every associated side effect within the therapeutic dosage. Approximately 33% of the drugs can pass this phase and move on to the one. Phase three revolves around the effectiveness of the new intervention and determines if it is worth to clinical practice [70–72].

5.3 Dosage programming

For modern targeted oncology drugs, a nuanced approach that prioritizes both efficacy and long-term tolerability of the drug is needed to optimize treatment outcomes for cancer patients because solely focusing on the maximum tolerated dose (MTD) in dose-finding trials is insufficient. The ideal dose should maximize clinical benefit while minimizing the long-term impact on quality of life. Conventional dosage trials are used to neglect crucial information that could lead to a more effective and better-tolerated dosage regimen. Delaying dosage optimization until after drug approval has significant drawbacks; for example, there is a risk of large numbers of patients receiving a poorly tolerated dose or one that doesn't provide optimal benefit. Plus, conducting postapproval clinical trials comparing different doses is complex and time-consuming; therefore, it is of great importance to identify the nanoparticle's therapeutic index—the margin between efficacious dosages and toxic concentrations—to rationally design the dosage. The FDA acknowledges that the optimal method for determining the best dose depends on several factors: the drug's class, the target patient population, and any preexisting knowledge about the drug's dosing characteristics. Instead of mandating a single approach, they strongly encourage sponsors to discuss their planned dose optimization strategies with the FDA early in the clinical development process, preferably during formal meetings. The pharmacokinetic plan for dose-finding trials must be robust enough to characterize the drug's pharmacokinetics after the first dose and at a steady state (after repeated doses) for each dose tested. This includes assessing parameters like linearity (is the drug's behavior proportional to the dose?), absorption, distribution, and elimination. The relationship between dose, drug exposure, and clinical responses (safety, efficacy, and activity) should be evaluated early and iteratively. Appropriate metrics for safety (e.g., lab results, adverse events) and activity (e.g., tumor response, biomarkers) should be selected based on the stage of development, the drug itself, and the disease being treated. Covariates (factors influencing the response) should be analyzed to identify potential differences in subpopulations [73–75].

5.4 Approvals

Positive Phase three outcomes typically form the basis of regulatory submissions for market approval. Shortly thereafter, the sponsors submit a new drug application (NDA) form, which covers a comprehensive safety and manufacturing data along with the results of all the animal and clinical tests in complete detail. Later on (almost 60 days), the NDA is reviewed by an assigned team from a specific

division of the FDA known as the Center for Drug Evaluation and Research (CDER). The CDER team includes physicians, chemists, pharmacologists, and other parties to evaluate the veracity of the achieved findings and proposed labeling. If the advantages of the new drug outweigh the observed and potential risks, the manufacturing facilities will be visited by FDA inspectors to decide whether to approve it or not. Once the drug receives approval from the FDA or other organizations, it enters the postmarketing surveillance, which is the final phase. The fourth phase is designed for monitoring the rare adverse effects and evaluating the drug's performance across broader patient populations and extended usage periods. Through this phase, important questions like what the effects of the drug are on certain groups with specific conditions (e.g., children or pregnant women) or whether it is safe to be used with other drugs are answered by taking complementary tests [76–79].

6. Future perspectives

Looking forward, the collaborative efforts between academia, industry, and regulatory individuals are essential to commercialize and establish standardized protocols for clinical applications. The burgeoning field of plasmonics is also becoming increasingly interdisciplinary, with researchers combining plasmonics with other areas like immunotherapy, gene therapy, and nanorobotics, and is poised to revolutionize the conventional drug delivery systems market along with the imaging and diagnostics. With the plasmonic systems finding their way into the market, large pharmaceutical companies and generic drug manufacturers are undoubtedly the most affected sectors. Additionally, it goes without saying that medical Imaging companies that solely focus on existing contrast agents and imaging technologies without incorporating plasmonics could face competition and reduced demand. On the contrary, Biotech firms investing in drug development and expertise to integrate plasmonic systems into their therapeutic pipelines will be well-positioned to gain market share. As of 2024, the global market revenue of plasmonics is USD 15.54 billion and is predicted to reach USD 41.55 billion by the end of 2031 with a compound annual growth rate (CAGR) of 15.09%. One of the mature applications of plasmonics is SPR-based sensors that have been commercialized for their real-time monitoring and point-of-care applications, but all the plasmonic drug carriers are still in the clinical phases [80,81].

CYT-6091, also known as Aurimune, developed by Cytimmune Inc, is a colloidal plasmonic-based nanocarrier for targeting solid tumor cells. It consists of 30 nm gold NPs functionalized with thiolated polyethylene glycol and recombinant human tumor necrosis factor alpha (rhTNF) as an anticancer. 6 h after the first injection to mice, a concentrated amount of TNF in cancerous cells was noticed, and surprisingly, the accumulation in healthy tissues was negligible or near zero. A minor fever as a side effect was witnessed in rabbits and dogs; nevertheless, it didn't induce hypotension, which was a major drawback in the former attempts. CYT-6091 has victoriously finished the first phase of clinical trials and has currently started phase II, sponsored by the National Cancer Institute (NCI) [82].

The potential of plasmonic nanoparticles extends beyond the delivery of chemotherapeutics, with a growing interest in their application for immunotherapy and multimodal regimens. Researchers have demonstrated the efficacy of plasmonic nanoparticles in delivering immunomodulatory drugs. A successful example is C19-A3 AuNP, a nanoagent designed for the treatment of type 1 diabetes and is composed of the human proinsulin peptide (C19-A3) anchored to the surface of ultrasmall gold nanoparticles (AuNP) with a diameter of less than 5 nm. In 2016, phase I began with three

doses administered at 4-week intervals to the subjects. The results indicated that patients with type 1 diabetes tolerated the C19-A3-AuNP doses well, with no evidence of systemic hypersensitivity observed [83,84].

The convergence of plasmonic nanoscience with immunological engineering opens up new windows for new vaccines and modern therapies. **EMX-001 (naNO-DENGUE)** is a dengue fever vaccine developed by Emergex Vaccines Holding. In phase I, healthy volunteers were administered intradermal microneedle injection containing different doses (2.5 and 7.5 nmol total peptide), which was successfully tolerated and adverse events were reported to be mild and transient. Overall, the hopeful clinical results provide proof-of-concept that Emergex's new nanovaccine is a potential candidate against the dengue virus in the near future [84].

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CHAPTER

Nonmetallic nanoparticles: A therapeutic platform for nonmalignant disorders

23

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1. Introduction to nonmetallic nanoparticles

This section introduces the fundamental concept of nonmetallic nanoparticles and their importance in biomedical applications, especially in treating nonmalignant disorders. It lays the groundwork by defining what these nanoparticles are and classifying them into different types. The section will also provide a broader context of how these nanoparticles are integrated into therapeutic platforms.

1.1 Definition and classification of nonmetallic nanoparticles

Nonmetallic nanoparticles are ultrafine particles, typically ranging from 1 to 100 nm in size, composed of materials other than metals. These materials may include polymers, ceramics, carbon-based compounds such as graphene and carbon nanotubes, as well as various types of oxides and silicates. Due to their small size and high surface area, nonmetallic nanoparticles exhibit unique physical and chemical properties that differ significantly from their bulk counterparts [1–3].

These nanoparticles can be classified in several ways. Based on composition, there are **polymeric nanoparticles**, made from synthetic or natural polymers, widely used in drug delivery and biomedical applications; **ceramic nanoparticles**, composed of inorganic materials such as silica or titanium dioxide, often employed in coatings and electronics; and **carbon-based nanoparticles**, such as carbon nanotubes and graphene, known for their exceptional strength and conductivity [4,5].

Nonmetallic nanoparticles are also categorized by their **structure**. **Nanodots** are spherical particles typically only a few nanometers in diameter, frequently used in imaging and sensing. **Nanofibers**, with fibrous structures at nanometer-scale diameters, are common in filtration and tissue engineering. **Nanoplates**, flat, two-dimensional structures, are utilized in material science due to their unique surface properties [2,6,7].

In terms of synthesis, there are two main approaches: **top-down methods**, which involve breaking down larger materials into nanoparticles through processes such as milling or lithography, and **bottom-up methods**, which rely on assembling nanoparticles from smaller units using chemical processes such as sol–gel methods or chemical vapor deposition [8–11].

Finally, nanoparticles can be classified by their **functionality**. **Functionalized nanoparticles** have surfaces modified with specific chemical groups to enhance their properties for targeted applications,

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while **bioactive nanoparticles** are designed to interact with biological systems, commonly used in drug delivery systems or as diagnostic agents [12].

1.2 Importance in biomedical applications, particularly for nonmalignant disorders

Nonmetallic nanoparticles are becoming increasingly essential in biomedical applications, particularly for nonmalignant disorders, due to their unique properties and versatile functionalities [13]. One of their most significant advantages lies in their ability to facilitate **targeted drug delivery**. These nanoparticles are engineered to transport therapeutic agents directly to specific sites within the body, enhancing treatment efficacy for conditions such as diabetes, autoimmune diseases, and chronic inflammatory disorders, while minimizing unwanted side effects [14].

Beyond their therapeutic applications, nonmetallic nanoparticles also play a crucial role in **diagnostic imaging**. They improve the contrast and sensitivity of imaging techniques such as MRI and ultrasound, aiding in the early detection and monitoring of nonmalignant conditions such as cardiovascular diseases and neurological disorders [15–17].

Some nonmetallic nanoparticles even possess inherent **therapeutic properties** or can be functionalized to enhance their activity. For example, polymeric nanoparticles are used for the gradual release of antiinflammatory drugs, providing sustained relief for chronic conditions such as arthritis. In **regenerative medicine**, these nanoparticles are incorporated into scaffolds to promote cell growth and tissue regeneration, offering promising solutions for disorders such as osteoporosis and tendon injuries [18,19].

Moreover, nonmetallic nanoparticles hold substantial potential for **immunomodulation** in the treatment of autoimmune disorders. They can be designed to either enhance or suppress specific immune functions, making them valuable in managing conditions such as lupus and rheumatoid arthritis. This ability to modulate immune responses opens up new therapeutic possibilities for immune-related diseases [20,21].

Additionally, the **safety and biocompatibility** of nonmetallic nanoparticles, particularly those derived from biocompatible materials such as certain polymers, make them sui for long-term use in treating chronic conditions. Their favorable safety profile enhances their potential for widespread applications in biomedical settings [22,23].

In summary, nonmetallic nanoparticles are proving to be vital in the treatment of nonmalignant disorders by improving drug delivery, enhancing diagnostic imaging, supporting tissue regeneration, modulating immune responses, and providing safer therapeutic options. These capabilities contribute to better patient outcomes and hold significant promise in addressing a wide range of health challenges.

1.3 Overview of therapeutic platforms and their relevance

Therapeutic platforms represent a diverse range of systems and technologies designed to deliver treatments to patients both effectively and safely. These platforms can be categorized based on their mechanisms of action, applications, and the specific diseases they target.

One prominent platform is **small molecule therapies**, which involve chemical compounds capable of interacting with biological processes. These therapies are widely used for treating diseases such as cancer,

cardiovascular disorders, and infectious diseases. Their significance lies in the ability to design them for specific intracellular targets, making them highly effective in modulating biological pathways [24].

Biologics, which include proteins, antibodies, and vaccines derived from living organisms, form another crucial platform. Biologics are particularly important in the treatment of autoimmune disorders, cancers, and infectious diseases. Their specificity allows for targeted therapeutic action, often resulting in fewer side effects compared with traditional chemical-based therapies [25].

The emerging field of **gene therapy** offers an innovative approach by modifying or replacing genes within a patient's cells to treat or prevent disease. Gene therapy holds particular promise for genetic disorders, certain cancers, and specific viral infections, providing the potential for long-term correction of underlying genetic defects [25,26].

Cell therapy, another breakthrough platform, involves the use of living cells for therapeutic purposes. Stem cell therapy and regenerative medicine are key examples, especially in treating degenerative diseases and tissue injuries. The ability of these therapies to repair or replace damaged cells offers substantial potential for regenerative healthcare [27–29].

Nanomedicine utilizes nanoparticles for drug delivery, diagnostics, and imaging, enhancing the precision of treatments. By improving drug bioavailability and reducing toxicity, nanomedicine is particularly relevant in cancer therapies, and it holds promise for a wide range of medical applications [30,31].

Targeted therapy focuses on specific molecular pathways associated with diseases, primarily cancers. By honing in on these unique pathways, targeted therapies can maximize treatment efficacy while minimizing damage to healthy cells. This platform is especially important in personalized medicine, where therapies are tailored to the unique molecular profile of each patient's disease [32,33].

Finally, **combination therapies**, which use multiple therapeutic agents either simultaneously or in sequence, can boost treatment efficacy and reduce resistance. This approach is particularly important in the treatment of complex diseases such as cancer and chronic conditions where multiple biological pathways are involved.

The relevance of these therapeutic platforms lies in their ability to address diverse medical challenges through tailored approaches. They offer numerous benefits, including greater treatment specificity, fewer side effects, improved patient outcomes, and the potential to develop novel treatment strategies. As research continues to advance, the integration of these platforms is expected to revolutionize healthcare, enabling more effective disease management and personalized patient care.

2. Types of nonmetallic nanoparticles

This section categorizes and explains the types of nonmetallic nanoparticles, distinguishing between organic and inorganic nanoparticles. It discusses how each type functions, their unique characteristics, and their specific roles in drug delivery and other therapeutic areas. These nanoparticles are shown as a flowchart in Fig. 23.1 in the below.

2.1 Organic nanoparticles: Liposomes, micelles, dendrimers

This subsection focuses on **organic nanoparticles**, particularly liposomes, micelles, and dendrimers. You will define each of these nanoparticles, explaining their composition and structural characteristics.

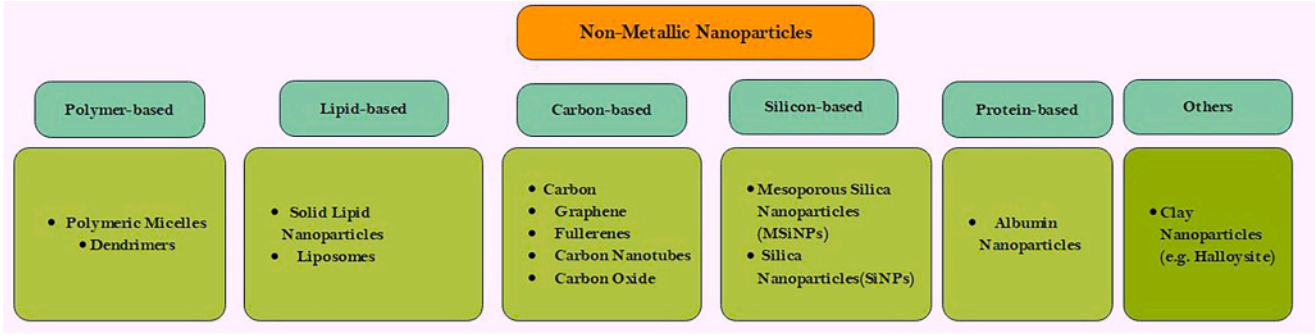


FIGURE 23.1 A flowchart listing examples of nonmetallic nanoparticles.

This flowchart includes the major categories and some specific examples under each group.

- **Liposomes:** Liposomes are microscopic, spherical vesicles composed of lipid bilayers, which provide a unique and versatile structure for encapsulating a wide range of therapeutic agents. These vesicles have the remarkable ability to encapsulate both hydrophilic drugs that dissolve in water and hydrophobic drugs that are fat-soluble. This dual capacity allows liposomes to be particularly effective in the formulation of various medications [34–37]. Due to their specialized properties, liposomes are extensively utilized in multiple fields, including drug delivery systems, gene therapy applications, and vaccine development processes. Their use in drug delivery is especially significant, as they enhance the stability and bioavailability of the encapsulated substances, ensuring that therapeutic agents remain effective throughout their delivery to target sites in the body. This multifaceted applicability underscores the importance of liposomes in modern medicine [38–40].
- **Micelles:** Micelles are unique amphiphilic structures characterized by having a hydrophobic core surrounded by hydrophilic components. This specific structure makes them particularly effective for solubilizing drugs that have poor solubility in water. Micelles play a crucial role in the pharmaceutical field, especially when it comes to enhancing the bioavailability of hydrophobic drugs, which often face challenges in being effectively absorbed by the body. By encapsulating these poorly soluble drugs within their hydrophobic core, micelles facilitate improved drug delivery and absorption, ultimately leading to enhanced therapeutic effects. Their ability to increase the solubility of such compounds underscores the significance of micelles in formulating effective drug delivery systems [41–43].
- **Dendrimers:** Dendrimers are organic nanoparticles with a branched, tree-like structure, synthesized through controlled chemical reactions that determine their size and functionality. They consist of a core molecule and multiple branching units, which can be made from various organic compounds and functionalized for specific applications. Dendrimers are categorized by generations, correlating with their size and structural complexity, and exhibit high uniformity, spherical shapes, and a large surface area that enhances their performance in drug delivery and nanomedicine. Their tailored properties make them suitable for applications in gene therapy, biosensors, and catalysis, providing versatile solutions in scientific and medical fields [44,45].

2.2 Inorganic nanoparticles: Silica nanoparticles, polymeric nanoparticles

In this part, the focus will be on inorganic nanoparticles, specifically silica nanoparticles and polymeric nanoparticles.

- **Silica Nanoparticles:** Silica nanoparticles are composed of silicon dioxide (SiO_2) and are synthesized through methods such as sol–gel processes or chemical vapor deposition. They can vary in size and morphology, offering features such as high surface area, porosity for drug delivery, chemical stability, and biocompatibility for biomedical uses [46].
- **Polymeric nanoparticles:** Polymeric nanoparticles are composed of either natural materials (such as proteins and polysaccharides) or synthetic polymers (such as PLA, PLGA, or PCL), and can be tailored for specific applications. They range in size from 20 nm to several micrometers, with shapes that vary based on the synthesis method [47]. Some nanoparticles feature a core–shell structure that encapsulates drugs while enhancing stability and controlled release. Their surfaces can be modified to improve interactions with biological systems and targeting

capabilities. Additionally, these nanoparticles are designed for controlled drug release and can be engineered for specific mechanical, thermal, and degradation properties, making them valuable in drug delivery, diagnostics, and various biomedical applications [48].

2.3 Comparison of properties and applications

This subsection presents a comparative analysis of the properties and applications of both organic and inorganic nanoparticles. The advantages and limitations of each type are highlighted, focusing on several key factors (Table 23.1).

- Biocompatibility and biodegradability
- Drug encapsulation and release efficiency
- Targeting capabilities
- Stability and toxicity

This table provides a clear and concise comparison of the properties and applications of organic and inorganic nanoparticles, highlighting their respective advantages and limitations.

3. Synthesis and characterization techniques

This section focuses on the processes used to create nonmetallic nanoparticles and the various tools and techniques employed to analyze their properties. It provides a comprehensive overview of the

Table 23.1 Here's a comparative analysis of organic and inorganic nanoparticles organized in a table format, focusing on their properties and applications.

Factor	Organic nanoparticles	Inorganic nanoparticles	References
Biocompatibility and biodegradability	Typically exhibit high biocompatibility; biodegradable, leading to reduced long-term toxicity.	Biocompatibility varies; some materials may require surface modifications to enhance compatibility. Often not biodegradable, leading to potential accumulation in the body.	[48]
Drug encapsulation and release efficiency	High drug encapsulation efficiency; allows for controlled and sustained release of therapeutic agents.	Can encapsulate drugs but may show lower release efficiency due to rigid structures and limited responsiveness to stimuli.	[49]
Targeting capabilities	Improved targeting through functionalization with ligands; specific delivery to tissues or cells is often achieved.	Targeting can be enhanced through surface modifications, but efficiency may be limited by complexity and properties of the nanoparticles.	[50]
Stability and toxicity	Stability can be influenced by environmental factors; lower toxicity generally associated with organic materials.	Generally stable under various conditions; however, concerns regarding toxicity and potential adverse effects necessitate careful evaluation.	[51]

methods for synthesizing these nanoparticles and the importance of accurately characterizing them to ensure they are suitable for therapeutic use.

3.1 Methods for synthesizing nonmetallic nanoparticles (e.g., chemical, physical, biological)

In this subsection, various approaches to synthesizing nonmetallic nanoparticles will be explored, categorized into *chemical, physical, and biological* methods.

3.1.1 Chemical methods

Chemical methods are widely used for synthesizing nonmetallic nanoparticles for biomedical applications, particularly in the treatment of nonmalignant disorders. Techniques such as sol–gel synthesis, chemical vapor deposition, and microemulsion techniques allow researchers to precisely control the size, shape, and composition of nanoparticles [52]. This level of control is crucial for optimizing drug delivery systems, as the physical and chemical properties of nanoparticles can significantly influence their behavior in biological environments. For example, sol–gel synthesis can be used to create silica nanoparticles that can encapsulate drugs for targeted delivery. These silica nanoparticles can be engineered to release their therapeutic payloads in a controlled manner, improving the effectiveness of treatments for conditions such as arthritis or chronic pain [53]. Similarly, polymeric nanoparticles synthesized through microemulsion techniques can be designed to carry antiinflammatory drugs, ensuring that they are released at the site of action over an extended period, thereby enhancing therapeutic outcomes. The ability to tailor these nanoparticles is essential for ensuring that they effectively encapsulate therapeutic agents and release them in a manner that maximizes their efficacy while minimizing side effects [54]. By delivering drugs at the right dose and at the appropriate time, chemical methods play a significant role in advancing targeted therapies for nonmalignant disorders. Overall, the careful engineering of nonmetallic nanoparticles through chemical synthesis represents a significant advancement in the development of innovative drug delivery formulations that can improve patient care and treatment effectiveness.

3.1.2 Physical methods

Physical methods employed for the synthesis of nonmetallic nanoparticles encompass a range of techniques, including high-energy ball milling, laser ablation, and sputtering. These methods utilize mechanical or thermal energy to break down bulk materials into nano-sized structures, allowing for the precise control of nanoparticle properties [55].

(a) High-Energy Ball Milling

High-energy ball milling involves the mechanical grinding of bulk materials in a rotating chamber, which results in the reduction of particle size. This method is advantageous for producing nanoparticles with controlled size and morphology [56].

- **Example in Biomedical Use**: In the context of creating silica nanoparticles, high-energy ball milling has been used to produce nanoparticles with tailored surface properties for drug delivery applications. Silica nanoparticles synthesized through this method can encapsulate therapeutic agents, enhancing their solubility and bioavailability. In particular, these silica nanoparticles have been investigated for delivering antiinflammatory drugs in the treatment of

chronic inflammatory diseases, such as rheumatoid arthritis, by targeting inflamed tissues and providing localized therapy [57].

(b) Laser Ablation

Laser ablation is a technique that uses focused laser energy to vaporize a target material, leading to the formation of nanoparticles in a surrounding medium. This method allows for the synthesis of nanoparticles with specific characteristics by controlling laser parameters [58].

- **Example in Biomedical Use**: Laser ablation has been effectively employed to produce gold and silica nanoparticles that can be functionalized for biomedical applications. For instance, silica nanoparticles created through this method have shown promise in delivering RNA-based therapeutics for genetic disorders. By encapsulating small interfering RNA (siRNA) within silica nanoparticles, researchers have been able to target gene expression more effectively, which is particularly useful in conditions such as cystic fibrosis, where gene correction is necessary [59].

(c) Sputtering

Sputtering is a physical vapor deposition technique where energetic particles displace atoms from a target material, which then condense to form nanoparticles on a substrate. This method is often used to create thin films but can also be adapted for nanoparticle synthesis [60].

- **Example in Biomedical Use**: Sputtering has been utilized to produce metal oxide nanoparticles, such as titanium dioxide (TiO2) and zinc oxide (ZnO), which have applications in drug delivery systems. TiO2 nanoparticles, for instance, can be employed in the treatment of skin inflammatory disorders. Their photocatalytic properties enable them to enhance the effectiveness of photodynamic therapy, where they assist in generating reactive oxygen species to combat inflammatory skin conditions effectively [61].

While physical methods for synthesizing nonmetallic nanoparticles are less commonly utilized in therapeutic applications due to scalability challenges and limitations in industrial production, they prove to be highly effective for generating specific inorganic nanoparticles. The unique characteristics imparted by these physical synthesis techniques make the resulting nanoparticles particularly valuable in biomedical contexts, especially for nonmalignant disorders. By capitalizing on the strengths of physical methods, researchers can continue to explore and expand the potential uses of nonmetallic nanoparticles across various applications in the biomedical field.

3.1.3 Biological methods

Biological methods for synthesizing nonmetallic nanoparticles encompass a range of environmentally friendly approaches that utilize biological organisms—such as bacteria, plants, and enzymes—to facilitate the synthesis process. This innovative method presents a sustainable and nontoxic alternative to the more traditional techniques commonly employed for nanoparticle synthesis.

One significant advantage of biological synthesis is that it often leads to the production of nanoparticles that exhibit enhanced biocompatibility, making them more suitable for use in biological environments. The use of biological materials can impart unique properties to the nanoparticles, enhancing their interaction with biological systems, which is particularly beneficial for therapeutic applications in the medical field [62].

Here's a table organizing the information on the different biological methods for synthesizing nonmetallic nanoparticles, including their applications and examples (Table 23.2).

Table 23.2 Examples of biological methods.

Method	Application	Example	References
Plant-based synthesis	The use of plant extracts to synthesize nanoparticles is gaining popularity due to their rich content of phytochemicals that can act as reducing and stabilizing agents.	Silver nanoparticles synthesized from extracts of *Azadirachta indica* (neem) have shown antiinflammatory properties, making them suitable for treating inflammatory diseases. Research has demonstrated that these nanoparticles can reduce inflammation in animal models of arthritis.	[63]
Bacterial synthesis	Certain bacteria possess the ability to synthesize nanoparticles intracellularly or extracellularly through metabolic processes.	*Pseudomonas aeruginosa* has been utilized to produce gold nanoparticles that exhibit antioxidant properties. These nanoparticles can be applied in the treatment of skin disorders, as they have shown promise in promoting wound healing and reducing inflammation in models of diabetic ulcers.	[64]
Enzymatic synthesis	Enzymes can facilitate the reduction of metal ions and the stabilization of nanoparticles, providing a controlled synthesis environment.	Enzymatically produced chitosan nanoparticles have been explored for drug delivery in treating nonmalignant disorders such as asthma. These nanoparticles can encapsulate bronchodilators, enhancing their therapeutic efficacy and targeting the delivery of the medication directly to the lungs, thus improving treatment outcomes in asthmatic patients.	[63]

The reduced toxicity associated with biological synthesis is crucial, as it lessens potential adverse effects when nanoparticles are introduced to living organisms. Nanoparticles produced through biological methods often exhibit lower cytotoxicity compared with those synthesized through conventional chemical routes, making them more suitable for in vivo applications.

Additionally, the lower environmental impact of biological synthesis methods makes them an appealing choice compared with conventional approaches that may generate harmful byproducts. This aligns with the growing demand for sustainable practices in science and medicine [65].

As a result, biological synthesis is gaining traction for medical applications, positioning itself as an important area of research and development in the quest for safer and more effective therapeutic

solutions. By harnessing the power of nature, researchers can develop nanoparticles that meet both safety and efficacy standards while contributing to a more sustainable future. This trend towards green chemistry not only enhances the therapeutic potential of nanoparticles but also aligns with global efforts to minimize environmental impact.

3.2 Characterization techniques (e.g., TEM, SEM, DLS, FTIR)

Once nanoparticles are synthesized, it's critical to thoroughly **characterize** them to ensure they meet the necessary size, shape, surface properties, and functionality for therapeutic use. This subsection focuses on the most common **characterization techniques** used to analyze nonmetallic nanoparticles.

3.2.1 Transmission electron microscopy (TEM)

Transmission Electron Microscopy (TEM) is a powerful imaging technique that utilizes a beam of electrons transmitted through an ultra-thin specimen to produce high-resolution images. It provides a magnification that can reach up to millions of times, allowing researchers to visualize the fine details of nanoparticles at the nanoscale [66].

3.2.1.1 Visualizing internal structure and morphology

TEM provides high-resolution images that reveal the internal structure and morphology of nanoparticles. This capability is crucial for understanding how the physical characteristics of nanoparticles influence their behavior in biological systems. For instance, researchers can assess whether nanoparticles possess a hollow structure, core–shell configuration, or specific surface modification [67]. These features can significantly affect how drugs are encapsulated, released, and targeted within the body.

3.2.1.2 Assessing size and uniformity

Uniformity in size and shape is critical for the therapeutic efficacy of nanoparticles. TEM allows researchers to measure the dimensions of individual nanoparticles accurately. For example, in drug delivery applications, nanoparticles that are too large may be cleared quickly from the bloodstream or may not penetrate target tissues effectively [68]. Conversely, nanoparticles that are too small may not accumulate sufficiently at the target site. By using TEM to monitor the size distribution of nanoparticles, researchers can optimize formulations to achieve the desired therapeutic profiles.

3.2.1.3 Evaluating drug loading and release mechanisms

TEM can be employed to observe how drugs are loaded into nanoparticles and how they are released in a controlled manner. By visualizing the interaction between drugs and the nanoparticle matrix, researchers can refine loading techniques to maximize drug encapsulation efficiency [69]. For instance, in the treatment of inflammatory diseases, TEM can help visualize the loading of antiinflammatory drugs into lipid-based nanoparticles, ensuring that the drugs remain effectively entrapped until they reach the target site. This controlled release can lead to improved therapeutic outcomes while minimizing side effects.

3.2.1.4 Understanding interactions with biological systems

TEM can also be used to study the interactions between nanoparticles and biological components, such as cells and tissues. Understanding these interactions is vital for predicting how nanoparticles will

behave in vivo and their potential therapeutic effectiveness [70]. For example, TEM can reveal how polymeric nanoparticles interact with immune cells, which is essential when designing drug delivery systems for autoimmune disorders. By assessing how these nanoparticles are taken up by immune cells, researchers can optimize their formulations to enhance therapeutic delivery while minimizing adverse immune responses.

3.2.2 Scanning electron microscopy (SEM): Overview of SEM

Scanning Electron Microscopy (SEM) is a powerful imaging technique that allows for the examination of the surface morphology and topography of nanoparticles at high magnification and resolution. By utilizing a focused beam of electrons, SEM generates detailed images that provide insights into the size, shape, and surface characteristics of nanoparticles [71]. This information is crucial for optimizing nanoparticles for therapeutic applications, especially in the targeted delivery of drugs for nonmalignant disorders.

3.2.2.1 Understanding surface properties

The surface properties of nanoparticles play a critical role in their interactions with biological systems. SEM enables researchers to visualize the surface roughness, texture, and any functional groups present on the nanoparticle surface. These characteristics can significantly influence the stability, dispersion, and bioavailability of nanoparticles in physiological environments. For example: For liposomal nanoparticles designed for delivering antiinflammatory drugs, SEM can reveal the presence of surface modifications (e.g., PEGylation) that enhance circulation time and reduce opsonization by the immune system [72,73]. This information helps optimize liposome formulations for improved therapeutic efficacy.

3.2.2.2 Determining overall shape and size

The shape and size of nanoparticles are critical factors affecting their pharmacokinetics and cellular uptake. SEM provides precise measurements of these dimensions, allowing researchers to tailor nanoparticles for specific therapeutic goals [74]. For instance, smaller nanoparticles may penetrate tissues more easily, while larger ones may be more suitable for targeted delivery. For example: In the development of polymeric nanoparticles for the treatment of inflammatory bowel disease (IBD), SEM can be used to determine the optimal size that ensures effective delivery to the inflamed intestinal mucosa [75]. By analyzing the size distribution, researchers can modify the synthesis parameters to achieve the desired size, enhancing drug accumulation at the target site.

3.2.2.3 Evaluating morphological changes during synthesis

During the synthesis of nonmetallic nanoparticles, various parameters (e.g., temperature, pH, and precursor concentration) can influence the morphology of the resulting particles. SEM allows for the real-time monitoring of these changes, enabling researchers to optimize synthesis conditions to achieve uniform and reproducible nanoparticle characteristics. For example: In the synthesis of silica nanoparticles for drug delivery applications, SEM can be employed to assess the effects of different synthesis conditions on particle shape (e.g., spherical versus elongated) [76,77]. Understanding these morphological variations can help tailor the particles for specific drug delivery applications, such as enhancing the encapsulation efficiency of hydrophobic drugs.

3.2.2.4 Assessing surface functionalization

Surface functionalization is often employed to enhance the targeting capability of nanoparticles. SEM can be utilized to visualize the success of these modifications, ensuring that targeting ligands (e.g., antibodies, peptides) are properly attached to the nanoparticle surface. For example: For targeting genetic disorders, nanoparticles may be functionalized with ligands that specifically bind to receptors on diseased cells. SEM can be used to confirm the presence and uniform distribution of these ligands on the nanoparticle surface, optimizing the design for effective gene delivery [78].

3.2.3 Dynamic light scattering (DLS)

DLS is used to measure the size distribution of nanoparticles in solution. This technique is important for understanding the stability of nanoparticle suspensions and ensuring consistent particle size, which is critical for drug delivery efficacy [79].

3.2.3.1 Measurement of size distribution

DLS measures the Brownian motion of particles in suspension and correlates this motion with particle size. By analyzing the scattered light from the nanoparticles, DLS can determine the hydrodynamic radius of particles, which is essential for understanding how they behave in biological environments [80]. For example: For nanoparticles intended for drug delivery in inflammatory diseases, such as rheumatoid arthritis, achieving a specific size range (typically between 100 and 200 nm) is crucial. Particles within this range can evade the immune system, prolong circulation time, and enhance accumulation at target sites. DLS allows researchers to confirm that their nanoparticles consistently fall within this optimal size range [81].

3.2.3.2 Understanding stability of nanoparticle suspensions

The stability of nanoparticle suspensions is vital for maintaining drug efficacy and avoiding aggregation or sedimentation, which can compromise therapeutic effects. DLS helps assess the stability of nanoparticle formulations by measuring changes in size over time. For example: In gene therapy for genetic disorders such as cystic fibrosis, nanoparticles must remain stable in solution to effectively deliver genetic material to target cells. By using DLS, researchers can monitor the stability of liposomal formulations containing siRNA, ensuring that they remain uniformly dispersed and functional during storage and administration [67,82].

3.2.3.3 Ensuring consistent particle size

Consistency in particle size is critical for ensuring predictable drug release profiles and biodistribution. Variability in size can lead to differences in pharmacokinetics and therapeutic outcomes. For example: In the development of polymeric nanoparticles for delivering antiinflammatory drugs to treat chronic conditions such as inflammatory bowel disease (IBD), DLS can be employed during the formulation process to ensure that the particles have a uniform size [83]. This uniformity helps to achieve controlled drug release and improved targeting of inflamed tissues, leading to better treatment efficacy and reduced side effects.

3.2.3.4 Optimization of drug loading and release profiles

DLS can assist in optimizing drug loading and release profiles by analyzing how the size of nanoparticles changes upon drug encapsulation. Changes in size can indicate successful loading or

aggregation, guiding formulation adjustments. For example: When formulating silica nanoparticles for delivering corticosteroids in the treatment of asthma, DLS can be used to assess the effect of drug loading on nanoparticle size. If significant aggregation occurs upon loading, adjustments can be made to the formulation (e.g., altering surfactants or polymer concentrations) to improve stability and ensure effective delivery [84,85].

3.2.4 Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique used to identify chemical bonds and functional groups present on the surface of nanoparticles. This technique provides critical information regarding the chemical composition and functionalization of nanoparticles, which is essential for optimizing their therapeutic applications, particularly in drug delivery for nonmalignant disorders [86].

3.2.4.1 Characterization of functional groups

FTIR can detect and characterize various functional groups on nanoparticle surfaces. The presence of specific functional groups (e.g., –OH, -NH2, –COOH) can influence how nanoparticles interact with biological tissues and drugs. For example: In drug delivery systems targeting inflammatory diseases, nanoparticles may be functionalized with carboxyl (-COOH) or amine (-NH2) groups to enhance their interaction with the targeted cells. FTIR can confirm these functional groups' presence, ensuring that the nanoparticles are optimally designed for enhanced cellular uptake [87].

3.2.4.2 Confirmation of drug loading

FTIR can be employed to confirm the successful loading of therapeutic agents onto nanoparticles. By comparing the FTIR spectra of the drug-loaded nanoparticles with that of the free drug, one can identify specific peaks associated with the drug, indicating its presence on the nanoparticle surface. For example: In the development of liposomal formulations for delivering antiinflammatory drugs such as dexamethasone, FTIR can be used to verify that the drug is adequately encapsulated within the liposome. The absence of characteristic peaks of the drug in the spectrum of the unloaded liposome, coupled with the presence of these peaks in the drug-loaded liposome spectrum, confirms successful loading [88,89].

3.2.4.3 Optimization of coatings for targeted delivery

FTIR is instrumental in evaluating and optimizing surface coatings or modifications aimed at enhancing the targeting ability of nanoparticles. Surface modifications often involve attaching ligands, antibodies, or other targeting moieties that can specifically bind to receptors on target cells. For example: When developing silica nanoparticles for targeted delivery of anticancer agents in inflammatory diseases, FTIR can be utilized to confirm the attachment of targeting ligands (e.g., folic acid) to the nanoparticle surface. The presence of characteristic absorption bands corresponding to the targeting ligand in the FTIR spectrum indicates successful functionalization, which enhances the nanoparticle's ability to interact selectively with folate receptor-expressing cells [90,91].

3.2.4.4 Stability assessment

FTIR can also help assess the stability of nanoparticles during storage or under varying environmental conditions. Changes in the FTIR spectra over time may indicate chemical degradation or instability,

prompting necessary modifications to the formulation. For example: In developing polymeric nanoparticles for the sustained release of drugs in chronic inflammatory diseases, FTIR can be used to monitor the stability of the nanoparticles over time [92]. By evaluating the spectra at different time points, researchers can determine if any degradation or changes in functional groups occur, ensuring that the nanoparticles remain effective throughout their shelf life.

3.3 Importance of characterization in determining therapeutic efficacy

Characterization involves the comprehensive analysis and assessment of therapeutic agents, examining their physical, chemical, and biological properties. This process is vital for determining the efficacy of a treatment for several key reasons.

First, characterization helps researchers understand the mechanisms of action, providing insights into how a therapeutic agent interacts with biological systems at the molecular level. This knowledge is essential for predicting the effectiveness of the therapy in treating specific conditions. Moreover, detailed characterization ensures the purity and quality of therapeutic agents by identifying impurities that could impact their safety or performance [93]. This is crucial to ensure that the treatment is free from contaminants and maintains a high standard of quality.

Characterization also plays a significant role in assessing dosage and delivery methods. By analyzing pharmacokinetics—how the body absorbs, distributes, metabolizes, and excretes the therapeutic agent—researchers can develop optimal dosing strategies and delivery methods to ensure that the treatment reaches its target in effective concentrations [94]. Additionally, it helps predict biological responses across different patient populations, taking into account genetic, environmental, and health-related factors. This information is critical for developing personalized medicine approaches, ensuring that treatments are tailored to individual needs.

Furthermore, characterization is necessary for regulatory compliance. Therapeutic agents must meet stringent characterization requirements to gain approval from health authorities, ensuring that the treatment adheres to regulatory guidelines, which in turn facilitates successful clinical trials and market entry [95]. Finally, characterization aids in optimizing the formulation of the therapeutic agent, improving stability, bioavailability, and overall effectiveness.

In summary, characterization is fundamental to determining the therapeutic efficacy of agents. It provides valuable insights into their mechanisms of action, ensures safety and quality, aids in appropriate dosing, predicts patient responses, ensures compliance with regulatory standards, and guides the development of optimized formulations, ultimately leading to better therapeutic outcomes.

4. Mechanisms of action

This section examines the interactions between nonmetallic nanoparticles and biological systems, the strategies used to target specific tissues or cells, and the processes involved in cellular uptake and intracellular function. Understanding these mechanisms is essential for optimizing nanoparticles for therapeutic applications, ensuring they efficiently reach and act on intended biological targets.

4.1 How nonmetallic nanoparticles interact with biological systems

In biological systems, nonmetallic nanoparticles interact in complex ways, largely influenced by their surface chemistry, the formation of a protein corona, and their biodistribution and circulation properties.

Surface chemistry and functionalization play a crucial role in determining how nanoparticles behave in a biological environment. Surface modifications, such as altering the charge, hydrophobicity, or the addition of specific functional groups, can dramatically affect their interaction with cells and tissues [96]. By fine-tuning these properties, researchers can improve the nanoparticles' compatibility with biological systems, minimize immune detection, and enhance their ability to target specific cells or organs. A common strategy to evade the immune system, for example, is coating nanoparticles with polyethylene glycol (PEG). This process, known as "stealthing," creates a protective barrier around the nanoparticles, preventing immune recognition and clearance. This allows the nanoparticles to remain in circulation longer, improving their chances of reaching the intended target site, which is especially important in drug delivery applications [97].

Once nanoparticles enter the body, they are rapidly covered by a layer of proteins, forming what is known as a **protein corona**. This corona significantly influences how nanoparticles interact with biological systems, as it alters their original surface properties [98]. The composition of the protein corona depends on the nanoparticles' characteristics and the type of biological fluid they encounter, such as blood or tissue fluid. The corona affects nanoparticle recognition by cells, their distribution within the body, and their overall safety profile. Understanding the formation and composition of the protein corona is vital for developing nanoparticles with enhanced targeting abilities, especially for applications such as drug delivery, medical imaging, and theranostics [99]. Researchers are exploring ways to manipulate protein corona formation through surface modifications and environmental controls, making nanoparticles more selective and effective in biomedical applications.

Biodistribution and circulation of nanoparticles are key determinants of their therapeutic efficacy and safety. Factors such as particle size, shape, and surface charge heavily influence how nanoparticles are distributed in the body. Smaller nanoparticles have better tissue penetration, such as crossing the blood–brain barrier, while larger ones are often more rapidly cleared by organs such as the liver or kidneys [100]. Spherical nanoparticles tend to distribute more evenly and are less likely to be recognized by the immune system compared with nonspherical ones, which may interact differently with cells due to their shape. Surface charge also plays a significant role; neutral nanoparticles usually have longer circulation times, while positively charged particles may be taken up more readily by cells but are often cleared faster. The route of administration—whether intravenous, oral, or topical—also impacts the biodistribution [101,102]. For example, nanoparticles administered intravenously face different clearance and distribution pathways than those delivered orally or through the skin. Researchers can modify surface properties and employ ligand-based targeting to control how nanoparticles travel and accumulate in the body, making them more effective for specific therapeutic uses, such as in targeted drug delivery or precision diagnostics.

These key factors—surface chemistry, protein corona formation, and biodistribution—are fundamental to the design and application of nonmetallic nanoparticles in medicine. By understanding and manipulating these interactions, researchers can create more efficient, selective, and safe nanoparticle-based therapies.

4.2 Targeting mechanisms: Passive versus active targeting

This subsection covers the two main strategies for targeting specific tissues or cells: **passive targeting** and **active targeting.** Each has its own set of advantages and is used based on the therapeutic goals. Passive targeting and active targeting represent two distinct strategies for enhancing the precision and efficacy of nanoparticle-based therapies, as shown in Fig. 23.2.

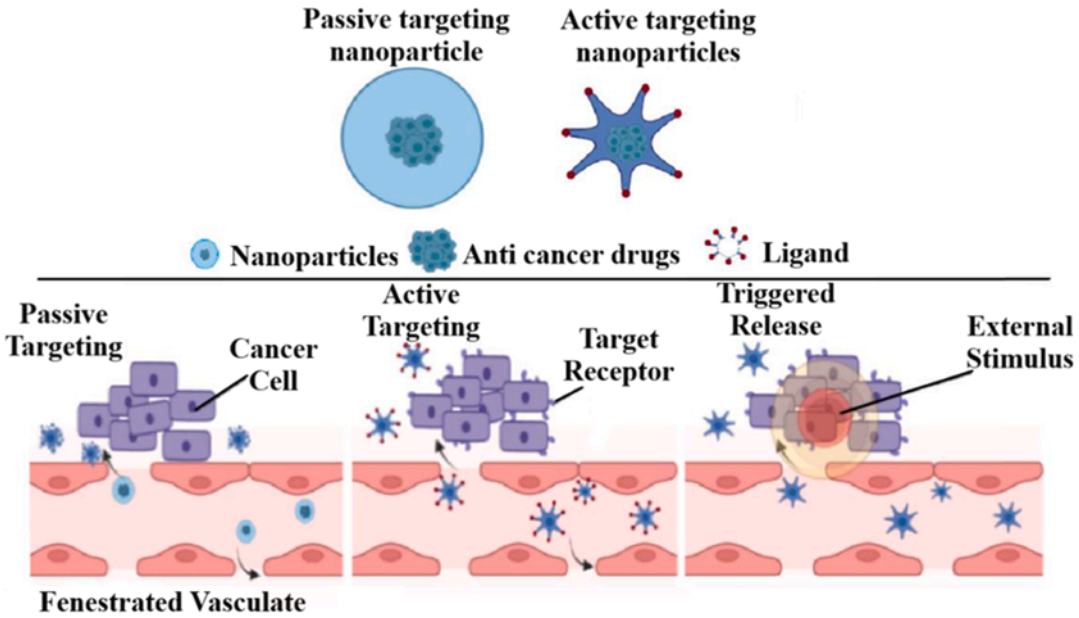


FIGURE 23.2 Mechanism of passive and active targeting in nanoparticle-based drug delivery systems [103].

The scheme illustrates the difference between passive and active targeting using nanoparticles for drug delivery, particularly in cancer therapy. In passive targeting, nanoparticles accumulate at the tumor site due to the enhanced permeability and retention (EPR) effect caused by the fenestrated vasculature of the tumor. In active targeting, nanoparticles are functionalized with ligands that bind to specific receptors on cancer cells, allowing for more precise targeting. The final stage shows triggered drug release upon exposure to an external stimulus, enhancing the specificity and effectiveness of the therapeutic action.

Passive targeting leverages the Enhanced Permeability and Retention (EPR) effect, a phenomenon observed in pathological conditions such as cancer, inflammation, and cardiovascular diseases, where leaky blood vessels allow larger molecules, including nanoparticles, to accumulate in the surrounding tissues [104]. This strategy is particularly useful for treating chronic inflammatory conditions and cardiovascular diseases, as it enables the passive delivery of therapeutic agents to affected areas without the need for specific targeting mechanisms [105]. While passive targeting offers simplicity and broad applicability across various diseases, it lacks the precision of active targeting, which may result in off-target effects and variability in therapeutic outcomes among patients. Despite these limitations, the use of passive targeting through the EPR effect presents a promising approach for the development of novel therapies aimed at nonmalignant disorders [106].

In contrast, **active targeting** enhances the specificity of nanoparticle delivery by modifying the nanoparticles with ligands that bind to receptors overexpressed on target cells. This method provides several advantages, including improved specificity, increased therapeutic efficacy, and the potential for personalized medicine tailored to individual patient profiles [107]. Active targeting is being explored for applications in cancer therapy, neurological disorders, and inflammatory diseases, allowing for the targeted delivery of therapeutic agents directly to diseased cells or tissues. However, challenges such

as the complexity of designing targeted nanoparticles, higher production costs, and concerns regarding in vivo stability persist [108]. Nonetheless, ongoing research continues to refine these strategies, and active targeting remains a highly promising avenue for improving the safety and effectiveness of nanoparticle-based therapies in both malignant and nonmalignant disorders (Table 23.3).

4.3 Cellular uptake and intracellular mechanisms

This subsection delves into how nanoparticles are internalized by cells and the subsequent intracellular pathways they utilize to deliver therapeutic agents. Understanding these mechanisms has shown in Fig. 23.3 is critical for designing nanoparticles that can effectively navigate cellular environments to reach their intended targets.

Cellular uptake mechanisms involve the processes by which cells internalize substances from their environment, which is essential for functions such as nutrient absorption, signaling, and immune responses. Nanoparticles primarily enter cells through **endocytosis**, a process that can occur through several pathways. **Clathrin-mediated endocytosis** is one such pathway, where the formation of clathrin-coated vesicles facilitates the engulfing of extracellular substances. Another pathway is **caveolae-mediated endocytosis**, which involves cholesterol-rich invaginations that transport nanoparticles into cells. Additionally, **macropinocytosis**, a nonspecific process, enables the uptake of extracellular fluid and particles. For immune cells such as macrophages, **phagocytosis** is the primary mechanism, allowing them to engulf larger particles or pathogens. **Pinocytosis**, on the other hand, involves the intake of small fluid droplets. These diverse mechanisms play a critical role in determining how effectively nanoparticles are internalized by cells, a key consideration when designing drug delivery systems that aim to improve therapeutic uptake [116].

Once inside the cell, nanoparticles must navigate complex **intracellular trafficking** routes to reach their target sites and release their therapeutic payloads. Following internalization through endocytosis, nanoparticles typically move through endosomal compartments, where they face the challenge of escaping the endosomes to avoid degradation [117]. Several strategies have been developed to facilitate **endosomal escape**: pH-sensitive nanoparticles, for instance, are designed to release their contents in the acidic environment of endosomes, while degradable nanoparticles break down under specific intracellular conditions to release their cargo [118]. Other nanoparticles are engineered to disrupt cellular membranes, allowing the release of therapeutic agents directly into the cytoplasm. Targeting specific organelles such as the nucleus or mitochondria can be enhanced by modifying

Table 23.3 Comparison of passive and active targeting.

No.	Feature	Passive targeting	Active targeting	References
1	Mechanism	Exploits EPR effect	Requires specific ligands	[109,110]
2	Specificity	Less specific	Highly specific	[111,112]
3	Simplicity	Simple	More complex	[110,113]
4	Applicability	Broad range of diseases	Limited to diseases with known target receptors	[110,114]
5	Efficacy	Can vary	Generally higher	[112,113]

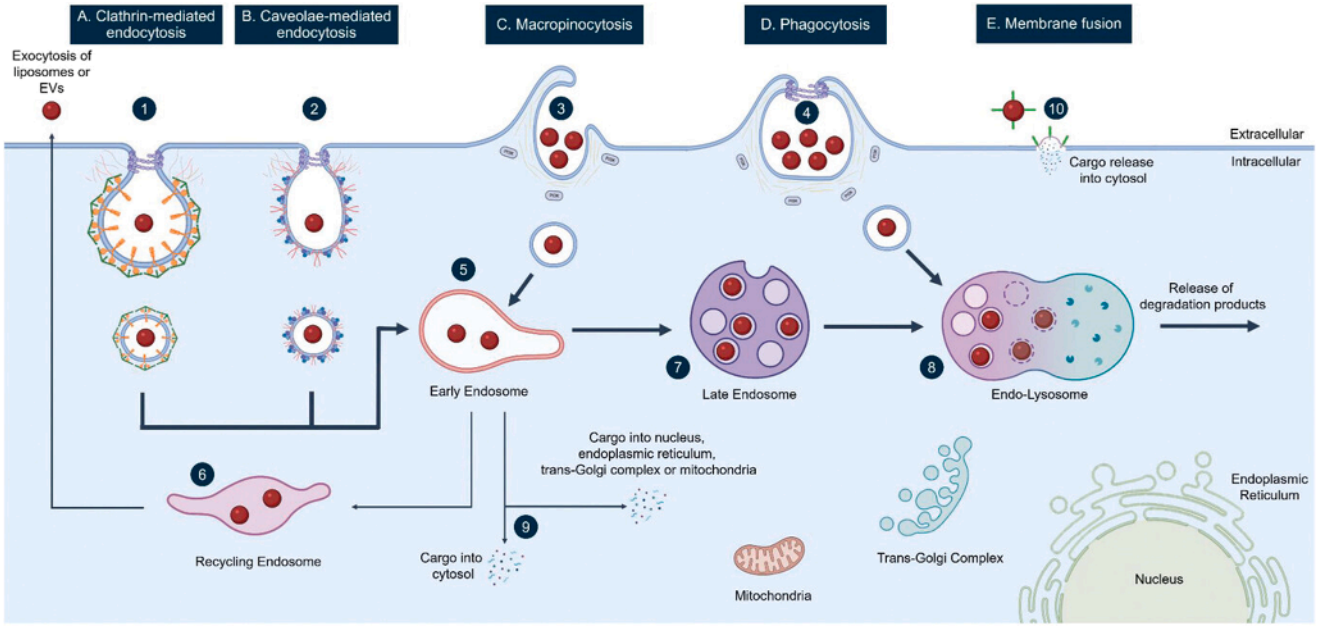


FIGURE 23.3 Mechanisms of nanoparticle uptake and intracellular trafficking [115].

The figure illustrates the various pathways of nanoparticle endocytosis and intracellular processing. Pathways include clathrin-mediated endocytosis (A), caveolae-mediated endocytosis (B), macropinocytosis (C), phagocytosis (D), and membrane fusion (E). Nanoparticles or extracellular vesicles (EVs) enter cells through these routes and are processed through early endosomes (5) and late endosomes (7). Some nanoparticles are recycled back to the membrane through recycling endosomes (6), while others merge with endo-lysosomes (8) for degradation. Cargo can be released into the cytosol (9) for intracellular distribution, including transport to the nucleus, endoplasmic reticulum, trans-Golgi complex, or mitochondria. Alternatively, nanoparticles can fuse with the membrane (10) to release cargo directly into the cytosol.

nanoparticles with ligands that direct them to these cellular components. Release mechanisms can also be controlled through stimuli such as temperature, light, or enzymatic activity. Effective intracellular trafficking and controlled release are crucial for therapies such as gene therapy, where the successful delivery of DNA or RNA depends on escaping endosomal degradation [119].

At the target site, nanoparticles can exert their **therapeutic action** through various mechanisms, depending on the payload and the disease being treated. **RNA interference (RNAi)**, for example, uses nanoparticles to deliver small interfering RNA (siRNA) that silences disease-causing genes, offering potential treatments for conditions such as neurodegenerative diseases. Similarly, nanoparticles are being used in **enzyme replacement therapy** to correct metabolic disorders by delivering functional enzymes directly to deficient cells [120,121]. **Targeted drug delivery** systems aim to minimize side effects by concentrating therapeutic agents specifically in diseased tissues, while **protein delivery** nanoparticles are employed in diseases such as diabetes, where they help in the controlled release of insulin [122]. **Gene therapy** also benefits from nanoparticles that deliver genetic material to correct mutations or introduce new genes, offering promising treatments for genetic disorders [123]. The efficacy of these therapies depends on the ability of nanoparticles to efficiently release their payloads, maintain the stability of the therapeutic agent, and achieve sufficient cellular uptake to produce the desired therapeutic effect.

By understanding and leveraging these processes, researchers can design more effective nanoparticle-based therapies for a wide range of nonmalignant disorders.

5. Therapeutic applications in nonmalignant disorders

This section explores the various nonmalignant disorders that can benefit from nanoparticle-based therapies. It delves into real-world examples of nanoparticles being used for treatment, illustrating their mechanisms for enhancing drug delivery and improving therapeutic outcomes.

5.1 Overview of nonmalignant disorders (e.g., diabetes, inflammatory diseases, neurological disorders, etc.)

Nonmalignant disorders encompass a broad range of chronic health conditions that, while not cancerous, can profoundly impact a person's quality of life, functionality, and overall health. Among these are diabetes, inflammatory diseases, and neurological disorders, each representing significant public health challenges due to their prevalence, complexity, and long-term consequences [124].

Diabetes is a metabolic disorder characterized by elevated blood glucose levels, resulting from insufficient insulin production or ineffective insulin action. The two main types are **Type 1 diabetes**, an autoimmune condition where the immune system attacks the insulin-producing beta cells in the pancreas, usually manifesting in childhood or adolescence and requiring lifelong insulin therapy, and **Type 2 diabetes**, the more common form, often associated with lifestyle factors such as obesity and physical inactivity. In Type 2, the body becomes resistant to insulin, leading to elevated glucose levels, managed through lifestyle changes, oral medications, and sometimes insulin [125]. Common complications of diabetes include cardiovascular disease (increased risk of heart disease, stroke, and hypertension), neuropathy (nerve damage causing pain, tingling, or loss of sensation, particularly in the feet), retinopathy (damage to the retina, potentially causing vision impairment or blindness), and kidney disease (diabetic nephropathy, which can lead to kidney failure if untreated) [126].

Inflammatory diseases are marked by chronic inflammation that affects various systems within the body, significantly impairing daily functioning and quality of life. **Rheumatoid arthritis (RA)** is a prime example, an autoimmune disorder causing chronic joint inflammation, pain, and eventual joint destruction, with the potential to affect other organs and cause systemic symptoms such as fatigue and fever [127]. Another example is **inflammatory bowel disease (IBD)**, which includes conditions such as Crohn's disease and ulcerative colitis. These result in chronic gastrointestinal inflammation, leading to symptoms such as abdominal pain, diarrhea, fatigue, and weight loss. **Psoriasis**, a chronic skin condition characterized by red, itchy, scaly patches due to systemic inflammation, is also part of this category. Managing inflammatory disorders often involves a combination of medications (such as NSAIDs, corticosteroids, and biologics), lifestyle modifications, and physical therapy to alleviate symptoms and prevent complications [128,129].

Neurological disorders involve impairments in the nervous system, affecting brain function, movement, and overall neurological health. **Multiple sclerosis (MS)**, for instance, is a disease in which the immune system attacks the protective sheath (myelin) surrounding nerves, leading to a wide range of symptoms, including weakness, coordination difficulties, vision problems, and cognitive changes [130]. **Parkinson's disease** is a progressive disorder primarily affecting movement, marked by tremors, stiffness, slowness of movement, and postural instability due to the degeneration of dopamine-producing neurons. Similarly, **Alzheimer's disease**, a form of dementia, causes cognitive decline, memory loss, and behavioral changes, primarily in older adults, though the progression and impact can vary. **Epilepsy**, another significant neurological disorder, is characterized by recurrent, unprovoked seizures, necessitating careful diagnosis and management to minimize its impact on daily life [131].

In conclusion, nonmalignant disorders such as diabetes, inflammatory diseases, and neurological conditions represent significant public health challenges due to their widespread impact and the complexity of managing them. Effective management often requires a multidisciplinary approach that includes medical treatment, lifestyle modifications, rehabilitation, and ongoing supportive care. Early diagnosis and personalized treatment strategies are essential in improving patient outcomes and enhancing the quality of life for individuals affected by these chronic conditions.

Here are the real-world examples from some Studies in Clinical Applications, focusing on their effectiveness, lessons learned, and overall impact on patient outcomes.

5.2 Silica nanoparticles in drug delivery for inflammatory disorders

The analysis by Namiot et al. (2023) highlights the significant role of nanoparticles in clinical trials, particularly lipid-based nanoparticles in vaccine development for COVID-19 and mesoporous silica nanoparticles (MSNs) in drug delivery. MSNs are noted for their tunable surface properties and large surface area, which enhance drug bioavailability, targeting, and controlled release, making them effective carriers for localized treatments, such as in chronic inflammation and cancer. Clinical studies indicate that MSNs improve drug delivery efficiency, allowing for targeted therapy in conditions such as rheumatoid arthritis, thus reducing required dosages and minimizing side effects. Key insights emphasize the importance of optimizing nanoparticle characteristics for better cellular uptake and ensuring biocompatibility for safe elimination. Overall, MSNs have positively impacted patient outcomes by alleviating symptoms and enhancing quality of life in chronic inflammatory disorders, suggesting they hold promise for improving therapeutic efficacy and patient well-being [132].

5.3 Carbon-based nanoparticles in neurological applications

The study by Kayoung Kim et al. (2020) in Nature Communications demonstrates the potential of carbon nanotubes (CNTs) for the precise clinical diagnosis of Alzheimer's disease (AD). The researchers developed a sensor array utilizing densely aligned CNTs for the multiplexed detection of key AD biomarkers in human plasma, achieving high sensitivity (90%), selectivity (90%), and accuracy (88.6%). This sensor outperforms traditional diagnostic methods, such as neuropsychological tests and neuroimaging, while offering a minimally invasive approach through plasma sampling. The CNTs' structure enhances signal transduction and allows simultaneous measurement of multiple biomarkers, improving diagnostic accuracy. Key insights from the study emphasize the need for optimization of CNT alignment and packing density to enhance sensitivity and reduce false positives. The findings suggest that CNT-based diagnostic tools could facilitate earlier and more accurate detection of AD, enabling timely therapeutic interventions and improving patient care. Overall, the study highlights the clinical relevance of CNTs in neurodiagnostics and their potential to transform routine AD screening [133].

5.4 Lipid-based nanoparticles in respiratory disorders

The study by Ellenmae W. X. Leong and Ruowen Ge investigates lipid nanoparticles (LNPs) as carriers for inhaled therapies, specifically for treating respiratory diseases such as COVID-19. LNPs are effective in delivering small molecules, nucleic acids, and proteins directly to the lungs, which enhances drug absorption and reduces side effects in the rest of the body. By encasing therapeutics in LNPs, higher drug concentrations can be achieved in lung tissues, crucial for effectively treating respiratory conditions.

The research emphasizes the importance of carefully designing LNP formulations to ensure drug stability and optimize delivery to the lungs. It also identifies challenges such as mucosal clearance and the need for targeted delivery to lung cells, which are vital for the success of LNP inhalation therapies. The LNP system shows promise for more efficient treatments for respiratory diseases, potentially benefiting patients with chronic conditions such as asthma or acute infections by providing quicker and more focused therapeutic effects [134].

5.5 Exosomes in autoimmune disorders

The study by Romina Heydari and colleagues examines the use of exosomes as potential diagnostic and therapeutic tools for rheumatoid arthritis (RA), a chronic inflammatory joint disease. Exosomes are effective in RA because they transport genetic material, proteins, and lipids between cells, influencing inflammation and immune responses. Notably, exosomes from mesenchymal stem cells (MSCs) have immunomodulatory properties that can be beneficial for treatment. Exosomes are promising for diagnosing RA by carrying microRNAs (miRNAs) that serve as biomarkers for the disease. MSC-derived exosomes have also shown the ability to reduce inflammation in RA models, indicating their therapeutic potential. The study highlights the need to understand the varied contents of exosomes and their target cells. It emphasizes further research on the diagnostic and therapeutic use of exosomal miRNAs and other molecules. A major challenge is the standardization of methods for isolating and characterizing exosomes for clinical use. Utilizing exosomes' capabilities could allow for earlier RA detection and improved management of inflammation and joint damage. This approach may lead to more personalized treatment plans and better long-term outcomes for patients, particularly in slowing disease progression and minimizing associated disabilities [135].

5.6 Mechanisms by which nanoparticles enhance drug delivery and efficacy

This subsection explains the mechanisms by which nonmetallic nanoparticles enhance drug delivery, targeting, and efficacy, making them highly advantageous in treating nonmalignant disorders. One key mechanism is **controlled release**, where nanoparticles such as polymeric or liposomal types are designed to deliver drugs in a sustained manner over time. This is particularly beneficial for managing chronic diseases such as diabetes, where consistent delivery of medications such as insulin helps maintain stable drug levels in the body. As a result, the need for frequent dosing is reduced, and the risk of side effects is minimized, improving patient compliance [136].

Another important mechanism is **improved bioavailability**, where nanoparticles increase the solubility and absorption of drugs that are poorly water-soluble. For example, micelles can encapsulate hydrophobic drugs, enhancing their uptake and effectiveness. This is especially critical in treating conditions such as inflammatory diseases, where high concentrations of the drug need to reach the site of inflammation for optimal therapeutic outcomes [137].

Targeted delivery is another key advantage of nanoparticles. Nanoparticles can be engineered to direct drugs specifically to targeted tissues or cells, improving treatment precision. This can occur through **active targeting**, where nanoparticles are functionalized with ligands that bind to receptors on specific cells, such as brain cells, allowing them to cross the blood–brain barrier for effective treatment of neurological disorders. Alternatively, **passive targeting** leverages the enhanced permeability and retention (EPR) effect, which enables nanoparticles to accumulate in inflamed areas or tissues with leaky blood vessels, as seen in conditions such as rheumatoid arthritis or Crohn's disease [138].

Nanoparticle-based drug delivery also significantly contributes to **reduced toxicity**. By directing drugs to specific sites in the body, nanoparticles minimize off-target effects, which is especially important in chronic conditions requiring long-term treatment, such as asthma or rheumatoid arthritis. Encapsulating toxic medications in nanoparticles can further protect healthy tissues, lowering the risk of adverse side effects [139].

Finally, the use of **multifunctional nanoparticles** is emerging as a powerful approach in therapy. These nanoparticles can be engineered to deliver multiple therapeutic agents or combine treatment with diagnostic capabilities, a concept known as theranostics. For instance, a single nanoparticle could deliver an antiinflammatory drug while also carrying imaging agents that allow clinicians to track disease progression or monitor treatment effectiveness in real-time. This multifunctional capability offers a comprehensive approach to treating complex disorders [140].

6. Benefits of nonmetallic nanoparticles

This section will highlight the key advantages of using nonmetallic nanoparticles in drug delivery and therapy, particularly for nonmalignant disorders. It will focus on the improvements these nanoparticles offer over conventional therapeutic approaches, showcasing their potential to enhance patient outcomes and minimize side effects.

6.1 Improved bioavailability and solubility

One of the most significant benefits of nonmetallic nanoparticles is their ability to **improve the bioavailability** and **solubility** of therapeutic agents. Many drugs, particularly those with hydrophobic properties, have limited solubility in water and, as a result, poor bioavailability when administered

through traditional methods [141]. Nonmetallic nanoparticles can overcome this limitation through the following mechanisms.

6.1.1 Encapsulation of hydrophobic drugs

Many drugs for managing non-cancer-related disorders, such as diabetes, are hydrophobic (poorly soluble in water). Nanoparticles such as micelles and liposomes can encapsulate these hydrophobic drugs within their core, allowing them to stay stable in water-based environments such as blood. For diabetic patients, effective glucose management often requires hydrophobic drugs (e.g., certain oral antidiabetics or insulin analogs). By using nanoparticles for encapsulation, these drugs remain stable and bioavailable, improving their absorption and ensuring they reach their target site. This enhanced solubility increases their effectiveness in controlling blood glucose levels [142].

6.1.2 Improved absorption and distribution

Nanoparticles enhance the solubility of poorly water-soluble drugs, improving their ability to cross biological barriers such as the gastrointestinal tract and the blood–brain barrier. This better absorption leads to higher bioavailability, so more of the drug reaches the intended tissues. For diabetes, delivering drugs effectively through the gastrointestinal tract is crucial for oral medications. Nanoparticles can help deliver insulin and other glucose-lowering agents in oral forms, overcoming poor absorption rates. This enhances the likelihood of the drug reaching its target, improving glucose regulation and offering a more comfortable alternative to injections [143].

6.1.3 Extended circulation time

Nanoparticles can be designed to evade the immune system's rapid clearance, allowing them to remain in the bloodstream for longer periods. This extended circulation increases the chance of drugs reaching target tissues, resulting in a more sustained therapeutic effect. In diabetes management, stable blood glucose levels are essential throughout the day. By prolonging the circulation of insulin or other antidiabetic drugs, nanoparticles can provide a sustained release, reducing the need for frequent dosing. This mimics the body's natural insulin release more closely, leading to more consistent blood glucose control and lowering the risk of hypoglycemic episodes [144].

6.1.4 Conclusion: improving diabetes therapy

Improving diabetes therapy can be enhanced through the use of nonmetallic nanoparticles. These nanoparticles offer innovative drug delivery systems that increase the efficacy and specificity of diabetes medications. They can help in targeting insulin or other therapeutics directly to cells, improving uptake and reducing side effects. Additionally, nonmetallic nanoparticles may facilitate better monitoring of glucose levels and allow for controlled release of medications, leading to more stable blood sugar management. Integrating these advanced technologies into diabetes treatment can lead to more effective management strategies, improved patient adherence, and ultimately better health outcomes for individuals with diabetes.

6.2 Reduction in side effects compared to conventional therapies

Nonmetallic nanoparticles offer a critical advantage in significantly reducing the side effects commonly associated with conventional therapies. Traditional drug delivery methods, such as oral or

systemic administration, often lead to widespread distribution of the drug throughout the body, resulting in off-target effects and toxicity in healthy tissues. Nanoparticles provide a solution to this challenge by enabling more precise and controlled drug delivery [145].

These nanoparticles achieve targeted delivery by being engineered to specifically interact with diseased cells or tissues, thus reducing the quantity of drug that affects healthy cells. For instance, active targeting involves attaching ligands to the surface of nanoparticles, which then bind to specific receptors on diseased cells, including inflamed tissues or neurons affected by neurodegenerative disorders. This targeted approach ensures that therapeutic agents are delivered directly to the affected sites, thereby minimizing the risk of side effects in other areas of the body [146].

Moreover, nanoparticles can be designed for controlled or sustained release of their therapeutic payloads. This characteristic diminishes the need for frequent dosing while maintaining a consistent drug concentration in the bloodstream. By avoiding the peaks and troughs typically seen with conventional drug administration, nanoparticles help prevent fluctuations in drug levels that can lead to side effects. This aspect is particularly beneficial for chronic conditions such as diabetes or rheumatoid arthritis, where stable drug levels are crucial for reducing adverse reactions [147].

Another significant benefit of nanoparticle-based therapies is the reduction in systemic toxicity. Nonmetallic nanoparticles can encapsulate potent drugs, such as corticosteroids or immunosuppressants, delivering them specifically to the site of disease. This targeted strategy minimizes the exposure of healthy tissues to the drug, thereby lowering the risk of systemic toxicity. For example, in inflammatory diseases, nanoparticles can concentrate the drug in inflamed tissues, shielding healthy areas from unnecessary exposure and alleviating common side effects such as immunosuppression or gastrointestinal irritation [148].

Overall, nonmetallic nanoparticles represent a powerful platform for enhancing the precision and safety of therapeutic interventions, especially in the treatment of nonmalignant disorders.

6.3 Enhanced therapeutic outcomes through targeted delivery

Targeted delivery systems in cancer therapy enhance therapeutic outcomes by improving the efficacy and specificity of drug administration. These systems direct therapeutic agents precisely to tumor sites, minimizing systemic side effects associated with traditional treatments. Key mechanisms include precision targeting through ligand-receptor interactions, the use of cell-penetrating peptides (CPPs) to enhance intracellular uptake, and the exploitation of the enhanced permeability and retention (EPR) effect for selective drug accumulation in tumors. Additionally, advanced systems allow for controlled and sustained release of drugs, improving patient compliance and therapeutic action. Clinically, targeted delivery reduces adverse effects, increases therapeutic efficacy, enables personalized medicine tailored to individual patient profiles, and supports combination therapies that address tumor heterogeneity. Overall, these innovations represent significant advancements in the management of cancer, promising improved patient outcomes [149].

7. Challenges and limitations

While nonmetallic nanoparticles offer many benefits for therapeutic applications, their clinical translation faces several significant challenges and limitations. This section will cover the key obstacles that need to be addressed to realize the full potential of nanoparticle-based therapies, focusing on biocompatibility, stability, and regulatory issues.

7.1 Biocompatibility and toxicity concerns

One of the primary challenges in developing nanoparticle-based therapies is ensuring biocompatibility and managing potential toxicity. Although nonmetallic nanoparticles are generally considered safer than their metallic counterparts, several factors can still lead to toxicity or adverse biological responses.

A major concern is the **activation of the immune system**, as nanoparticles may be recognized as foreign substances by the body, triggering immune responses. This can result in inflammation or the rapid clearance of nanoparticles from the bloodstream, which diminishes their therapeutic effectiveness. In some cases, nanoparticles may cause allergic reactions or chronic immune activation, especially if they accumulate in tissues over time [150].

Another issue arises from the **toxicity of degradation products**. Certain nonmetallic nanoparticles are designed to degrade within the body, but the by-products of this degradation can sometimes be toxic. For example, while polymeric nanoparticles typically degrade into biocompatible molecules, at higher concentrations these degradation products may still cause toxicity. Organic nanoparticles, such as dendrimers, can also induce cytotoxicity if their surface chemistry is not properly optimized for biocompatibility [151].

Furthermore, the **long-term biocompatibility** of nanoparticles remains a significant concern. The effects of prolonged exposure are not yet fully understood, particularly for chronic disorders that require repeated nanoparticle administration. Nanoparticles that are not efficiently cleared from the body may accumulate in organs such as the liver or spleen, potentially leading to adverse effects over time [152].

To address these concerns, ongoing research is focused on engineering nanoparticles with **optimal surface chemistry** and **controlled degradation profiles** to reduce immune activation and minimize toxicity, ensuring their safety and efficacy in therapeutic applications.

7.2 Stability and reproducibility issues

Another major challenge in the development and commercialization of nonmetallic nanoparticles lies in ensuring their stability during storage and use, as well as achieving reproducibility in their manufacturing processes. These challenges are critical because any instability or variability can compromise the safety and efficacy of the final product.

Nanoparticles must maintain their **physical and chemical stability**, preserving their size, shape, and functional properties both during storage and after administration. Factors such as temperature fluctuations, pH changes, and interactions with biological fluids can lead to issues such as aggregation or degradation. For instance, liposomes may become unstable and release their cargo prematurely, or polymeric nanoparticles might degrade in the bloodstream before reaching their target, reducing their therapeutic effectiveness [153].

In addition to stability, **reproducibility in manufacturing** presents a significant hurdle. Consistent, large-scale production of nanoparticles with precise control over size, shape, and surface properties is challenging. Even minor variations in particle size or surface charge can drastically influence nanoparticle behavior within the body, affecting their biodistribution, targeting efficiency, and therapeutic efficacy. Variability between batches can complicate regulatory approval processes and limit the consistency required for clinical applications [154].

Furthermore, **shelf-life** is a crucial consideration. Nanoparticles must remain stable for extended periods to be commercially viable, but many formulations degrade or lose functionality over time. Maintaining stability may require special storage conditions, such as low temperatures or inert environments, to ensure a reasonable shelf-life for widespread distribution. These stability and reproducibility challenges underscore the complexity of developing reliable nanoparticle-based therapies for nonmalignant disorders [155].

7.3 Regulatory hurdles in nanoparticle-based therapies

The regulatory approval process for nanoparticle-based therapies is particularly complex due to the novel properties and multifunctional nature of these systems, which do not fit neatly into existing frameworks for conventional drugs or biologics. One of the key challenges is the **uncertainty in classification**. Regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), often struggle to categorize nanoparticle-based products. Depending on their composition and function, nanoparticles might be classified as drugs, biologics, medical devices, or even a combination of these categories. This ambiguity complicates the regulatory pathway. For example, nanoparticles that serve both diagnostic and therapeutic purposes (known as theranostics) may fall under multiple regulatory categories, leading to more stringent and complicated approval requirements [156,157].

Another significant hurdle is the **lack of standardized testing protocols** for assessing the safety, efficacy, and quality of nanoparticle formulations. The diverse nature of nanoparticles makes it difficult to develop universal guidelines. Currently, there is no standardized set of testing protocols to evaluate these therapies, making it challenging for regulatory agencies to consistently assess nanoparticle-based treatments across various studies. As a result, robust preclinical and clinical data are required, yet the uniqueness of each nanoparticle system makes the creation of comprehensive guidelines an ongoing challenge [158].

Moreover, the **preclinical and clinical evaluation** of nanoparticle-based therapies is often more complex and time-consuming than that of conventional treatments. These systems require thorough testing in preclinical models (such as animals) and in clinical trials to assess pharmacokinetics, toxicity, and therapeutic efficacy. Due to the intricate nature of nanoparticles, the testing is often extensive and expensive, which can significantly delay regulatory approval. Complicating matters further, animal models frequently fail to accurately predict how nanoparticles will behave in human systems, making the translation from preclinical to clinical testing problematic [159].

Safety concerns also present a major regulatory challenge. Nanoparticles, particularly those designed for sustained release or that degrade over time, must undergo rigorous scrutiny to ensure their long-term biocompatibility. Regulatory agencies often require extended safety evaluations to address potential risks, such as the accumulation of nanoparticles in tissues or organs over time. Furthermore, the degradation products of nanoparticles must be shown to be nontoxic, adding another layer of complexity to the safety evaluation process. These factors contribute to the longer timelines and higher costs associated with gaining regulatory approval for nanoparticle-based therapies [160].

8. Current research trends

This section will explore the latest developments and research trends in the field of nonmetallic nanoparticles, particularly as they pertain to their applications in treating nonmalignant disorders. It

will cover recent technological advancements, innovations in drug delivery systems, and future directions for research.

8.1 Recent advancements in nonmetallic nanoparticle technology

In recent years, significant advancements in the technology surrounding nonmetallic nanoparticles have emerged, driven by the demand for more effective therapeutic solutions. One major development is **nanoparticle engineering**, where innovations in design and synthesis have led to improved control over size, shape, and surface modifications. Advanced techniques such as layer-by-layer assembly and 3D printing now allow researchers to create nanoparticles with tailored characteristics for targeted drug delivery and controlled release. For instance, optimizing the size of nanoparticles can enhance their cellular uptake and distribution, while specific surface modifications facilitate targeted binding to diseased tissues, improving the precision and efficacy of treatments [161].

Another area of progress is the development of **multifunctional nanoparticles**, which can serve multiple roles, such as diagnostic imaging and therapeutic delivery, known as theragnostic. These nanoparticles are designed to carry both imaging agents and drugs, enabling real-time monitoring of treatment efficacy and localization of the drug within the body. For example, silica nanoparticles can be modified to carry imaging agents alongside antiinflammatory drugs, allowing simultaneous visualization and treatment of inflammatory diseases, which is a critical step forward in personalized medicine [162].

Additionally, a growing focus on **biodegradable nanoparticles** has been observed, where recent research emphasizes creating nanoparticles that degrade safely into nontoxic by-products after delivering their therapeutic payload. This innovation aligns with the increasing demand for biocompatible, sustainable, and patient-friendly treatment options. Materials such as PLGA (poly(lactic-co-glycolic acid)) and chitosan are being widely studied for their potential in biodegradable nanoparticle formulations, offering safer alternatives for long-term therapies while minimizing environmental impact [163].

8.2 Innovations in drug formulation and delivery systems

The field of drug formulation and delivery is rapidly evolving, with new strategies being developed to enhance the effectiveness of therapeutic agents. Key innovations include **nanoemulsions** and **nanocrystals**, which are gaining attention for their ability to improve the solubility and bioavailability of poorly soluble drugs. Nanoemulsions consist of tiny droplets of oil dispersed in water, allowing for enhanced absorption of lipophilic drugs. Similarly, nanocrystals are submicron-sized drug particles that offer improved dissolution rates, leading to increased bioavailability [164].

Another exciting trend is the development of **stimuli-responsive nanoparticles** that can release their therapeutic payloads in response to specific environmental triggers, such as pH changes, temperature variations, or the presence of specific biomolecules. For instance, nanoparticles designed to release antiinflammatory drugs specifically at the site of inflammation can significantly enhance therapeutic efficacy while minimizing side effects [165].

Moreover, there is a growing trend towards utilizing **combination therapies** delivered through nanoparticle systems, particularly for complex conditions such as diabetes or inflammatory diseases. By codelivering multiple therapeutic agents that target different pathways or mechanisms, researchers

aim to improve treatment efficacy and reduce the risk of resistance. Nanoparticles can be engineered to encapsulate various drugs, allowing for simultaneous or sequential release tailored to the patient's needs. This innovative approach not only optimizes therapeutic outcomes but also represents a significant advancement in the field of drug delivery systems [166].

8.3 Future directions in research for nonmalignant disorders

As the field of nonmetallic nanoparticles continues to evolve, several promising future directions are emerging for research aimed at addressing nonmalignant disorders. One significant trend is the move toward personalized nanomedicine. Future studies may increasingly focus on tailoring nanoparticle formulations to individual patient profiles, optimizing drug delivery based on genetic, biochemical, or metabolic characteristics. This personalized approach aims to enhance treatment efficacy and minimize adverse effects, especially for chronic conditions that require long-term management.

Another important area of focus will be the clinical translation of nanoparticle technologies. While numerous preclinical studies have demonstrated the efficacy of nonmetallic nanoparticles, transitioning these findings into clinical applications presents ongoing challenges. Future research will likely prioritize developing scalable production methods and establishing rigorous clinical trial protocols to evaluate safety and efficacy comprehensively. Collaborations between academic institutions, industry partners, and regulatory bodies will be essential to overcome barriers and facilitate the successful translation of these innovative therapies into clinical settings [167].

Researchers are also expanding their exploration of new therapeutic areas for nonmetallic nanoparticles, particularly in treating a broader range of nonmalignant disorders. This includes rare diseases and conditions with high unmet medical needs, such as neurological disorders, autoimmune diseases, and chronic inflammatory conditions. By leveraging the unique properties of nanoparticles, researchers aim to address the complexities of these diseases more effectively [168].

Finally, as the use of nanoparticles expands, there will be a growing emphasis on understanding their environmental impact and ensuring sustainable practices in their production and disposal. Future research may increasingly explore biodegradable and eco-friendly materials for nanoparticle synthesis. This shift aligns with global efforts to reduce environmental pollution and promote sustainability within the healthcare sector. Together, these future directions highlight the potential of nonmetallic nanoparticles to significantly improve the management of nonmalignant disorders while addressing important considerations for health and environmental sustainability.

9. Regulatory framework for nanoparticle therapeutics

The regulatory landscape for nanoparticle-based therapeutics is complex and continues to evolve as research in this area advances. This section will provide a comprehensive overview of the regulatory bodies involved, the challenges faced during the approval process, and notable case examples of approved nonmetallic nanoparticle therapies.

9.1 Overview of regulatory bodies and guidelines

Various regulatory bodies worldwide oversee the development, approval, and monitoring of nanoparticle-based therapies, ensuring their safety and efficacy in medical applications. The U.S. Food

and Drug Administration (FDA) serves as the primary regulatory agency in the United States, responsible for the safety and efficacy of medical products, including drugs, biologics, and medical devices. Within the FDA, the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) play crucial roles in reviewing nanoparticle formulations. The FDA has issued guidance documents that outline considerations for the development and approval of nanoparticle-based products, emphasizing the importance of comprehensive characterization, safety assessments, and efficacy studies [169,170].

Similarly, the European Medicines Agency (EMA) oversees the evaluation of medicinal products in the European Union. The EMA provides guidelines for the development and evaluation of nanomedicines, with the Committee for Medicinal Products for Human Use (CHMP) responsible for assessing the safety and efficacy of nanoparticle therapeutics. Additionally, the EMA has developed specific guidelines for the risk assessment of nanomedicines, addressing critical aspects such as toxicity, exposure, and environmental impact [171].

The World Health Organization (WHO) also plays a vital role by providing international guidance on health-related matters, including the use of nanoparticles in medicine. The WHO promotes global collaboration to develop standardized guidelines and best practices for the safety assessment and regulation of nanomedicines [172].

In addition to these prominent organizations, various countries have their regulatory authorities, such as Health Canada and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA), which establish guidelines for the approval of nanoparticle-based therapies [173]. Together, these regulatory bodies work to create a framework that ensures the responsible development and use of nanomedicine while addressing the unique challenges posed by nanoparticle technology.

9.2 Challenges in regulatory approval of nanoparticle-based therapies

The regulatory approval process for nanoparticle-based therapies faces several unique challenges that arise from the novel properties of nanoparticles and their diverse applications. One of the primary challenges is the complexity of nanoparticle formulations. These therapeutics often consist of intricate mixtures with varying compositions, sizes, shapes, and surface modifications. This complexity complicates the standardization of testing protocols and makes it difficult to evaluate their safety and efficacy consistently. Regulatory agencies require comprehensive characterization data to assess the potential risks associated with these formulations [174].

Additionally, there is a lack of standardized testing protocols for nanoparticle safety assessments. Currently, no universally accepted framework exists, which leads to inconsistencies in testing methodologies across different studies. Regulatory bodies mandate data on biocompatibility, toxicity, pharmacokinetics, and long-term effects of nanoparticles. However, the absence of standardized testing complicates the evaluation process and hinders the ability to draw reliable conclusions regarding safety and efficacy [175].

Uncertainty about the long-term effects of nanoparticles also poses significant challenges. Given their novel nature, there is limited understanding of their long-term impacts on human health. Regulatory agencies remain cautious about approving therapies that may present unknown toxicological profiles or unexpected interactions with biological systems over extended periods [176].

Furthermore, regulatory classification issues contribute to the complexities of the approval process. Nanoparticle-based products can often be ambiguous in classification, as they may fall into different

categories, such as drugs, biologics, or medical devices. This ambiguity can lead to confusion regarding the applicable regulatory pathways and requirements, which prolongs the approval process and creates additional hurdles for developers [177].

Finally, there is a need for extensive preclinical and clinical data to support the safety and efficacy of nanoparticle therapies. Regulatory agencies typically require substantial evidence before approval, which can be resource-intensive and time-consuming. This poses particular challenges for small companies or academic researchers attempting to navigate the intricate regulatory landscape. Together, these factors contribute to a complex and often prolonged regulatory approval process for nanoparticle-based therapies.

9.3 Case examples of approved nonmetallic nanoparticle therapies

Despite the challenges in regulatory approval, several nonmetallic nanoparticle therapies have successfully received clinical use approval, demonstrating their potential in enhancing therapeutic outcomes. One notable example is **Doxil** (Doxorubicin Liposome Injection), which is a pegylated liposomal formulation of doxorubicin. Approved by the FDA for the treatment of breast cancer, ovarian cancer, and Kaposi's sarcoma, Doxil enhances the drug's stability and alters its distribution. This leads to reduced side effects and improved therapeutic outcomes, showcasing the advantages of nanoparticle-based formulations [178].

Another significant product is **Abraxane** (Paclitaxel Albumin-bound Particles), an albumin-bound nanoparticle formulation of paclitaxel. Approved for treating breast cancer, non-small-cell lung cancer, and pancreatic cancer, Abraxane improves the solubility of paclitaxel and facilitates its targeted delivery to tumor tissues. This targeted approach results in enhanced efficacy and reduced toxicity compared with traditional formulations [179].

Onivyde (Irinotecan Liposome Injection) is yet another example, specifically designed for the treatment of metastatic pancreatic cancer. This liposomal formulation enhances the bioavailability of irinotecan and minimizes side effects, ultimately leading to improved patient outcomes [180].

Additionally, **Vectibix** (Panitumumab) illustrates the integration of nonmetallic nanoparticles in targeted therapy for colorectal cancer. This monoclonal antibody linked to nanoparticles enhances the targeting and delivery of biologics, contributing to improved therapeutic efficacy in patients [181].

Moreover, nanoparticle-based vaccines have shown promising results in both preclinical and clinical studies, particularly for infectious diseases. For instance, various nanoparticle formulations have been developed for vaccines against influenza and COVID-19, demonstrating their potential to elicit rapid and effective immune responses. These examples collectively highlight the successful integration of nonmetallic nanoparticles into clinical practice, paving the way for their broader application in therapeutics [182].

10. Commercialization and market potential

The commercialization of nonmetallic nanoparticles represents a rapidly growing sector within the broader field of nanomedicine. This section will explore the current commercial landscape, identify key players and their contributions, and analyze the economic impact and future market trends for nanoparticle-based therapeutics.

10.1 Overview of the commercial landscape for nonmetallic nanoparticles

The commercial landscape for nonmetallic nanoparticles is characterized by significant investment, innovation, and a growing number of applications in healthcare.

One key aspect of this landscape is **market growth**. The global nanomedicine market, which encompasses nonmetallic nanoparticles, is experiencing robust expansion. This growth is driven by several factors, including the increasing prevalence of chronic diseases, advancements in nanotechnology, and a heightened focus on personalized medicine. Market analysts project that the nanomedicine sector will reach several billion dollars in the coming years, indicating strong commercial potential. Another important feature is the **diverse applications** of nonmetallic nanoparticles. These nanoparticles are being explored for a wide range of uses, including drug delivery, imaging, diagnostics, and therapeutics. This versatility allows companies to target multiple sectors within the healthcare industry, making nonmetallic nanoparticles particularly appealing for commercialization [183].

In addition, regulatory progress is enhancing the commercial landscape. As regulatory agencies develop clearer guidelines for nanoparticle-based products, the approval process is becoming more streamlined. This increased regulatory clarity encourages investment and development in this area, attracting pharmaceutical companies and startups to explore and commercialize novel nanoparticle therapies [184].

Finally, **collaborations and partnerships** play a vital role in the commercialization of nonmetallic nanoparticles. These efforts often involve collaborations between academic institutions, research organizations, and industry stakeholders. Such partnerships facilitate knowledge transfer, accelerate the development of new therapies, and help bring innovative products to market more efficiently [185].

Together, these aspects illustrate a dynamic and promising commercial landscape for nonmetallic nanoparticles, highlighting their potential to transform healthcare and improve patient outcomes.

10.2 Key players in the market and their products

Several key players in the pharmaceutical and biotechnology industries are actively involved in the development and commercialization of nonmetallic nanoparticles. Notable companies include (Table 23.4).

10.3 Economic impact and future market trends

The commercialization of nonmetallic nanoparticles carries significant economic implications, influencing healthcare costs, patient access to innovative therapies, and overall market dynamics. One major advantage of these nanoparticles is their potential to make therapies more cost-effective. By enhancing drug bioavailability and efficacy, nonmetallic nanoparticles can reduce the overall treatment costs associated with chronic diseases. This not only improves therapeutic outcomes but also reduces side effects, leading to decreased long-term healthcare expenditures, which makes these treatments more accessible and financially viable for healthcare systems and patients alike [195].

The rise of nanomedicine has also attracted substantial investment from venture capital firms, government agencies, and pharmaceutical companies, bolstering research, development, and commercialization efforts and fueling the growth of nanoparticle-based therapies in the market. Additionally, emerging markets are becoming increasingly significant in this landscape. As global healthcare demands evolve, companies are looking to regions with rising healthcare needs and limited

access to advanced treatments as potential markets for affordable nanoparticle-based therapies. These regions represent new opportunities to provide innovative treatments to populations previously underserved by advanced healthcare technologies [196].

Looking to the future, several trends are expected to shape the nonmetallic nanoparticle market. A key area of focus is personalized medicine, where tailoring nanoparticle therapies to individual patient profiles can enhance treatment effectiveness and outcomes. Progress in regulatory frameworks is also anticipated, as clearer and more supportive regulations may expedite approval processes, facilitating faster market entry for new nanoparticle-based products. Additionally, combination therapies—where nanoparticles codeliver multiple therapeutic agents—are gaining traction, offering promising solutions for complex diseases. Furthermore, the application of nonmetallic nanoparticles in diagnostics is expanding, allowing for early disease detection and efficient monitoring, which strengthens their role in modern healthcare and broadens their impact on patient care [197].

Table 23.4 Key players in the nanomedicine market and their nanoparticle-based products.

Company	Focus area	Notable products/Technologies	References
Pfizer Inc.	mRNA vaccines and therapeutics	**COVID-19 vaccine**: Developed with BioNTech, utilizes lipid nanoparticles (LNPs) with the ionizable lipid ALC-0315 for stability and immune response enhancement.	[186]
Moderna, Inc.	mRNA technology for vaccines	**COVID-19 vaccine**: Uses LNPs with ionizable lipid SM-102, optimized for intramuscular delivery. Expanding LNP platform for pulmonary delivery targeting respiratory diseases.	[187]
Bristol Myers Squibb	Oncology and immunology	**Abraxane**: Nanoparticle albumin-bound paclitaxel formulation that improves drug delivery to tumors while reducing side effects.	[188]
Johnson & Johnson	Oncology and infectious diseases	Developing nanoparticle-based therapies, including viral vectors and lipid nanoparticles for vaccine delivery.	[189]
Sanofi SA	Vaccines and biologics	Developing nanoparticle-based vaccines to enhance immune responses against various pathogens.	[190]
Alnylam Pharmaceuticals	RNA interference (RNAi) therapeutics	**Patisiran (Onpattro)**: First approved siRNA drug delivered through LNPs, targeting hereditary transthyretin-mediated amyloidosis.	[191]
Nanospectra Biosciences, Inc.	Cancer therapy	Uses gold nanoshells for photothermal therapy, targeting cancer cells while minimizing damage to surrounding healthy tissue.	[192]
Arrowhead Pharmaceuticals	RNA therapeutics	Develops RNA interference therapies utilizing LNPs to deliver therapeutic RNA molecules directly into cells.	[193]
Nanobiotix	Oncology	**NBTXR3**: Nanoparticle designed to enhance radiation therapy effectiveness by increasing tumor response.	[194]

11. Future perspectives and innovations

As the field of nonmetallic nanoparticles continues to evolve, numerous innovations and emerging technologies promise to enhance their therapeutic potential. This section will explore future perspectives in this domain, including technological advancements, the potential for combination therapies, and the role of personalized medicine in improving patient outcomes.

11.1 Emerging technologies in the field of nonmetallic nanoparticles

This table provides a structured overview of key technological advancements and their transformative role in enhancing nonmetallic nanoparticles for therapeutic applications (Table 23.5).

Table 23.5 The emerging technologies and their potential impact on the future of nonmetallic nanoparticles in therapeutics.

Emerging technology	Description	Potential impact	Example application	References
Smart nanoparticles	Nanoparticles that respond to specific stimuli (pH, temperature, light) for controlled drug release.	Enhances therapeutic efficacy by ensuring targeted, localized drug release, reducing side effects.	pH-sensitive nanoparticles releasing drugs in acidic tumor environments.	[24]
Hybrid nanoparticles	Combining different types of nanoparticles (organic and inorganic) to create systems with enhanced properties.	Improves stability, drug loading, and targeting capabilities for more effective treatments.	Hybrid nanoparticles enhancing drug delivery to inflamed tissues in arthritis.	[198]
Nanoparticle coating technologies	Surface modification of nanoparticles with targeting ligands, antibodies, or peptides for selective targeting.	Increases precision of therapeutic interventions by targeting specific cells or tissues.	Antibody-coated nanoparticles targeting cancer cell markers.	[199]
3D printing of nanoparticles	Integration of 3D printing technology for customized nanoparticle-based drug delivery systems.	Enables rapid prototyping and development of tailored drug delivery devices with specific properties.	3D-printed nanoparticle-based patches for transdermal drug delivery.	[200]
AI in nanoparticle design	Use of AI and machine learning to predict nanoparticle behavior and optimize formulations.	Accelerates discovery and optimization of nanoparticle systems, leading to more efficient and effective treatments.	AI-guided design of nanoparticles for improved brain drug delivery.	[201]

11.2 Potential for combination therapies (e.g., nanoparticles with biologics)

Combination therapies that incorporate nonmetallic nanoparticles alongside biologics or other therapeutic modalities hold great promise for enhancing treatment outcomes. These therapies leverage the unique properties of nanoparticles to create synergistic effects with biologics, such as monoclonal antibodies or RNA-based therapies, thereby improving therapeutic efficacy [202]. For instance, nanoparticles can act as carriers for biologics, enhancing their stability, solubility, and bioavailability, which is crucial for maximizing their effectiveness [203].

Moreover, nanoparticles facilitate the targeted delivery of multiple therapeutic agents to specific sites within the body. This capability allows for the simultaneous delivery of a chemotherapeutic agent and a targeted antibody, effectively attacking cancer cells from multiple angles and enhancing overall treatment efficacy. Such targeted approaches are essential for optimizing therapeutic outcomes and minimizing off-target effects [204].

In addition to improving delivery and efficacy, the incorporation of nanoparticles in combination therapies can help overcome drug resistance mechanisms that are often encountered in cancer treatment. By delivering multiple drugs that possess different mechanisms of action, nanoparticles have the potential to circumvent the pathways cancer cells utilize to evade therapy. This multifaceted approach can lead to more effective treatment strategies and better patient outcomes [108].

Furthermore, the combination of nanoparticles with vaccine antigens or adjuvants represents a significant advancement in vaccine development. Nanoparticles can serve as delivery vehicles that enhance the stability and presentation of vaccine components, leading to more robust and lasting immune responses. This synergy not only improves the effectiveness of vaccines but also has the potential to address various infectious diseases and cancer [205].

11.3 Role of personalized medicine in enhancing therapeutic efficacy

Personalized medicine focuses on tailoring medical treatment to the individual characteristics of each patient, and nonmetallic nanoparticles play a significant role in this paradigm. One of the key aspects of personalized medicine is the biomarker-driven approach. By integrating nonmetallic nanoparticles with biomarker identification, personalized treatment strategies can be developed. This involves utilizing nanoparticles that specifically target biomarkers associated with a patient's disease, allowing for customized therapies that improve efficacy while minimizing adverse effects.

Advances in genetic profiling also contribute to personalized medicine by enabling the identification of patient-specific mutations or genetic predispositions that affect drug response. Nonmetallic nanoparticles can be designed to deliver tailored therapies that take these individual genetic factors into account, resulting in more effective treatment strategies. Additionally, personalized medicine promotes adaptive treatment strategies, wherein therapies are adjusted based on the patient's response. Nonmetallic nanoparticles, with their potential for controlled and targeted drug delivery, facilitate real-time adjustments to treatment plans based on ongoing monitoring of patient outcomes [206].

Moreover, the development of patient-centric nanoparticle formulations that consider individual preferences and lifestyles can significantly enhance treatment adherence and satisfaction. Engaging patients in the design and implementation of nanoparticle-based therapies not only fosters a sense of ownership but also leads to better health outcomes. In this context, personalized medicine is instrumental in enhancing the therapeutic efficacy of nonmetallic nanoparticles, highlighting how these innovations can lead to more tailored and effective treatment options [207].

12. Ethical considerations

As the application of nonmetallic nanoparticles in medicine advances, several ethical considerations arise. This section will explore the ethical implications of using nanoparticles in healthcare, public perception of nanotechnology, and addressing concerns about long-term effects and safety.

12.1 Ethical implications of using nanoparticles in medicine

The integration of nanoparticles into medical applications presents a range of ethical implications that must be carefully considered. First and foremost is the issue of informed consent. Patients need to be adequately informed about the nature of nanoparticle-based therapies, including the potential benefits, risks, and unknowns associated with these treatments. Given the complexity of nanoparticle technology, ensuring that patients fully understand what they are consenting to can pose significant challenges. This necessitates clear and effective communication from healthcare providers to facilitate informed decision-making [208].

Equity in access is another critical consideration. The potentially high cost of nanoparticle-based therapies could exacerbate existing healthcare disparities, raising ethical concerns about access and affordability. It is imperative that ethical frameworks address these issues to ensure that all patients, regardless of their socioeconomic status, have equitable access to these advanced therapies [209]. Research ethics also play a vital role in the development of nanoparticles, which often involves extensive preclinical research, including animal testing. Ethical guidelines must be established to ensure that such research is conducted responsibly, striking a balance between the pursuit of scientific advancement and the welfare of research animals [209].

Moreover, the dual-use concerns of nanoparticle technology warrant attention. The potential applications of these technologies extend beyond therapeutic uses to areas such as surveillance or military applications. Ethical discussions must therefore address the dual-use nature of nanoparticle technologies and the potential consequences of their misuse. In this context, it is essential to explore these ethical implications in detail, emphasizing the importance of robust ethical frameworks to guide the development and application of nanoparticle technologies in medicine. Such frameworks can help navigate the complexities associated with these advancements, ensuring that they are utilized responsibly and equitably in healthcare.

12.2 Public perception and acceptance of nanotechnology in healthcare

Public perception is vital for the acceptance and use of nanotechnology in healthcare, and several key factors shape this perception. One of the most significant aspects is awareness and understanding. Many individuals have limited knowledge of nanotechnology and its medical applications, which can lead to misconceptions and fears about nanoparticles. To foster greater acceptance, increasing education and outreach efforts is essential, as improved understanding can alleviate resistance to the implementation of nanotechnology in medical treatments.

Another crucial factor is media representation. The way nanotechnology is portrayed in the media significantly impacts public perception. Positive articles that highlight the benefits and advancements of nanoparticles can enhance acceptance, while sensationalized reporting on potential risks may instill doubt and skepticism among the public. Therefore, responsible media coverage is important in shaping a balanced perspective [210].

Trust in science and healthcare professionals also plays a pivotal role in the acceptance of new technologies. The public's confidence in scientists and healthcare providers influences their willingness to embrace innovative solutions. Transparency regarding research findings, regulatory processes, and clinical applications can help build trust and address public concerns, creating a supportive environment for nanotechnology adoption [211].

Additionally, ethical and social considerations significantly impact how the public views the use of nanotechnology in healthcare. Engaging communities in discussions about the ethical implications and addressing societal concerns can lead to a more informed and balanced perspective. By involving various stakeholders in these conversations, it is possible to foster a greater understanding of the potential benefits and challenges associated with nanoparticles in healthcare [212].

12.3 Addressing concerns about long-term effects and safety

Addressing the long-term safety and effects of nanoparticles in medical applications is essential, requiring a multifaceted approach to mitigate potential risks. **Rigorous long-term safety studies** are paramount to thoroughly evaluate the biodistribution, toxicity, and biocompatibility of nanoparticles over extended periods. These studies aim to confirm that the use of nanoparticles in medicine does not present unforeseen health risks, ensuring their safe integration into clinical settings [213].

Strong regulatory oversight is also critical. Regulatory bodies must implement comprehensive guidelines for assessing nanoparticle-based therapies, focusing on safety and efficacy benchmarks before approval. Additionally, postapproval monitoring is essential to detect any emerging safety concerns once these therapies are in widespread use, facilitating swift response if issues arise [184].

Transparency and data sharing are equally important for building public confidence in nanoparticle-based treatments. Researchers and developers should commit to openly sharing information on safety, efficacy, and any adverse effects associated with nanoparticle applications. This openness fosters trust among healthcare professionals and the public, contributing to greater acceptance and understanding of these technologies [209].

Finally, **patient education** is crucial for empowering patients to make informed decisions regarding their treatment. By providing clear, accessible information about the potential risks and benefits of nanoparticle-based therapies, healthcare providers can foster open communication and ensure that patients feel actively involved in decisions about their healthcare. This collaborative approach prioritizes patient safety and fosters trust, paving the way for responsible and effective adoption of nanoparticle technologies in medicine [214].

13. Conclusion

The exploration of nonmetallic nanoparticles as a therapeutic platform for nonmalignant disorders reveals a promising landscape filled with innovation and potential. Throughout this chapter, we have examined key areas, beginning with a comprehensive understanding of nonmetallic nanoparticles, their classifications, and their importance in biomedical applications. We have also explored the methods of synthesizing these nanoparticles and the critical role of characterization techniques in ensuring their efficacy and safety for therapeutic use. Understanding the mechanisms by which nonmetallic nanoparticles interact with biological systems and their targeting strategies has further illuminated their therapeutic potential.

In terms of applications, nonmetallic nanoparticles show great promise in treating a range of nonmalignant disorders, including diabetes, inflammatory diseases, and neurological conditions. However, challenges such as biocompatibility, toxicity, and regulatory hurdles remain significant barriers that need to be addressed before these nanoparticles can be fully integrated into clinical practice. Despite these obstacles, the potential for future advancements is immense. Emerging technologies and combination therapies offer new avenues for enhancing the efficacy of nonmetallic nanoparticles.

Ethical considerations also play a crucial role in the development of this field. Addressing public perception, long-term safety concerns, and fostering trust among patients and healthcare providers are essential for the successful integration of nanoparticles into mainstream medicine. The vast potential of nonmetallic nanoparticles lies in their ability to improve drug delivery and patient outcomes, overcoming the limitations of conventional therapies.

Moving forward, interdisciplinary collaboration is key. Researchers, clinicians, regulatory bodies, and industry stakeholders must work together to translate nanoparticle technologies from the laboratory to the clinic. To achieve this, we must prioritize funding for innovative research and actively engage the public to address ethical concerns and enhance understanding. By fostering an environment of openness and cooperation, the development of nonmetallic nanoparticles can align with both patient needs and societal values.

As research continues to advance, nonmetallic nanoparticles have the potential to transform the treatment landscape for nonmalignant disorders, offering new hope to patients. The commitment to advancing this field holds the promise of delivering groundbreaking therapies that can significantly improve the quality of life for countless individuals. Embracing this challenge with enthusiasm and determination will pave the way for a future of innovative, life-changing treatments.

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CHAPTER

Nonmetallic stimuli-responsive nanocarriers in the treatment of noncancerous disease

24

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1. Introduction to nonmetallic nanoparticles

This section introduces the fundamental concept of nonmetallic nanoparticles and their importance in biomedical applications, especially in treating non-malignant disorders. It lays the groundwork by defining what these nanoparticles are and classifying them into different types. The section will also provide a broader context of how these nanoparticles are integrated into therapeutic platforms.

1.1 Definition and classification of nonmetallic nanoparticles

Nonmetallic nanoparticles are ultrafine particles, typically ranging from 1 to 100 nm in size, composed of materials other than metals. These materials may include polymers, ceramics, carbon-based compounds like graphene and carbon nanotubes, as well as various types of oxides and silicates. Due to their small size and high surface area, nonmetallic nanoparticles exhibit unique physical and chemical properties that differ significantly from their bulk counterparts[1–3].

These nanoparticles can be classified in several ways. Based on composition, there are **polymeric nanoparticles**, made from synthetic or natural polymers, widely used in drug delivery and biomedical applications; **ceramic nanoparticles**, composed of inorganic materials like silica or titanium dioxide, often employed in coatings and electronics; and **carbon-based nanoparticles**, such as carbon nanotubes and graphene, known for their exceptional strength and conductivity [4,5].

Nonmetallic nanoparticles are also categorized by their **structure**. **Nanodots** are spherical particles typically only a few nanometers in diameter, frequently used in imaging and sensing. **Nanofibers**, with fibrous structures at nanometer-scale diameters, are common in filtration and tissue engineering. **Nanoplates**, flat, two-dimensional structures, are utilized in material science due to their unique surface properties [2,6,7].

In terms of synthesis, there are two main approaches: **top-down methods**, which involve breaking down larger materials into nanoparticles through processes like milling or lithography, and **bottom-up methods**, which rely on assembling nanoparticles from smaller units using chemical processes such as sol-gel methods or chemical vapor deposition[8–11].

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Lastly, nanoparticles can be classified by their functionality. **Functionalized nanoparticles** have surfaces modified with specific chemical groups to enhance their properties for targeted applications, while **bioactive nanoparticles** are designed to interact with biological systems, commonly used in drug delivery systems or as diagnostic agents [12].

1.2 Importance in biomedical applications, particularly for non-malignant disorders

Nonmetallic nanoparticles are becoming increasingly essential in biomedical applications, particularly for non-malignant disorders, due to their unique properties and versatile functionalities [13]. One of their most significant advantages lies in their ability to facilitate **targeted drug delivery**. These nanoparticles are engineered to transport therapeutic agents directly to specific sites within the body, enhancing treatment efficacy for conditions such as diabetes, autoimmune diseases, and chronic inflammatory disorders, while minimizing unwanted side effects [14].

Beyond their therapeutic applications, nonmetallic nanoparticles also play a crucial role in **diagnostic imaging**. They improve the contrast and sensitivity of imaging techniques like MRI and ultrasound, aiding in the early detection and monitoring of non-malignant conditions such as cardiovascular diseases and neurological disorders[15–17].

Some nonmetallic nanoparticles even possess inherent **therapeutic properties** or can be functionalized to enhance their activity. For example, polymeric nanoparticles are used for the gradual release of anti-inflammatory drugs, providing sustained relief for chronic conditions like arthritis. In **regenerative medicine**, these nanoparticles are incorporated into scaffolds to promote cell growth and tissue regeneration, offering promising solutions for disorders such as osteoporosis and tendon injuries [18,19].

Moreover, nonmetallic nanoparticles hold substantial potential for **immunomodulation** in the treatment of autoimmune disorders. They can be designed to either enhance or suppress specific immune functions, making them valuable in managing conditions such as lupus and rheumatoid arthritis. This ability to modulate immune responses opens up new therapeutic possibilities for immune-related diseases [20,21].

Additionally, the safety and biocompatibility of nonmetallic nanoparticles, particularly those derived from biocompatible materials like certain polymers, make them suitable for long-term use in treating chronic conditions. Their favorable safety profile enhances their potential for widespread applications in biomedical settings [22,23].

In summary, nonmetallic nanoparticles are proving to be vital in the treatment of non-malignant disorders by improving drug delivery, enhancing diagnostic imaging, supporting tissue regeneration, modulating immune responses, and providing safer therapeutic options. These capabilities contribute to better patient outcomes and hold significant promise in addressing a wide range of health challenges.

1.3 Overview of therapeutic platforms and their relevance

Therapeutic platforms represent a diverse range of systems and technologies designed to deliver treatments to patients both effectively and safely. These platforms can be categorized based on their mechanisms of action, applications, and the specific diseases they target.

One prominent platform is **small molecule therapies**, which involve chemical compounds capable of interacting with biological processes. These therapies are widely used for treating diseases such as cancer, cardiovascular disorders, and infectious diseases. Their significance lies in the ability to design them for specific intracellular targets, making them highly effective in modulating biological pathways [24].

Biologics, which include proteins, antibodies, and vaccines derived from living organisms, form another crucial platform. Biologics are particularly important in the treatment of autoimmune disorders, cancers, and infectious diseases. Their specificity allows for targeted therapeutic action, often resulting in fewer side effects compared to traditional chemical-based therapies [25].

The emerging field of **gene therapy** offers an innovative approach by modifying or replacing genes within a patient's cells to treat or prevent disease. Gene therapy holds particular promise for genetic disorders, certain cancers, and specific viral infections, providing the potential for long-term correction of underlying genetic defects [25,26].

Cell therapy, another breakthrough platform, involves the use of living cells for therapeutic purposes. Stem cell therapy and regenerative medicine are key examples, especially in treating degenerative diseases and tissue injuries. The ability of these therapies to repair or replace damaged cells offers substantial potential for regenerative healthcare[27–29].

Nanomedicine utilizes nanoparticles for drug delivery, diagnostics, and imaging, enhancing the precision of treatments. By improving drug bioavailability and reducing toxicity, nanomedicine is particularly relevant in cancer therapies, and it holds promise for a wide range of medical applications [30,31].

Targeted therapy focuses on specific molecular pathways associated with diseases, primarily cancers. By honing in on these unique pathways, targeted therapies can maximize treatment efficacy while minimizing damage to healthy cells. This platform is especially important in personalized medicine, where therapies are tailored to the unique molecular profile of each patient's disease [32,33].

Lastly, **combination therapies**, which use multiple therapeutic agents either simultaneously or in sequence, can boost treatment efficacy and reduce resistance. This approach is particularly important in the treatment of complex diseases like cancer and chronic conditions where multiple biological pathways are involved.

The relevance of these therapeutic platforms lies in their ability to address diverse medical challenges through tailored approaches. They offer numerous benefits, including greater treatment specificity, fewer side effects, improved patient outcomes, and the potential to develop novel treatment strategies. As research continues to advance, the integration of these platforms is expected to revolutionize healthcare, enabling more effective disease management and personalized patient care.

2. Types of nonmetallic nanoparticles

This section categorizes and explains the types of nonmetallic nanoparticles, distinguishing between organic and inorganic nanoparticles. It discusses how each type functions, their unique characteristics, and their specific roles in drug delivery and other therapeutic areas. These nanoparticles are shown as a flowchart in the Fig. 24.1 in the below.

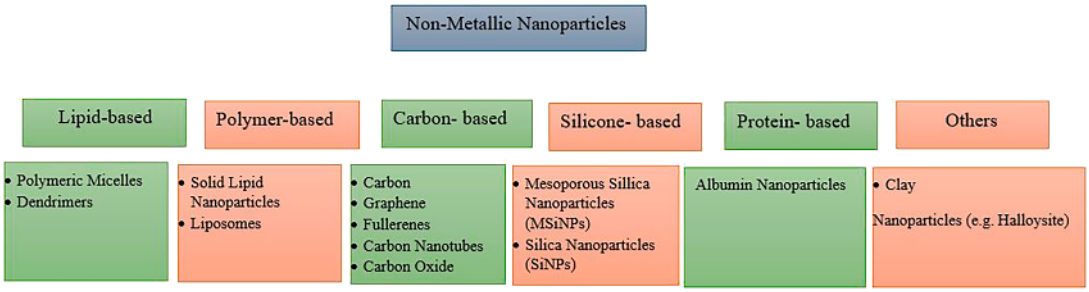


FIGURE 24.1

A flowchart listing examples of nonmetallic nanoparticles.
This flowchart includes the major categories and some specific examples under each group.

2.1 Organic nanoparticles: Liposomes, micelles, dendrimers

This subsection focuses on **organic nanoparticles**, particularly liposomes, micelles, and dendrimers. You will define each of these nanoparticles, explaining their composition and structural characteristics:

- **Liposomes:** Liposomes are microscopic, spherical vesicles composed of lipid bilayers, which provide a unique and versatile structure for encapsulating a wide range of therapeutic agents. These vesicles have the remarkable ability to encapsulate both hydrophilic drugs that dissolve in water and hydrophobic drugs that are fat-soluble. This dual capacity allows liposomes to be particularly effective in the formulation of various medications [34–37] Due to their specialized properties, liposomes are extensively utilized in multiple fields, including drug delivery systems, gene therapy applications, and vaccine development processes. Their use in drug delivery is especially significant, as they enhance the stability and bioavailability of the encapsulated substances, ensuring that therapeutic agents remain effective throughout their delivery to target sites in the body. This multifaceted applicability underscores the importance of liposomes in modern medicine[38–40].
- **Micelles:** Micelles are unique amphiphilic structures characterized by having a hydrophobic core surrounded by hydrophilic components. This specific structure makes them particularly effective for solubilizing drugs that have poor solubility in water. Micelles play a crucial role in the pharmaceutical field, especially when it comes to enhancing the bioavailability of hydrophobic drugs, which often face challenges in being effectively absorbed by the body. By encapsulating these poorly soluble drugs within their hydrophobic core, micelles facilitate improved drug delivery and absorption, ultimately leading to enhanced therapeutic effects. Their ability to increase the solubility of such compounds underscores the significance of micelles in formulating effective drug delivery systems[41–43].

Dendrimers: Dendrimers are organic nanoparticles with a branched, tree-like structure, synthesized through controlled chemical reactions that determine their size and functionality. They consist of a core molecule and multiple branching units, which can be made from various organic compounds and functionalized for specific applications. Dendrimers are categorized by generations, correlating

with their size and structural complexity, and exhibit high uniformity, spherical shapes, and a large surface area that enhances their performance in drug delivery and nanomedicine. Their tailored properties make them suitable for applications in gene therapy, biosensors, and catalysis, providing versatile solutions in scientific and medical fields [44,45].

2.2 Inorganic nanoparticles: Silica nanoparticles, polymeric nanoparticles

In this part, the focus will be on inorganic nanoparticles, specifically silica nanoparticles and polymeric nanoparticles.

Silica Nanoparticles: Silica nanoparticles are composed of silicon dioxide (SiO_2) and are synthesized through methods like sol-gel processes or chemical vapor deposition. They can vary in size and morphology, offering features such as high surface area, porosity for drug delivery, chemical stability, and biocompatibility for biomedical uses [46].

Polymeric nanoparticles: Polymeric nanoparticles are composed of either natural materials (like proteins and polysaccharides) or synthetic polymers (such as PLA, PLGA, or PCL), and can be tailored for specific applications. They range in size from20 nm to several micrometers, with shapes that vary based on the synthesis method [47]. Some nanoparticles feature a core-shell structure that encapsulates drugs while enhancing stability and controlled release. Their surfaces can be modified to improve interactions with biological systems and targeting capabilities. Additionally, these nanoparticles are designed for controlled drug release and can be engineered for specific mechanical, thermal, and degradation properties, making them valuable in drug delivery, diagnostics, and various biomedical applications(49).

2.3 Comparison of properties and applications

This subsection presents a comparative analysis of the properties and applications of both organic and inorganic nanoparticles. The advantages and limitations of each type are highlighted, focusing on several key factors and showed in Table 24.1.

2.3.1 Biocompatibility and biodegradability

- Drug encapsulation and release efficiency
- Targeting capabilities
- Stability and toxicity

This table provides a clear and concise comparison of the properties and applications of organic and inorganic nanoparticles, highlighting their respective advantages and limitations.

3. Synthesis and characterization techniques

This section focuses on the processes used to create nonmetallic nanoparticles and the various tools and techniques employed to analyze their properties. It provides a comprehensive overview of the methods for synthesizing these nanoparticles and the importance of accurately characterizing them to ensure they are suitable for therapeutic use.

Table 24.1 Here is a comparative analysis of organic and inorganic nanoparticles organized in a table format, focusing on their properties and applications.

Factor	Organic nanoparticles	Inorganic nanoparticles	Ref.
Biocompatibility and biodegradability	Typically exhibit high biocompatibility; biodegradable, leading to reduced long-term toxicity.	Biocompatibility varies; some materials may require surface modifications to enhance compatibility. Often not biodegradable, leading to potential accumulation in the body.	[48]
Drug encapsulation and release efficiency	High drug encapsulation efficiency; allows for controlled and sustained release of therapeutic agents.	Can encapsulate drugs but may show lower release efficiency due to rigid structures and limited responsiveness to stimuli.	[49]
Targeting capabilities	Improved targeting through functionalization with ligands; specific delivery to tissues or cells is often achieved.	Targeting can be enhanced via surface modifications, but efficiency may be limited by complexity and properties of the nanoparticles.	[50]
Stability and toxicity	Stability can be influenced by environmental factors; lower toxicity generally associated with organic materials.	Generally stable under various conditions; however, concerns regarding toxicity and potential adverse effects necessitate careful evaluation.	[51]

3.1 Methods for synthesizing nonmetallic nanoparticles (e.g., chemical, physical, biological)

In this subsection, various approaches to synthesizing nonmetallic nanoparticles will be explored, categorized into *chemical, physical, and biological* methods.

3.1.1 Chemical methods

Chemical methods are widely used for synthesizing nonmetallic nanoparticles for biomedical applications, particularly in the treatment of non-malignant disorders. Techniques such as sol-gel synthesis, chemical vapor deposition, and microemulsion techniques allow researchers to precisely control the size, shape, and composition of nanoparticles [52]. This level of control is crucial for optimizing drug delivery systems, as the physical and chemical properties of nanoparticles can significantly influence their behavior in biological environments. For example, sol-gel synthesis can be used to create silica nanoparticles that can encapsulate drugs for targeted delivery. These silica nanoparticles can be engineered to release their therapeutic payloads in a controlled manner, improving the effectiveness of treatments for conditions like arthritis or chronic pain [53]. Similarly, polymeric nanoparticles synthesized through microemulsion techniques can be designed to carry anti-inflammatory drugs, ensuring that they are released at the site of action over an extended period, thereby enhancing

therapeutic outcomes. The ability to tailor these nanoparticles is essential for ensuring that they effectively encapsulate therapeutic agents and release them in a manner that maximizes their efficacy while minimizing side effects [54]. By delivering drugs at the right dose and at the appropriate time, chemical methods play a significant role in advancing targeted therapies for non-malignant disorders. Overall, the careful engineering of nonmetallic nanoparticles through chemical synthesis represents a significant advancement in the development of innovative drug delivery formulations that can improve patient care and treatment effectiveness.

3.1.2 Physical methods

3.1.2.1 High-energy ball milling

High-energy ball milling involves the mechanical grinding of bulk materials in a rotating chamber, which results in the reduction of particle size. This method is advantageous for producing nanoparticles with controlled size and morphology [55].

Example in Biomedical Use: In the context of creating silica nanoparticles, high-energy ball milling has been used to produce nanoparticles with tailored surface properties for drug delivery applications. Silica nanoparticles synthesized via this method can encapsulate therapeutic agents, enhancing their solubility and bioavailability. In particular, these silica nanoparticles have been investigated for delivering anti-inflammatory drugs in the treatment of chronic inflammatory diseases, such as rheumatoid arthritis, by targeting inflamed tissues and providing localized therapy [56].

3.1.2.2 Laser ablation

Laser ablation is a technique that uses focused laser energy to vapourize a target material, leading to the formation of nanoparticles in a surrounding medium. This method allows for the synthesis of nanoparticles with specific characteristics by controlling laser parameters [57].

Example in Biomedical Use: Laser ablation has been effectively employed to produce gold and silica nanoparticles that can be functionalized for biomedical applications. For instance, silica nanoparticles created through this method have shown promise in delivering RNA-based therapeutics for genetic disorders. By encapsulating small interfering RNA (siRNA) within silica nanoparticles, researchers have been able to target gene expression more effectively, which is particularly useful in conditions like cystic fibrosis, where gene correction is necessary [58].

3.1.2.3 Sputtering

Sputtering is a physical vapor deposition technique where energetic particles displace atoms from a target material, which then condense to form nanoparticles on a substrate. This method is often used to create thin films but can also be adapted for nanoparticle synthesis [59].

Example in Biomedical Use: Sputtering has been utilized to produce metal oxide nanoparticles, such as titanium dioxide (TiO2) and zinc oxide (ZnO), which have applications in drug delivery systems. TiO2 nanoparticles, for instance, can be employed in the treatment of skin inflammatory disorders. Their photocatalytic properties enable them to enhance the effectiveness of photodynamic therapy, where they assist in generating reactive oxygen species to combat inflammatory skin conditions effectively [60].

While physical methods for synthesizing nonmetallic nanoparticles are less commonly utilized in therapeutic applications due to scalability challenges and limitations in industrial production, they prove to be highly effective for generating specific inorganic nanoparticles. The unique characteristics

imparted by these physical synthesis techniques make the resulting nanoparticles particularly valuable in biomedical contexts, especially for non-malignant disorders. By capitalizing on the strengths of physical methods, researchers can continue to explore and expand the potential uses of nonmetallic nanoparticles across various applications in the biomedical field.

3.1.3 Biological methods

Biological methods for synthesizing nonmetallic nanoparticles encompass a range of environmentally friendly approaches that utilize biological organisms—such as bacteria, plants, and enzymes—to facilitate the synthesis process. This innovative method presents a sustainable and non-toxic alternative to the more traditional techniques commonly employed for nanoparticle synthesis.

One significant advantage of biological synthesis is that it often leads to the production of nanoparticles that exhibit enhanced biocompatibility, making them more suitable for use in biological environments. The use of biological materials can impart unique properties to the nanoparticles, enhancing their interaction with biological systems, which is particularly beneficial for therapeutic applications in the medical field [61].

Here is Table 24.2 organizing the information on the different biological methods for synthesizing nonmetallic nanoparticles, including their applications and examples:

Table 24.2 Examples of biological methods.

Method	Application	Example	Ref.
Plant-based synthesis	The use of plant extracts to synthesize nanoparticles is gaining popularity due to their rich content of phytochemicals that can act as reducing and stabilizing agents.	Silver nanoparticles synthesized from extracts of *Azadirachta indica* (neem) have shown anti-inflammatory properties, making them suitable for treating inflammatory diseases. Research has demonstrated that these nanoparticles can reduce inflammation in animal models of arthritis.	[62]
Bacterial synthesis	Certain bacteria possess the ability to synthesize nanoparticles intracellularly or extracellularly through metabolic processes.	*Pseudomonas aeruginosa* has been utilized to produce gold nanoparticles that exhibit antioxidant properties. These nanoparticles can be applied in the treatment of skin disorders, as they have shown promise in promoting wound healing and reducing inflammation in models of diabetic ulcers.	[63]
Enzymatic synthesis	Enzymes can facilitate the reduction of metal ions and the stabilization of nanoparticles, providing a controlled synthesis environment.	Enzymatically produced chitosan nanoparticles have been explored for drug delivery in treating non-malignant disorders such as asthma. These nanoparticles can encapsulate bronchodilators, enhancing their therapeutic efficacy and targeting the delivery of the medication directly to the lungs, thus improving treatment outcomes in asthmatic patients.	[62]

The reduced toxicity associated with biological synthesis is crucial, as it lessens potential adverse effects when nanoparticles are introduced to living organisms. Nanoparticles produced through biological methods often exhibit lower cytotoxicity compared to those synthesized through conventional chemical routes, making them more suitable for in vivo applications.

Additionally, the lower environmental impact of biological synthesis methods makes them an appealing choice compared to conventional approaches that may generate harmful byproducts. This aligns with the growing demand for sustainable practices in science and medicine [64].

As a result, biological synthesis is gaining traction for medical applications, positioning itself as an important area of research and development in the quest for safer and more effective therapeutic solutions. By harnessing the power of nature, researchers can develop nanoparticles that meet both safety and efficacy standards while contributing to a more sustainable future. This trend toward green chemistry not only enhances the therapeutic potential of nanoparticles but also aligns with global efforts to minimize environmental impact.

3.2 Characterization techniques (e.g., TEM, SEM, DLS, FTIR)

Once nanoparticles are synthesized, it's critical to thoroughly **characterize** them to ensure they meet the necessary size, shape, surface properties, and functionality for therapeutic use. This subsection focuses on the most common **characterization techniques** used to analyze nonmetallic nanoparticles:

3.2.1 Transmission electron microscopy (TEM)

Transmission Electron Microscopy (TEM) is a powerful imaging technique that utilizes a beam of electrons transmitted through an ultra-thin specimen to produce high-resolution images. It provides a magnification that can reach up to millions of times, allowing researchers to visualize the fine details of nanoparticles at the nanoscale [65].

3.2.1.1 Visualizing internal structure and morphology

TEM provides high-resolution images that reveal the internal structure and morphology of nanoparticles. This capability is crucial for understanding how the physical characteristics of nanoparticles influence their behavior in biological systems. For instance, researchers can assess whether nanoparticles possess a hollow structure, core-shell configuration, or specific surface modification [66]. These features can significantly affect how drugs are encapsulated, released, and targeted within the body.

3.2.1.2 Assessing size and uniformity

Uniformity in size and shape is critical for the therapeutic efficacy of nanoparticles. TEM allows researchers to measure the dimensions of individual nanoparticles accurately. For example, in drug delivery applications, nanoparticles that are too large may be cleared quickly from the bloodstream or may not penetrate target tissues effectively [67]. Conversely, nanoparticles that are too small may not accumulate sufficiently at the target site. By using TEM to monitor the size distribution of nanoparticles, researchers can optimize formulations to achieve the desired therapeutic profiles.

3.2.1.3 Evaluating drug loading and release mechanisms

TEM can be employed to observe how drugs are loaded into nanoparticles and how they are released in a controlled manner. By visualizing the interaction between drugs and the nanoparticle matrix, researchers can refine loading techniques to maximize drug encapsulation efficiency [68]. For instance, in the treatment of inflammatory diseases, TEM can help visualize the loading of anti-inflammatory drugs into lipid-based nanoparticles, ensuring that the drugs remain effectively entrapped until they reach the target site. This controlled release can lead to improved therapeutic outcomes while minimizing side effects.

3.2.1.4 Understanding interactions with biological systems

TEM can also be used to study the interactions between nanoparticles and biological components, such as cells and tissues. Understanding these interactions is vital for predicting how nanoparticles will behave in vivo and their potential therapeutic effectiveness [69]. For example, TEM can reveal how polymeric nanoparticles interact with immune cells, which is essential when designing drug delivery systems for autoimmune disorders. By assessing how these nanoparticles are taken up by immune cells, researchers can optimize their formulations to enhance therapeutic delivery while minimizing adverse immune responses.

3.2.2 Scanning electron microscopy (SEM): Overview of SEM

Scanning Electron Microscopy (SEM) is a powerful imaging technique that allows for the examination of the surface morphology and topography of nanoparticles at high magnification and resolution. By utilizing a focused beam of electrons, SEM generates detailed images that provide insights into the size, shape, and surface characteristics of nanoparticles [70]. This information is crucial for optimizing nanoparticles for therapeutic applications, especially in the targeted delivery of drugs for non-malignant disorders.

3.2.2.1 Understanding surface properties

The surface properties of nanoparticles play a critical role in their interactions with biological systems. SEM enables researchers to visualize the surface roughness, texture, and any functional groups present on the nanoparticle surface. These characteristics can significantly influence the stability, dispersion, and bioavailability of nanoparticles in physiological environments. Forexample: For liposomal nanoparticles designed for delivering anti-inflammatory drugs, SEM can reveal the presence of surface modifications (e.g., PEGylation) that enhance circulation time and reduce opsonization by the immune system [71,72]. This information helps optimize liposome formulations for improved therapeutic efficacy.

3.2.2.2 Determining overall shape and size

The shape and size of nanoparticles are critical factors affecting their pharmacokinetics and cellular uptake. SEM provides precise measurements of these dimensions, allowing researchers to tailor nanoparticles for specific therapeutic goals [73]. For instance, smaller nanoparticles may penetrate tissues more easily, while larger ones may be more suitable for targeted delivery. For example: In the development of polymeric nanoparticles for the treatment of inflammatory bowel disease (IBD), SEM can be used to determine the optimal size that ensures effective delivery to the inflamed intestinal

mucosa [74]. By analyzing the size distribution, researchers can modify the synthesis parameters to achieve the desired size, enhancing drug accumulation at the target site.

3.2.2.3 Evaluating morphological changes during synthesis

During the synthesis of nonmetallic nanoparticles, various parameters (e.g., temperature, pH, and precursor concentration) can influence the morphology of the resulting particles. SEM allows for the real-time monitoring of these changes, enabling researchers to optimize synthesis conditions to achieve uniform and reproducible nanoparticle characteristics. For example: In the synthesis of silica nanoparticles for drug delivery applications, SEM can be employed to assess the effects of different synthesis conditions on particle shape (e.g., spherical vs. elongated) [75,76]. Understanding these morphological variations can help tailor the particles for specific drug delivery applications, such as enhancing the encapsulation efficiency of hydrophobic drugs.

3.2.2.4 Assessing surface functionalization

Surface functionalization is often employed to enhance the targeting capability of nanoparticles. SEM can be utilized to visualize the success of these modifications, ensuring that targeting ligands (e.g., antibodies, peptides) are properly attached to the nanoparticle surface. For example: For targeting genetic disorders, nanoparticles may be functionalized with ligands that specifically bind to receptors on diseased cells. SEM can be used to confirm the presence and uniform distribution of these ligands on the nanoparticle surface, optimizing the design for effective gene delivery [77].

3.2.3 Dynamic light scattering (DLS)

DLS is used to measure the size distribution of nanoparticles in solution. This technique is important for understanding the stability of nanoparticle suspensions and ensuring consistent particle size, which is critical for drug delivery efficacy [78].

3.2.3.1 Measurement of size distribution

DLS measures the Brownian motion of particles in suspension and correlates this motion with particle size. By analyzing the scattered light from the nanoparticles, DLS can determine the hydrodynamic radius of particles, which is essential for understanding how they behave in biological environments [79]. For example: For nanoparticles intended for drug delivery in inflammatory diseases, such as rheumatoid arthritis, achieving a specific size range (typically between 100 and 200 nm) is crucial. Particles within this range can evade the immune system, prolong circulation time, and enhance accumulation at target sites. DLS allows researchers to confirm that their nanoparticles consistently fall within this optimal size range [80].

3.2.3.2 Understanding stability of nanoparticle suspensions

The stability of nanoparticle suspensions is vital for maintaining drug efficacy and avoiding aggregation or sedimentation, which can compromise therapeutic effects. DLS helps assess the stability of nanoparticle formulations by measuring changes in size over time. For example: In gene therapy for genetic disorders such as cystic fibrosis, nanoparticles must remain stable in solution to effectively deliver genetic material to target cells. By using DLS, researchers can monitor the stability of liposomal formulations containing siRNA, ensuring that they remain uniformly dispersed and functional during storage and administration [66,81].

3.2.3.3 Ensuring consistent particle size

Consistency in particle size is critical for ensuring predictable drug release profiles and biodistribution. Variability in size can lead to differences in pharmacokinetics and therapeutic outcomes. For example: In the development of polymeric nanoparticles for delivering anti-inflammatory drugs to treat chronic conditions like inflammatory bowel disease (IBD), DLS can be employed during the formulation process to ensure that the particles have a uniform size [82]. This uniformity helps to achieve controlled drug release and improved targeting of inflamed tissues, leading to better treatment efficacy and reduced side effects.

3.2.3.4 Optimization of drug loading and release profiles

DLS can assist in optimizing drug loading and release profiles by analyzing how the size of nanoparticles changes upon drug encapsulation. Changes in size can indicate successful loading or aggregation, guiding formulation adjustments. For example: When formulating silica nanoparticles for delivering corticosteroids in the treatment of asthma, DLS can be used to assess the effect of drug loading on nanoparticle size. If significant aggregation occurs upon loading, adjustments can be made to the formulation (e.g., altering surfactants or polymer concentrations) to improve stability and ensure effective delivery [83,84].

3.2.4 Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique used to identify chemical bonds and functional groups present on the surface of nanoparticles. This technique provides critical information regarding the chemical composition and functionalization of nanoparticles, which is essential for optimizing their therapeutic applications, particularly in drug delivery for non-malignant disorders [85].

3.2.4.1 Characterization of functional groups

FTIR can detect and characterize various functional groups on nanoparticle surfaces. The presence of specific functional groups (e.g., –OH, -NH2, –COOH) can influence how nanoparticles interact with biological tissues and drugs. For example: In drug delivery systems targeting inflammatory diseases, nanoparticles may be functionalized with carboxyl (-COOH) or amine (-NH2) groups to enhance their interaction with the targeted cells. FTIR can confirm these functional groups' presence, ensuring that the nanoparticles are optimally designed for enhanced cellular uptake [86].

3.2.4.2 Confirmation of drug loading

FTIR can be employed to confirm the successful loading of therapeutic agents onto nanoparticles. By comparing the FTIR spectra of the drug-loaded nanoparticles with that of the free drug, one can identify specific peaks associated with the drug, indicating its presence on the nanoparticle surface. For example: In the development of liposomal formulations for delivering anti-inflammatory drugs like dexamethasone, FTIR can be used to verify that the drug is adequately encapsulated within the liposome. The absence of characteristic peaks of the drug in the spectrum of the unloaded liposome, coupled with the presence of these peaks in the drug-loaded liposome spectrum, confirms successful loading [87,88].

3.2.4.3 Optimization of coatings for targeted delivery

FTIR is instrumental in evaluating and optimizing surface coatings or modifications aimed at enhancing the targeting ability of nanoparticles. Surface modifications often involve attaching ligands, antibodies, or other targeting moieties that can specifically bind to receptors on target cells. For example: When developing silica nanoparticles for targeted delivery of anti-cancer agents in inflammatory diseases, FTIR can be utilized to confirm the attachment of targeting ligands (e.g., folic acid) to the nanoparticle surface. The presence of characteristic absorption bands corresponding to the targeting ligand in the FTIR spectrum indicates successful functionalization, which enhances the nanoparticle's ability to interact selectively with folate receptor-expressing cells [89,90].

3.2.4.4 Stability assessment

FTIR can also help assess the stability of nanoparticles during storage or under varying environmental conditions. Changes in the FTIR spectra over time may indicate chemical degradation or instability, prompting necessary modifications to the formulation. For example: In developing polymeric nanoparticles for the sustained release of drugs in chronic inflammatory diseases, FTIR can be used to monitor the stability of the nanoparticles over time [91]. By evaluating the spectra at different time points, researchers can determine if any degradation or changes in functional groups occur, ensuring that the nanoparticles remain effective throughout their shelf life.

3.3 Importance of characterization in determining therapeutic efficacy

Characterization involves the comprehensive analysis and assessment of therapeutic agents, examining their physical, chemical, and biological properties. This process is vital for determining the efficacy of a treatment for several key reasons.

First, characterization helps researchers understand the mechanisms of action, providing insights into how a therapeutic agent interacts with biological systems at the molecular level. This knowledge is essential for predicting the effectiveness of the therapy in treating specific conditions. Moreover, detailed characterization ensures the purity and quality of therapeutic agents by identifying impurities that could impact their safety or performance [92]. This is crucial to ensure that the treatment is free from contaminants and maintains a high standard of quality.

Characterization also plays a significant role in assessing dosage and delivery methods. By analyzing pharmacokinetics—how the body absorbs, distributes, metabolizes, and excretes the therapeutic agent—researchers can develop optimal dosing strategies and delivery methods to ensure that the treatment reaches its target in effective concentrations [93]. Additionally, it helps predict biological responses across different patient populations, taking into account genetic, environmental, and health-related factors. This information is critical for developing personalized medicine approaches, ensuring that treatments are tailored to individual needs.

Furthermore, characterization is necessary for regulatory compliance. Therapeutic agents must meet stringent characterization requirements to gain approval from health authorities, ensuring that the treatment adheres to regulatory guidelines, which in turn facilitates successful clinical trials and market entry [94]. Finally, characterization aids in optimizing the formulation of the therapeutic agent, improving stability, bioavailability, and overall effectiveness.

In summary, characterization is fundamental to determining the therapeutic efficacy of agents. It provides valuable insights into their mechanisms of action, ensures safety and quality, aids in appropriate dosing, predicts patient responses, ensures compliance with regulatory standards, and guides the development of optimized formulations, ultimately leading to better therapeutic outcomes.

4. Mechanisms of action

This section examines the interactions between nonmetallic nanoparticles and biological systems, the strategies used to target specific tissues or cells, and the processes involved in cellular uptake and intracellular function. Understanding these mechanisms is essential for optimizing nanoparticles for therapeutic applications, ensuring they efficiently reach and act on intended biological targets.

4.1 How nonmetallic nanoparticles interact with biological systems

In biological systems, nonmetallic nanoparticles interact in complex ways, largely influenced by their surface chemistry, the formation of a protein corona, and their biodistribution and circulation properties.

Surface chemistry and functionalization play a crucial role in determining how nanoparticles behave in a biological environment. Surface modifications, such as altering the charge, hydrophobicity, or the addition of specific functional groups, can dramatically affect their interaction with cells and tissues [95]. By fine-tuning these properties, researchers can improve the nanoparticles' compatibility with biological systems, minimize immune detection, and enhance their ability to target specific cells or organs. A common strategy to evade the immune system, for example, is coating nanoparticles with polyethylene glycol (PEG). This process, known as "stealthing," creates a protective barrier around the nanoparticles, preventing immune recognition and clearance. This allows the nanoparticles to remain in circulation longer, improving their chances of reaching the intended target site, which is especially important in drug delivery applications [96].

Once nanoparticles enter the body, they are rapidly covered by a layer of proteins, forming what is known as a **protein corona**. This corona significantly influences how nanoparticles interact with biological systems, as it alters their original surface properties [97]. The composition of the protein corona depends on the nanoparticles' characteristics and the type of biological fluid they encounter, such as blood or tissue fluid. The corona affects nanoparticle recognition by cells, their distribution within the body, and their overall safety profile. Understanding the formation and composition of the protein corona is vital for developing nanoparticles with enhanced targeting abilities, especially for applications like drug delivery, medical imaging, and theranostics [98]. Researchers are exploring ways to manipulate protein corona formation through surface modifications and environmental controls, making nanoparticles more selective and effective in biomedical applications.

Biodistribution and circulation of nanoparticles are key determinants of their therapeutic efficacy and safety. Factors such as particle size, shape, and surface charge heavily influence how nanoparticles are distributed in the body. Smaller nanoparticles have better tissue penetration, such as crossing the blood-brain barrier, while larger ones are often more rapidly cleared by organs like the liver or kidneys [99]. Spherical nanoparticles tend to distribute more evenly and are less likely to be recognized by the immune system compared to non-spherical ones, which may interact differently with cells due to their

shape. Surface charge also plays a significant role; neutral nanoparticles usually have longer circulation times, while positively charged particles may be taken up more readily by cells but are often cleared faster. The route of administration—whether intravenous, oral, or topical—also impacts the biodistribution [100,101]. For example, nanoparticles administered intravenously face different clearance and distribution pathways than those delivered orally or through the skin. Researchers can modify surface properties and employ ligand-based targeting to control how nanoparticles travel and accumulate in the body, making them more effective for specific therapeutic uses, such as in targeted drug delivery or precision diagnostics.

These key factors—surface chemistry, protein corona formation, and biodistribution—are fundamental to the design and application of nonmetallic nanoparticles in medicine. By understanding and manipulating these interactions, researchers can create more efficient, selective, and safe nanoparticle-based therapies.

4.2 Targeting mechanisms: Passive versus active targeting

This subsection covers the two main strategies for targeting specific tissues or cells: **passive targeting** and **active targeting.** Each has its own set of advantages and is used based on the therapeutic goals. Passive targeting and active targeting represent two distinct strategies for enhancing the precision and efficacy of nanoparticle-based therapies, as shown in Fig. 24.2.

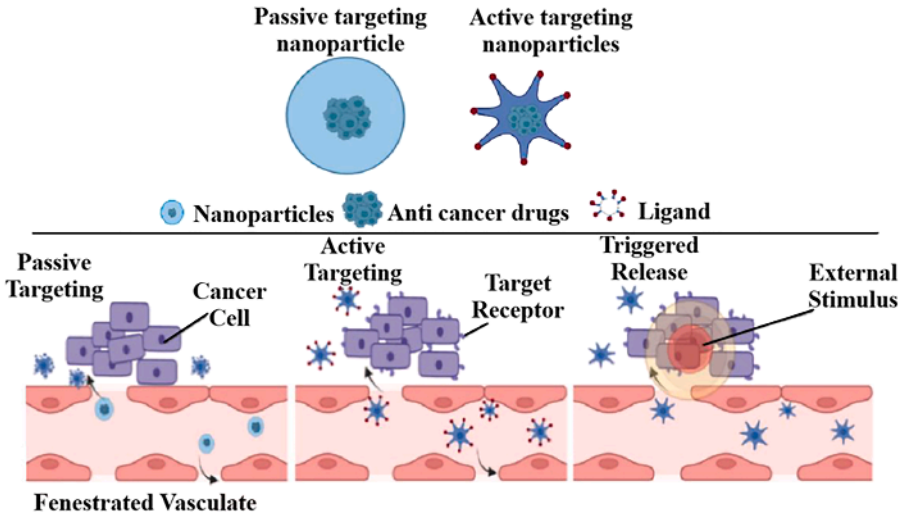


FIGURE 24.2

Mechanism of passive and active targeting in nanoparticle-based drug delivery systems [102].
The scheme illustrates the difference between passive and active targeting using nanoparticles for drug delivery, particularly in cancer therapy. In passive targeting, nanoparticles accumulate at the tumor site due to the enhanced permeability and retention (EPR) effect caused by the fenestrated vasculature of the tumor. In active targeting, nanoparticles are functionalized with ligands that bind to specific receptors on cancer cells, allowing for more precise targeting. The final stage shows triggered drug release upon exposure to an external stimulus, enhancing the specificity and effectiveness of the therapeutic action.

Passive targeting leverages the Enhanced Permeability and Retention (EPR) effect, a phenomenon observed in pathological conditions such as cancer, inflammation, and cardiovascular diseases, where leaky blood vessels allow larger molecules, including nanoparticles, to accumulate in the surrounding tissues [103]. This strategy is particularly useful for treating chronic inflammatory conditions and cardiovascular diseases, as it enables the passive delivery of therapeutic agents to affected areas without the need for specific targeting mechanisms [104]. While passive targeting offers simplicity and broad applicability across various diseases, it lacks the precision of active targeting, which may result in off-target effects and variability in therapeutic outcomes among patients. Despite these limitations, the use of passive targeting through the EPR effect presents a promising approach for the development of novel therapies aimed at non-malignant disorders [105].

In contrast, **active targeting** enhances the specificity of nanoparticle delivery by modifying the nanoparticles with ligands that bind to receptors overexpressed on target cells. This method provides several advantages, including improved specificity, increased therapeutic efficacy, and the potential for personalized medicine tailored to individual patient profiles [106]. Active targeting is being explored for applications in cancer therapy, neurological disorders, and inflammatory diseases, allowing for the targeted delivery of therapeutic agents directly to diseased cells or tissues. However, challenges such as the complexity of designing targeted nanoparticles, higher production costs, and concerns regarding in vivo stability persist [107]. Nonetheless, ongoing research continues to refine these strategies, and active targeting remains a highly promising avenue for improving the safety and effectiveness of nanoparticle-based therapies in both malignant and non-malignant disorders. In addition, Table 24.3 presents a comparison between passive and active targeting approaches along with their respective features.

4.3 Cellular uptake and intracellular mechanisms

This subsection delves into how nanoparticles are internalized by cells and the subsequent intracellular pathways they utilize to deliver therapeutic agents. Understanding these mechanisms has shown in Fig. 24.3 is critical for designing nanoparticles that can effectively navigate cellular environments to reach their intended targets.

Cellular uptake mechanisms involve the processes by which cells internalize substances from their environment, which is essential for functions like nutrient absorption, signaling, and immune responses. Nanoparticles primarily enter cells through **endocytosis**, a process that can occur via several

Table 24.3 Comparison of passive and active targeting.

No.	Feature	Passive targeting	Active targeting	Ref.
1	Mechanism	Exploits EPR effect	Requires specific ligands	[108,109]
2	Specificity	Less specific	Highly specific	[110,111]
3	Simplicity	Simple	More complex	[109,112]
4	Applicability	Broad range of diseases	Limited to diseases with known target receptors	[109,113]
5	Efficacy	Can vary	Generally higher	[111,112]

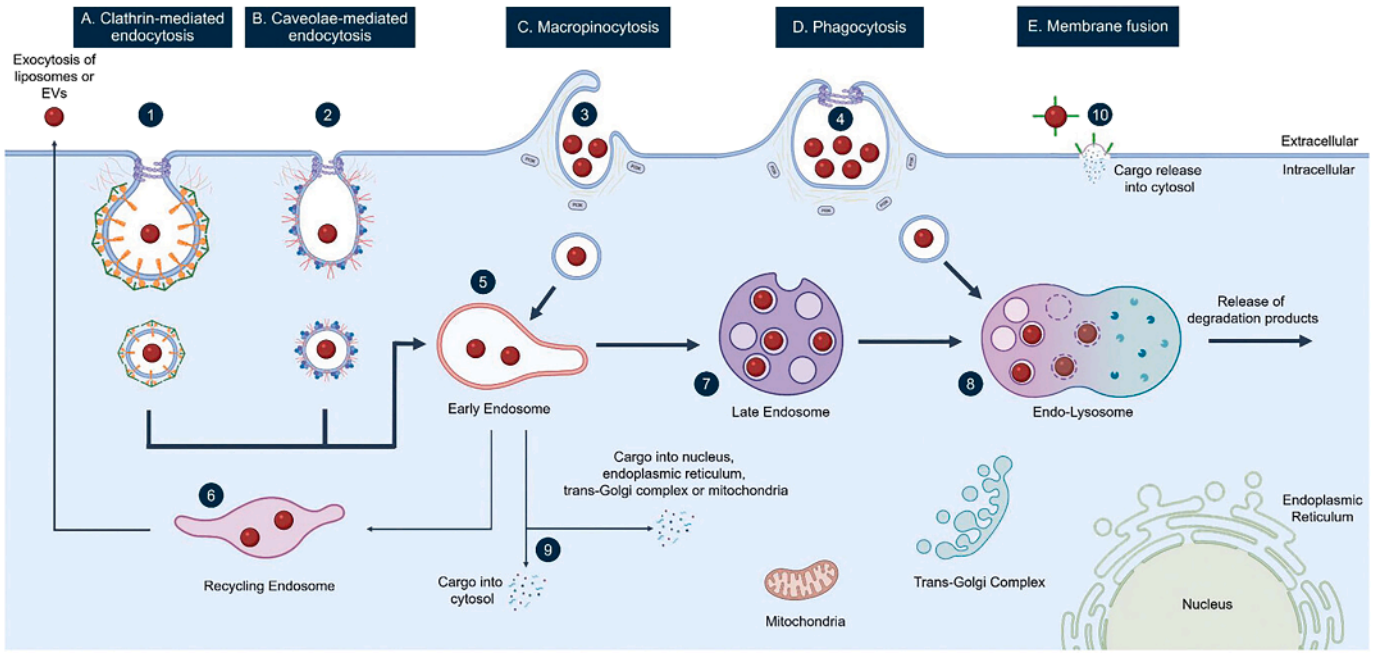


FIGURE 24.3

Mechanisms of nanoparticle uptake and intracellular trafficking [114].
The figure illustrates the various pathways of nanoparticle endocytosis and intracellular processing. Pathways include clathrin-mediated endocytosis (A), caveolae-mediated endocytosis (B), macropinocytosis (C), phagocytosis (D), and membrane fusion (E). Nanoparticles or extracellular vesicles (EVs) enter cells through these routes and are processed via early endosomes (5) and late endosomes (7). Some nanoparticles are recycled back to the membrane via recycling endosomes (6), while others merge with endo-lysosomes (8) for degradation. Cargo can be released into the cytosol (9) for intracellular distribution, including transport to the nucleus, endoplasmic reticulum, trans-Golgi complex, or mitochondria. Alternatively, nanoparticles can fuse with the membrane (10) to release cargo directly into the cytosol.

pathways. **Clathrin-mediated endocytosis** is one such pathway, where the formation of clathrin-coated vesicles facilitates the engulfing of extracellular substances. Another pathway is **caveolae-mediated endocytosis**, which involves cholesterol-rich invaginations that transport nanoparticles into cells. Additionally, **macropinocytosis**, a non-specific process, enables the uptake of extracellular fluid and particles. For immune cells like macrophages, **phagocytosis** is the primary mechanism, allowing them to engulf larger particles or pathogens. **Pinocytosis**, on the other hand, involves the intake of small fluid droplets. These diverse mechanisms play a critical role in determining how effectively nanoparticles are internalized by cells, a key consideration when designing drug delivery systems that aim to improve therapeutic uptake [115].

Once inside the cell, nanoparticles must navigate complex **intracellular trafficking** routes to reach their target sites and release their therapeutic payloads. Following internalization via endocytosis, nanoparticles typically move through endosomal compartments, where they face the challenge of escaping the endosomes to avoid degradation [116]. Several strategies have been developed to facilitate **endosomal escape**: pH-sensitive nanoparticles, for instance, are designed to release their contents in the acidic environment of endosomes, while degradable nanoparticles break down under specific intracellular conditions to release their cargo [117]. Other nanoparticles are engineered to disrupt cellular membranes, allowing the release of therapeutic agents directly into the cytoplasm. Targeting specific organelles such as the nucleus or mitochondria can be enhanced by modifying nanoparticles with ligands that direct them to these cellular components. Release mechanisms can also be controlled through stimuli like temperature, light, or enzymatic activity. Effective intracellular trafficking and controlled release are crucial for therapies such as gene therapy, where the successful delivery of DNA or RNA depends on escaping endosomal degradation [118].

At the target site, nanoparticles can exert their **therapeutic action** through various mechanisms, depending on the payload and the disease being treated. **RNA interference (RNAi)**, for example, uses nanoparticles to deliver small interfering RNA (siRNA) that silences disease-causing genes, offering potential treatments for conditions like neurodegenerative diseases. Similarly, nanoparticles are being used in **enzyme replacement therapy** to correct metabolic disorders by delivering functional enzymes directly to deficient cells [119,120]. **Targeted drug delivery** systems aim to minimize side effects by concentrating therapeutic agents specifically in diseased tissues, while **protein delivery** nanoparticles are employed in diseases such as diabetes, where they help in the controlled release of insulin [121]. **Gene therapy** also benefits from nanoparticles that deliver genetic material to correct mutations or introduce new genes, offering promising treatments for genetic disorders [122]. The efficacy of these therapies depends on the ability of nanoparticles to efficiently release their payloads, maintain the stability of the therapeutic agent, and achieve sufficient cellular uptake to produce the desired therapeutic effect.

By understanding and leveraging these processes, researchers can design more effective nanoparticle-based therapies for a wide range of non-malignant disorders.

5. Therapeutic applications in non-malignant disorders

This section explores the various non-malignant disorders that can benefit from nanoparticle-based therapies. It delves into real-world examples of nanoparticles being used for treatment, illustrating their mechanisms for enhancing drug delivery and improving therapeutic outcomes.

5.1 Overview of non-malignant disorders (e.g., diabetes, inflammatory diseases, neurological disorders, etc.)

Non-malignant disorders encompass a broad range of chronic health conditions that, while not cancerous, can profoundly impact a person's quality of life, functionality, and overall health. Among these are diabetes, inflammatory diseases, and neurological disorders, each representing significant public health challenges due to their prevalence, complexity, and long-term consequences [123].

Diabetes is a metabolic disorder characterized by elevated blood glucose levels, resulting from insufficient insulin production or ineffective insulin action. The two main types are **Type 1 diabetes**, an autoimmune condition where the immune system attacks the insulin-producing beta cells in the pancreas, usually manifesting in childhood or adolescence and requiring lifelong insulin therapy, and **Type 2 diabetes**, the more common form, often associated with lifestyle factors such as obesity and physical inactivity. In Type 2, the body becomes resistant to insulin, leading to elevated glucose levels, managed through lifestyle changes, oral medications, and sometimes insulin [124]. Common complications of diabetes include cardiovascular disease (increased risk of heart disease, stroke, and hypertension), neuropathy (nerve damage causing pain, tingling, or loss of sensation, particularly in the feet), retinopathy (damage to the retina, potentially causing vision impairment or blindness), and kidney disease (diabetic nephropathy, which can lead to kidney failure if untreated) [125].

Inflammatory diseases are marked by chronic inflammation that affects various systems within the body, significantly impairing daily functioning and quality of life. Rheumatoid arthritis (RA) is a prime example, an autoimmune disorder causing chronic joint inflammation, pain, and eventual joint destruction, with the potential to affect other organs and cause systemic symptoms such as fatigue and fever [126]. Another example is **inflammatory bowel disease (IBD)**, which includes conditions like Crohn's disease and ulcerative colitis. These result in chronic gastrointestinal inflammation, leading to symptoms such as abdominal pain, diarrhea, fatigue, and weight loss. **Psoriasis**, a chronic skin condition characterized by red, itchy, scaly patches due to systemic inflammation, is also part of this category. Managing inflammatory disorders often involves a combination of medications (such as NSAIDs, corticosteroids, and biologics), lifestyle modifications, and physical therapy to alleviate symptoms and prevent complications [127,128].

Neurological disorders involve impairments in the nervous system, affecting brain function, movement, and overall neurological health. **Multiple sclerosis (MS)**, for instance, is a disease in which the immune system attacks the protective sheath (myelin) surrounding nerves, leading to a wide range of symptoms, including weakness, coordination difficulties, vision problems, and cognitive changes [129]. **Parkinson's disease** is a progressive disorder primarily affecting movement, marked by tremors, stiffness, slowness of movement, and postural instability due to the degeneration of dopamine-producing neurons. Similarly, **Alzheimer's disease**, a form of dementia, causes cognitive decline, memory loss, and behavioral changes, primarily in older adults, though the progression and impact can vary. **Epilepsy**, another significant neurological disorder, is characterized by recurrent, unprovoked seizures, necessitating careful diagnosis and management to minimize its impact on daily life [130].

In conclusion, non-malignant disorders such as diabetes, inflammatory diseases, and neurological conditions represent significant public health challenges due to their widespread impact and the complexity of managing them. Effective management often requires a multidisciplinary approach that includes medical treatment, lifestyle modifications, rehabilitation, and ongoing supportive care. Early

diagnosis and personalized treatment strategies are essential in improving patient outcomes and enhancing the quality of life for individuals affected by these chronic conditions.

Here are the real-world examples from some Studies in Clinical Applications, focusing on their effectiveness, lessons learned, and overall impact on patient outcomes.

5.2 Silica nanoparticles in drug delivery for inflammatory disorders

The analysis by Namiot et al. (2023) highlights the significant role of nanoparticles in clinical trials, particularly lipid-based nanoparticles in vaccine development for COVID-19 and mesoporous silica nanoparticles (MSNs) in drug delivery. MSNs are noted for their tunable surface properties and large surface area, which enhance drug bioavailability, targeting, and controlled release, making them effective carriers for localized treatments, such as in chronic inflammation and cancer. Clinical studies indicate that MSNs improve drug delivery efficiency, allowing for targeted therapy in conditions like rheumatoid arthritis, thus reducing required dosages and minimizing side effects. Key insights emphasize the importance of optimizing nanoparticle characteristics for better cellular uptake and ensuring biocompatibility for safe elimination. Overall, MSNs have positively impacted patient outcomes by alleviating symptoms and enhancing quality of life in chronic inflammatory disorders, suggesting they hold promise for improving therapeutic efficacy and patient well-being [131].

5.3 Carbon-based nanoparticles in neurological applications

The study by Kayoung Kim et al. (2020) in Nature Communications demonstrates the potential of carbon nanotubes (CNTs) for the precise clinical diagnosis of Alzheimer's disease (AD). The researchers developed a sensor array utilizing densely aligned CNTs for the multiplexed detection of key AD biomarkers in human plasma, achieving high sensitivity (90%), selectivity (90%), and accuracy (88.6%). This sensor outperforms traditional diagnostic methods, such as neuropsychological tests and neuroimaging, while offering a minimally invasive approach through plasma sampling. The CNTs' structure enhances signal transduction and allows simultaneous measurement of multiple biomarkers, improving diagnostic accuracy. Key insights from the study emphasize the need for optimization of CNT alignment and packing density to enhance sensitivity and reduce false positives. The findings suggest that CNT-based diagnostic tools could facilitate earlier and more accurate detection of AD, enabling timely therapeutic interventions and improving patient care. Overall, the study highlights the clinical relevance of CNTs in neurodiagnostics and their potential to transform routine AD screening [132].

5.4 Lipid-based nanoparticles in respiratory disorders

The study by Ellenmae W. X. Leong and Ruowen Ge investigates lipid nanoparticles (LNPs) as carriers for inhaled therapies, specifically for treating respiratory diseases such as COVID-19. LNPs are effective in delivering small molecules, nucleic acids, and proteins directly to the lungs, which enhances drug absorption and reduces side effects in the rest of the body. By encasing therapeutics in LNPs, higher drug concentrations can be achieved in lung tissues, crucial for effectively treating respiratory conditions.

The research emphasizes the importance of carefully designing LNP formulations to ensure drug stability and optimize delivery to the lungs. It also identifies challenges like mucosal clearance and the need for targeted delivery to lung cells, which are vital for the success of LNP inhalation therapies. The LNP system shows promise for more efficient treatments for respiratory diseases, potentially benefiting patients with chronic conditions like asthma or acute infections by providing quicker and more focused therapeutic effects [133].

5.5 Exosomes in autoimmune disorders

The study by Romina Heydari and colleagues examines the use of exosomes as potential diagnostic and therapeutic tools for rheumatoid arthritis (RA), a chronic inflammatory joint disease. Exosomes are effective in RA because they transport genetic material, proteins, and lipids between cells, influencing inflammation and immune responses. Notably, exosomes from mesenchymal stem cells (MSCs) have immunomodulatory properties that can be beneficial for treatment. Exosomes are promising for diagnosing RA by carrying microRNAs (miRNAs) that serve as biomarkers for the disease. MSC-derived exosomes have also shown the ability to reduce inflammation in RA models, indicating their therapeutic potential. The study highlights the need to understand the varied contents of exosomes and their target cells. It emphasizes further research on the diagnostic and therapeutic use of exosomal miRNAs and other molecules. A major challenge is the standardization of methods for isolating and characterizing exosomes for clinical use. Utilizing exosomes' capabilities could allow for earlier RA detection and improved management of inflammation and joint damage. This approach may lead to more personalized treatment plans and better long-term outcomes for patients, particularly in slowing disease progression and minimizing associated disabilities [134].

5.6 Mechanisms by which nanoparticles enhance drug delivery and efficacy

This subsection explains the mechanisms by which nonmetallic nanoparticles enhance drug delivery, targeting, and efficacy, making them highly advantageous in treating non-malignant disorders. One key mechanism is **controlled release**, where nanoparticles like polymeric or liposomal types are designed to deliver drugs in a sustained manner over time. This is particularly beneficial for managing chronic diseases such as diabetes, where consistent delivery of medications like insulin helps maintain stable drug levels in the body. As a result, the need for frequent dosing is reduced, and the risk of side effects is minimized, improving patient compliance [135].

Another important mechanism is **improved bioavailability**, where nanoparticles increase the solubility and absorption of drugs that are poorly water-soluble. For example, micelles can encapsulate hydrophobic drugs, enhancing their uptake and effectiveness. This is especially critical in treating conditions like inflammatory diseases, where high concentrations of the drug need to reach the site of inflammation for optimal therapeutic outcomes [136].

Targeted delivery is another key advantage of nanoparticles. Nanoparticles can be engineered to direct drugs specifically to targeted tissues or cells, improving treatment precision. This can occur through **active targeting**, where nanoparticles are functionalized with ligands that bind to receptors on specific cells, such as brain cells, allowing them to cross the blood-brain barrier for effective treatment of neurological disorders. Alternatively, **passive targeting** leverages the enhanced permeability and

retention (EPR) effect, which enables nanoparticles to accumulate in inflamed areas or tissues with leaky blood vessels, as seen in conditions like rheumatoid arthritis or Crohn's disease [137].

Nanoparticle-based drug delivery also significantly contributes to **reduced toxicity**. By directing drugs to specific sites in the body, nanoparticles minimize off-target effects, which is especially important in chronic conditions requiring long-term treatment, such as asthma or rheumatoid arthritis. Encapsulating toxic medications in nanoparticles can further protect healthy tissues, lowering the risk of adverse side effects [138].

Lastly, the use of **multi-functional nanoparticles** is emerging as a powerful approach in therapy. These nanoparticles can be engineered to deliver multiple therapeutic agents or combine treatment with diagnostic capabilities, a concept known as theranostics. For instance, a single nanoparticle could deliver an anti-inflammatory drug while also carrying imaging agents that allow clinicians to track disease progression or monitor treatment effectiveness in real-time. This multi-functional capability offers a comprehensive approach to treating complex disorders [139].

6. Benefits of nonmetallic nanoparticles

This section will highlight the key advantages of using nonmetallic nanoparticles in drug delivery and therapy, particularly for non-malignant disorders. It will focus on the improvements these nanoparticles offer over conventional therapeutic approaches, showcasing their potential to enhance patient outcomes and minimize side effects.

6.1 Improved bioavailability and solubility

One of the most significant benefits of nonmetallic nanoparticles is their ability to improve the bioavailability and solubility of therapeutic agents. Many drugs, particularly those with hydrophobic properties, have limited solubility in water and, as a result, poor bioavailability when administered through traditional methods [140]. Nonmetallic nanoparticles can overcome this limitation through the following mechanisms:

6.1.1 Encapsulation of hydrophobic drugs

Many drugs for managing non-cancer-related disorders, like diabetes, are hydrophobic (poorly soluble in water). Nanoparticles such as micelles and liposomes can encapsulate these hydrophobic drugs within their core, allowing them to stay stable in water-based environments like blood. For diabetic patients, effective glucose management often requires hydrophobic drugs (e.g., certain oral antidiabetics or insulin analogs). By using nanoparticles for encapsulation, these drugs remain stable and bioavailable, improving their absorption and ensuring they reach their target site. This enhanced solubility increases their effectiveness in controlling blood glucose levels [141].

6.1.2 Improved absorption and distribution

Nanoparticles enhance the solubility of poorly water-soluble drugs, improving their ability to cross biological barriers like the gastrointestinal tract and the blood-brain barrier. This better absorption leads to higher bioavailability, so more of the drug reaches the intended tissues. For diabetes, delivering drugs effectively through the gastrointestinal tract is crucial for oral medications. Nanoparticles can help deliver insulin and other glucose-lowering agents in oral forms, overcoming poor absorption

rates. This enhances the likelihood of the drug reaching its target, improving glucose regulation and offering a more comfortable alternative to injections [142].

6.1.3 Extended circulation time

Nanoparticles can be designed to evade the immune system's rapid clearance, allowing them to remain in the bloodstream for longer periods. This extended circulation increases the chance of drugs reaching target tissues, resulting in a more sustained therapeutic effect. In diabetes management, stable blood glucose levels are essential throughout the day. By prolonging the circulation of insulin or other antidiabetic drugs, nanoparticles can provide a sustained release, reducing the need for frequent dosing. This mimics the body's natural insulin release more closely, leading to more consistent blood glucose control and lowering the risk of hypoglycemic episodes [143].

6.1.4 Conclusion: Improving diabetes therapy

Improving diabetes therapy can be enhanced through the use of nonmetallic nanoparticles. These nanoparticles offer innovative drug delivery systems that increase the efficacy and specificity of diabetes medications. They can help in targeting insulin or other therapeutics directly to cells, improving uptake and reducing side effects. Additionally, nonmetallic nanoparticles may facilitate better monitoring of glucose levels and allow for controlled release of medications, leading to more stable blood sugar management. Integrating these advanced technologies into diabetes treatment can lead to more effective management strategies, improved patient adherence, and ultimately better health outcomes for individuals with diabetes.

6.2 Reduction in side effects compared to conventional therapies

Nonmetallic nanoparticles offer a critical advantage in significantly reducing the side effects commonly associated with conventional therapies. Traditional drug delivery methods, such as oral or systemic administration, often lead to widespread distribution of the drug throughout the body, resulting in off-target effects and toxicity in healthy tissues. Nanoparticles provide a solution to this challenge by enabling more precise and controlled drug delivery [144].

These nanoparticles achieve targeted delivery by being engineered to specifically interact with diseased cells or tissues, thus reducing the quantity of drug that affects healthy cells. For instance, active targeting involves attaching ligands to the surface of nanoparticles, which then bind to specific receptors on diseased cells, including inflamed tissues or neurons affected by neurodegenerative disorders. This targeted approach ensures that therapeutic agents are delivered directly to the affected sites, thereby minimizing the risk of side effects in other areas of the body [145].

Moreover, nanoparticles can be designed for controlled or sustained release of their therapeutic payloads. This characteristic diminishes the need for frequent dosing while maintaining a consistent drug concentration in the bloodstream. By avoiding the peaks and troughs typically seen with conventional drug administration, nanoparticles help prevent fluctuations in drug levels that can lead to side effects. This aspect is particularly beneficial for chronic conditions like diabetes or rheumatoid arthritis, where stable drug levels are crucial for reducing adverse reactions [146].

Another significant benefit of nanoparticle-based therapies is the reduction in systemic toxicity. Nonmetallic nanoparticles can encapsulate potent drugs, such as corticosteroids or immunosuppressants, delivering them specifically to the site of disease. This targeted strategy minimizes the exposure

of healthy tissues to the drug, thereby lowering the risk of systemic toxicity. For example, in inflammatory diseases, nanoparticles can concentrate the drug in inflamed tissues, shielding healthy areas from unnecessary exposure and alleviating common side effects like immunosuppression or gastrointestinal irritation [147].

Overall, nonmetallic nanoparticles represent a powerful platform for enhancing the precision and safety of therapeutic interventions, especially in the treatment of non-malignant disorders.

6.3 Enhanced therapeutic outcomes through targeted delivery

Targeted delivery systems in cancer therapy enhance therapeutic outcomes by improving the efficacy and specificity of drug administration. These systems direct therapeutic agents precisely to tumor sites, minimizing systemic side effects associated with traditional treatments. Key mechanisms include precision targeting through ligand-receptor interactions, the use of cell-penetrating peptides (CPPs) to enhance intracellular uptake, and the exploitation of the enhanced permeability and retention (EPR) effect for selective drug accumulation in tumors. Additionally, advanced systems allow for controlled and sustained release of drugs, improving patient compliance and therapeutic action. Clinically, targeted delivery reduces adverse effects, increases therapeutic efficacy, enables personalized medicine tailored to individual patient profiles, and supports combination therapies that address tumor heterogeneity. Overall, these innovations represent significant advancements in the management of cancer, promising improved patient outcomes [148].

7. Challenges and limitations

While nonmetallic nanoparticles offer many benefits for therapeutic applications, their clinical translation faces several significant challenges and limitations. This section will cover the key obstacles that need to be addressed to realize the full potential of nanoparticle-based therapies, focusing on biocompatibility, stability, and regulatory issues.

7.1 Biocompatibility and toxicity concerns

One of the primary challenges in developing nanoparticle-based therapies is ensuring biocompatibility and managing potential toxicity. Although nonmetallic nanoparticles are generally considered safer than their metallic counterparts, several factors can still lead to toxicity or adverse biological responses.

A major concern is the **activation of the immune system**, as nanoparticles may be recognized as foreign substances by the body, triggering immune responses. This can result in inflammation or the rapid clearance of nanoparticles from the bloodstream, which diminishes their therapeutic effectiveness. In some cases, nanoparticles may cause allergic reactions or chronic immune activation, especially if they accumulate in tissues over time [149].

Another issue arises from the **toxicity of degradation products**. Certain nonmetallic nanoparticles are designed to degrade within the body, but the by-products of this degradation can sometimes be toxic. For example, while polymeric nanoparticles typically degrade into biocompatible molecules, at higher concentrations these degradation products may still cause toxicity. Organic nanoparticles, such

as dendrimers, can also induce cytotoxicity if their surface chemistry is not properly optimized for biocompatibility [150].

Furthermore, the long-term biocompatibility of nanoparticles remains a significant concern. The effects of prolonged exposure are not yet fully understood, particularly for chronic disorders that require repeated nanoparticle administration. Nanoparticles that are not efficiently cleared from the body may accumulate in organs like the liver or spleen, potentially leading to adverse effects over time [151].

To address these concerns, ongoing research is focused on engineering nanoparticles with **optimal surface chemistry** and **controlled degradation profiles** to reduce immune activation and minimize toxicity, ensuring their safety and efficacy in therapeutic applications.

7.2 Stability and reproducibility issues

Another major challenge in the development and commercialization of nonmetallic nanoparticles lies in ensuring their stability during storage and use, as well as achieving reproducibility in their manufacturing processes. These challenges are critical because any instability or variability can compromise the safety and efficacy of the final product.

Nanoparticles must maintain their physical and chemical stability, preserving their size, shape, and functional properties both during storage and after administration. Factors such as temperature fluctuations, pH changes, and interactions with biological fluids can lead to issues like aggregation or degradation. For instance, liposomes may become unstable and release their cargo prematurely, or polymeric nanoparticles might degrade in the bloodstream before reaching their target, reducing their therapeutic effectiveness [152].

In addition to stability, **reproducibility in manufacturing** presents a significant hurdle. Consistent, large-scale production of nanoparticles with precise control over size, shape, and surface properties is challenging. Even minor variations in particle size or surface charge can drastically influence nanoparticle behavior within the body, affecting their biodistribution, targeting efficiency, and therapeutic efficacy. Variability between batches can complicate regulatory approval processes and limit the consistency required for clinical applications [153].

Furthermore, **shelf-life** is a crucial consideration. Nanoparticles must remain stable for extended periods to be commercially viable, but many formulations degrade or lose functionality over time. Maintaining stability may require special storage conditions, such as low temperatures or inert environments, to ensure a reasonable shelf-life for widespread distribution. These stability and reproducibility challenges underscore the complexity of developing reliable nanoparticle-based therapies for non-malignant disorders [154].

7.3 Regulatory hurdles in nanoparticle-based therapies

The regulatory approval process for nanoparticle-based therapies is particularly complex due to the novel properties and multifunctional nature of these systems, which do not fit neatly into existing frameworks for conventional drugs or biologics. One of the key challenges is the **uncertainty in classification**. Regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), often struggle to categorize nanoparticle-based products. Depending on their composition and function, nanoparticles might be classified as drugs, biologics,

medical devices, or even a combination of these categories. This ambiguity complicates the regulatory pathway. For example, nanoparticles that serve both diagnostic and therapeutic purposes (known as theranostics) may fall under multiple regulatory categories, leading to more stringent and complicated approval requirements [155,156].

Another significant hurdle is the **lack of standardized testing protocols** for assessing the safety, efficacy, and quality of nanoparticle formulations. The diverse nature of nanoparticles makes it difficult to develop universal guidelines. Currently, there is no standardized set of testing protocols to evaluate these therapies, making it challenging for regulatory agencies to consistently assess nanoparticle-based treatments across various studies. As a result, robust preclinical and clinical data are required, yet the uniqueness of each nanoparticle system makes the creation of comprehensive guidelines an ongoing challenge [157].

Moreover, the **preclinical and clinical evaluation** of nanoparticle-based therapies is often more complex and time-consuming than that of conventional treatments. These systems require thorough testing in preclinical models (such as animals) and in clinical trials to assess pharmacokinetics, toxicity, and therapeutic efficacy. Due to the intricate nature of nanoparticles, the testing is often extensive and expensive, which can significantly delay regulatory approval. Complicating matters further, animal models frequently fail to accurately predict how nanoparticles will behave in human systems, making the translation from preclinical to clinical testing problematic [158].

Safety concerns also present a major regulatory challenge. Nanoparticles, particularly those designed for sustained release or that degrade over time, must undergo rigorous scrutiny to ensure their long-term biocompatibility. Regulatory agencies often require extended safety evaluations to address potential risks, such as the accumulation of nanoparticles in tissues or organs over time. Furthermore, the degradation products of nanoparticles must be shown to be non-toxic, adding another layer of complexity to the safety evaluation process. These factors contribute to the longer timelines and higher costs associated with gaining regulatory approval for nanoparticle-based therapies [159].

8. Current research trends

This section will explore the latest developments and research trends in the field of nonmetallic nanoparticles, particularly as they pertain to their applications in treating non-malignant disorders. It will cover recent technological advancements, innovations in drug delivery systems, and future directions for research.

8.1 Recent advancements in nonmetallic nanoparticle technology

In recent years, significant advancements in the technology surrounding nonmetallic nanoparticles have emerged, driven by the demand for more effective therapeutic solutions. One major development is **nanoparticle engineering**, where innovations in design and synthesis have led to improved control over size, shape, and surface modifications. Advanced techniques like layer-by-layer assembly and 3D printing now allow researchers to create nanoparticles with tailored characteristics for targeted drug delivery and controlled release. For instance, optimizing the size of nanoparticles can enhance their cellular uptake and distribution, while specific surface modifications facilitate targeted binding to diseased tissues, improving the precision and efficacy of treatments [160].

Another area of progress is the development of **multi-functional nanoparticles**, which can serve multiple roles, such as diagnostic imaging and therapeutic delivery, known as theragnostic. These nanoparticles are designed to carry both imaging agents and drugs, enabling real-time monitoring of treatment efficacy and localization of the drug within the body. For example, silica nanoparticles can be modified to carry imaging agents alongside anti-inflammatory drugs, allowing simultaneous visualization and treatment of inflammatory diseases, which is a critical step forward in personalized medicine [161].

Additionally, a growing focus on **biodegradable nanoparticles** has been observed, where recent research emphasizes creating nanoparticles that degrade safely into non-toxic by-products after delivering their therapeutic payload. This innovation aligns with the increasing demand for biocompatible, sustainable, and patient-friendly treatment options. Materials such as PLGA (poly(lactic-co-glycolic acid)) and chitosan are being widely studied for their potential in biodegradable nanoparticle formulations, offering safer alternatives for long-term therapies while minimizing environmental impact [162].

8.2 Innovations in drug formulation and delivery systems

The field of drug formulation and delivery is rapidly evolving, with new strategies being developed to enhance the effectiveness of therapeutic agents. Key innovations include **nanoemulsions** and **nanocrystals**, which are gaining attention for their ability to improve the solubility and bioavailability of poorly soluble drugs. Nanoemulsions consist of tiny droplets of oil dispersed in water, allowing for enhanced absorption of lipophilic drugs. Similarly, nanocrystals are submicron-sized drug particles that offer improved dissolution rates, leading to increased bioavailability [163].

Another exciting trend is the development of **stimuli-responsive nanoparticles** that can release their therapeutic payloads in response to specific environmental triggers, such as pH changes, temperature variations, or the presence of specific biomolecules. For instance, nanoparticles designed to release anti-inflammatory drugs specifically at the site of inflammation can significantly enhance therapeutic efficacy while minimizing side effects [164].

Moreover, there is a growing trend towards utilizing **combination therapies** delivered through nanoparticle systems, particularly for complex conditions like diabetes or inflammatory diseases. By co-delivering multiple therapeutic agents that target different pathways or mechanisms, researchers aim to improve treatment efficacy and reduce the risk of resistance. Nanoparticles can be engineered to encapsulate various drugs, allowing for simultaneous or sequential release tailored to the patient's needs. This innovative approach not only optimizes therapeutic outcomes but also represents a significant advancement in the field of drug delivery systems [165].

8.3 Future directions in research for non-malignant disorders

As the field of nonmetallic nanoparticles continues to evolve, several promising future directions are emerging for research aimed at addressing non-malignant disorders. One significant trend is the move toward personalized nanomedicine. Future studies may increasingly focus on tailoring nanoparticle formulations to individual patient profiles, optimizing drug delivery based on genetic, biochemical, or metabolic characteristics. This personalized approach aims to enhance treatment efficacy and minimize adverse effects, especially for chronic conditions that require long-term management.

Another important area of focus will be the clinical translation of nanoparticle technologies. While numerous preclinical studies have demonstrated the efficacy of nonmetallic nanoparticles, transitioning these findings into clinical applications presents ongoing challenges. Future research will likely prioritize developing scalable production methods and establishing rigorous clinical trial protocols to evaluate safety and efficacy comprehensively. Collaborations between academic institutions, industry partners, and regulatory bodies will be essential to overcome barriers and facilitate the successful translation of these innovative therapies into clinical settings [166].

Researchers are also expanding their exploration of new therapeutic areas for nonmetallic nanoparticles, particularly in treating a broader range of non-malignant disorders. This includes rare diseases and conditions with high unmet medical needs, such as neurological disorders, autoimmune diseases, and chronic inflammatory conditions. By leveraging the unique properties of nanoparticles, researchers aim to address the complexities of these diseases more effectively [167].

Lastly, as the use of nanoparticles expands, there will be a growing emphasis on understanding their environmental impact and ensuring sustainable practices in their production and disposal. Future research may increasingly explore biodegradable and eco-friendly materials for nanoparticle synthesis. This shift aligns with global efforts to reduce environmental pollution and promote sustainability within the healthcare sector. Together, these future directions highlight the potential of nonmetallic nanoparticles to significantly improve the management of non-malignant disorders while addressing important considerations for health and environmental sustainability.

9. Regulatory framework for nanoparticle therapeutics

The regulatory landscape for nanoparticle-based therapeutics is complex and continues to evolve as research in this area advances. This section will provide a comprehensive overview of the regulatory bodies involved, the challenges faced during the approval process, and notable case examples of approved nonmetallic nanoparticle therapies.

9.1 Overview of regulatory bodies and guidelines

Various regulatory bodies worldwide oversee the development, approval, and monitoring of nanoparticle-based therapies, ensuring their safety and efficacy in medical applications. The U.S. Food and Drug Administration (FDA) serves as the primary regulatory agency in the United States, responsible for the safety and efficacy of medical products, including drugs, biologics, and medical devices. Within the FDA, the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) play crucial roles in reviewing nanoparticle formulations. The FDA has issued guidance documents that outline considerations for the development and approval of nanoparticle-based products, emphasizing the importance of comprehensive characterization, safety assessments, and efficacy studies [168,169].

Similarly, the European Medicines Agency (EMA) oversees the evaluation of medicinal products in the European Union. The EMA provides guidelines for the development and evaluation of nanomedicines, with the Committee for Medicinal Products for Human Use (CHMP) responsible for assessing the safety and efficacy of nanoparticle therapeutics. Additionally, the EMA has developed

specific guidelines for the risk assessment of nanomedicines, addressing critical aspects such as toxicity, exposure, and environmental impact [170].

The World Health Organization (WHO) also plays a vital role by providing international guidance on health-related matters, including the use of nanoparticles in medicine. The WHO promotes global collaboration to develop standardized guidelines and best practices for the safety assessment and regulation of nanomedicines [171].

In addition to these prominent organizations, various countries have their regulatory authorities, such as Health Canada and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA), which establish guidelines for the approval of nanoparticle-based therapies [172]. Together, these regulatory bodies work to create a framework that ensures the responsible development and use of nanomedicine while addressing the unique challenges posed by nanoparticle technology.

9.2 Challenges in regulatory approval of nanoparticle-based therapies

The regulatory approval process for nanoparticle-based therapies faces several unique challenges that arise from the novel properties of nanoparticles and their diverse applications. One of the primary challenges is the complexity of nanoparticle formulations. These therapeutics often consist of intricate mixtures with varying compositions, sizes, shapes, and surface modifications. This complexity complicates the standardization of testing protocols and makes it difficult to evaluate their safety and efficacy consistently. Regulatory agencies require comprehensive characterization data to assess the potential risks associated with these formulations [173].

Additionally, there is a lack of standardized testing protocols for nanoparticle safety assessments. Currently, no universally accepted framework exists, which leads to inconsistencies in testing methodologies across different studies. Regulatory bodies mandate data on biocompatibility, toxicity, pharmacokinetics, and long-term effects of nanoparticles. However, the absence of standardized testing complicates the evaluation process and hinders the ability to draw reliable conclusions regarding safety and efficacy [174].

Uncertainty about the long-term effects of nanoparticles also poses significant challenges. Given their novel nature, there is limited understanding of their long-term impacts on human health. Regulatory agencies remain cautious about approving therapies that may present unknown toxicological profiles or unexpected interactions with biological systems over extended periods [175].

Furthermore, regulatory classification issues contribute to the complexities of the approval process. Nanoparticle-based products can often be ambiguous in classification, as they may fall into different categories, such as drugs, biologics, or medical devices. This ambiguity can lead to confusion regarding the applicable regulatory pathways and requirements, which prolongs the approval process and creates additional hurdles for developers [176].

Lastly, there is a need for extensive preclinical and clinical data to support the safety and efficacy of nanoparticle therapies. Regulatory agencies typically require substantial evidence before approval, which can be resource-intensive and time-consuming. This poses particular challenges for small companies or academic researchers attempting to navigate the intricate regulatory landscape. Together, these factors contribute to a complex and often prolonged regulatory approval process for nanoparticle-based therapies.

9.3 Case examples of approved nonmetallic nanoparticle therapies

Despite the challenges in regulatory approval, several nonmetallic nanoparticle therapies have successfully received clinical use approval, demonstrating their potential in enhancing therapeutic outcomes. One notable example is **Doxil** (Doxorubicin Liposome Injection), which is a pegylated liposomal formulation of doxorubicin. Approved by the FDA for the treatment of breast cancer, ovarian cancer, and Kaposi's sarcoma, Doxil enhances the drug's stability and alters its distribution. This leads to reduced side effects and improved therapeutic outcomes, showcasing the advantages of nanoparticle-based formulations [177].

Another significant product is **Abraxane** (Paclitaxel Albumin-bound Particles), an albumin-bound nanoparticle formulation of paclitaxel. Approved for treating breast cancer, non-small cell lung cancer, and pancreatic cancer, Abraxane improves the solubility of paclitaxel and facilitates its targeted delivery to tumor tissues. This targeted approach results in enhanced efficacy and reduced toxicity compared to traditional formulations [178].

Onivyde (Irinotecan Liposome Injection) is yet another example, specifically designed for the treatment of metastatic pancreatic cancer. This liposomal formulation enhances the bioavailability of irinotecan and minimizes side effects, ultimately leading to improved patient outcomes [179].

Additionally, **Vectibix** (Panitumumab) illustrates the integration of nonmetallic nanoparticles in targeted therapy for colorectal cancer. This monoclonal antibody linked to nanoparticles enhances the targeting and delivery of biologics, contributing to improved therapeutic efficacy in patients [180].

Moreover, nanoparticle-based vaccines have shown promising results in both preclinical and clinical studies, particularly for infectious diseases. For instance, various nanoparticle formulations have been developed for vaccines against influenza and COVID-19, demonstrating their potential to elicit rapid and effective immune responses. These examples collectively highlight the successful integration of nonmetallic nanoparticles into clinical practice, paving the way for their broader application in therapeutics [181].

10. Commercialization and market potential

The commercialization of nonmetallic nanoparticles represents a rapidly growing sector within the broader field of nanomedicine. This section will explore the current commercial landscape, identify key players and their contributions, and analyze the economic impact and future market trends for nanoparticle-based therapeutics.

10.1 Overview of the commercial landscape for nonmetallic nanoparticles

The commercial landscape for nonmetallic nanoparticles is characterized by significant investment, innovation, and a growing number of applications in healthcare.

One key aspect of this landscape is **market growth**. The global nanomedicine market, which encompasses nonmetallic nanoparticles, is experiencing robust expansion. This growth is driven by several factors, including the increasing prevalence of chronic diseases, advancements in nanotechnology, and a heightened focus on personalized medicine. Market analysts project that the nanomedicine sector will reach several billion dollars in the coming years, indicating strong commercial potential. Another important feature is the **diverse applications** of nonmetallic nanoparticles. These

nanoparticles are being explored for a wide range of uses, including drug delivery, imaging, diagnostics, and therapeutics. This versatility allows companies to target multiple sectors within the healthcare industry, making nonmetallic nanoparticles particularly appealing for commercialization [182].

In addition, regulatory progress is enhancing the commercial landscape. As regulatory agencies develop clearer guidelines for nanoparticle-based products, the approval process is becoming more streamlined. This increased regulatory clarity encourages investment and development in this area, attracting pharmaceutical companies and startups to explore and commercialize novel nanoparticle therapies [183].

Finally, **collaborations and partnerships** play a vital role in the commercialization of nonmetallic nanoparticles. These efforts often involve collaborations between academic institutions, research organizations, and industry stakeholders. Such partnerships facilitate knowledge transfer, accelerate the development of new therapies, and help bring innovative products to market more efficiently [184].

Together, these aspects illustrate a dynamic and promising commercial landscape for nonmetallic nanoparticles, highlighting their potential to transform healthcare and improve patient outcomes.

10.2 Key players in the market and their products

Several key players in the pharmaceutical and biotechnology industries are actively involved in the development and commercialization of nonmetallic nanoparticles. Notable companies have showed in the following Table 24.4.

Table 24.4 Key players in the nanomedicine market and their nanoparticle-based products.

Company	Focus area	Notable products/Technologies	Ref.
Pfizer Inc.	mRNA vaccines and therapeutics	**COVID-19 vaccine**: Developed with BioNTech, utilizes lipid nanoparticles (LNPs) with the ionizable lipid ALC-0315 for stability and immune response enhancement.	[185]
Moderna, Inc.	mRNA technology for vaccines	**COVID-19 vaccine**: Uses LNPs with ionizable lipid SM-102, optimized for intramuscular delivery. Expanding LNP platform for pulmonary delivery targeting respiratory diseases.	[186]
Bristol myers squibb	Oncology and immunology	**Abraxane**: Nanoparticle albumin-bound paclitaxel formulation that improves drug delivery to tumors while reducing side effects.	[187]
Johnson and johnson	Oncology and infectious diseases	Developing nanoparticle-based therapies, including viral vectors and lipid nanoparticles for vaccine delivery.	[188]
Sanofi SA	Vaccines and biologics	Developing nanoparticle-based vaccines to enhance immune responses against various pathogens.	[189]

Continued

Table 24.4 Key players in the nanomedicine market and their nanoparticle-based products.—cont'd

Company	Focus area	Notable products/Technologies	Ref.
Alnylam pharmaceuticals	RNA interference (RNAi) therapeutics	**Patisiran (Onpattro)**: First approved siRNA drug delivered via LNPs, targeting hereditary transthyretin-mediated amyloidosis.	[190]
Nanospectra biosciences, Inc.	Cancer therapy	Uses gold nanoshells for photothermal therapy, targeting cancer cells while minimizing damage to surrounding healthy tissue.	[191]
Arrowhead pharmaceuticals	RNA therapeutics	Develops RNA interference therapies utilizing LNPs to deliver therapeutic RNA molecules directly into cells.	[192]
Nanobiotix	Oncology	**NBTXR3**: Nanoparticle designed to enhance radiation therapy effectiveness by increasing tumor response.	[193]

10.3 Economic impact and future market trends

The commercialization of nonmetallic nanoparticles carries significant economic implications, influencing healthcare costs, patient access to innovative therapies, and overall market dynamics. One major advantage of these nanoparticles is their potential to make therapies more cost-effective. By enhancing drug bioavailability and efficacy, nonmetallic nanoparticles can reduce the overall treatment costs associated with chronic diseases. This not only improves therapeutic outcomes but also reduces side effects, leading to decreased long-term healthcare expenditures, which makes these treatments more accessible and financially viable for healthcare systems and patients alike [194].

The rise of nanomedicine has also attracted substantial investment from venture capital firms, government agencies, and pharmaceutical companies, bolstering research, development, and commercialization efforts and fueling the growth of nanoparticle-based therapies in the market. Additionally, emerging markets are becoming increasingly significant in this landscape. As global healthcare demands evolve, companies are looking to regions with rising healthcare needs and limited access to advanced treatments as potential markets for affordable nanoparticle-based therapies. These regions represent new opportunities to provide innovative treatments to populations previously underserved by advanced healthcare technologies [195].

Looking to the future, several trends are expected to shape the nonmetallic nanoparticle market. A key area of focus is personalized medicine, where tailoring nanoparticle therapies to individual patient profiles can enhance treatment effectiveness and outcomes. Progress in regulatory frameworks is also anticipated, as clearer and more supportive regulations may expedite approval processes, facilitating faster market entry for new nanoparticle-based products. Additionally, combination therapies—where nanoparticles co-deliver multiple therapeutic agents—are gaining traction, offering promising solutions for complex diseases. Furthermore, the application of nonmetallic nanoparticles in diagnostics is expanding, allowing for early disease detection and efficient monitoring, which strengthens their role in modern healthcare and broadens their impact on patient care [196].

11. Future perspectives and innovations

As the field of nonmetallic nanoparticles continues to evolve, numerous innovations and emerging technologies promise to enhance their therapeutic potential. This section will explore future perspectives in this domain, including technological advancements, the potential for combination therapies, and the role of personalized medicine in improving patient outcomes.

11.1 Emerging technologies in the field of nonmetallic nanoparticles

Emerging technologies are rapidly transforming the field of nonmetallic nanoparticles, offering new opportunities for improved therapeutic efficacy and personalized medicine. Table 24.5 summarizes the key emerging technologies and their anticipated contributions to the future of nonmetallic

Table 24.5 The emerging technologies and their potential impact on the future of nonmetallic nanoparticles in therapeutics.

Emerging technology	Description	Potential impact	Example application	Ref.
Smart nanoparticles	Nanoparticles that respond to specific stimuli (pH, temperature, light) for controlled drug release.	Enhances therapeutic efficacy by ensuring targeted, localized drug release, reducing side effects.	pH-sensitive nanoparticles releasing drugs in acidic tumor environments.	[24]
Hybrid nanoparticles	Combining different types of nanoparticles (organic and inorganic) to create systems with enhanced properties.	Improves stability, drug loading, and targeting capabilities for more effective treatments.	Hybrid nanoparticles enhancing drug delivery to inflamed tissues in arthritis.	[197]
Nanoparticle coating technologies	Surface modification of nanoparticles with targeting ligands, antibodies, or peptides for selective targeting.	Increases precision of therapeutic interventions by targeting specific cells or tissues.	Antibody-coated nanoparticles targeting cancer cell markers.	[198]
3D printing of nanoparticles	Integration of 3D printing technology for customized nanoparticle-based drug delivery systems.	Enables rapid prototyping and development of tailored drug delivery devices with specific properties.	3D-printed nanoparticle-based patches for transdermal drug delivery.	[199]
AI in nanoparticle design	Use of AI and machine learning to predict nanoparticle behavior and optimize formulations.	Accelerates discovery and optimization of nanoparticle systems, leading to more efficient and effective treatments.	AI-guided design of nanoparticles for improved brain drug delivery.	[200]

nanoparticle-based therapies. This table provides a structured overview of key technological advancements and their transformative role in enhancing nonmetallic nanoparticles for therapeutic applications.

11.2 Potential for combination therapies (e.g., nanoparticles with biologics)

Combination therapies that incorporate nonmetallic nanoparticles alongside biologics or other therapeutic modalities hold great promise for enhancing treatment outcomes. These therapies leverage the unique properties of nanoparticles to create synergistic effects with biologics, such as monoclonal antibodies or RNA-based therapies, thereby improving therapeutic efficacy [201]. For instance, nanoparticles can act as carriers for biologics, enhancing their stability, solubility, and bioavailability, which is crucial for maximizing their effectiveness [202].

Moreover, nanoparticles facilitate the targeted delivery of multiple therapeutic agents to specific sites within the body. This capability allows for the simultaneous delivery of a chemotherapeutic agent and a targeted antibody, effectively attacking cancer cells from multiple angles and enhancing overall treatment efficacy. Such targeted approaches are essential for optimizing therapeutic outcomes and minimizing off-target effects [203].

In addition to improving delivery and efficacy, the incorporation of nanoparticles in combination therapies can help overcome drug resistance mechanisms that are often encountered in cancer treatment. By delivering multiple drugs that possess different mechanisms of action, nanoparticles have the potential to circumvent the pathways cancer cells utilize to evade therapy. This multifaceted approach can lead to more effective treatment strategies and better patient outcomes [107].

Furthermore, the combination of nanoparticles with vaccine antigens or adjuvants represents a significant advancement in vaccine development. Nanoparticles can serve as delivery vehicles that enhance the stability and presentation of vaccine components, leading to more robust and lasting immune responses. This synergy not only improves the effectiveness of vaccines but also has the potential to address various infectious diseases and cancer [204].

11.3 Role of personalized medicine in enhancing therapeutic efficacy

Personalized medicine focuses on tailoring medical treatment to the individual characteristics of each patient, and nonmetallic nanoparticles play a significant role in this paradigm. One of the key aspects of personalized medicine is the biomarker-driven approach. By integrating nonmetallic nanoparticles with biomarker identification, personalized treatment strategies can be developed. This involves utilizing nanoparticles that specifically target biomarkers associated with a patient's disease, allowing for customized therapies that improve efficacy while minimizing adverse effects.

Advances in genetic profiling also contribute to personalized medicine by enabling the identification of patient-specific mutations or genetic predispositions that affect drug response. Nonmetallic nanoparticles can be designed to deliver tailored therapies that take these individual genetic factors into account, resulting in more effective treatment strategies. Additionally, personalized medicine promotes adaptive treatment strategies, wherein therapies are adjusted based on the patient's response. Nonmetallic nanoparticles, with their potential for controlled and targeted drug delivery, facilitate real-time adjustments to treatment plans based on ongoing monitoring of patient outcomes [205].

Moreover, the development of patient-centric nanoparticle formulations that consider individual preferences and lifestyles can significantly enhance treatment adherence and satisfaction. Engaging patients in the design and implementation of nanoparticle-based therapies not only fosters a sense of ownership but also leads to better health outcomes. In this context, personalized medicine is instrumental in enhancing the therapeutic efficacy of nonmetallic nanoparticles, highlighting how these innovations can lead to more tailored and effective treatment options [206].

12. Ethical considerations

As the application of nonmetallic nanoparticles in medicine advances, several ethical considerations arise. This section will explore the ethical implications of using nanoparticles in healthcare, public perception of nanotechnology, and addressing concerns about long-term effects and safety.

12.1 Ethical implications of using nanoparticles in medicine

The integration of nanoparticles into medical applications presents a range of ethical implications that must be carefully considered. First and foremost is the issue of informed consent. Patients need to be adequately informed about the nature of nanoparticle-based therapies, including the potential benefits, risks, and unknowns associated with these treatments. Given the complexity of nanoparticle technology, ensuring that patients fully understand what they are consenting to can pose significant challenges. This necessitates clear and effective communication from healthcare providers to facilitate informed decision-making [207].

Equity in access is another critical consideration. The potentially high cost of nanoparticle-based therapies could exacerbate existing healthcare disparities, raising ethical concerns about access and affordability. It is imperative that ethical frameworks address these issues to ensure that all patients, regardless of their socioeconomic status, have equitable access to these advanced therapies [208]. Research ethics also play a vital role in the development of nanoparticles, which often involves extensive preclinical research, including animal testing. Ethical guidelines must be established to ensure that such research is conducted responsibly, striking a balance between the pursuit of scientific advancement and the welfare of research animals [208].

Moreover, the dual-use concerns of nanoparticle technology warrant attention. The potential applications of these technologies extend beyond therapeutic uses to areas such as surveillance or military applications. Ethical discussions must therefore address the dual-use nature of nanoparticle technologies and the potential consequences of their misuse. In this context, it is essential to explore these ethical implications in detail, emphasizing the importance of robust ethical frameworks to guide the development and application of nanoparticle technologies in medicine. Such frameworks can help navigate the complexities associated with these advancements, ensuring that they are utilized responsibly and equitably in healthcare.

12.2 Public perception and acceptance of nanotechnology in healthcare

Public perception is vital for the acceptance and use of nanotechnology in healthcare, and several key factors shape this perception. One of the most significant aspects is awareness and understanding. Many individuals have limited knowledge of nanotechnology and its medical applications, which can

lead to misconceptions and fears about nanoparticles. To foster greater acceptance, increasing education and outreach efforts is essential, as improved understanding can alleviate resistance to the implementation of nanotechnology in medical treatments.

Another crucial factor is media representation. The way nanotechnology is portrayed in the media significantly impacts public perception. Positive articles that highlight the benefits and advancements of nanoparticles can enhance acceptance, while sensationalized reporting on potential risks may instill doubt and skepticism among the public. Therefore, responsible media coverage is important in shaping a balanced perspective [209].

Trust in science and healthcare professionals also plays a pivotal role in the acceptance of new technologies. The public's confidence in scientists and healthcare providers influences their willingness to embrace innovative solutions. Transparency regarding research findings, regulatory processes, and clinical applications can help build trust and address public concerns, creating a supportive environment for nanotechnology adoption [210].

Additionally, ethical and social considerations significantly impact how the public views the use of nanotechnology in healthcare. Engaging communities in discussions about the ethical implications and addressing societal concerns can lead to a more informed and balanced perspective. By involving various stakeholders in these conversations, it is possible to foster a greater understanding of the potential benefits and challenges associated with nanoparticles in healthcare [211].

12.3 Addressing concerns about long-term effects and safety

Addressing the long-term safety and effects of nanoparticles in medical applications is essential, requiring a multifaceted approach to mitigate potential risks. **Rigorous long-term safety studies** are paramount to thoroughly evaluate the biodistribution, toxicity, and biocompatibility of nanoparticles over extended periods. These studies aim to confirm that the use of nanoparticles in medicine does not present unforeseen health risks, ensuring their safe integration into clinical settings [212].

Strong regulatory oversight is also critical. Regulatory bodies must implement comprehensive guidelines for assessing nanoparticle-based therapies, focusing on safety and efficacy benchmarks prior to approval. Additionally, post-approval monitoring is essential to detect any emerging safety concerns once these therapies are in widespread use, facilitating swift response if issues arise [183].

Transparency and data sharing are equally important for building public confidence in nanoparticle-based treatments. Researchers and developers should commit to openly sharing information on safety, efficacy, and any adverse effects associated with nanoparticle applications. This openness fosters trust among healthcare professionals and the public, contributing to greater acceptance and understanding of these technologies [208].

Finally, **patient education** is crucial for empowering patients to make informed decisions regarding their treatment. By providing clear, accessible information about the potential risks and benefits of nanoparticle-based therapies, healthcare providers can foster open communication and ensure that patients feel actively involved in decisions about their healthcare. This collaborative approach prioritizes patient safety and fosters trust, paving the way for responsible and effective adoption of nanoparticle technologies in medicine [213].

13. Conclusion

The exploration of nonmetallic nanoparticles as a therapeutic platform for non-malignant disorders reveals a promising landscape filled with innovation and potential. Throughout this chapter, we have examined key areas, beginning with a comprehensive understanding of nonmetallic nanoparticles, their classifications, and their importance in biomedical applications. We have also explored the methods of synthesizing these nanoparticles and the critical role of characterization techniques in ensuring their efficacy and safety for therapeutic use. Understanding the mechanisms by which nonmetallic nanoparticles interact with biological systems and their targeting strategies has further illuminated their therapeutic potential.

In terms of applications, nonmetallic nanoparticles show great promise in treating a range of non-malignant disorders, including diabetes, inflammatory diseases, and neurological conditions. However, challenges such as biocompatibility, toxicity, and regulatory hurdles remain significant barriers that need to be addressed before these nanoparticles can be fully integrated into clinical practice. Despite these obstacles, the potential for future advancements is immense. Emerging technologies and combination therapies offer new avenues for enhancing the efficacy of nonmetallic nanoparticles.

Ethical considerations also play a crucial role in the development of this field. Addressing public perception, long-term safety concerns, and fostering trust among patients and healthcare providers are essential for the successful integration of nanoparticles into mainstream medicine. The vast potential of nonmetallic nanoparticles lies in their ability to improve drug delivery and patient outcomes, overcoming the limitations of conventional therapies.

Moving forward, interdisciplinary collaboration is key. Researchers, clinicians, regulatory bodies, and industry stakeholders must work together to translate nanoparticle technologies from the laboratory to the clinic. To achieve this, we must prioritize funding for innovative research and actively engage the public to address ethical concerns and enhance understanding. By fostering an environment of openness and cooperation, the development of nonmetallic nanoparticles can align with both patient needs and societal values.

As research continues to advance, nonmetallic nanoparticles have the potential to transform the treatment landscape for non-malignant disorders, offering new hope to patients. The commitment to advancing this field holds the promise of delivering groundbreaking therapies that can significantly improve the quality of life for countless individuals. Embracing this challenge with enthusiasm and determination will pave the way for a future of innovative, life-changing treatments.

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PART

VI Recent advances and regulatory aspects of stimuli-responsive nanocarriers

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CHAPTER

Clinical translation, challenges, biological toxicity, and safety issues associated with stimuli-responsive nanocarriers

25

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1. Introduction

Nanocarriers have been introduced via the development of material science and nanotechnology to deliver chemotherapy drugs, nucleic acids, proteins, and contrast agents, presenting tremendous promise over traditional therapeutics and diagnostic agents. Nanocarrier-drug complexes or nanomedicines such as liposomes and polymer nanoparticles (NPs) have been widely studied. Furthermore, nanocarriers can be incorporated with stimuli-responsive components to enhance the bioavailability and targeting efficiency of drugs at specific sites. Several nanomedicines have been approved for clinical application. However, compared with the rate of publications, preliminary prosperity of nanomedicine studies, and preclinical investigations, the clinical approval of nanomedicines faces a declining tendency, which discourages some scientists, who become pessimistic about the prospect of nanomedicines. It can be seen that there is a wide gap between technical revolution and clinical translation due to the technical and cost challenges in scale-up and production, and low treatment efficacy in clinical trials. Furthermore, the delayed development of clinical nanomedicines may be detrimental to patients. Therefore, we introduce and discuss the challenges and obstacles in regulatory approval and reevaluate the opportunities for next-generation clinical nanomedicines with advanced functionalities.

The need for thorough preclinical and toxicology studies for drug approval implemented by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) is undeniable. The role of such tests is to ascertain that new pharmaceuticals are safe and effective before reaching human clinical trials.

Preclinical research begins with in vitro studies. These studies use cell cultures to assess the drug's biological activity. These methods include a variety of assays, such as cytotoxicity, receptor binding, and enzyme inhibition. The commonly employed in vitro tests need to evaluate various aspects of drug

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safety and efficacy. For example, the release rate of a drug from topical formulations can be evaluated by in vitro release testing (IVRT). IVRT can play a great part in comparing a proposed generic product with its reference standard to establish bioequivalence. In-Vitro Permeation Testing (IVPT) evaluates the permeation of a drug through the skin or other membranes. IVPT thus ensures that the drug reaches its intended site of action, which is particularly important for topical and transdermal products. Cytochrome P450 (CYP) enzymes play significant roles in drug metabolism. Enzyme assays are conducted to assess drug potential in CYP enzyme inhibition/induction. This helps predict drug-drug interactions and metabolic stability. Drug transporter assays evaluate how a medication interacts with various transport proteins, which may have an impact on how well the drug is absorbed, distributed, and excreted. Additionally, cell viability and cytotoxicity assays measure the effect of a drug on cell health. Providing critical insights into the potential toxicity of the drug is an important outcome of cytotoxicity tests.

Following the in vitro studies, in vivo studies are conducted to evaluate the drug's effects in animal models. These studies encompass pharmacokinetics, which examines the absorption, distribution, metabolism, and excretion of the drug; pharmacodynamics, which explores the drug's mechanism of action; and initial toxicity studies. In vivo testing is indispensable for several reasons. Not only does it provide a comprehensive whole-body assessment, but insights into how a drug interacts with the entire organism, including its metabolism, absorption, distribution, and excretion, are offered via in vivo testing. Unlike in vitro studies, the complex interactions within a living system are considered during in vivo tests. The drug's impact in a clinical setting is, therefore, better exhibited in vivo. Moreover, the identification of potential side effects, optimal dosing regimens, and overall therapeutic potential is undertaken by in vivo tests.

Understanding complex biological processes can be done in animal-based preclinical research. Mice and rats are common animals employed in research. Mice are valuable models for various diseases and drug testing. They are widely used due to their genetic similarity to humans and ease of handling. On the other hand, detailed physiological studies can be possible with rats because of their larger size in comparison to mice. Nonhuman primates share significant genetic similarities with humans and have been used in developing vaccines for diseases such as polio. Rabbits are utilized for cardiovascular and ocular research, as well as toxicity studies. Ensuring that results from animal studies effectively translate to humans involves several major steps.

1.1 Selection of model

Complicated interactions of human ailments are difficult to replicate, and animal models often oversimplify diseases. Animal models with the most similarity to human physiology and genetics are chosen by researchers. For improved translation, nonhuman primates or genetically modified mice with humanized genes can be used.

1.2 Dosage conversion

Equivalent doses between animals and humans can be determined using body surface area or metabolic rates. Conversion of doses helps ensure similar drug exposure between lab animals and human subjects.

1.3 Pharmacokinetics studies

Drug absorption, distribution, metabolism, and excretion in animals are studied via PK research. Dosing regimens for human trials are therefore guided by such data. Animal-based drug metabolism is different from the metabolism of human subjects, and human-specific enzymes affect drug efficacy and safety.

1.4 Toxicological investigations

Adverse drug reaction evaluation happens in animals. Dose-response relationships help safe dosing prediction in humans.

1.5 Studies on effectiveness

Potential clinical benefits are provided by insights that are gathered from drug efficacy assessment in animal models. However, differences in disease progression may affect efficacy evaluation.

1.6 Considerations related to species

Data interpretation related to interspecies variations (e.g., metabolism, immune response) is of major importance regarding understanding and adjustment of such differences between species.

1.7 Clinical trials

Safety, efficacy, and optimal dosing can be validated via human trials (phases I-III). Real-world patient diversity provides critical information.

1.8 Surrogate endpoints and biomarkers

Biomarkers that correlate with clinical outcomes should be identified along with surrogate endpoints (e.g., tumor shrinkage). Such elements aid early assessment.

1.9 Ethical and regulatory compliance

Researchers adhere to ethical guidelines and regulatory requirements. Institutional Animal Care and Use Committees (IACUCs) oversee animal studies.

1.10 Translational research centers

Collaboration across disciplines (clinicians, scientists, and industry) needs to happen to bridge the gap. Translational centers focus on producing community-relevant science.

While animal models provide important insights, no model perfectly replicates human complexity. Researchers keep refining methods to enhance translation and minimize discrepancies.

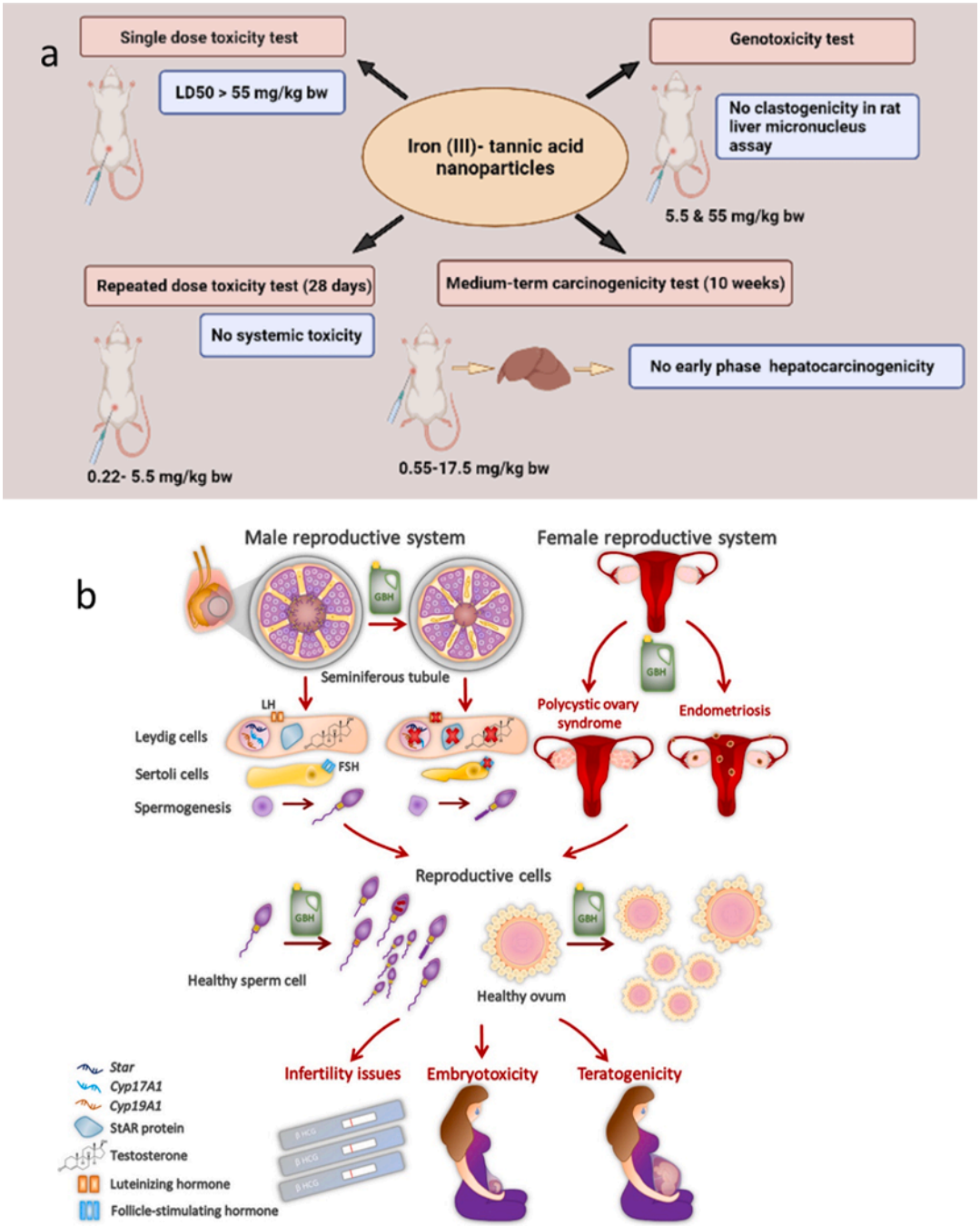


FIGURE 25.1

(a) some toxicity assessment studies of nanoparticles with permission from Ref. [1]. (b) reproductive system toxicity with permission from Ref. [2]

From C.B. Hlaing, A. Chariyakornkul, C. Pilapong, C. Punvittayagul, S. Srichairatanakool, R. Wongpoomchai, Assessment of systemic toxicity, genotoxicity, and early phase hepatocarcinogenicity of iron (III)-tannic acid nanoparticles in rats, Nanomaterials, 12 (7) (2022), 1040, https://doi.org/10.3390/nano12071040.

2. Toxicology studies

Various studies can determine the toxicity of newly developed drugs and nanoparticles (Fig. 25.1) [1].

Acute Toxicity Research:

Objective: Determination of the effects of a single dose of the drug.

Methods: Administration of the drug to model animals needs to happen, and animal observation for immediate toxic effects would be the next step.

Studies on Repeated Dose Toxicity:

Objective: Assessment of repeated dose regimen effects over a period (subacute, subchronic, and chronic toxicity).

Methods: Upon administration of the drug on a day-to-day basis for weeks to months, subject monitoring needs to occur for toxic effect determination.

Studies on Genotoxicity:

Objective: Evaluation of drug potential for causing genetic mutations.

Methods: There are important assays for genotoxicity evaluation, like the Ames test and chromosomal aberration tests.

Genetic mutation. The potential of drugs can be assessed with such tests. Drug carcinogenic risk can thus be predicted.

Carcinogenicity Evaluations:

Objective: Determination of the potential for a drug to cause cancer.

Methods: Long-term studies need to be undertaken in rodents to observe tumor development.

Reproductive and Developmental Toxicity Assessments (Fig. 25.1b) [2]:

Objective: Detection of the drug's effects on reproduction and development.

Methods: Fertility, embryonic development, and teratogenicity need to be monitored in such assessments.

Pharmacological Safety Assessments:

Objective: Investigation of the drug's effects on vital physiological functions.

Methods: Cardiovascular, respiratory, and central nervous systems, alongside important systems and organs in the body, need to be observed for drug effects and pharmacological evaluations.

Several critical steps are involved in the regulatory submission process for drug approval by the FDA and EMA. The safety and efficacy of new drugs are therefore guaranteed before proceeding to human clinical trials by regulatory agencies. Submission of an Investigational New Drug (IND) application to the FDA or an Investigational Medicinal Product Dossier (IMPD) to the EMA is the beginning of the drug discovery journey. The importance of this step is the presentation of detailed preclinical data to the regulatory authorities. The contents of these submissions are compiled to show the drug's potential benefits and safety profile and include comprehensive study reports, protocols, and safety data.

Upon submission of the IND or IMPD, a thorough evaluation of the presented data is conducted by regulatory agencies. The rigorous review process is directed and aimed at ensuring that the drug is safe

for human trials. If the data support the drug's safety and efficacy, the authorities approve proceeding to clinical trials. This step is key as it ensures that only drugs with favorable safety and efficacy move forward to human testing.

Many similarities and some distinct differences are present in the drug approval processes of the FDA and EMA. The FDA operates under a direct authority to approve drug products in a wide range of products, including medicines, medical devices, cosmetics, and food and feeds, which are regulated by this agency. In contrast, the EMA provides nonbinding recommendations to the European Commission (EC), which makes the final decision. In addition to regulating human and veterinary medicines, medical device evaluations are other results of EMA participation in regulatory procedures. When it comes to approval pathways, a single drug approval pathway is utilized by the FDA, with advisory committees providing recommendations. The EMA, however, has four approval pathways: centralized, national, mutual recognition, and decentralized. Certain types of medicines, such as those for rare diseases or biotechnology products, require a centralized procedure.

Both agencies offer accelerated approval processes to facilitate the availability of drugs for serious conditions. To ensure faster approval, the FDA's Accelerated Approval pathway allows for the use of surrogate endpoints in clinical trials. Temporary approval is granted based on a positive benefit-risk assessment by EMA's Conditional Marketing Authorization. Additional data may be required post-approval for such short-term approval. Industry consultation might be a different aspect between the FDA and EMA. FDA and EMA consultations take place throughout the drug development process with industry stakeholders. EMA consultation with industry follows a more structured approach compared to the FDA, in that multiple committees and working groups are involved. The regulatory frameworks of both agencies hold postmarketing surveillance as an important element. Both the FDA and EMA have extensive protocols in place to monitor the effectiveness and safety of approved medications. However, the specific regulations and reporting requirements can be different between the two agencies.

Regional differences also play a key role in the regulatory process. The FDA operation region would be solely within the United States, while the European Union, Norway, Iceland, and Liechtenstein are covered by the EMA. The UK created the Medicines and Healthcare Products Regulatory Agency (MHRA) as its own regulatory agency after Brexit. These differences in regulatory processes can significantly affect the strategies pharmaceutical companies use when seeking approval in both regions. Understanding these distinctions is crucial for drug approval and makes sure that new therapies reach patients in need.

The FDA's role is to protect public health while promoting innovation. Products are FDA-regulated under two main statutes: the Food, Drug, and Cosmetic Act (FDCA) for chemically synthesized drugs and devices and the Public Health Service Act (PHSA) for biologically derived therapeutics. FDA categorization of the products happens based on their mode of action: chemical (drug), mechanical (device), or biological. Nanotechnology products are often from multiple categories, combining chemical, mechanical, and biological modes of action. The combination product process was criticized not only for its difficulties in classifying products that combine multiple elements (chemical, biological, and mechanical) but also for a lack of transparency and consistency in decision-making. The goal of the 21st Century Cures Act of 2016 was to improve FDA procedures for these products' standardization and openness. The FDA often classifies nanotechnology-derived products as combination products. This agency assigns a primary regulatory route (drug, device, or biologic) to such products and adds specific requirements to ensure safety and efficacy.

Chemical, biological, and mechanical elements are integrated with nanotechnology combination products and often result in novel solutions across various fields. Here are some examples:

- Drug-Eluting Stents: Cardiovascular treatments benefit from these medical devices, and they are coated with nanoparticles that release drugs to prevent artery blockage.
- Nanoparticles Loaded with Cancer Therapies: These therapies combine nanoparticles with drugs to target and destroy cancer cells more effectively while minimizing damage to healthy cells.
- Nanoparticle-Enhanced Imaging Agents: Used in medical imaging, these agents improve the clarity and precision of diagnostic images.
- Nanotechnology embedded inside wound dressings: nanoparticle incorporation in such dressings can promote faster healing and reduce infection risks.
- Contact lenses loaded with nanoparticles: Controlled medication release for the treatment of ocular diseases happens by taking advantage of such drug delivery-intended lenses.

Diverse applications of nanotechnology in creating combination products that enhance treatment efficacy and patient outcomes are illustrated in these examples.

Approved examples include nanoformulations of cancer drugs (paclitaxel and doxorubicin), an immunosuppressant (sirolimus), and a topical emulsion (estradiol). The first approved nanodrug was a liposomal formulation of doxorubicin. Other approved devices include a tissue reinforcement device, a bone graft substitute, and a tissue-sealing system. A nano-based formulation of the hepatitis A vaccine has also been approved.

Caelyx's approval story can be of significance and interest for the examination of novel drug delivery system approval processes. Caelyx (pegylated liposomal doxorubicin) is a chemotherapy drug used to treat various types of cancer, including metastatic breast cancer, advanced ovarian cancer, multiple myeloma, and AIDS-related Kaposi's sarcoma. The European Commission granted Caelyx marketing authorization throughout the European Union on June 21, 1996.

Animal studies have been playing a major role in understanding the pharmacokinetics and toxicity of Caelyx. Here are some key findings:

PK studies: In animal models, extended circulation time and a reduced volume of distribution were observed with Caelyx compared to free doxorubicin. This results in higher drug concentrations in tumors.

Tissue Distribution: Studies indicated preferential accumulation of Caelyx in various implanted tumors and human tumor xenografts, enhancing drug concentrations in the tumor compared to free drug.

Toxicity Studies: A repeated-dose intravenous toxicity study in rodents had to be discontinued after eight doses due to high mortality related to skin toxicity and poor general health of the animals. Animal studies conducted on this formulation are.

1. **Cardiotoxicity**: Although cardiac toxicity was significant due to known issues with doxorubicin, the level of toxicity in rabbits showed a reduction when compared to conventional doxorubicin formulations.
2. **Dermal Toxicity**: Serious skin inflammations and ulcers were observed in rats and dogs, which showed similarity to patient reactions. Such dermal toxicity was finally reduced by adjusting doses.
3. **Anaphylactoid Response**: Despite such reactions being less severe with Caelyx, acute reactions (e.g., hypotension and salivation) were observed in dogs and thus reduced by antihistamines.

4. **Local Toxicity**: Slight local irritation or tissue damage after extravasation was observed in subcutaneous studies; better tolerance was witnessed with the nanoformulation compared to the standard doxorubicin.
5. **Mutagenicity and Carcinogenicity**: No specific studies with Caelyx were conducted, but doxorubicin is known to be mutagenic and carcinogenic. Pegylated placebo liposomes are, however, not problematic in this area.
6. **Reproductive Toxicity**: Ovarian and testicular atrophy, decreased testicular weights, and hypospermia have been observed in mice, rats, and dogs. Reproductive health implications are therefore possible with these formulations.
7. **Nephrotoxicity**: Renal toxicity was observed at high doses in monkeys, as well as in rats and rabbits with lower doses of doxorubicin. These findings may, however, be irrelevant to patient risk.

When comparing the toxicity profiles of conventional doxorubicin (DOX) and its liposomal formulation, Caelyx (also known as Doxil in the US), both the EMA and the US Food and Drug Administration (FDA) have conducted extensive studies.

2.1 EMA findings

The EMA has documented that Caelyx, due to its pegylated liposomal formulation, shows reduced cardiotoxicity and myelosuppression compared to conventional doxorubicin. Specifically, in animal models, up to 50% more Caelyx could be administered without increasing the risk of cardiomyopathy. This is significant because cardiotoxicity is a major limiting factor in the use of conventional doxorubicin [3,4].

2.2 FDA findings

The FDA's Adverse Event Reporting System (FAERS) has also highlighted differences in toxicity profiles. Higher risks of myelosuppression, cardiotoxicity, and alopecia were witnessed with conventional doxorubicin when compared to Caelyx. However, Caelyx has a higher incidence of redness, swelling, and pain on the palms of the hands and soles of the feet (palmar-plantar erythrodysesthesia (PPE)) [5,6].

Pazenir (nanoparticle albumin-bound formulation of paclitaxel) was developed to improve the delivery and efficacy of paclitaxel, a well-known chemotherapy drug. The EMA approved Pazenir in 2019 for the treatment of metastatic breast cancer, nonsmall cell lung cancer, and metastatic adenocarcinoma of the pancreas. This approval was based on its bioequivalence to Abraxane, another nanoparticle albumin-bound paclitaxel, which had been authorized in the EU since 2008. The approval of Pazenir was a significant advancement in the treatment of various cancers. Some of the severe side effects associated with paclitaxel could be potentially reduced by this new formulation. While both drugs share similar toxicity profiles, improved targeting and possibly better tolerability have been reported with the nanoparticle albumin-bound formulation of Pazenir in patients.

1. Neurotoxicity: Pazenir shows a lower incidence of peripheral neuropathy compared to paclitaxel, as seen in rodent models, thanks to its nanoparticle albumin-bound formulation that increases tumor targeting and reduces effects in tissues other than the intended area. In nonhuman primates,

Pazenir presents fewer neurotoxic symptoms, such as reduced grip strength and altered gait, compared to paclitaxel.

2. Myelosuppression: Pazenir and paclitaxel both induce myelosuppression (presented as reductions in leukocyte and platelet counts) in a rodent model, though the severity may be slightly less in the nanoformulation. In comparison to paclitaxel, dogs given Pazenir show myelosuppression but with a potentially better recovery profile.
3. Gastrointestinal Toxicity: Reduction of gastrointestinal (GI) toxicity, with less severe mucositis and diarrhea in rodents, was reported in Pazenir compared to paclitaxel, likely due to improved drug delivery and reduced systemic exposure. Superior gastrointestinal tolerability and reduced instances of vomiting and diarrhea are exhibited in nonhuman primates.

The animal toxicity studies present that while both paclitaxel and Pazenir share common toxicities, the nanoparticle albumin-bound formulation of Pazenir offers some advantages. These include reduced neurotoxicity and gastrointestinal toxicity.

Based on the data above and the detailed toxicity profiles of doxorubicin and paclitaxel and their liposomal formulations (Caelyx and Pazenir), it is evident that the nanoformulation exhibits distinct characteristics compared to the conventional form. Separate investigation and validation are needed for each of these drugs due to their unique properties. In recent years, the development and application of nano-compounds, as well as the reformulation of existing drugs, have gained significant attention. Numerous studies have been conducted for the introduction of various nanoproducts into the pharmaceutical market. One of the most important aspects of nanocompounds is their inherent differences from conventional compounds. These differences result in distinct toxicity profiles, pharmacokinetics, and pharmacodynamics. Consequently, it is imperative to treat these nanocompounds as new drugs and conduct comprehensive evaluation and validation. Despite the clear differences between nanocompounds and their conventional counterparts, these major dissimilarities have not been fully considered in many regulatory practices. A review of the nanocompound dossiers approved by the EMA and FDA reveals that certain toxicity tests are sometimes overlooked, with reliance placed on the toxicity data of conventional formulations. This suboptimal approach warrants reconsideration.

The table below presents the nanoparticle guidelines released by organizations such as the FDA (US Food and Drug Administration), EMA, MHLW (Ministry of Health, Labor and Welfare of Japan), and India.

The table Table 25.1 indicates that, in contrast to conventional pharmaceuticals, there are very few guidelines related to nanoparticles. Given the increasing application of nanoparticles, new guidelines and instructions in this field are expected to be published as soon as possible to reduce misunderstandings and enhance product repeatability and efficiency. Currently, most guidelines are related to liposomes, which might be because there are more liposomal products on the market than other nanoparticles.

Although smart nanoparticle drug delivery systems have more advantages over conventional chemotherapy, there are serious concerns about their toxicity. Conventional drug toxicity assays may not be sufficient to assess nanoparticle toxicity, and the lack of a standard list of necessary tests makes it difficult to assess their safety. Nanoparticles can trigger the immune system's response, resulting in inflammation, allergic/autoimmune reactions, and hypersensitivity reactions following repeated exposure to PEGylated formulations.

Table 25.1 Nanoparticle guidelines released by organizations.

Guideline	year
FDA - Guidance - drug products, including biological products, that contain nanomaterials. Guidance for industry	2022
Guidelines for the evaluation of Nanopharmaceuticals in India	2019
FDA - liposome drug products chemistry, manufacturing, and controls; human pharmacokinetics and bioavailability; and Labeling documentation. Guidance for industry	2018
MHLW - guideline for the development of liposome drug products (2016)	2016
MHLW - Reflection paper on nucleic acids (siRNA)-loaded nanotechnology-based drug products (2015)	2016
FDA - Guidance for industry considering whether an FDA-regulated product involves the application of nanotechnology	2014
MHLW/EMA - Reflection paper on the development of block copolymer micelle medicinal products	2014
EMA - Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovative medicinal product	2013
EMA - Reflection paper on surface coatings: General issues for consideration regarding parenteral administration of coated nanomedicine products	2013
EMA - Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovative liposomal product	2013
Second regulatory review on Nanopharmaceuticals, European Union, 2012, nanomaterials: Commission proposes 'case by case approach' to assessment	2012
EU (SCENIHR) - risk assessment of products of nanotechnologies; 2009	2009

Evaluation of bacterial endotoxin contamination in nanomaterials requires special attention due to their ability to distort toxicity and efficacy studies.

Nanoparticles can potentially cause cytotoxic effects such as oxidative stress, membrane damage, and interference with cellular processes. To understand how different properties of nanoparticles influence their biological response and develop standard criteria for toxicity assessment, comprehensive material characterization is necessary. The size and surface charge of nanocarriers are particularly significant factors that affect their toxicity. The toxicity of small nanoparticles is higher, and they have a wider distribution, but larger ones tend to accumulate in organs such as the liver and spleen. Nanoparticles can cause DNA damage and cell death by crossing barriers and entering cells. The toxicity of nanoparticles can also be influenced by their shape, as spherical nanoparticles are more prone to cellular engulfment. Surface charge plays an important role in interactions with biological systems. Positive nanoparticles have higher toxicity because they have a greater ability to penetrate cells and a higher ability to opsonize. Chemical composition and biodegradation also determine toxicity. Biocompatible materials such as liposomes and biodegradable nanopolymers such as PLA and PLGA are preferred to minimize toxicity, while toxic synthetic components should be avoided.

Biodegradable nanopolymers like PLA and PLGA are used to minimize toxicity. Nanoparticles can accumulate in tissues to toxic levels if not properly degraded by the body. High or repeated doses can overwhelm the body's clearance systems, leading to redistribution to other organs. For cancer treatment, nanocarriers need stable shells to prevent premature drug release and improve biocompatibility. The route of administration also affects nanoparticle toxicity due to changes in their distribution and

behavior in the body. Unintentional accumulation of nanocarriers in healthy tissues should be avoided to prevent toxicity. Microbubbles, used for drug delivery, can accumulate in the liver and spleen, releasing drugs at toxic levels. Long-term biocompatibility data are often lacking, hindering clinical translation [7,8].

3. Recommendations for improved evaluation

To ensure the safety and efficacy of nanocompounds, it is essential to:

1. Conduct Comprehensive Toxicity Studies: Perform thorough toxicity assessments specific to the nanocompound, independent of the conventional drug's data.
2. Evaluate Pharmacokinetics and Pharmacodynamics: Investigate the unique pharmacokinetic and pharmacodynamic profiles of nanocompounds to understand their behavior in biological systems.
3. Implement Rigorous Regulatory Standards: Update regulatory frameworks to mandate separate and detailed evaluations for nanocompounds, treating them as new entities.

By adopting these recommendations, regulatory bodies can better ensure the safe integration of nano-compounds into clinical practice, ultimately benefiting patient outcomes and advancing the field of nanomedicine.

4. Challenges

Recent advancements in the field of stimuli-responsive nanocarriers have focused on addressing the challenges related to their stability, targeting, controlled release, and biocompatibility Table 25.2. Here are some notable developments:

1. Advanced Characterization Techniques: Improved analytical methods, such as high-resolution electron microscopy and dynamic light scattering, have enhanced the precise characterization of nanocarriers, ensuring better control over their size, shape, and surface properties.
2. Biodegradable and Biocompatible Materials: The development of new biodegradable polymers and biocompatible materials has reduced the toxicity and improved the safety profile of nanocarriers. Examples include polylactic-co-glycolic acid (PLGA) and polyethylene glycol (PEG)-based nanocarriers.
3. Enhanced Targeting Strategies: Advances in ligand-receptor targeting, such as the use of antibodies, peptides, and aptamers, have improved the specificity and efficiency of nanocarrier targeting to tumor cells. This has been complemented by the use of external stimuli like magnetic fields to guide nanocarriers.
4. Stimuli-Responsive Polymers: The design of novel stimuli-responsive polymers that respond more precisely to environmental changes, such as pH, temperature, and enzymes, has improved the controlled release of drugs. These polymers can be engineered to degrade or change conformation in response to specific stimuli.
5. Multifunctional Nanocarriers: The development of multifunctional nanocarriers that combine multiple stimuli-responsive mechanisms has enhanced their efficacy. For example, combining pH and temperature responsiveness in a single nanocarrier can provide more precise control over drug release.

Table 25.2 Challenges and potential solutions in nanomedicines.

Challenge	Description	Impact	Potential solutions
Premature drug release	High drug loading capacity (DLC) can lead to premature leakage at neutral pH, reducing the effectiveness of the drug delivery system.	Reduced drug targeting specificity and potential damage to healthy cells, reducing overall therapeutic index.	Carrier optimization to increase stability at neutral pH, such as adding stronger surface coatings or optimizing drug binding chemistry.
Insufficient pH Differentiation in soft Tumors[a]	Slight differences in pH between healthy cells and cancer cells often fail targeted drug release into the cancer environment, complicating in vitro targeting of tumor cells.	Inadequate drug release into the tumor environment results in less than optimal therapeutic effects.	Designing carriers that discriminate and respond to subtle pH differences or combining pH sensitivity with other stimuli, such as enzymatic or temperature changes
Hypoxic tumor microenvironment in solid Tumors[a]	Tumors often have a hypoxic microenvironment with low pH, which can affect the performance of pH-responsive nanocarriers.	The degree of hypoxia and vascular density can affect the efficiency of drug delivery.	Development of nanocarriers that are more sensitive to pH changes and incorporate targeting ligands to enhance delivery to hypoxic regions.
Inconsistent drug release rates	In vitro studies have revealed that prolonged drug release does not always correlate with increased cytotoxicity, which highlights the need for precise control over drug release timing.	Delayed therapeutic effect and potential development of drug resistance in tumor cells due to subtherapeutic dosing.	Make adjustments to the drug release profile to ensure a rapid but controlled release that maintains therapeutic levels for the necessary duration. This could be achieved by utilizing multi-staged release mechanisms that allow for an initial rapid release followed by a sustained release phase.
Endosomal Escape	After nanocarriers are taken up by cells, they need to successfully escape from endosomes to prevent the degradation of their drug payloads; however, achieving efficient endosomal escape remains a significant challenge.	Poor therapeutic outcomes and underestimation of the nanocarrier's potential efficacy.	Incorporate endosome-disrupting agents or design nanocarriers that change surface charge or composition to enhance endosomal escape.

Table 25.2 Challenges and potential solutions in nanomedicines.—cont'd

Challenge	Description	Impact	Potential solutions
Cytotoxicity of Empty carriers	The nanocarriers themselves can exhibit cytotoxic effects on normal cells, complicating the evaluation of the therapeutic window in in vitro tests.	Complicates the assessment of the true therapeutic index and may lead to incorrect dosing or safety assessments.	Focus on biocompatible carrier materials, reduce carrier concentration in formulations, or surface-coat carriers with biocompatible polymers to mitigate inherent toxicity.
Variability in cell Internalization	Differences in cell line characteristics and nanocarrier surface properties can lead to inconsistent uptake and internalization, which can affect in vitro efficacy results.	Unpredictable treatment outcomes and difficulties in standardizing treatment protocols for different types of cancer.	Customize nanocarrier surface properties (e.g., charge and hydrophobicity) to match specific cell line characteristics or use targeting ligands specific to the cancer type [9].

[a] *Typically include benign tumors like lipomas and fibromas, which are often softer to the touch due to their composition.*
[b] *Include both benign and malignant tumors that form a solid mass, such as carcinomas and sarcomas.*

6. In Vivo Imaging and Tracking: Advances in imaging techniques, such as fluorescence and magnetic resonance imaging (MRI), have enabled real-time tracking of nanocarriers in vivo. This helps in understanding their biodistribution, clearance, and interaction with biological systems.
7. Scalable Manufacturing Processes: Innovations in manufacturing technologies, such as microfluidics and nanoprecipitation, have improved the scalability and reproducibility of nanocarrier production, facilitating their clinical translation.

These advancements are paving the way for more effective and safer stimuli-responsive nanocarriers (Fig. 25.2) [10], enhancing their potential for clinical applications in cancer therapy and beyond. The following section outlines several challenges associated with utilizing these nanomaterials in cellular studies.

4.1 pH-responsive nanocarriers

These nanocarriers respond to the acidic environment of tumor tissue or intracellular organelles and release their drug. Polymeric micelles and liposomes are common pH-sensitive nanoparticles (Fig. 25.2) [11,12].

pH-responsive nanocarriers offer several advantages and disadvantages in drug delivery systems Table 25.3 [13].

4.2 Ultrasound-responsive nanocarriers

These use ultrasound waves (US) to trigger drug release Table 25.4. They are often used in combination with other stimulus-responsive mechanisms to enhance precision [14].

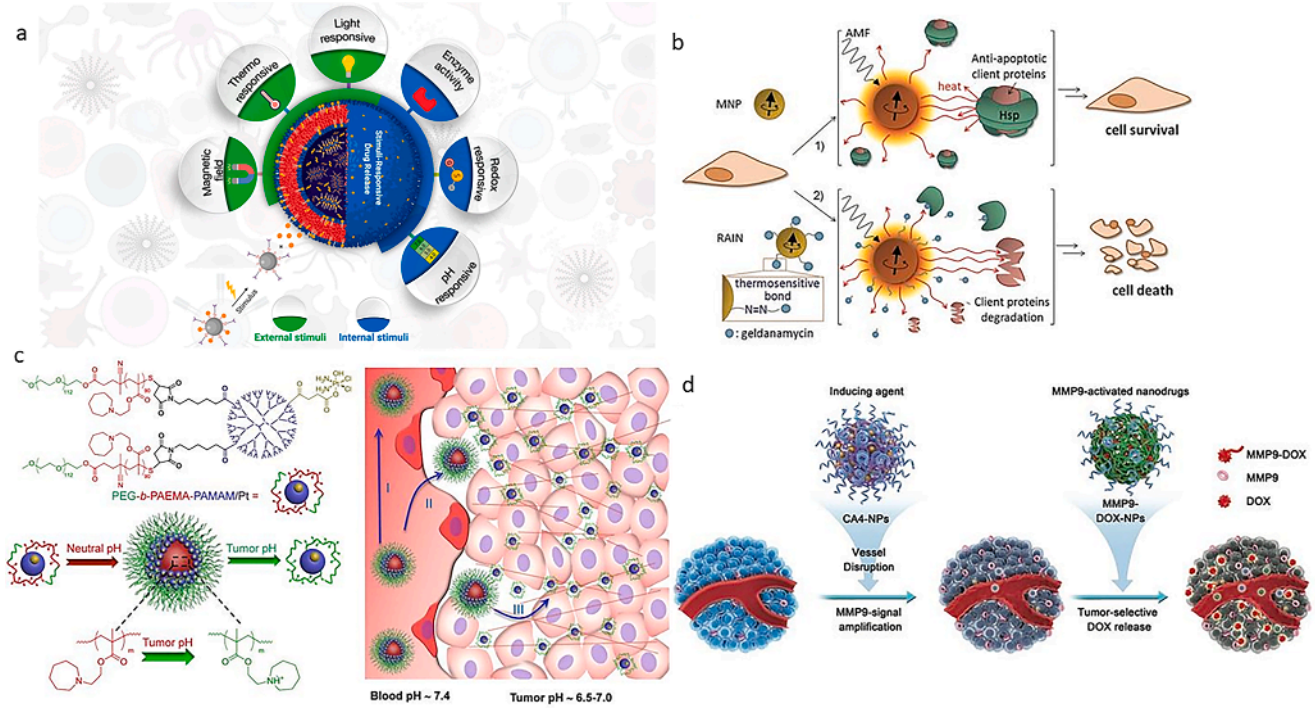


FIGURE 25.2

Overview of the stimuli-responsive nanoparticles.

(a) Overview of the stimuli-responsive nanoparticles with permission from Ref. [9], responsiveness mechanisms in (b) magnetic-responsive, (c) pH-responsive, and (d) enzyme-responsive nanostructures reprinted with permission from Ref. [10].

From N. Kaushik, S.B. Borkar, S.K. Nandanwar, P.K. Panda, E.H. Choi, N. Kumar Kaushik, Nanocarrier cancer therapeutics with functional stimuli-responsive mechanisms, J. Nanobiotechnol. 20 (1) (2022), https://doi.org/10.1186/s12951-022-01364-2.

Table 25.3 Advantages and disadvantages of pH-responsive drug delivery systems.

Advantages	Disadvantages
Targeted drug release:	Stability issues:
PH-sensitive nanocarriers, by releasing the drug in the organ at the desired pH, increase efficacy while reducing side effects caused by off-target release.	Maintaining stability under physiological conditions while simultaneously releasing the drug in an environment with the desired pH is challenging.
Enhanced drug solubility:	Complex Synthesis:
These nanocarriers enhance the solubility of hydrophobic drugs, leading to improved drug delivery	The design and synthesis of pH-responsive nanocarriers can be complex and costly.
Controlled release:	Potential toxicity:
They offer controlled and sustained drug release, which can improve patient compliance and treatment outcomes.	Biocompatibility and toxicity studies are essential due to the risk of toxicity posed by nanomaterials.
Ersatility:	Regulatory Hurdles:
Depending on the pH of the target tissue, responsive nanocarriers can be designed for that purpose.	The regulatory process for approval can be time-consuming and requires extensive data on safety and efficacy.

Ultrasound-responsive nanocarriers are an innovative approach to cancer treatment, offering several advantages and some disadvantages Table 25.5 [15,16].

Some challenges associated with ultrasound-responsive nanoparticles are depicted in Fig. 25.3 [17].

4.3 Magnetic-responsive nanocarriers

Magnetic-responsive nanocarriers (Fig. 25.2) [11], also known as MNPs, are nanoparticles engineered for very specific applications, like drug targeting, among other biomedical purposes. These are normally prepared with magnetic materials, including iron oxide, which can be guided to certain locations within the body by the use of an external magnetic field. This targeting mode of action minimizes side effects and enhances the effectiveness of the treatments (Table 25.6), (Table 25.7) [10,18,19].

4.4 Thermal-responsive nanocarriers

Temperature variation causes thermal-responsive nanocarriers to release their payload and is one of the leading hopefuls for targeted drug delivery. In the therapeutic area of cancer, hyperthermia, or HT, can be described as the practice of raising the temperature in tumor tissues to cause many different biological responses (Table 25.8). Further details on this technology are presented because it addresses various aspects.

4.5 Categories of hyperthermia

1. **Mild Hyperthermia** (40–42°C):
   It induces reversible physiological changes. This may improve the delivery of chemotherapeutic agents and oxygen to the tumor by enhancing blood flow and reoxygenation.

Table 25.4 Ultrasound-responsive nanocarriers, challenges and solutions.

Challenge	Description	Impact	Potential solutions
Temperature increase from the US waves	A propagating US wave carries energy, which can partly be absorbed by the tissue in the form of heat, leading to a temperature increase in the respective area.	Temperature increase can cause tissue burn and necrosis due to protein denaturation. Temperature sensitivity varies between tissues.	Use of thermal Index (TI) to estimate potential temperature elevation. Control initial pressure and frequency to manage temperature increase. Apply mild hyperthermia (below 43°C) to reduce cell death. Use strong hyperthermia (above 43°C) for tumor tissue ablation. Utilize mild hyperthermia to enhance blood flow and drug release from thermo-sensitive nanocarriers.
Mechanical effects of the US.	The mechanical effects caused by the US can be grouped into noncavitational effects and acoustic cavitation.	Noncavitational effects like acoustic radiation forces can increase the convective transport of drugs. Shear forces can widen intracellular spaces, enhancing nanoparticle penetration.	Utilize acoustic radiation forces for drug transport. Apply shear forces to enhance nanoparticle penetration.
	Acoustic cavitation involves the formation and/or oscillation of gas bubbles in a fluid, which can be endogenous or exogenous.	Stable cavitation creates fluid flow (microstreaming) with velocities and shear forces that can break particles or permeabilize cells. Inertial cavitation involves rapid bubble growth and collapse, potentially causing significant tissue damage.	Use stable cavitation for controlled microstreaming and cell permeabilization. Manage inertial cavitation to avoid tissue damage.
Bilayer Sonophore effect	A nonthermal, noncavitational US effect based on the interaction of US pressure waves with	Short-term disruption of membrane integrity allows substances to cross the plasma membrane.	Utilize the bilayer sonophore effect for targeted drug delivery. Manage US parameters to control membrane

Table 25.4 Ultrasound-responsive nanocarriers, challenges and solutions.—cont'd

Challenge	Description	Impact	Potential solutions
	bilayer membranes causes fluctuations between compression and rarefaction phases.	Alteration of cell morphology due to compression or stretching of the cytoskeleton. Stimulation of gated ion channels, changes the intracellular electrolyte distribution. Reduction of membrane fluidity due to lipid peroxidation.	disruption and cell morphology changes. Explore methods for reducing lipid peroxidation and maintaining membrane fluidity.

Table 25.5 Advantages and some disadvantages of ultrasound-responsive nanocarriers.

Advantages	Disadvantages
Targeted drug delivery:	Heat generation:
Increases tumor site accumulation while minimizing systemic side effects.	Thermal damage to surrounding healthy tissues can occur if it is not properly controlled.
Controlled release:	Limited Depth penetration:
Makes it possible to deliver drugs on demand through ultrasound.	The penetration depth of high-frequency ultrasound waves is limited.
Enhanced penetration:	Complexity of treatment:
Treatment efficacy can be enhanced by increasing the permeability of cell membranes and tissues.	Specialized equipment and precise control of ultrasound parameters are necessary.
NonInvasive:	Potential for Cavitation:
Reduces the need for surgical interventions and their associated complications.	Can lead to unwanted tissue damage due to the risk of cavitation.
Versatility:	Regulatory and safety concerns:
It is able to be combined with various types of nanocarriers for various therapeutic agents.	Proving its safety requires extensive preclinical and clinical trials, which can be time-consuming and costly.

2. **Moderate Hyperthermia** (42–45°C):
   More pronounced physiological changes are caused. Alters protein structures and metabolic pathways, potentially making cancer cells more susceptible to treatments.
3. **Thermal Ablation** ($\geq$45°C):
   Results in irreparable damage and cell death. Used to destroy tumor tissues directly. However, several challenges must be overcome before their full potential can be realized [10,11].

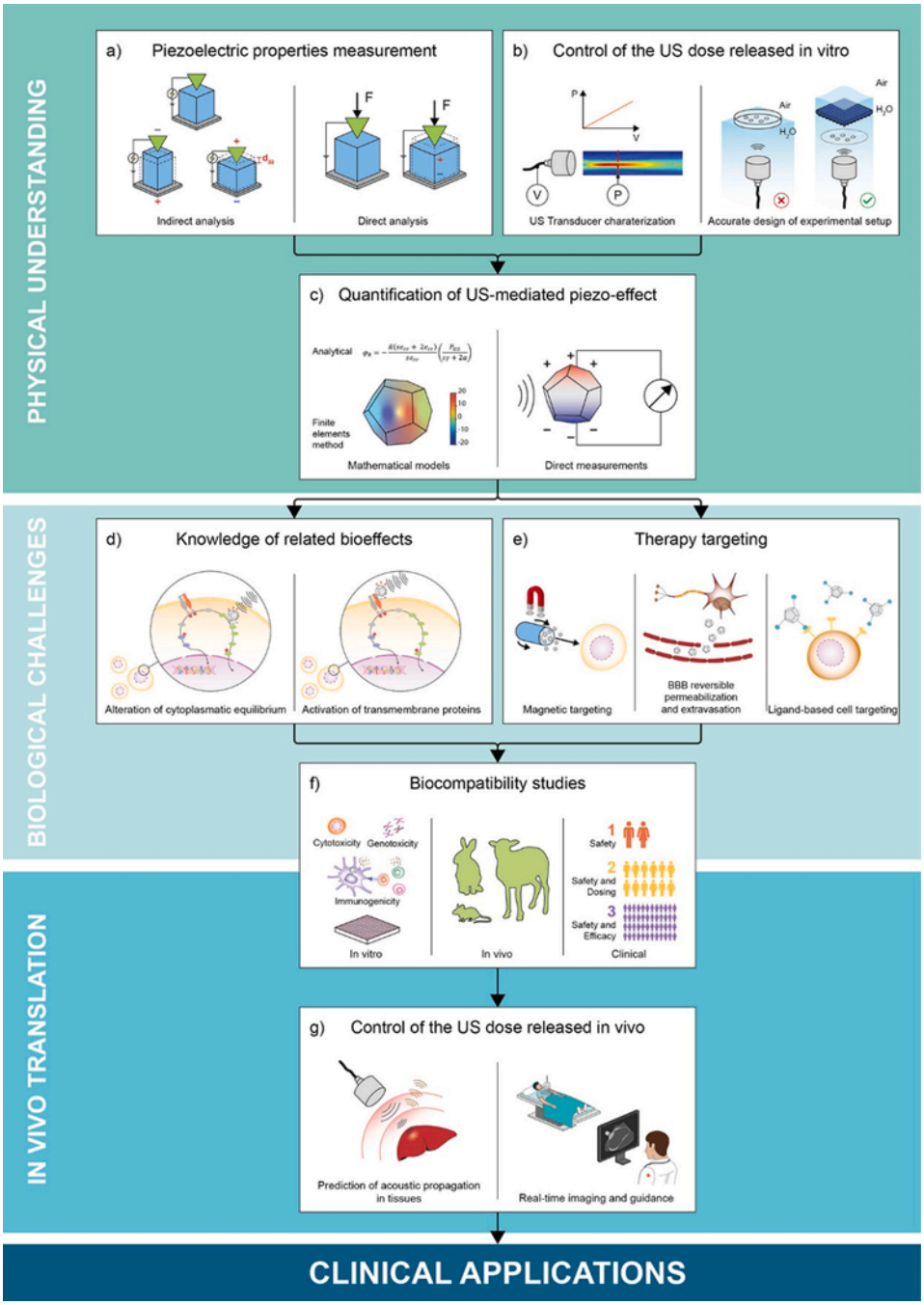


FIGURE 25.3

Some of the challenges related to ultrasound-responsive nanoparticles. With permission from ref. [17].

From A. Cafarelli, A. Marino, L. Vannozzi, J. Puigmartí-Luis, S. Pané, G. Ciofani, L. Ricotti, Piezoelectric nanomaterials activated by ultrasound: The pathway from discovery to future clinical adoption, ACS Nano, 15 (7) (2021), 11066–11086, https://doi.org/10.1021/acsnano.1c03087.

Table 25.6 Magnetic-responsive nanocarriers, challenges and potential solutions.

Challenge	Description	Impact	Potential solutions
Oxidative stress	Induction of oxidative stress in cells, resulting in cellular damage and apoptosis	Cellular damage, apoptosis	Surface modification with antioxidants and the application of biocompatible coatings
Inflammatory responses	Triggering of inflammatory responses upon exposure to MNPs	Tissue damage, inflammation	Surface modification, use of antiinflammatory agents
Genotoxicity	DNA damage caused by MNPs	Mutations, potential cancer risk	Rigorous genotoxicity testing, use of safer materials
Cellular uptake and accumulation	Variability in cellular uptake and potential accumulation in nontarget cells	Reduced efficacy, potential toxicity	Targeted delivery systems, controlled release mechanisms
Biodistribution and accumulation	Accumulation of MNPs in organs like the liver, spleen, and lungs	Organ toxicity, long-term health issues	Biodegradable materials, thorough biodistribution studies
Immune system activation	Activation of the immune system in animals	Adverse immune responses	Surface modification, use of immunosuppressive agents
Long-term effects	Unclear long-term effects of chronic exposure to MNPs	Unforeseen health issues	Prolonged in vivo investigations, ongoing surveillance
Biodegradability	Lack of biodegradability leads to prolonged retention in the body	Long-term toxicity	Development of biodegradable MNPs, regular clearance studies

4.6 Devices and technologies for thermal-responsive nanocarriers

Following are some of the important devices in thermal power supply for thermal-responsive nanocarriers, which help assure precise and controlled drug release. Some of the key devices and technologies are introduced herein:

1. **Laser** Lasers can easily produce localized heat with a high degree of precision. The use of near-infrared lasers is highly effective since they can go deep into tissues without significant destruction of the surrounding tissues. They are generally used in photothermal therapy, where the laser heats the nanocarriers and triggers drug release at the target site.
2. **Magnetic Hyperthermia**

This approach utilizes magnetic nanoparticles that, under the radiofrequency alternating magnetic fields, start heating up. This generated heat may provide a trigger for drug release from thermal-responsive nanocarriers. Most in vitro and in vivo studies concerning this approach have focused on cancer treatments by targeted destruction of tumor cells with minimal damage to healthy tissues.

Table 25.7 Advantages and disadvantages of magnetic-responsive nanocarriers.

Advantages	Disadvantages
Targeted drug delivery:	Potential toxicity:
Magnetic fields can guide the nanocarriers to particular sites, increasing their precision in drug delivery.	Accumulation in nontarget tissues may induce toxicity.
Controlled release:	Immune response:
The release of a drug can be controlled with the help of an external magnetic field for on-demand therapy.	Nanocarriers may elicit immunological responses, resulting in inflammation.
Enhanced imaging:	Complex manufacturing:
Magnetic properties improve the contrast in imaging techniques such as MRI.	The fabrication and surface modification of nanocarriers can be intricate and expensive.
Reduced side effects:	Biodistribution issues:
Targeted delivery minimizes exposure of drugs to healthy tissues, reducing side effects.	Disparate distribution within the body may result in ineffective targeting.
Multifunctionality:	Long-term effects:
Can be used for both therapy and diagnostics-theranostics.	Unidentified long-term consequences and possible chronic toxicity.

1. **Ultrasound Systems**
   High-intensity focused ultrasound provides localized heat. The process of focusing ultrasound waves on a small area raises the temperature, which initiates drug release from nanocarriers. Employed in thermal-responsive nanocarriers for targeted drug delivery and tumor ablation.
2. **Radiofrequency Ablation**
   RF ablation is one such technique that utilizes electromagnetic waves for the production of heat. Heating of nanocarriers by this technique may be utilized for drug release. This is one of the common methods in tumor treatments, where it is required to provide very localized heating in order not to damage the healthy tissues around the tumor site.

4.7 Biological effects

Increased Blood Flow and Reoxygenation: This increases the delivery of therapeutic agents as well as oxygen, thereby enhancing the efficacy of treatments such as chemotherapy and radiotherapy.

Conformational Change of Proteins: Heat can denature the proteins, which may impede their function and, therefore, could cause cell death of cancer cells.

Altered Metabolic Pathways: Changes in metabolism could make the cancer cells more sensitive to treatment.

Antitumor Immunity: Hyperthermia acts by affecting the antitumor immune response, a process leading to the recognition and destruction of tumor cells.

Mechanisms of Inhibited DNA Repair: The application of heat can stop the mechanisms of the cancerous cells that repair damaged DNA and, in doing so, render them more helpless for treatments that inflict DNA damage.

Table 25.8 Thermal-responsive nanocarriers, challenges and potential solutions.

Challenge	Description	Impact	Potential solutions
Precise temperature control	Challenges in attaining and sustaining the precise temperature necessary for drug release.	Fluctuations in body temperature and the surrounding environment can influence performance. .	Advancement of sophisticated temperature-sensing technology and targeted heating techniques.
Stability and Shelf-life	Ensuring stability during storage and transportation to prevent premature release.	Risk of premature drug release before reaching the target site.	Formulation improvements and use of stabilizing agents.
Scalability and manufacturing	Developing cost-effective and scalable manufacturing processes.	Challenges in commercial viability and large-scale production.	Investment in scalable manufacturing technology and process enhancement.
Thermal Sensitivity	Varying sensitivity of cells to temperature changes.	Potential harm to healthy cells during therapeutic procedures.	Advancement of accurate temperature regulation systems and targeted heating strategies.
Protein Misfolding	Unfolding and aggregation of proteins due to heat stress.	Formation of toxic aggregates impairing cellular functions.	Use of heat shock proteins and molecular chaperones to prevent misfolding.
Maintaining Homeostasis	Balancing protective heat shock response with normal cellular functions.	Extended thermal stress can result in cellular damage and apoptosis.	Regulation of heat shock response and stress management strategies.

In a number of cases, hyperthermia is combined with other treatment modes, like chemotherapy and radiotherapy, to enhance their results. The temperature and length of time need to be precisely controlled so that optimum therapeutic benefit can be achieved under minimum destruction of the normal tissues around.

4.8 Enzymatic-stimuli nanocarriers

Here is a table (Table 25.9) summarizing the challenges associated with enzymatic-stimuli nanocarriers, along with their descriptions, impacts, and potential solutions (Fig. 25.2) [11,20]:

Nanomedicines are a very heterogeneous class of drug products, including polymer-drug conjugates, polymer-protein conjugates, protein-based nanoparticles, polymeric micelles, inorganic nanoparticles, and different lipid-based nanoparticulate drugs, with PEGylated liposomes being a prime example. In the last few decades, thousands of different nanomedicine formulations have been designed and evaluated for a wide range of diseases. Of these, approximately 50 are approved for clinical application, with hundreds more in clinical trials involving administration to patients. To give an idea of the variety and range of nanomedicines, the following are examples:

Table 25.9 Enzymatic-stimuli nanocarriers, challenges, and potential solutions.

Challenge	Description	Impact	Potential solutions
Enzyme specificity	Challenges in selectively targeting particular enzymes without impacting others.	Off-target effects and reduced efficacy.	Develop nanocarriers with enhanced selectivity utilizing advanced molecular methodologies.
Enzyme activity variability	Variability in enzymatic activity among different patients or tissues.	Inconsistent therapeutic outcomes.	Personalized medicine approaches and patient-specific enzyme profiling.
Cellular uptake	Variability in how different cell types internalize nanocarriers.	Inconsistent drug delivery and therapeutic effects.	Enhancement of nanocarrier surface characteristics to improve cellular uptake by target cells.
In vivo stability	Nanocarriers may exhibit instability within the intricate biological milieu of live organisms.	Premature degradation and loss of therapeutic efficacy.	Development of more robust nanocarrier designs and use of protective coatings.
Stability of nanocarriers	Nanocarriers can break down prior to arriving at the target site.	Reduced drug delivery efficiency.	Use of stabilizing agents and protective coatings.
Controlled release	Difficulty in achieving precise control over drug release.	Potential for premature or delayed drug release.	Engineering nanocarriers with responsive release mechanisms.
Biocompatibility	Potential for immune response or toxicity.	Adverse side effects and reduced patient compliance.	Use of biocompatible and biodegradable materials.
Scalability	Challenges in large-scale production of nanocarriers.	High production costs and limited availability.	Development of cost-effective and scalable manufacturing processes.
Regulatory approval	Stringent regulatory requirements for new nanocarrier systems.	Delays in clinical translation and market entry.	Early engagement with regulatory bodies and thorough preclinical testing.

1. Abraxane (Paclitaxel Albumin-bound) [Protein-based nanoparticle]. Also approved for breast cancer, nonsmall cell lung cancer, and pancreatic cancer. Albumin nanoparticles are helpful in enhancing the solubility and delivery of the drug paclitaxel.
2. Onivyde (irinotecan liposome injection) is a liposomal formulation, and it is for the treatment of metastatic pancreatic cancer that is said to improve irinotecan stability, increasing its bioavailability as a result of the formulation into lipid nanoparticles.

3. Moderna COVID-19 Vaccine: Lipid Nanoparticle—Due to the similarity with the Pfizer-BioNTech vaccine, lipid nanoparticles are used to carry mRNA encoding the spike protein of SARS-CoV-2 [21].

These examples highlight the versatility and effect of nanomedicine in current healthcare. The amelioration of main developmental challenges, with a focus on commercial feasibility, clinical issues, and preclinical and pharmaceutical aspects, will help better position nanomedicine projects for success, early investment, and commercial interest. Because every nanomedicine requires multiyear and highly expensive preclinical and clinical testing, all need evaluation for their commercial and practical feasibility related to their primary target indication before any development is begun. This would cover not only considerations of the potential to improve patient benefit but also assessments of the size of the eventual patient population.

Improved Patient Benefit: Nanomedicines can bring about critical improvements over existing therapies, for example, increasing efficacy, lowering toxicity, and improving the convenience of the administration route.

Doxil: pegylated liposomal doxorubicin—this nanomedicine formulation reduces the cardiotoxicity of doxorubicin and permits targeted delivery to cancerous cells, thereby improving patient outcomes.

Abraxane-nanoparticle albumin-bound paclitaxel is a formulation that increases paclitaxel's solubility and delivery, enhancing its efficacy with reduced side effects in the treatment of cancer.

These benefits can justify a significant price premium when the product hits the market. The clearer the benefit, the easier it will be to justify this premium and thereby attract investors and commercial interest.

Market Potential: The eventual number of patients who would make use of nanomedicine determines the market potential and sales. For example, the COVID-19 vaccines by Pfizer-BioNTech and Moderna, using lipid nanoparticles to deliver mRNA, have been administered to millions in the world so far, thus giving a hint of the potential that exists for effective nanomedicines.

Challenges in Adoption: Despite the benefits, challenges remain in adopting nanomedicines to replace traditional therapies. In many cases, when a simple, inexpensive tablet is to be replaced by an advanced nanomedicine requiring parenteral administration, there may be some resistance on the part of physicians and patients. This would especially be so if the new treatment is considerably more expensive and administered in an outpatient clinic.

Cost Issues: Due to the relatively high cost of research and development, as well as in the production process of nanomedicines, an obvious improvement in either the disease-free survival or incidence of side effects will need to exist for the majority of new drug entities to become commercially feasible. For instance, therapeutic options, particularly in oncology treatment like those by Doxil and Abraxane, could very well be rationalized against their clinical benefit with improved outcomes for their patients, leading to minimal incidence of serious adverse drug reactions. Issues to ensure marketability need to start at the very beginning when nanomedicine is being developed. This guarantees products that offer considerable patient advantage and a feasible path to commercial success.

The evidence for better efficacy or safety for nanomedicines is hard to prove and requires large, long, and capital-intensive clinical trials. That brings us to the second challenge: feasibility in clinical development, including proper clinical study design issues.

Clinical Trial Design: To demonstrate adequate clinical potential for a new nanomedicine in pivotal trials, endpoints need to be selected that reflect the expected patient benefits and are valued by society (government bodies) and payors (insurance). Whereas proving superiority over the standard of care

may be comparatively easy, demonstrating a clinically relevant benefit, including but not limited to the modulation of disease better, maintaining remission longer, and delaying disease progression and/or disability, in general, requires multiple and/or very large studies. Doxil has been engineered to decrease cardiotoxicity and also enable targeted delivery to tumor cells more effectively. Its development required extensive clinical trials documenting a superior safety and efficacy profile as compared to the conventional formulation of doxorubicin. Abraxane increases the solubility and delivery of paclitaxel, thereby giving superior efficacy with fewer side effects associated with the treatment of cancer. Clinical trials had to be conducted that proved its superiority to conventional paclitaxel, both in efficacy and safety.

Endpoints and Trial Design: When the objective is to reduce toxicity, such as mitigating costly side effects or preventing allergic reactions, these benefits must be demonstrated while maintaining equal efficacy. Achieving this typically necessitates a large trial design. The size of the trial population and the classification of patients are critical in this context and are determined by the expected changes in endpoint results and the study's objectives. This emphasizes the importance of carefully evaluating these factors in phase 2 studies and implementing measures to reduce variability in clinical trials.

Variability and patient inclusion criteria: The application of strict standards in the selection of inclusion criteria leads to the inclusion of a limited population of eligible patients in the trials. For instance, the Pfizer-BioNTech and Moderna COVID-19 vaccines, which use lipid nanoparticles to deliver mRNA to the site of action, had to address these issues during rapid development and large-scale clinical trials to reduce side effects in the target community.

Biomarkers: An accurate and precise selection of biomarkers produced in response to drugs, including nanodrugs, is one of the methods to evaluate the response and effectiveness of treatment, which can somewhat reduce the impact of variation, although it does not solve the problem. Many new drug products, including nanomedicines, now come with their specific biomarkers, such as histopathological or novel biomarkers, which have been identified and quantified early in development. For example, by specific biomarkers of acute and chronic myocardial toxicity, targeted treatments such as Doxil and Abraxane were evaluated and compared with traditional drugs. In clinical development and research, study design, endpoints, patient diversity, and use of biomarkers should be considered. By addressing these challenges early in development, developers can ensure that their nanomedicine products not only provide significant benefits to patients but also have a clear path to commercialization (Fig. 25.4) [22].

Translating cellular experiments into animal models and subsequently into clinical studies for stimuli-responsive nanocarriers presents several challenges; each section concludes with proposed solutions to rectify the identified issues.

- Biological Complexity:
  Cell models are often unable to represent the full complexity of living organisms. While they provide valuable insights into signaling pathways and mechanisms of drug action at the cellular and molecular levels, they lack the complex interactions present in whole organisms, such as immune responses, interactions between organs, and the tumor microenvironment. Results from cell studies may not be consistent with animal studies. The results may be encouraging at the cellular level, but in the animal model, efficacy may not be observed, and drug interaction with the animal may lead to an unexpected immune response or toxicity in the animal. For example, PEGylated nanoparticles designed to evade the immune system can sometimes induce an immune

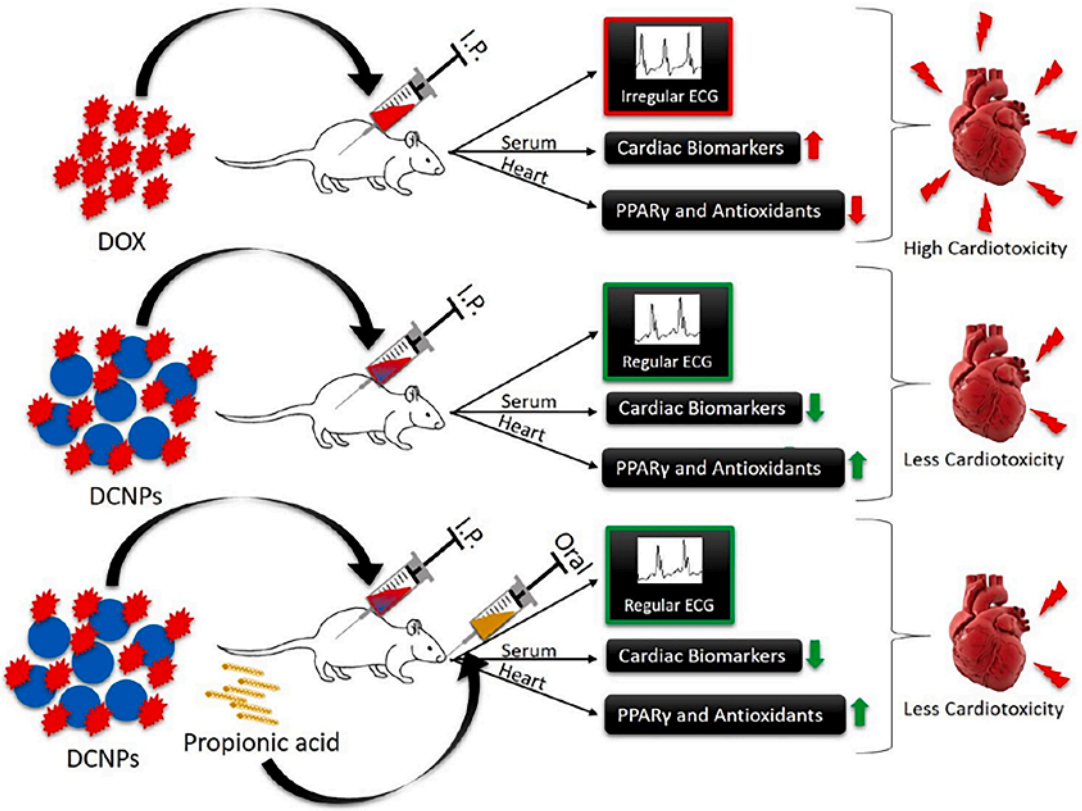


FIGURE 25.4

Myocardial toxicity of doxorubicin relieved with nanoparticles as evaluated by biomarkers, reprinted with permission from [22].

From D. Siva, Subramanian A., D. Rajesh, G. Archunan, P. Padmanabhan, B. Gulyás, S. Achiraman, Mollification of doxorubicin (DOX)-mediated cardiotoxicity using conjugated chitosan nanoparticles with supplementation of propionic acid, Nanomaterials, 12 (3) (2022), 502, https://doi.org/10.3390/nano12030502.

response in the body. It causes allergic reactions and complicates the translation of these results into clinical studies.

To overcome these problems and get closer to the real body environment, more sophisticated laboratory models that have been used in recent years, such as 3D cell cultures and organ systems on a chip, can be used, which better mimic the complexity of human tissues. Organs. Another solution is to conduct studies in several animal models with different species to obtain a wide range of biological responses. This helps to understand how different species react to nanocarriers.

- Reproducibility and Variability:
  Following cellular research, animal models introduce biological heterogeneity that can affect the reproducibility of results. Although these animal studies have a better degree of accuracy because

of the complexity of animals in comparison to cells, they frequently have less precision than cell experiments. Differences in genetics, species, physiology, metabolic systems, and environmental factors between animals and humans can lead to contradictory results. A study might, for instance, show that a certain nanocarrier effectively delivers drugs to tumor cells created in mice. However, efficacy has not been observed in humans. Before starting clinical studies based on existing standards, there is a need for preclinical tests on two different mammalian species. To overcome these challenges, it is very important to develop and follow standardized and globally harmonized protocols, taking into account the physicochemical properties of nanomaterials and their differences from traditional drugs for preparation and clinical trials. Making changes to and modifying current protocols will help reduce interspecies variability and ensure more consistent and predictable results. In addition, the use of animal models with genetic variation can better predict human responses and increase the effectiveness in clinical phases.

- Ethical and Regulatory Constraints:
  According to the 3Rs, ethical considerations and legal requirements should be taken into account, and the amount and type of animal testing should be limited as much as possible. This limitation could affect clinical studies in humans. Because animal studies are limited, less data is available to researchers, and translation of animal results to humans becomes a challenge. Regulatory bodies such as the FDA and EMA require extensive safety and efficacy data in cells and animals before approving new treatments for human trials. This often involves multiple animal studies to assess toxicity and efficacy, which can be ethically challenging, costly, and time-consuming. One of the final solutions in preclinical studies is the use of primates, which is currently considered a challenging study detail. One of the principles of 3R is the use of alternative methods (replacement), such as in-silico modeling and simulation, the use of computer models, and artificial intelligence to complete animal studies and reduce the number of animals used in studies. However, the replacement of these methods should be done by interacting with regulatory bodies in the early stages of development and actively investigating any ethical concerns during studies.
- Predictive Validity:
  The translation of data obtained from animal studies to humans has always been discussed. Only a small percentage of treatments that were effective in animal studies are successfully translated to human applications and find therapeutic use in humans. Approximately only about 5% of therapeutic interventions effective in animals receive regulatory approval to enter the human clinical stage. Despite promising preclinical results, many nanocarriers fail in clinical trials due to unanticipated side effects or lack of efficacy. Part of this failure is due to their physicochemical differences; for example, Doxil, a liposomal formulation of doxorubicin, has reduced cardiotoxicity in animal models but challenges clinical use due to issues such as hand-foot syndrome.
  As mentioned in the above sections, to improve the translation of preclinical findings and turn them into clinical successes, it is very important to identify and validate biomarkers that can predict clinical outcomes based on preclinical data. However, these biomarkers in animal and human studies also have differences that should be taken into account in the research and development stages. Investing in translational research, including pilot clinical trials and comparative experimental designs, can also increase the likelihood of success.

- Nanocarrier-Specific Challenges:
  Nanocarriers are considered complex pharmaceutical systems due to their specific physicochemical conditions. Stimuli-responsive nanocarriers add another layer of complexity to these drug delivery systems because they are also sensitive to external stimuli and cause drug release from them, so maintaining the stability of the external environment in this class of drugs is a challenge. Factors such as stability, release kinetics, and targeting efficiency should be fully evaluated in animal models before clinical translation. For example, pH-sensitive liposomes designed to release drugs in the acidic tumor microenvironment must maintain their stability at a physiological pH of 7.4 while efficiently releasing their drug in an acidic environment such as the tumor environment. Any changes in the path of drug delivery cause these systems to fail.

  To address these challenges, it is important to design nanocarriers with tunable properties for stability in the bloodstream and controlled release at the target site. This includes extensive studies on the complete physicochemical properties of nanocarriers and the characteristics of external stimuli, such as the intensity and duration of exposure to the stimulus, as well as the application of the stimulus in animal studies and clinical environments to understand their behavior in different conditions in the in vitro and in vivo environments. Stimuli-responsive nanocarriers, such as pH-sensitive or temperature-sensitive nanoparticles, should be rigorously tested for stability, release kinetics, and targeting efficiency in relevant animal models.
- Translational Gaps:
  There are often gaps in the translation process due to differences in species, scale, dosage, and administration routes between preclinical and clinical studies. Filling these gaps requires optimization and accurate validation of preclinical methods. Dosing methods and treatment regimens used in animal models often differ significantly from human regimens. For example, a nanocarrier that requires repeated dosing in mice may require a different schedule in humans due to differences in pharmacokinetics, like rate of metabolism and clearance [23–25]. To address these challenges, it is essential to: Optimize dosing regimens: Conduct comprehensive pharmacokinetic (P.K.) and pharmacodynamic (PD) studies in animals to determine the appropriate dosing schedules for humans.

  Develop scalable and reproducible manufacturing processes: By maintaining stability and quality, it is ensured that there is a possibility of producing nanocarriers on an industrial scale and in mass production.

  By focusing on these areas, we can improve the translation of nanomedicine from preclinical studies to clinical applications and ultimately increase the predictability of preclinical studies. Addressing these challenges requires a multidisciplinary approach between researchers of various fields, clinicians, and regulatory institutions that combines advanced modeling techniques and strong experimental designs and generates reliable results. In addition to the inherent toxicity of the active pharmaceutical ingredient (API), nanomedicines can cause toxicity at three levels beyond that.

4.9 Biodistribution and local overexposure

First, changing drugs into their nanoforms often dramatically alters the biodistribution of the drug molecules. This altered distribution can lead to the accumulation of drugs in specific organs in the body, potentially causing local overload and organ-specific toxicity. One of these examples is the accumulation of nanoparticles in the lymphatic organs, which ultimately leads to immune responses

and allergic reactions. One of the drugs available in the market is the liposomal form of doxorubicin, which causes drug accumulation in the spleen and liver and causes hepatotoxicity and splenomegaly. Similarly, some polymer-coated drugs preferentially accumulate in the kidneys, raising concerns about nephrotoxicity. From this category of materials, we can mention nanoparticles coated with PEG. To reduce these risks, it is very important to conduct preliminary pharmacokinetic and preclinical biodistribution studies. These studies should evaluate how the drug is distributed throughout the body and identify organs at risk of overexposure. To assess organ-specific toxicity and drug exposure, the animal should be exposed to different doses of the drug, followed by extensive histopathological examinations and established clinical chemistry protocols. Considering that the drugs have nano-shaped carriers, these carriers should be used in different doses as control groups in studies. In this way, side effects and toxicity caused by them are also identified and help to translate preclinical data more effectively.

4.10 Excipients and unproven safety

Secondly, some of the toxic effects of nanomaterials are related to their excipients, some of which have not been studied yet. They are inactive ingredients used as carriers for the active ingredients of a drug. These materials are generally considered safe, but when they are placed next to nanomaterials, chemical and molecular changes occur in them, which can cause unpredictable side effects. For example, surfactants used in the formulation of nanocarriers, such as polysorbate 80, may cause hypersensitivity and anaphylaxis reactions in some patients. In addition to studying nanomaterials, extensive studies should also be done on their carriers. Their biodistribution and pharmacokinetics will be investigated, and toxicological studies and histopathological analysis will also be helpful.

4.11 Immunological responses

One of the important safety issues related to nanomaterials is the immunological responses caused by their consumption. These responses are often not well recognized in animal studies and can include hypersensitivity reactions that, although rare, can be severe and sometimes life-threatening. PEGylated liposomes are one of the activators of the inflammatory system and the complement cascade in humans, which leads to complement activation-associated allergy in some patients. Another effect of nanomaterials is their interaction with blood cells; gold nanoparticles may react with platelets and cause thrombocytopenia in severe cases. Detection of these side effects in preclinical phases is done by laboratory assays that evaluate complement binding and cellular interactions. In addition, preclinical immunological studies in larger animals, such as pigs, are recommended because they provide a more accurate prediction of human immune responses and are more immunologically similar to humans. These studies can help identify potential hypersensitivity reactions and other immunological side effects before clinical trials in humans.

Nanomedicines have complexity in formulation and pharmaceutical form, which causes unique challenges for them. One of these issues is the interindividual difference in humans and the variation in the rate of response to treatment and the occurrence of side effects. This variability is often more pronounced for stimuli-nanocarriers, as their efficacy is highly dependent on multiple factors that can vary significantly between animal models and human patients.

5. Key factors affecting nanomedicine efficacy

Pharmacokinetics and Tissue Distribution: The purpose of using nanomedicines is to improve the pharmacokinetic properties and tissue distribution of traditional medicines. In preclinical studies, the results may be promising, and the drug accumulates at the intended site of action, but these studies are not predictive of clinical outcomes in humans. In humans, differences in metabolism, immune response, and organ function can alter these parameters, resulting in reduced efficacy. The speed and mode of drug metabolism in different species have significant differences from each other, which ultimately have a significant impact on the effectiveness and side effects of the drug. A study on PEGylated liposomal doxorubicin showed promising results in mice, but similar results were not obtained in human trials due to differences in drug clearance rates and immune system interactions [26].

Target Site Accumulation and Penetration: The ability of nanomedicines to accumulate and penetrate the target tissue is very important for their effectiveness. The heterogeneity of the tumor and its microenvironment in humans has a significant effect on the amount of drug absorption by the tumor. Tumors with dense or poorly vascularized stroma may impede the delivery of nanoparticles and reduce therapeutic efficacy. The tumor microenvironment is one of the key factors in the response to treatment, especially in stimuli-nanocarriers, where environmental factors such as pH affect drug secretion, and this factor can cause different treatment responses in patients. In this situation, it will be challenging to control the environment and predict the treatment response.

Drug Release at the Target Site: Controlled drug release mechanisms are designed to ensure that the active drug is released at the site of action in a controlled and specific manner, and to reduce side effects as much as possible and increase effectiveness. However, the tumor microenvironment in humans differs from animal models and affects the amount of drug released from the nanocarrier in response to stimuli. Changes in factors such as pH level, enzyme activity, and environmental factors such as UV radiation, temperature, and cellular uptake can lead to inconsistent drug release profiles that affect the therapeutic efficacy of nanomedicines. A study on pH-sensitive liposomes for doxorubicin delivery showed effective drug release in acidic tumor environments in mice, but the results were not reproducible in clinical studies and were inconsistent among animals [27].

5.1 Importance of tumor microenvironment

The tumor microenvironment plays a critical role in the efficacy of nanomedicines. Key aspects include:

- Tumor Vascularization: Vascularization of tumors plays a vital role in drug delivery to the tumor, but the vascularization of human tumors is often heterogeneous, and this affects the distribution of nanoparticles in the tumor and the amount of drug uptake. The amount of angiogenesis in different areas of the tumor is even different, and in the center of the tumor, the amount of angiogenesis is less, and the acidity is more; as a result, it will be more difficult to deliver drugs to this area. This variety can lead to differences in the effective way to reach and penetrate the nanomedicine into the tumor tissue. A comparative study of vascular-targeted nanoparticles has shown significant differences in vascular density between mouse models and human tumors, ultimately leading to differences in efficacy.
- Stroma and Macrophage Population: Another effective factor in the tumor microenvironment is the presence of stromal barriers and the population of macrophages, which can affect the release

and removal of drugs from the environment. In humans, the variability of these factors in different tumors can lead to inconsistent therapeutic responses. For example, tumors with high densities of tumor-associated macrophages (TAMs) may increase nanoparticle retention, while those with low TAM densities may decrease efficacy.

5.2 Need for biomarkers and companion diagnostics

In order to predict the therapeutic effects of nanomedicines, there is a need to develop specific biomarkers that determine the response to treatment; on the other hand, these biomarkers can help patients choose the appropriate nanomedicine and personalized treatment. Biomarkers that show levels of tumor vascularization, macrophage density, and extracellular matrix composition can guide the selection of appropriate nanodrug formulations. Companion diagnostics can help monitor nanoparticle distribution and drug release in real time, enabling personalized therapeutic settings. Another advantage of using biomarkers is the easier translation of preclinical to clinical results, although the type and level of expression of some biomarkers in animal and human models differ from each other, and this should be taken into account during studies. This approach will lead to more predictable and effective cancer treatments, ultimately enhancing patient outcomes.

The final challenge in the development of nanomedicinal drugs is the proper management of chemistry, manufacturing, and quality control (CMC). Unlike conventional drug products, the in vivo performance of nanomedicines—such as biodistribution, target accumulation, and drug availability at pathological and nonpathological sites—depends directly on the physicochemical properties of the nanoparticles that contain the drug. This makes the management of CMC particularly critical.

For any medicinal product, quality control is essential, but for nanomedicines, it involves additional layers of complexity. Critical quality attributes such as particle size, size distribution, surface charge, morphology, drug encapsulation, and release must be tightly controlled. For example, one of the changes made in doxorubicin in the pharmaceutical form of Doxil is the particle size and surface modification, which reduces its cardiotoxicity and increases the targeting of cancer cells. Quality control studies ensure that these properties remain constant during production because any change in them will lead to changes in the patient's clinical condition. Formulation, particle size, and drug loading are important to improve solubility and delivery. Strict quality control measures are taken to maintain these characteristics. In the case of stimuli-nanocarrier, the external stimulus with the same characteristics during the treatment can have a significant effect on the patient's response.

It is important to consider critical quality characteristics in formulation design. To ensure optimal performance, narrow specifications must be defined for the nanodrug and its external stimulus. For example, Pfizer-BioNTech and Moderna's COVID-19 vaccines use lipid nanoparticles to deliver mRNA. Particle size, surface characteristics, and encapsulation efficiency are critical to their efficiency and stability and require strict quality control from the very beginning. Manufacturing processes for nanomedicines must be robust, scalable, and compliant with Good Manufacturing Practice (GMP) guidelines. Implementation of quality by design (QbD) principles is also recommended. It includes in-process controls to monitor key quality attributes during compounding, allowing the adjustment of critical process parameters such as temperature and pressure. For example:

Onivyde (liposomal irinotecan): This nanomedicine requires precise control over particle size and drug release rates. The manufacturing process includes in-process controls to ensure these attributes are maintained.

It is important to evaluate the composition, excipients, type and intensity of the external stimulus, and key quality attributes early in the design phase, preferably before clinical testing in animal models. This helps to avoid costly subsequent formulation processes and repeat studies. For example, impurities related to sterility and manufacturing should be considered early to avoid issues that could necessitate a review of the entire clinical data package.

Developing scalable manufacturing methods is usually done at a later stage, but being aware of potential early warnings can save time and resources. For example, the large-scale production of COVID-19 vaccines required initial consideration of scalability to meet global demand. A detailed and comprehensive investigation of aspects of chemistry, fabrication, and control from the beginning of nanomedicine development is very important for successful translation. By addressing these challenges, developers can ensure that their nanomedicine products are not only effective and safe but also commercially viable.

6. Conclusions

The challenges of stimuli-responsive nanocarriers were discussed based on responsive systems such as pH-responsive, ultrasound-responsive, magnetic-responsive, thermal-responsive, and enzymatic-stimuli nanocarriers in this chapter, and potential solutions have been proposed to facilitate the clinical translation of nanomedicines for interdisciplinary scientists. Addressing these challenges requires a multidisciplinary approach between researchers of various fields, clinicians, and regulatory institutions that combines advanced modeling techniques and strong experimental designs and generates reliable results. It is essential to conduct comprehensive toxicity studies, evaluate pharmacokinetics and pharmacodynamics, tissue distribution, target site accumulation and penetration, drug release at the target site, need for biomarkers and companion diagnostics, and implement rigorous regulatory standards to ensure the safety and efficacy of nanocompounds. Considering these recommendations, regulatory bodies can better ensure the safe integration of nano-compounds into clinical practice, ultimately benefiting patient outcomes and advancing the field of nanomedicine by addressing the challenges related to their stability, targeting, controlled release, and biocompatibility.

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CHAPTER

Recent updates on clinical trials associated with stimuli-responsive nanocarriers

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1. Introduction

Nanocarriers are promising tools in diagnostics, therapeutics, and theranostics [1–3]. Various types have been developed, with some advancing to clinical trials. These systems enhance bioavailability and therapeutic efficacy while reducing the adverse effects of treatments on healthy tissues [4,5]. Nanocarriers, including liposomes, polymer nanoparticles (NPs) [6], and inorganic NPs, have been designed with features such as surface functionalization (e.g., PEGylation or ligands) to improve circulation time and targeting abilities [7]. However, issues like the unpredictable ligand-receptor interactions, drug release mechanisms, and achieving precise drug delivery to specific sites remain [8–10]. To address this, stimuli-responsive nanocarriers have been developed.

Stimuli-responsive NPs have garnered significant attention in recent decades. This concept originated in the 1970s with the development of thermo-responsive liposomes for enhanced drug release in tumors [11]. Stimuli-responsive nanocarriers alter their structure in response to environmental stimuli, triggering drug release, amplifying imaging signals or locally increasing the temperature.

Stimuli for triggering drug release can be either endogenous or exogenous [12]. In most cases, endogenous stimuli are associated with specific pathophysiological conditions, such as pH, enzyme activity, oxygen concentration, biomolecules (such as glucose), electronic balance, and redox potential [8]. In contrast, exogenous stimuli trigger the nanocarriers remotely, including light, magnetic fields, ultrasound (US), temperature, and irradiation [9,10]. Although endogenous stimuli are closely linked to disease progression stages, external stimuli are controllable and less influenced by individual variability [13]. However, internal stimuli do not need a remote apparatus [8]. These smart delivery systems incorporate various carrier types—such as hydrogels, micelles, NPs, and liposomes—along with stimulus-responsiveness and targeting capabilities. [14]. They provide advantages such as reduced toxicity, controlled release, and enhanced cellular uptake. The fabrication of these carriers depends on specific chemical bonds, functional groups, and polymers that enable their responsive functions [15].

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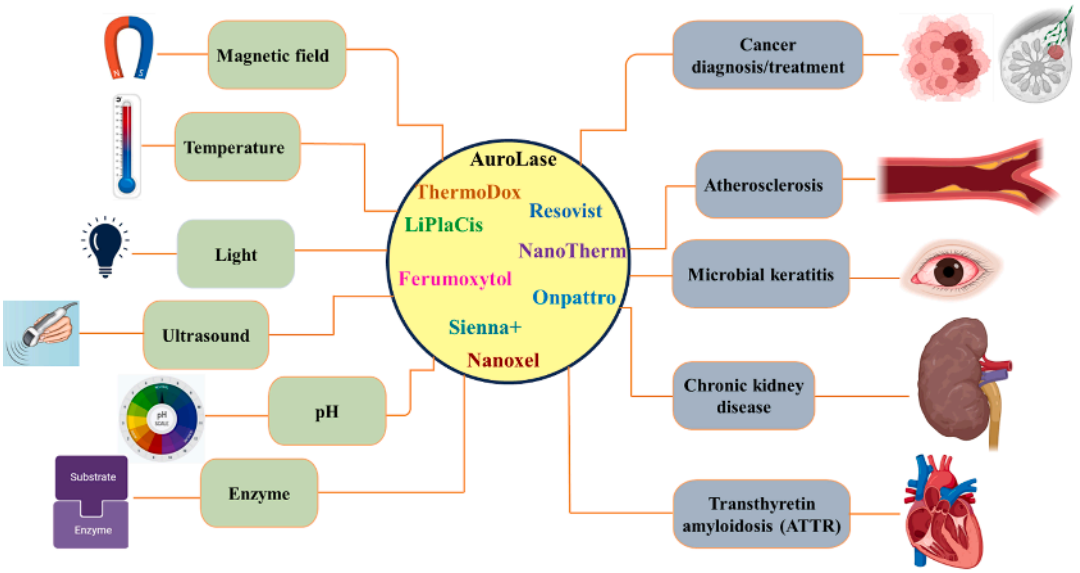


FIGURE 26.1

Exogenous/endogenous stimuli-responsive nanocarriers currently under investigation in clinical trials.

Carriers activated by a single stimulus are termed single-stimulus-responsive carriers, while those that respond to multiple stimuli are referred to as multi-stimuli-responsive carriers [16]. Nanocarriers that respond to dual or multiple stimuli have garnered increasing attention in recent years as they offer significant advantages over those triggered by a single stimulus [8,16,17]. Multistimuli responsive NPs enable more precise control of drug delivery to targeted disease sites as they release the load in the presence of more than one stimuli [18]. Additionally, these NPs may be designed for sequential release of drug which increases timing and dosing control [19].

Over the past decade, extensive preclinical research has focused on developing and optimizing stimuli-responsive nanocarriers to create stable, site-specific therapeutic, diagnostic, and theranostic agents (Fig. 26.1). However, several challenges remain in translating these advances into clinical applications. Some clinical trials may have successfully addressed the challenges of applying responsive nanocarriers in clinical settings. In the following sections, we will provide a brief overview of both internal and external stimuli-responsive nanocarriers, followed by an in-depth review of their associated clinical trials. Lastly, we will discuss the key concerns and prospects of these nanocarriers.

2. External stimuli

2.1 Light-responsive nanocarriers

The light-responsive nanocarriers utilize light as a trigger to control drug release with high spatial and temporal precision within targeted cells or tissues. Traditional drug delivery systems rely on UV

(200–400 nm) or visible light (400–650 nm), which activates processes like photolysis and photoisomerization. However, these methods face limitations, such as poor tissue penetration and autofluorescence of some endogenous molecules such as hemoglobin [20,21]. Recent innovations focus on near-infrared (NIR) light (650–900 nm penetrates 2–3 cm in soft tissue) to overcome these challenges, which uses lower-energy photons to facilitate drug release. Key mechanisms like two-photon absorption and photon upconversion drive these advances [22]. Light is widely employed as an external trigger due to its non-invasive nature, cleanliness, and the ability to switch it on and off quickly [23]. As a result, various organic and inorganic NPs have been developed both preclinically and clinically for applications such as light-responsive drug delivery, photothermal therapy, and photodynamic therapy [24,25]. Two primary challenges that have hindered the clinical advancement of light-responsive NPs are the limited ability of light to penetrate deep tissues and the requirement for precise site-specific irradiation [22,24]. The exact depth of penetration depends on factors like wavelength, tissue composition, and tissue scattering properties [26]. Nonetheless, certain nanoformulations have proven successful in patient treatment.

AuroLase (Nanospectra Biosciences, Inc., Houston, TX) is an FDA-approved gold-silica nanoshells (GSNs) used to treat focal prostate lesions. The particles are prepared with a silica core and gold shell. Following intravenous administration, GSNs accumulate in solid tumors due to the enhanced permeability of blood vessels (Enhanced Permeability and Retention (EPR) effect). When exposed to a NIR laser, the concentrated GSNs in the tumor tissues generate sufficient hyperthermia to induce necrosis.

The clinical safety of AuroLase was confirmed in a study by Stern et al. in 2016 [27]. In this research, nanoshells were synthesized following the method outlined by Oldenburg et al. [28]. Briefly, aminated silica NPs were suspended in gold colloids in the presence of certain reactants to form nanoshells. The resulting particles had a shell thickness of 12–15 nm and an optical absorption peak between 780 and 800 nm. To prevent serum proteins from accumulating on the particle surface, GSNs were pPEGylated and suspended in a 10% trehalose solution to ensure iso-osmotic conditions for injection. The optical density of the final formulation was 100 ± 5 at 800 nm and the final particle size was reported 150 nm. The GSNs colloidal solution was filtered, sterilized, and then administered. Twenty-two prostate cancer patients, confirmed by biopsy and scheduled for prostatectomy (complete removal of the prostate gland), received NP infusions before undergoing radical prostatectomy (RRP). Of these, 15 patients also underwent laser ablation a day after the infusion but before RRP. The ablation involved applying a laser at 3.0–5.0 W for 3–4 min per treatment site. The optical fibers were directed within the cortex of the left and right hemispheres of the prostate by a transrectal US probe. A thermocouple was affixed to the US probe and placed against the anterior rectal wall at the level of the prostate. No temperature increase was detected in the surrounding tissues during the procedure. As the study focused on safety, it did not report whether the procedure affected tumor size. The researchers suggested that further studies are needed to determine whether this procedure could serve as an alternative to conventional methods. Over 6 months, patients were monitored at nine-time points, with adverse events recorded and comprehensive blood, urine, and immunological tests performed. Two NP-related adverse events were noted: an allergic reaction and a transient burning sensation of the epigastrium experienced by 2 patients during infusion. No significant changes were observed in blood tests, urinalysis, or rectal wall temperature. The findings were consistent with previous research and confirmed the safety of systemically administered gold nanoshell particles for use in focal prostate ablation therapies.

Post approval, some clinical trials investigated the clinical safety of GSNs combined with magnetic resonance imaging/US (MRI/US) fusion imaging (NCT02680535, NCT04240639). In a study [29] the feasibility and safety of GSNs followed with laser ablation and combined with MRI/US fusion imaging was conducted in 16 patients with low-to intermediate-risk localized prostate cancer. The goal of this procedure was to achieve regional control of prostate cancer while minimizing patient morbidity and enhancing functional outcomes.

Following GSN infusion and precise laser ablation, patients underwent multiparametric MRI (mpMRI) at 48–72 hours. mpMRI is a technique designed to produce a detailed three-dimensional image of the prostate. It combines T2-weighted imaging, diffusion-weighted imaging, dynamic contrast-enhanced imaging, and when required, MR spectroscopy. This integration allows for improved accuracy in assessing and diagnosing prostate conditions [29].

Then patients underwent mpMRI/US targeted fusion biopsies at 3 and 12 months, as well as a standard 12-core systematic biopsy at 12 months. The treatment was completed in 94% of patients, with no significant changes in urinary or sexual function. One patient experienced transient epigastric pain during the GSN infusion and did not complete the procedure. All patients demonstrated signs of coagulative necrosis upon repeat biopsy. At the 3-month mark, 62.5% of the lesions within the ablation zones were found negative for tumors. By the 12-month assessment, this proportion increased to 87.5% of lesions showing no tumor presence in the ablation areas.

The study concluded that the treatment protocol is feasible and safe in men with low- or intermediate-risk localized prostate cancer, with no major complications observed. This procedure holds promise as a potential alternative to prostatectomy for certain patients in the near future.

Two additional clinical trials evaluated the effectiveness of AuroLase in photothermal therapy for patients with refractory and/or recurrent head and neck tumors (NCT00848042) and primary and/or metastatic lung tumors (NCT01679470) [30]. In the lung cancer study, patients received a single intravenous infusion of AuroLase, followed by dose escalation of laser radiation delivered via bronchoscopy. In the head and neck cancer study, participants received treatment with one or more laser ablations after an intravenous dose of AuroLase. Fifteen participants underwent laser ablation at varying densities and were monitored over 6 months. The head and neck study demonstrated a high safety profile with more than 80% tumour regression in some cases. The lung cancer study was terminated in 2016 with no results published [31].

GSNs are applied in the photothermal therapy of atherosclerosis. Atherosclerosis is a condition characterized by the Buildup of fatty deposits (plaque) along the arterial walls, which narrows the passage through which blood flows. This narrowing restricts oxygen delivery to essential organs, potentially leading to serious health issues. In a clinical trial (NCT01270139) [32], researchers assessed the safety and feasibility of two different delivery methods for GSNs in the photothermal treatment of atherosclerosis. In this clinical study, 180 patients were categorized into three groups to assess safety and feasibility of two NP delivery techniques and photothermal therapy. One group of 60 patients received silica-gold NPs in a bioengineered patch placed on the artery. The patch was composed of thinly sliced porous bovine pericardium, serving as a biological scaffold. Into this scaffold, layers of purified allogeneic stem cells, exhibiting an early-stage mesenchymal phenotype, were cultivated and allowed to grow. Initially, the stem cells were cultivated in a medium containing GSNs (60–70 nm). The patch was subsequently implanted on the epicardium through minimally invasive cardiac surgery. The patients underwent NIR laser ablation 7 days after NPs implantation. They used stem cells based on preclinical studies indicating that delivering NPs within stem cells led to

greater total and percent reductions in plaque volume. Additionally, stem cells may enhance vascularization (the formation of blood vessels in an organ or body part, either as a normal process or in response to a medical condition). The second group, consisting of 60 patients, received an intracoronary infusion of magnetic silica-gold NPs (90–150 nm) combined with CD68-targeted gas-filled albumin microbubbles and stem cells in a 1:1 ratio, using a magnetic navigation system. Prior to the procedure, the microbubbles and stem cells were pre-cultivated with magnetic NPs. The microbubbles were then ruptured using a Sonos 5500 machine (Agilent Technologies) equipped with an S3 transducer operating in ultraharmonic mode (transmitting at 1.3 MHz and receiving at 3.6 MHz) with a mechanical index of 1.5 and a depth of 4 cm. The AXIOM Artis dBC (Siemens) magnetic navigation system was employed to guide the NPs to the fat plaque precisely. This was achieved via two computer-controlled permanent external magnets that generated a 0.08 T magnetic field, allowing for precise directional control targeting the specific vessel and lesion. Finally, patients underwent NIR laser ablation to complete the procedure. The last 60 patients underwent XIENCE V stent (commercial drug containing stent) implantation.

Over 12 months, no life-threatening complications or instances of thrombosis were observed at the intervention site. Follow-up imaging after 12 months revealed that the percent plaque volume was reduced to 44.8% in the gold-silica NPs group, compared to 43.2% in the magnetic gold-silica NPs group and 22.7% in the control group. Thus, both NP delivery methods demonstrated efficacy in treating atherosclerosis and exhibited a safety profile appropriate for clinical application.

In 2021, an early-phase I clinical trial (NCT05268718) was initiated to evaluate safety and efficacy of a hollow gold-silver-copper oxide (Au-Ag-Cu_2O) composite nanogel, used in conjunction with photothermal therapy, for treating severe drug-resistant microbial keratitis (inflammation of the cornea). The study included three distinct participant groups. One group received treatment with the Au-Ag-Cu_2O composite nanogel (where the eyes were coated with the nanogel), followed by NIR irradiation (laser wavelength: 808 nm, power: 1.5 W/cm^2, temperature controlled at 40°C for 10 min). A second group was treated with 1% voriconazole eye drops, while the third group received normal saline. They claimed the activated Au-Ag-Cu_2O nanogel released silver ions, copper ions, and reactive oxygen species (ROS), which eradicate bacteria. To the best of our knowledge, no results from this study have been published to date [33].

A prospective randomized controlled trial (NCT05651815) assessed the efficacy of graphene-based photothermal adjuvant therapy (GPAT) in comparison to conventional treatment for patients with mild COVID-19 caused by the Omicron BF.7 variant [33,34]. Participants were randomly assigned to receive either the standard therapy combined with photothermal treatment or the standard therapy alone, and treatment outcomes were assessed based on symptom recovery, duration of hospitalization, viral negativity, and inflammatory markers. Patients receiving GPAT did not demonstrate superior clinical efficacy compared with conventional treatment alone. The GPAT group showed a longer median time to symptom recovery and longer hospital stay, although a reduction in abnormal IL-6 level changes was observed, suggesting a potential immunomodulatory effect rather than improved antiviral efficacy.

2.2 Temperature-responsive nanocarriers

Thermosensitive liposomes (TSLs) might represent the most advanced thermo responsive nanotechnologies, as evidenced by their involvement in numerous clinical trials [35]. ThermoDox is a

temperature-sensitive liposomal formulation of the chemotherapy drug doxorubicin (DOX), developed by Celsion Corporation. It is designed to treat primary liver cancer (hepatocellular carcinoma (HCC)) and recurring chest wall breast cancer. Composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), monostearoylphosphatidylcholine (MSPC), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N methoxy polyethylene glycol-2000 (DSPE-MPEG-2000) lipids, the formulation is engineered for precise thermal response, allowing for rapid drug release upon local hyperthermia (around 42°C) [36]. ThermoDox administration in combination with radiofrequency ablation (RFA), enhancing drug release at the tumor site [37].

A Phase III study (NCT 02112656) investigated whether ThermoDox can help treat HCC when paired with standardized RFA (sRFA). The trial was randomized, double-blind, and placebo-controlled, focusing on the safety and effectiveness of combining ThermoDox with sRFA versus sRFA with a placebo infusion. The study targeted patients with single HCC tumors between 3.0 and 7.0 cm in size, using a consistent treatment dwell time. For sRFA, the dwell time is defined as at least 45 minutes, measured from the first activation of the RFA probe to its removal after the final ablation cycle [38]. In a clinical trial, patients were randomly assigned to receive either 50 mg/m^2 of ThermoDox or a placebo via intravenous infusion over 30 minutes. The ThermoDox group took 20 mg of oral dexamethasone 24 hours prior to the infusion to reduce reactions, while the control group took a placebo. All patients received a blinded cocktail of medications before the infusion, and control participants received placebo treatments. The RFA procedure began at least 15 minutes after the infusion and concluded within 3 hours. 554 patients (277 in each group) were enrolled. 160 patients passed away, and 20 were withdrawn. Survival rates were similar between the ThermoDox + sRFA group and the placebo group. However, patients in the ThermoDox group had higher rates of leukopenia and neutropenia [38].

Other trials involving heat-sensitive nanocarriers have had mixed outcomes. For instance, an open-label Phase II study (NCT01464593) looked into using ThermoDox for metastatic colorectal cancer through regional liver therapy. This study included patients with unresectable liver metastases who were candidates for either RFA or microwave ablation. It required at least two ablation-plus-ThermoDox treatments per lesion, but due to poor enrollment, the trial was terminated early, and no data was collected [39].

Another trial (NCT04852367) explored combining focused US with ThermoDox for non-resectable pancreatic cancer. The approach involved delivering ThermoDox intravenously and using US to heat tumors, aiming to compare this method with standard DOX delivery. This Phase I, non-randomized safety study, known as PanDox, started in 2021. It measured DOX concentrations in tumor biopsies and used the JC200 Focused Ultrasound Tumor Therapeutic System (a CE-marked device from Haifu Medical). The approval of the study was withdrawn after a failed clinical trial [36,40,41].

The NCT02850419 study is a phase II trial, developed to evaluate the efficacy and tolerability of a heat-activated targeted therapy approach, radiotherapy, hyperthermia, and lyso-thermosensitive liposomal DOX, for treating loco-regional relapse in breast cancer patients. This study aimed to evaluate the application of a tri-modal approach with LTLD to improve outcomes: radiation, hyperthermia, and targeted release of a novel formulation of DOX. Unfortunately, this trial was withdrawn in 2020 after running for 3 years, with no further explanation provided [42].

Additionally, certain NPs that respond to magnetic fields, light, or US also exhibit sensitivity to temperature changes [43].

2.3 Ultrasound-responsive nanocarriers

The US generates acoustic waves with frequencies above 20 kHz, which require a medium like gas, liquid, or solid to travel through. In gases and liquids, the waves propagate longitudinally, while in solids, transversal waves can also occur due to shear stress. US waves possess common physical properties like attenuation, reflection, refraction, and absorption. The US source is typically a transducer containing a piezoelectric crystal that converts electrical signals into mechanical pressure waves. The US is a safe, portable, deep-penetrating, and cost-effective imaging modality, making it a promising approach for triggering drug delivery. US-responsive systems can facilitate real-time imaging and image-guided therapy [44]. Common US-responsive materials include polymer-coated bubbles and emulsions (such as microbubbles, nanobubbles, nanodroplets, and nanoemulsions), polymer vesicles, micelles, and hydrogels. These materials can deliver a variety of therapeutic agents, including small-molecule drugs, proteins, and DNA [45].

While nanocarriers have demonstrated promising results in preclinical studies, several challenges limit their progress toward clinical application. Issues such as the need for high-intensity US, poor stability, inconsistent activation efficiency between batches, and biodegradability remain significant barriers to their development [46]. Among these, biodegradability and adaptability have made US-responsive polymeric materials a focal point of research. These materials provide numerous potential applications, including tumor therapy, BBB disruption, transdermal drug delivery, thrombolysis, and infection treatment.

US-induced targeted delivery relies on mechanisms such as thermal effects, cavitation, and acoustic radiation forces. Controlled heating can release drugs from thermosensitive carriers; however, excessive heat poses the risk of damaging surrounding tissues. Cavitation, a process caused by the rapid expansion and collapse of microbubbles, enhances the permeability of cells and blood vessels, enabling more effective drug delivery. Meanwhile, acoustic radiation forces reduce the size of the nanocarriers, improving their ability to penetrate biological membranes. These mechanisms collectively underscore the potential of US-responsive systems, although further research is needed to address the limitations and ensure their safety and efficiency in clinical applications [47].

Lyso-thermosensitive liposomal DOX (LTLD) is the first heat-activated formulation of a liposomal drug carrier to be utilized in human clinical trials. Its novel means of DOX release under conditions of mild hyperthermia was developed at Duke University [48]. LTLD releases its drug payload at temperatures above 39·5°C. Liposomal formulations enhance the plasma pharmacokinetics of DOX, resulting in a plasma half-life in humans of about 1 h, much longer than free DOX, which has a half-life of only minutes. The active drug in LTLD is DOX hydrochloride. The chemical formula is C27H29NO11 HCl, and the molecular weight is 579.99 Da. LTLD combines DOX with also-TSLs that are made from three synthetic phospholipids: DPPC, MSPC, and DSPE-MPEG-2000 [49]. LTLD is produced as stable liposomes loaded with DOX and stored in solution form. In clinical trials, LTLD has been used exclusively in combination with minimally invasive RFA to enhance tumor margin treatment [48]. However, preclinical research demonstrated that applying focused US to generate mild hyperthermia non-invasively after systemic administration of LTLD can significantly increase the intratumoral delivery and distribution of DOX, potentially boosting its therapeutic effectiveness at a given systemic dose [50].

One clinical trial study of LTLD used the targeted release of also-thermosensitive liposomal DOX (TRODOX). This was a first phase clinical trial (NCT02181075) to investigate the feasibility of TRODOX in patients with liver tumors. This study directly quantified the DOX concentration before

and after US exposure from tumor biopsies using high-performance liquid chromatography (HPLC). Twenty-eight evaluable adult patients with incurable confirmed hepatic primary or secondary (metastatic) tumors participated in this trial. Patients under a general anesthetic received a single cycle of ThermoDox (50 mg/m^2), immediately followed by US-mediated hyperthermia in a single target liver tumor. US-mediated hyperthermia was achieved through US-guided focused US targeted at a single target using the Model JC200 Focused Ultrasound Tumor Therapeutic System (Haifu Medical, JC200), which is clinically approved (CE-marked) for tumor therapy [51].

This study consists of two parts. In Part 1, minimally invasive thermometry was performed using a thermistor or thermocouple implanted via a biopsy co-axial needle core to confirm US-induced hyperthermia. Part 2, on the other hand, was conducted without invasive thermometry better to simulate the intended clinical application of the technique. In Part 1, the total DOX concentration in the US-treated tumor, as determined by HPLC, is directly compared to the concentration measured in the same tumor before US exposure. For patients in Part 2, since a pre-US tumor biopsy is not available, the mean tumor concentration from Part 1 is used as a reference. The study aimed to demonstrate at least a two-fold increase in intratumoral DOX concentration, or a final concentration exceeding 10 μg/g, in at least 50% of patients whose tissue samples are evaluable by HPLC [51].

Ten patients were recruited for the analysis. The treatment led to an average 3.7-fold increase in DOX concentrations in tumor biopsies, rising from an estimated 2.34 μg/g (SD 0.93) immediately after drug infusion to 8.56 μg/g (SD 5.69) following focused US. A two-to ten-fold increase was observed in 70% of patients, meeting the primary endpoint. However, five patients experienced grade 4 transient neutropenia, and one patient had an extended hospital stay due to unexpected grade 1 confusion. Neutropenia, a condition marked by a reduced neutrophil count [52], occurred in one patient with grade 3 neutropenia (neutrophil count between 500 and 1000 per mm^3), five patients with grade 4 neutropenia (neutrophil count below 500 per mm^3), and one patient with grade 3 anemia (hemoglobin levels of 65–80 g/L). No treatment-related deaths were reported [50]. All adverse events are listed in Table 26.1 [50].

In another report of this trial (NCT02181075), 10 participants (age range 49–68 years; average age 60 years; six men and four women) enrolled. Ultrasonic heating of target tumors (treated volume: 11–73 cm^3 [mean ± standard deviation, 50 cm^3 ± 26]) was monitored in six participants by using a minimally invasive temperature sensor and four participants were treated without real-time thermometry. The study found no thermal ablation related to focused US investigated through MRI and biopsy, demonstrated the procedure's safety. Additionally, the insignificant difference between the actual and predicted US power revealed the feasibility of computational methods for focused US-mediated hyperthermia planning models for defining treatment parameters. The response assessment showed that the target tumor that received drug and focused US decreased by 36.4%, which was observed in positron emission tomography/computed tomography images taken 17 days after the intervention. In contrast, there was no substantial change in the volume of a similarly sized tumor that received the drug but did not undergo focused US treatment. This 10-participant study confirmed the feasibility of using focused US-mediated hyperthermia planning models to define treatment parameters that safely enabled targeted, noninvasive drug delivery to liver tumors while monitored with less expensive B-mode US guidance and without real-time thermometry [53].

2.4 Magnetic field-responsive nanocarriers

Iron oxide NPs, specifically Fe_3O_4 and Fe_2O_3, are among the most extensively studied magnetic NPs due to their ability to lose magnetization once the external magnetic field is removed [54]. These NPs

Table 26.1 Adverse events stratified by definite or probable causality.

	Grade 1-2	Grade3	grade4
Definitely or probably related to LTLD: Hematological toxic effects			
Neutropenia or Neutrophils decreased	0	1(10%)	5 (50%)
Anemia	0	1 (10%)	0
Urinary tract infection	1 (10%)	0	0
Definitely or probably related to LTLD: non-hematological toxic effects			
Alopecia	8 (80%)	0	0
Candida infection	1 (10%)	0	0
Fatigue or lethargy	4 (40%)	0	0
Nausea	2 (20%)	0	0
Vomiting	2 (20%)	0	0
Definitely or probably related to the procedure			
Abdominal pain	2 (20%)	0	0
Back pain	1 (10%)	0	0
Decreased appetite 1 (10%) 0 0	1 (10%)	0	0
Dysphonia 1 (10%) 0 0	1 (10%)	0	0
Erythema 1 (10%) 0 0	1 (10%)	0	0
Fatigue 1 (10%) 0 0	1 (10%)	0	0
Hepatic pain 1 (10%) 0 0	1 (10%)	0	0
Musculoskeletal chest pain	2 (20%)	0	0
Musculoskeletal pain or discomfort	6 (60%)	0	0
Nausea 1 (10%) 0 0	1 (10%)	0	0
Pain in extremity	2 (20%)	0	0
Skin discoloration	1 (10%)	0	0

No grade 5 adverse events occurred.

offer versatile applications in medical imaging, therapeutic interventions, diagnostics, and in the isolation of cells and large biomolecules [55]. Most magnetic NPs are coated with organic or inorganic materials to ensure stability, solubility, and biocompatibility while preventing aggregation [56]. Building on promising in vitro and in vivo study results, several magnetic NPs are currently undergoing clinical trials, with some already receiving clinical approval. In this session, we review some clinical trials of magnetic NPs.

Ferumoxtran-10 (Combidex/Sinerem) is a dextran-coated ultra-small superparamagnetic iron oxide (USPIO) NP (approximately 30 nm) that was clinically approved by the European Medicines Agency (EMA) in 2006 but withdrawn in 2007 [57].

Numerous clinical trials have investigated the safety of NPs. In a phase II trial conducted in 1999, 104 patients with liver or spleen abnormalities underwent MRI enhanced with ferumoxtran-10 at doses of 0.8, 1.1, and 1.7 mg Fe/kg. Adverse events were reported by 15% of participants, with 33 events documented. The most common side effects included dyspnea (3.8%), chest pain (2.9%), and rash (2.9%). Importantly, no serious adverse events occurred during the 48-hour observation period, and there were no clinically significant changes in vital signs, physical exams, or lab results, indicating that ferumoxtran-10 was a safe and well-tolerated MR contrast agent [58].

At 2007, the EMA's Committee for Medicinal Products for Human Use (CHMP) raised concerns and provisionally concluded that Sinerem could not be approved for diagnostic use in characterizing lymph nodes visualized by MRI to assess the spread of primary tumors in pelvic cancers. The CHMP's main concern was that Sinerem's effectiveness in enhancing MRI images was not sufficiently demonstrated. Reader evaluations showed inconsistencies regarding any improvement Sinerem might offer in detecting cancer spread to lymph nodes during MRI scans, making it unhelpful for managing patients with these conditions. Consequently, at the time of withdrawal, the CHMP determined that the benefits of Sinerem as a diagnostic tool for lymph node characterization in pelvic cancer patients were inadequately substantiated, and any potential advantages did not outweigh the associated risks.

Although Sinerem was withdrawn due to its lack of efficacy in imaging lymph nodes for pelvic cancer, researchers have since evaluated this nanoformulation for imaging other tissues. In a clinical trial in 2024 [59], researchers evaluated the image quality and vascular suppression of Ferumoxtran-10-enhanced magnetic resonance neurography (MRN) in comparison to gadolinium-enhanced MRN. The study involved 37 participants, with 17 receiving Ferumoxtran-10 and 20 receiving gadolinium as the contrast agent. Ferumoxtran-10 was administered via a 100 mL saline infusion at a dose of 0.13 mL per kilogram of body weight (20 mg/mL) over a 30–60-minute period. MRI scans of the pelvic region were performed 24–36 hours following the infusion. Three independent reviewers assessed the image quality of both the gadolinium and Ferumoxtran-10-enhanced sequences for different nerves (sciatic, femoral, obturator, pudendal, and lateral femoral cutaneous) using a 5-point Likert scale (ranging from 0-poor to 4-perfect).

The results indicated that inter-reader agreement (the degree of the reliability of the interpretation of medical images by the readers which is calculated using intraclass coefficients) for evaluating nerve image quality, was 0.76 for gadolinium-enhanced MRN and 0.89 for Ferumoxtran-10-enhanced MRN. Ferumoxtran-10 exhibited significantly better vascular suppression compared to gadolinium-enhanced MRN, with mean values of 2.70 ± 0.4 for Ferumoxtran-10 and 1.80 ± 0.5 for gadolinium ($P < .001$). Additionally, nerve visibility for all analyzed nerves was markedly improved in the Ferumoxtran-10-enhanced MRN scans. Fig. 26.2 shows the coronal 3D Short Tau Inversion Recovery Maximum Intensity Projection (STIR MIP) images. A coronal 3D STIR MIP image is a three-dimensional MRI scan processed to maximize the visibility of bright areas, with fat signals suppressed to enhance contrast. It's often used in medical imaging to detect lesions, inflammation, tumors, or fluid collections in soft tissues and bone structures.

Fig. 26.2a shows the 3D STIR MIP image of lumbosacral plexus following intravenous administration of gadolinium and Fig. 26.2b shows the coronal 3D STIR MIP image of the lumbosacral plexus following intravenous infusion of Ferumoxtran-10. Ferumoxtran-10 decreased noise and conceal almost all surrounding blood vessels. The results suggested that Ferumoxtran-10 may be an effective contrast agent for imaging the lumbosacral plexus, particularly in patients for whom Gadolinium is contraindicated.

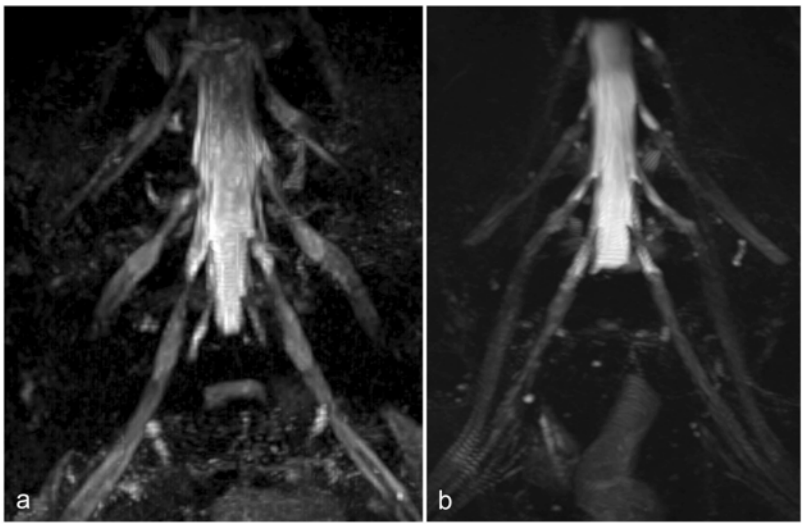


FIGURE 26.2

(a) A gadolinium-enhanced 3D STIR MIP image of lumbosacral plexus and (b) Ferumoxtran-10-enhanced 3D STIR MIP image of lumbosacral plexus.

Ferucarbotran (Resovist) is the second SPIO NP to receive clinical approval for use as a contrast agent in MRI for liver imaging. [60]. This contrast agent was clinically approved in some parts of Europe in 2001 but discontinued in 2009 in some countries [57] due to less enhancement compared to gadolinium-based agents [61]. Japan and Franc are two countries in which Resovist is still approved [62,63]. Resovist is a T2-weighted SPIO NP coated with carboxydextran in a size of about 45–60 nm that is primarily designed for liver imaging [64,65]. T2 (transverse relaxations) refers to the time it takes for protons to lose coherence with each other after being stimulated by a radiofrequency pulse [66]. These NPs are uptaken by the reticuloendothelial system and make the liver, spleen, lymph nodes, and bone marrow normal cells appear dark in MRI. However, tumor cells show high signal intensity and appear bright [65].

One of the earliest phase II clinical trials of Resovist was conducted in 1997 [67]. The study evaluated the safety, efficacy, and pharmacodynamics of Resovist, in MRI for patients with liver lesions. Thirty-three patients received different doses of Resovist (4, 8, or 16 μmol Fe/kg). MR images were taken before injection and at various intervals afterward, with blood samples analyzed for changes. No drug-related adverse events were observed. However, higher doses led to increased serum iron, ferritin, and clotting factor changes. The optimal imaging results were seen 40 minutes post-injection with the 8 μmol Fe/kg dose. Resovist was found to be safe and effective for enhancing lesion detection in MRI.

Ferumoxsil (Gastromark, Lumirem) is a siloxane-coated iron oxide NP (200–400 nm) [57,65]. Although the FDA approved Gastromark in 1996, the manufacturer discontinued it in 2012 due to financial losses, despite evidence of its effectiveness and safety [68]. This NP has a size of approximately 10 nm and a hydrodynamic diameter of about 300 nm. Siloxane, which is non-biodegradable

and insoluble, is suspended in viscosity-increasing agents such as starch and cellulose. As a negative oral contrast agent, Gastromark reduced the signal on T2-weighted images and enhanced the imaging of the gastrointestinal tract during MRI scans [69].

Ferumoxides (Endorem, Feridex I.V) is another dextran-coated SPIO NP (80–150 nm) that has been used in liver, spleen, and myocardial perfusion MRI. It was approved by FDA in 1996 but discontinued in 2008 [57,65] because of limited marketing potential [70]. In a 2002 clinical trial, researchers compared the effectiveness of ferumoxides and ferumoxtran-10 with gadolinium in MRI imaging of intracranial tumors [71]. Twenty patients involved in the study, all of the patients first underwent gadolinium-enhanced MRI at least 24 hours before but no more than 28 days before iron infusion. Subsequently, all the patients were administered 2.6 mg Fe/kg of ferumoxtran-10 diluted in 50 mL of normal saline, with imaging performed 24 hours post-infusion. Due to the minimal uptake of ferumoxides in the initial tests, ferumoxtran-10 was used exclusively for further evaluations. However, 3 patients did receive ferumoxides intravenously at the recommended clinical dose of 0.56 mg of iron per kilogram of body weight.

The results indicated that ferumoxides provided no improvement in image quality and showed minimal iron uptake in intracranial tumors. Ferumoxtran exhibited changes in signal intensity in most regions enhanced by gadolinium. Additionally, it showed enhancement in certain areas not observed with gadolinium, suggesting a distinct mechanism for crossing the blood-brain barrier (BBB), potentially different from the intercellular junctions.

Ferristene (Abdoscan) is is polystyrene-coated iron oxide NPs in size of about 3500 nm [55]. Abdoscan was clinically approved in Sweden in 1993 but discontinued in 2002 [57] due to a lack of profitability, despite being demonstrated as effective and safe [68] In a 2000 clinical trial [72], researchers compared imaging findings from conventional MRI with no contrast agents, ferristene-enhanced MRI, and a combination of ferristene/gadodiamide-enhanced MRI for staging of rectal carcinoma. The study included 29 participants, each receiving 23.4 mg/L of iron. To maintain the NPs in the rectum, they were mixed with microcrystalline cellulose and xanthan gum granules to increase viscosity and administered via a rectal tube. Before administering the contrast agent, sagittal and transverse T1-weighted images were obtained. After the rectal administration of the ferristene-based contrast material, sagittal T1-weighted images were acquired. Subsequently, gadodiamide was injected intravenously, and both sagittal and transverse T1-weighted images were captured.

Two experienced radiologists investigated number of detectable tumors; the quality of tumor delineation; and the presence of enlarged regional lymph nodes. Radiologic staging was conducted using the Dukes staging system, with the results categorized into two groups: Dukes A, representing carcinoma or benign tumors, and Dukes B, indicating carcinoma or more advanced stages. The contrast between pre- and post-contrast images was significant. In this study, the majority (25 out of 29) of tumors identified through manual palpation, proctoscopy, or barium studies were not visible on MR images taken before administering the ferristene-based contrast material. The increased diagnostic accuracy results from the ferristene formulation, which expands the bowel lumen, stretches the rectal wall, and creates a signal void in the luminal space, thereby enhancing tissue layer visibility. The addition of gadodiamide further increases signal intensity in the highly vascular mucosal layers, which appear as a distinct white line on T1-weighted images, while the muscular layer shows up as a dark band. This contrast aids in assessing tumor growth, as tumors appear darker than the enhanced mucosa, and any tumor spread into the mucosal layer is visible as a break in the bright structure. Thus,

ferristene/gadodiamide provided more precise tumor delineation than ferristene alone with the sensitivity of 100%; specificity of 70%; and accuracy of 90% in distinguishing tumor stages worse than Dukes A.

Ferumoxytol (Feraheme, Rienso) is a polyglucose sorbitol carboxymethyl ether-coated SPIO NP with a size of approximately 30 nm. It is utilized both as a contrast agent in MRI and for the treatment of iron deficiency anemia [57,65]. It was clinically approved in the US (2009) and Europe (2013) [55]. Postmarketing research on ferumoxytol, used to treat anemia, revealed that administering the drug at a dose of 510 mg over 17 seconds led to acute hypersensitivity reactions. This prompted the FDA to issue a black box warning in 2015. As a result, the dosing regimen was adjusted to infuse 510 mg over 15 minutes to reduce the risk of adverse reactions [73]. However, the data about diagnostic applications is positive [74–76].

In 2019, a retrospective, multicenter study examined the safety and adverse effects of ferumoxytol as an off-label contrast agent based on medical documents of patients [77]. Off-label use is the use of pharmaceutical drugs for an unapproved indication or in an unapproved age group, dosage, or route of administration. Adverse effects were assessed using the National Cancer Institute's Common Terminology Criteria for Adverse Events (grades 1–5). A total of 3215 patients had received ferumoxytol at doses ranging from 1 to 11 mg per kilogram of body weight between January 2003 and October 2018. The centers had reported no significant changes in vital signs or any life-threatening effects. These findings suggested that ferumoxytol was a safe option for use as a contrast agent in MRI.

In 2019, a clinical trial was conducted to determine the optimal ferumoxytol dose for MRI angiography in patients with chronic kidney disease [78]. Twenty-three patients underwent ferumoxytol-enhanced MRI using a 3.0-T Prisma MRI scanner, and image quality was assessed based on contrast-to-noise ratio (CNR) and signal-to-noise ratio (SNR) in the abdominal aorta and inferior vena cava. Each patient received intravenous doses of 0, 1, 2, 3, and 4 mg/kg of ferumoxytol. The findings revealed that a 3 mg/kg dose yielded at least 75% of the predicted peak CNR and SNR, resulting in images of excellent diagnostic quality (Fig. 26.3). However, administering doses beyond 4 mg/kg led to a progressive decline in signal intensity.

This SPIO has been applied in vasculature, cardiac, and tumor imaging in adults and children [79]. Additionally, Some active clinical trials in 2024, evaluate the ferumoxytol efficiency in MRI to predict stroke risk in ICAD (NCT05515874), evaluate vessel wall MRI techniques (NCT03084523), assess of renal vasculature and function (NCT05045872), phenotyping interstitial cystitis/bladder pain syndrome (NCT05811377), detect of intracardiac thrombus (NCT06146751), image atherosclerosis (NCT06551805), study tumor-associated macrophage infiltration in central nervous system tumors (NCT06572475), manage pancreatic cancer based on dynamic contrast enhanced MRI (NCT02070705), perform magnetic resonance angiography/venography on panvascular disease (NCT06136806), and development of radiation-free whole-body MR imaging technique (NCT01542879).

Sienna+ is a carboxydextran-coated iron oxide NP approved by the EMA in 2011 as an MRI contrast agent for imaging cancerous sentinel lymph nodes in breast cancer patients [80]. These NPs are detectable using the Sentimag magnetometer and have been utilized for sentinel lymph node biopsies. A phase II clinical trial compared the identification rate of Sienna+/Sentimag to that of the standard technique (radioisotope and blue dye or radioisotope alone) in sentinel lymph node biopsies. The study included 160 patients, all of whom received a subcutaneous injection of the brownish-black Sienna+ suspension into the breast, with 132 patients also receiving blue dye. Once administered, the NPs rapidly accumulated in sentinel lymph nodes, where they were detectable by MRI or

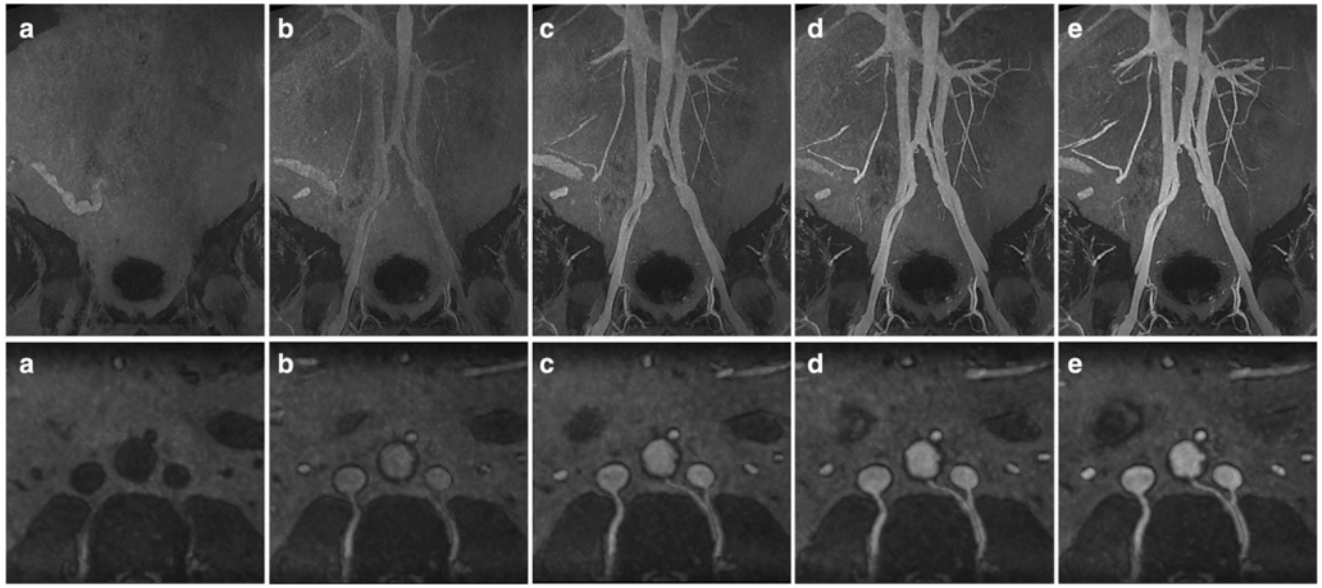


FIGURE 26.3

The coronal (upper panels) and axial (lower panels) images of abdominal and aortoiliac vasculature (a) before and after b: 1 mg/mL, c: 2 mg/mL, d: 3 mg/mL, and e: 4 mg/mL ferumoxytol administration.

intraoperatively with the Sentimag magnetometer. Identification rates were 95.0% using the standard technique and 94.4% with the magnetic technique. In conclusion, the magnetic method is shown to be as effective as the standard technique.

NanoTherm, a colloidal suspension of amino silane-coated iron oxide NPs (10–15 nm), received approval from the EMA in 2010 [57,81]. These magnetic NPs can be administered either intratumoral or interstitially [82]. After administration, they are heated via an alternating magnetic field, which either destroys cancer cells or sensitizes them to concurrent radiotherapy or chemotherapy, reducing the risk of recurrence. This formulation is indicated for the treatment of glioblastoma, prostate cancer, and pancreatic cancer [83].

In a phase II clinical trial, which began in April 2005 and stopped enrolling participants in September 2009, researchers assessed whether applying a new intratumoral thermotherapy technique using NanoTherm could enhance survival outcomes [84]. The primary focus of the study was overall survival following the diagnosis of the first tumor recurrence, with the secondary endpoint being overall survival from the time of the primary tumor diagnosis. The study included 59 participants, each receiving an average of 4 mL of NanoTherm suspension (112 mg/mL), which was approximately 0.28 mL per cubic centimeter of tumor volume. The magnetic fluid was administered under general anesthesia using neuronavigation, in a procedure similar to a brain needle biopsy. Patients underwent six hyperthermia sessions over 3 weeks, with each session lasting 1 hour. Stereotactic beam radiotherapy using a 6 MV Novalis system was administered immediately before or after each hyperthermia session. The average overall survival following the first tumor recurrence was 13.4 months, and survival after the primary tumor diagnosis averaged 23.2 months compared to 6.2 and 14.6 month had reported in previous studies respectively. Tumor volume at the beginning of the study was the only factor significantly correlated with subsequent survival. The results demonstrated that NanoTherm thermotherapy combined with reduced radiotherapy is safe and prolongs survival after the first tumor recurrence compared to conventional therapies in patients with recurrent glioblastoma. Two significant drawbacks of this new approach are, first, the need to remove all metal within 40 cm of the treatment area (including dental work), and second, the permanent prohibition of MRI for diagnosing future tumor progression. However, conventional MRI can still be used without restrictions outside the area of NP deposits, in accordance with current practices.

3. Internal stimuli

3.1 pH-responsive nanocarriers

pH-responsive nanocarriers are promising candidates for oral drug delivery as they protect drugs from degradation in the acidic parts of the gastrointestinal tract [85–87]. These carriers ensure targeted and efficient delivery by releasing the drug only at specific pH levels. On the other hand, since they can respond to varying pH conditions within the gastrointestinal tract, they are particularly suited for targeted drug delivery to the bowel [88–90]. Furthermore, since the pH of the tumor microenvironment is slightly acidic compared to the pH of normal tissues, pH-responsive nanosystems which release target at acidic consitions can prevent drug release in healthy tissues, minimizing unintended side effects and enhancing treatment precision [91–94, 181].

Compared to conventional nanosystems, pH-responsive nanocarriers undergo physical or chemical transformations in acidic environments, such as swelling, dissociation, or degradation. These changes

enable targeted drug release or degradation at specific sites and definite times, enhancing therapeutic precision [10,95]. The responsiveness is driven by the protonation of ionizable groups or acid hydrolysis of chemical bonds and may involve hydrophobic interactions, π-π stacking, hydrogen bonding, or ionic bonding within the nanostructure [96].

While numerous studies have been published on pH-sensitive NPs, few have successfully advanced to clinical trials or commercialization due to limitations in clinical effectiveness, safety concerns, or challenges in scaling up for industrial production. The efficacy of these NPs can be limited by factors such as insufficient levels of stimuli, delayed chemical bond cleavage, low uptake in target cells, high accumulation of serum proteins around the NP, and challenges with payload release [97–99].

In 2020, researchers at Al-Azhar University registered a Phase I clinical trial (NCT03774680) of a pH-sensitive polymeric NP on ClinicalTrials.gov. The project aimed to develop polymeric NPs loaded with the anti-cancer drug Cetuximab for oral delivery. These NPs, created using the solvent evaporation method with ethylcellulose polymer, were designed to release the drug at a pH above 6.8, ensuring stability in the stomach (pH 1.5) to avoid toxicity. They specifically targeted colorectal cancer cells (pH changes from 6.4 in the ascending colon to 7.0 in the descending colon) using octreotide, a somatostatin receptor agonist, which binds to somatostatin receptors overexpressed in these cells. However, no published results from this trial are currently available, and the status of the project is unknown in ClinicalTrials.gov.

NC-6300 (NanoCarrier) is a PEG-poly aspartate block copolymer micelle, chemically bonded to epirubicin through a hydrazone bond [100]. This bond is designed to break in the acidic microenvironment of solid tumors, enabling targeted drug release at the tumor site. The primary side effect of epirubicin is cardiotoxicity, a risk that is mitigated when delivered in nanoformulations [101,102].

A first-in-human phase I clinical trial of NC-6300 was conducted in 2017 [103]. Patients aged 41 to 72 with advanced or recurrent solid tumors unresponsive to standard therapies were enrolled in the study. NC-6300 was administered as a 10-minute intravenous infusion every 3 weeks, with escalating doses ranging from 15 to 225 mg/m^2. The suitability of doses exceeding 60 mg/m^2 was assessed using a Bayesian continual reassessment model. Treatment-emergent adverse events and tumor responses were evaluated according to internationally accepted guidelines. All 19 patients treated with NC-6300 were evaluated for safety, and no deaths occurred. Reduced neutrophil and white blood cell counts were the most common treatment-related adverse events. Four serious adverse events were reported in two patients, but only a Grade 3 retinal hemorrhage at the 225 mg/m^2 dose was linked to NC-6300. Major hematologic toxicities (in 25% or more of patients) included decreased neutrophil counts (74% for any grade, 53% for Grade 3 or 4), white blood cell counts (74%, 53%), and anemia (42%, 32%). Non-hematologic toxicities included malaise, nausea, anorexia, alopecia, and vomiting, with no cardiac events or hand-foot syndrome observed. Decreased blood counts were more frequent at doses of 100 mg/m^2 or higher, while nausea, vomiting, and anorexia occurred at lower doses without increased severity at higher doses. No unexpected adverse events were observed with NC-6300 monotherapy.

The pharmacokinetics of NC-6300 were assessed after the first dose. Total epirubicin circulated in the plasma for about 8 hours, then declined with a half-life of 23–27 hours. The pharmacokinetic parameters (The definite integral of a drug's concentration in blood plasma over time, along with the peak concentration of the drug in the blood, cerebrospinal fluid, or target organ following administration) showed linear dose proportionality between 15 and 225 mg/m^2. The volume of distribution was close to blood volume, suggesting minimal distribution outside the bloodstream. A rapid increase

in free epirubicin at the end of infusion likely resulted from a small free epirubicin component in the formulation, reaching peak concentration about 8 hours after dosing. In this study, one patient with breast cancer (100 mg/m^2 group) showed a partial response, while stable disease was observed in 10 patients across dose levels from 15 to 255 mg/m^2. Stable disease lasted between 54 and 561 days, with 57% of patients at the maximum tolerated dose (MTD) experiencing stable disease for 78–561 days. This trial demonstrated that NC-6300 was well-tolerated in patients with different solid tumors and showed lower toxicity compared to conventional epirubicin formulations.

Two other clinical trials have warranted the safety and further investigation of NC-6300. In the first study [104], researchers assessed the safety, pharmacokinetics, efficacy, and impact on quality of life following NC-6300 monotherapy in a phase 1b dose-escalation trial. The study was designed based on the Bayesian continuous reassessment method and involved 29 patients with advanced, metastatic, or unresectable solid tumors. NC-6300 was administered on the first day of each 21-day cycle, with dose escalations ranging from 125 mg/m^2 to 215 mg/m^2, based on the epirubicin-equivalent dosage. This study observed eight dose-limiting toxicity (DLT) events in six patients at doses of 170 mg/m^2 or higher, primarily involving grade 4 thrombocytopenia (low platelet count) and febrile neutropenia (fever and low neutrophil count). All DLTs were managed with dose reductions or required no intervention, except for one case of grade 4 thrombocytopenia at 215 mg/m^2. The MTD was established at 185 mg/m^2, and the recommended Phase II dose was set at 150 mg/m^2, with no DLTs observed at these levels. All 29 participants experienced at least one adverse event, though there were no treatment-related deaths. Common adverse events across all doses included nausea, neutropenia, fatigue, and anemia. The most frequent grade 3–4 adverse events in the entire cohort were neutropenia (59%), anemia (24%), thrombocytopenia (24%), and febrile neutropenia (21%). Non-hematologic grade 3 or higher toxicities were rare. Cardiac toxicity occurred in four subjects, including one case of grade 3 tachycardia. Among subjects receiving a cumulative dose of 900 mg/m^2 or more, only one experienced grade 1 tachycardia, while the rest had no cardiac toxicity.

Twenty-eight subjects were assessed for tumor response, one was excluded due to a lack of post-treatment data. The Disease Control Rate (DCR) was 64% (which includes those with stable disease (SD), observed in 54% of subjects and the overall objective response rate (ORR) of 11%) while 36% experienced progressive disease (PD).In the subset of patients with soft-tissue sarcoma, ORR was higher at 18%, with a DCR of 76%, SD of 59%, and PD of 24%. Partial responses were noted in cases of angiosarcoma and endometrial stromal sarcoma. A leiomyosarcoma patient at 170 mg/m^2 showed significant tumor shrinkage but developed a new lesion, and a melanoma patient, with prior treatment achieved stable disease for 9 months. The results demonstrated that the MTD of NC-6300 was higher than conventional epirubicin with manageable side effects and preliminary signs of efficacy, particularly in angiosarcoma.

In a follow-up study, the researchers conducted an expansion cohort based on the previous findings to further assess the efficacy of NC-6300 in treating angiosarcoma [105]. This study included patients aged 18 or older with histologically confirmed angiosarcoma that was ineligible for treatment via surgery or radiotherapy. NC-6300 was administered intravenously at a dose of 150 mg/m^2 on the first day of a 21-day cycle, with treatment continuing until disease progression or unacceptable toxicity. Disease progression was assessed every 6 weeks using RECIST v1.1 criteria (a standard way to measure the response of a tumor to treatment which assesses target lesion, new lesions, and overall response), with the primary focus being on median progression-free survival (mPFS). The most common adverse events of any severity, regardless of their connection to NC-6300, included

neutropenia (90%), fatigue, thrombocytopenia (60% each), and nausea (50%). The most common grade 3 and 4 adverse events were neutropenia (80%), thrombocytopenia (40%), and both anemia and leukopenia (20% each). mPFS for all participants was 5.4 months, with those who had prior anthracycline treatment showing an mPFS of 3.8 months and those without prior anthracycline exposure showing an mPFS of 8.2 months. The findings aligned with previous research and reaffirmed the safety profile of NC-6300. Additionally, NC-6300 demonstrated promising anti-tumor activity, especially in angiosarcoma patients without prior anthracycline treatment.

Onpattro (Patisiran) is a liposomal siRNA formulation approved by the FDA in 2019. This formulation is administered intravenously and reduces transthyretin (TTR) production. TTR is a protein that carries thyroxine and retinol throughout the body. Elevated levels of TTR can lead to misfolding and aggregation into amyloid fibrils, which deposit in various tissues, particularly affecting the peripheral nervous system and heart. At physiological pH, TTR remains stable; however, at lower pH levels, it can become destabilized. This destabilization promotes the aggregation of TTR monomers into insoluble fibrils, which can lead to the development of ATTR, a progressive disorder that primarily affects the heart and nervous system [106]. Patisiran liposomes are composed of ionizable cationic lipids (DLin-MC3-DMA), phospholipid, cholesterol, and polyethylene glycol-modified lipids (PEG2000-C-DMG) [107]. Once patisiran enters the bloodstream, the PEG-modified lipids are replaced by serum proteins, primarily apolipoprotein E. This protein interacts with the cholesterol components of the lipid complex, guiding the drug to the liver. Hepatocytes then absorb the ApoE-coated liposomes and direct them to the endosome [108]. DLin-MC3-DMA, a pH-sensitive ionizable lipid within the NP, becomes positively charged in the acidic endosomal environment, initiating an osmotic pressure difference that ultimately ruptures the endosome [109]. Released siRNA reduces TTR by inhibiting the mRNA translation [110]. Reduced TTR levels has been shown to alleviate neuropathy associated with elevated TTR significantly.

A phase III randomized controlled trial was conducted involving Patisiran. The trial enrolled 225 patients aged 18–85 years, all diagnosed with hereditary ATTR with peripheral neuropathy, and a Neuropathy Impairment Score ranging from 5 to 130 [111]. Participants were recruited from 19 countries. They were randomly assigned in a 2:1 ratio to receive either patisiran (0.3 mg per kilogram of body weight) or a placebo, given intravenously over approximately 80 minutes every 3 weeks for a total of 18 months. The results showed that those who received patisiran demonstrated better clinical outcomes than those in the placebo group. Throughout the trial, patients in the placebo group experienced a progressive decline in sensorimotor and autonomic functions, which resulted in more severe symptoms and a reduction in their ability to perform daily activities. In contrast to the placebo group, patients treated with patisiran exhibited either stabilization or improvement in disease. Remarkably, 8% of patients in the patisiran group showed improvement in their polyneuropathy disability score (score 0 for no impairment to 4 for confined to a wheelchair or bedridden) after 18 months, while no improvements were observed in the placebo group. This included patients transitioning from assisted to unassisted walking, a significant milestone for those with hereditary transthyretin amyloidosis, reflecting the positive impact of patisiran on gait speed.

In 2024, three clinical trials registered on ClinicalTrials.gov are currently in the recruitment phase, focusing on the effects of patisiran in patients with hereditary ATTR. The first study (NCT05040373) is a cohort study aimed at evaluating the impact of patisiran on pregnancy complications in women exposed to the drug 12 weeks before their most recent menstrual period or at any time during pregnancy. The second study (NCT05873868) seeks to assess the morphological and functional cardiac

changes in patients with hereditary ATTR and stage 1 or 2 polyneuropathy who are treated with either patisiran or vutrisiran. Finally, the third study (NCT05023889) will assess the effectiveness and safety of patisiran in patients with wild-type hereditary ATTR and symptomatic polyneuropathy, specifically focusing on its effects on neurological impairment and quality of life.

Nanoxel, a paclitaxel (PTX)-loaded micellar formulation consisting of PVP-b-PNIPAM (Polyvinylpyrrolidone-block- Poly N-isopropylacrylamide) block copolymers, is cremophor-free and water-soluble. Cremophor is commonly used as a formulation vehicle for poorly water-soluble drugs like PTX. However, its use has been linked to several serious side effects, including severe allergic-like reactions, hyperlipidemia (high lipids or fats in blood), abnormal lipoprotein profiles, erythrocyte aggregation, and peripheral neuropathy (the injury to nerves outside the brain and spinal cord). While the pharmacokinetics of Cremophor is dose-dependent, its clearance is significantly affected by the duration of infusion. Therefore, excluding Cremophor from drug formulations can help minimize these adverse effects [112]. It is important to note that **Nanoxel-M** is a micellar formulation of docetaxel encapsulated in methoxy-poly(ethylene glycol)-block-poly(D, L-lactide) (mPEG-PDLLA). However, it is not designed to be stimuli-responsive [113].

Nanoxel, developed by Dabur Pharma Ltd, is a PVP-b-PNIPAAM NP formulation of PTX with a size range of 80–100 nm that is activated by the low pH of the tumor microenvironment, leading to PNIPAAM degradation and drug release, and received DCGI approval in 2008 for treating breast cancer, non-small cell lung cancer (NSCLC) and acquired immunodeficiency syndrome (AIDS) related Kaposi Sarcoma [114]. PTX is gradually released into the tumor microenvironment due to the surface erosion of PNIPAM. Once released, it stabilizes microtubules, leading to the arrest of the cell cycle at the G2/M phase. This disruption effectively inhibits tumor cell growth [115]. Nanoxel differs from other clinically approved PTX-loaded polymer micelles, such as Genexol-polymeric NP micelle (Genexol-PM) and Abraxane, in two key ways. First, unlike Abraxane, which is not stimuli-responsive, Nanoxel offers enhanced responsiveness. Second, while Nanoxel is provided as a liquid formulation requiring storage at 2–8°C, Genexol-PM and Abraxane are lyophilized powders that can be stored at room temperature [116].

In a pre-approval phase I dose-escalation study [117], researchers evaluated the MTD, safety profile, and pharmacokinetics of Nanoxel (DO/NDR/02) when administered to patients with refractory or metastatic solid tumors. Twenty-three patients in this study received Nanoxel as a 1-h infusion at doses ranging from 135 to 375 mg/m^2, with a maximum of six treatment cycles. Blood samples were collected during cycles 1 and 2 for pharmacokinetic and toxicity analysis, and PTX Concentrations were determined using a validated HPLC technique. No cases of serious allergic reactions, febrile neutropenia (fever with low white blood cell count), or significant nerve damage were observed. The main non-hematological toxicities included grade 3 infection (without neutropenia) at 135 mg/m^2 and grade 3 diarrhea at 300 and 375 mg/m^2. Major hematological toxicities were grade 4 neutropenia at 135 and 375 mg/m^2. The MTD was established at 375 mg/m^2. One-third of the patients showed improvement, and half of the patients with advanced breast cancer responded well to the treatment. The pharmacokinetic analysis indicated that there was a direct relationship between the drug concentration in the blood over time and the dose given, up to a maximum of 375 mg/m^2. The results of the study suggest the safety of Nanoxel for further investigations.

In 2013, some researchers evaluated the clinical and economic results of using Nanoxel in India [118]. They presented three retrospective analyses carried out in a real-world clinical setting, not a controlled clinical trial environment, along with an economic evaluation. The first analysis focused on

the incidence of infusion reactions associated with Nanoxel. The second compared the frequency of other adverse events between patients treated with Nanoxel and those receiving alternative medications. The third contrasted the effectiveness of Nanoxel versus conventional PTX in managing gastroesophageal tumors. Additionally, the economic model estimated the potential financial impact of Nanoxel compared to conventional PTX for treating solid tumors in India.

The first analysis focused on infusion reactions in 84 patients with various cancers, revealing no hypersensitivity reactions after administering 596 infusions without premedication. The second analysis compared adverse events between 115 patients, with 83 receiving Nanoxel and 32 receiving conventional PTX (Table 26.2). It found that while both groups experienced common toxicities, nausea and vomiting were significantly more prevalent in the PTX group, whereas neutropenia was more frequent in the Nanoxel group. The third analysis assessed effectiveness in patients with gastroesophageal tumors, showing a median survival of 22 months for Nanoxel compared to 12 months for conventional PTX, although this difference was not statistically significant. Economically, Nanoxel treatment resulted in cost savings of approximately 21,580 Indian rupees (USD 471) per 3-week cycle due to lower administration costs and fewer adverse events. Overall, the study suggests that Nanoxel may offer a promising and cost-effective alternative to conventional PTX, potentially improving patient outcomes, though further research is needed to optimize its use.

ONM-100 is a micelle-based formulation consisting of a diblock copolymer of PEG and a poly (methyl methacrylate) derivative, covalently linked to a functionalized IndoCyanine Green (ICG) fluorophore. In acidic environments, the ICG dissociates from the micelle and becomes unquenched, making ONM-100 an ideal candidate for identifying solid tumors during fluorescence-guided surgeries. Several clinical trials have assessed the safety and efficacy of ONM-100 for imaging various solid tumors.

In a clinical trial [119], the safety, pharmacokinetics, imaging feasibility, and range of safe doses of ONM-100 for intra-operative imaging in patients with head and neck squamous cell carcinoma (HNSCC), breast cancer , esophageal cancer, and colorectal cancer were evaluated. From March to December 2018, a total of 30 patients were enrolled in a phase I dose-finding study. During phase Ia, 15 patients with HNSCC and breast cancer received five different intravenous doses of ONM-100: 0.3 mg/kg, 0.5 mg/kg, 0.8 mg/kg, 1.0 mg/kg, and 1.2 mg/kg. In phase Ib, 15 patients with four types of cancer, were administered a single intravenous dose of ONM-100 (1.2 mg/kg), identified as the optimal dose in phase Ia, 24 ± 8 hours before surgery.

All 30 participants completed the study, with ONM-100 demonstrating good tolerability. No dose-limiting toxicities or serious drug-related adverse events were reported. Four possible grade 1 adverse events were observed in the 0.3 mg/kg cohort. Blood counts up to day 10 revealed no abnormalities linked to the imaging agent. ONM-100 plasma exposure correlated linearly with the dose, and the mean half-life in the 1.2 mg/kg cohort was 44.5 ± 15.8 hours. Pharmacokinetics were consistent across tumor types. The study demonstrated that ONM-100 fluorescence successfully detected viable tumors in 29 of 30 subjects, including 100% of tumor-positive surgical margins missed by standard-of-care surgery. It also identified five additional occult lesions. Sensitivity was 100% for both breast cancer and HNSCC, with specificities of 75% and 57%, respectively. While false positives included cases like ductal carcinoma in situ, these findings could still have clinical relevance. Limitations included challenges in visualizing deeper-seated tumors due to NIR imaging constraints. The minimally invasive procedure added minimal time to surgeries, highlighting ONM-100's potential to enhance surgical precision and outcomes.

Table 26.2 Comparison of adverse events between patients treated with Nanoxel and conventional paclitaxel across different system organ classes.

system organ class involved	Adverse event	Nanoxel group (No. of patients)	Adverse events reported (%)	Conventional Paclitaxel Group (No. of patients)	Adverse Events reported (%)	*P* value
Blood and lymphatic system disorders	Anemia	13	28 (14.43)	5	6 (11.76)	0.82
	Neutropenia	26	44 (22.68)	3	5 (9.80)	0.048
	Thrombocytopenia	1	20 (10.31)	2	2 (3.92)	0.267
	Angina	1	1 (0.52)	0	0 (0.00)	1
Cardiac disorders	Diarrhea	12	15 (7.73)	4	8 (15.69)	0.103
Gastrointestinal disorders	Dysphagia	2	2 (1.03)	0	0 (0.00)	1
	Hemetemesis	1	1 (0.52)	0	0 (0.00)	1
	Nausea	4	5 (2.58)	5	5 (9.80)	0.035
	Vomiting	11	19 (9.79)	4	11 (21.57)	0.03
	Intestinal obstruction	1	1 (0.52)	0	0 (0.00)	1
	Fever	10	13 (6.70)	1	1 (1.96)	0.311
General disorders and administration site conditions	Redness and edema at injection site	1	1 (0.52)	0	0 (0.00)	1
	Weakness	1	1 (0.52)	1	2 (3.92)	0.11
	Body ache	2	2 (1.03)	0	0 (0.00)	1
	Pedal edema	1	1 (0.52)	0	0 (0.00)	1
	Mucositis	2	4 (2.06)	0	0 (0.00)	0.582
	Herpes zooster	1	1 (0.52)	0	0 (0.00)	1
Infections and infestations	Dehydration	2	2 (1.03)	0	0 (0.00)	1
Metabolism and nutrition disorders	Hypokalemia	1	1 (0.52)	1	2 (3.92)	0.11
	Hyponatremia	1	1 (0.52)	0	0 (0.00)	1
	Hypoproteinaemia	1	1 (0.52)	0	0 (0.00)	1

Continued

Table 26.2 Comparison of adverse events between patients treated with Nanoxel and conventional paclitaxel across different system organ classes.—cont'd

system organ class involved	Adverse event	Nanoxel group (No. of patients)	Adverse events reported (%)	Conventional Paclitaxel Group (No. of patients)	Adverse Events reported (%)	*P* value
	Pain in extremities	2	9 (4.64)	0	0 (0.00)	0.21
Musculoskeletal disorders	Leptomeningeal-carcinomatosis	0	0 (0.00)	1	1 (1.96)	0.208
Neoplasms benign, malignant and unspecified	Neuropathy	6	11 (5.67)	0	0 (0.00)	0.126
Nervous system disorders	Burning of sole	1	1 (0.52)	0	0 (0.00)	1
	Convulsion	1	1 (0.52)	0	0 (0.00)	1
	Irrelevant talk	0	0 (0.00)	1	1 (1.96)	0.208
Psychiatric disorders	Cough	1	1 (0.52)	0	0 (0.00)	1
Respiratory, thoracic and mediastinal disorders	Pneumonia	1	1 (0.52)	0	0 (0.00)	1
	Dyspnea	1	1 (0.52)	0	0 (0.00)	1
	Erythmatous rash	1	1 (0.52)	0	0 (0.00)	1
Skin disorders	Itching	1	1 (0.52)	0	0 (0.00)	1
Vascular disorders	Deep vein thrombosis	2	2 (1.03)	0	0 (0.00)	1

3.2 Redox-responsive nanocarriers

Redox-responsive NPs typically contain functional groups or bonds such as diselenide, disulfide, platin conjugates, thioether, or thiol, sensitive to ROS and glutathione levels [120]. Preclinical studies have explored the use of redox-responsive nanocarriers in various applications, including tumor targeting, brain disease treatment, cardiovascular disease management, and as agents for anti-aging therapies [121,122]. Redox nanomaterials hold significant promise for disease prevention and treatment in preclinical research; however, their clinical application remains limited. Current research has mainly focused on in vitro studies and xenograft-based animal models. These experimental models, however, do not accurately replicate human tumors or other pathological conditions, especially metastatic tumors. Consequently, they fall short of simulating the cellular and tissue damage resulting from oxidative stress in the human body [121,123]. To our knowledge, no redox-responsive nanocarrier has yet advanced to clinical trials. Table 26.3 provides a summary of recent redox responsive nanocarriers (in vitro and in vivo) developed for tumor theranostics.

While redox-responsive nanoformulations have yet to progress to clinical trials, some redox-responsive prodrugs are currently undergoing clinical evaluation. For example, Evofosfamide (TH-302) is a redox-responsive prodrug containing a 2-nitroimidazole moiety linked to bromo-isophosphoramide mustard (Br-IPM). This drug is a substrate for certain cellular reductases, enzymes that catalyze chemical reductions. Under hypoxic conditions, which are common in tumors, reductase reduces TH-302, leading to the release of Br-IPM, a DNA cross-linking agent, or isophosphoramide mustard [144]. TH-302 first entered clinical trials in 2007. In a phase III clinical trial, the efficacy of TH-302 in combination with DOX was compared to DOX monotherapy. A total of 640 patients with soft tissue sarcoma were enrolled and randomly assigned to receive either DOX alone (75 mg/m^2 intravenously On the first day of each 21-day cycle, for a maximum of six cycles) or DOX (same dose and schedule) in combination with TH-302 (300 mg/m^2 intravenously on days 1 and 8 of every 21-day cycle for up to six cycles). The results indicated that combination therapy did not improve overall survival compared to DOX monotherapy [145].

Tirapazamine (TPZ) is another redox-responsive drug that damages DNA. This benzotriazine compound undergoes reduction by cellular reductase in hypoxia [146]. Although early results were promising, phase III clinical trials for TPZ were disappointing, showing no therapeutic advantage over standard chemoradiotherapy or chemotherapy alone [147]. However, the trial outcomes were influenced by the poor quality of radiotherapy, and when adjusted for this, the TPZ treatment arms demonstrated better performance. It was also hypothesized that the lack of efficacy was due to excessive drug consumption, which impaired extravascular transport. Additionally, TPZ's activation under relatively mild hypoxia, commonly found in the liver, gastrointestinal tract, and bone marrow, may have contributed to increased toxicity [148].

PR-104, a phosphate-based precursor to PR-104A, is a dinitrobenzamide mustard extensively studied in both preclinical and clinical settings. This hypoxia-activated prodrug includes an inactive nitrogen mustard group that is activated under conditions of severe hypoxia, leading to cell death through the formation of DNA cross-links [149]. In a phase I clinical trial, the efficacy of PR-104 in combination with sorafenib (a tyrosine kinase inhibitor) in patients with HCC [150]. At the PR-104 dose of 770 mg/m^2, six patients received treatment across 14 total cycles, with a median of 2 cycles per patient (range: 1–4). Following a dose reduction to 550 mg/m^2, eight patients were treated for a total of 19 cycles, with a median of 3 cycles each (range: 1–3). At both dose levels, the median daily

Table 26.3 Representative examples of redox-responsive nanocarriers used for tumor theranostics in vitro and in vivo.

Nanoplatforms	Types of NPs	Cargos	Therapeutic strategies and efficacy	Refs.
$Au@Cu_2 - {}_xS$	Nanoreactor	–	A robust Au@Cu2 − xS nanoreactor assembled by silk fibroin for enhanced intratumoral glucose depletion and redox dyshomeostasis	[124]
diPTX@ Fe&TA NPs	Heterozygous NPs	PTX	Multiple stimuli-responsive metal-phenolic network prodrug NPs for chemotherapy	[125]
DMP-NPs	Polymer	6-MP	Drug/carrier dual redox-responsive system for enhanced lymphoma therapy	[126]
PolyHPMA-DOX	Polymer	DOX/ Ce6	Chemo-photodynamic NPs with GSH-sensitive polymeric prodrug have a great potential as a chemo-photodynamic anti-cancer agent.	[127]
PDPP@D	Dendritic polymer	DOX	Facilitate the effective delivery of two anti-tumor drugs to the deep tumor tissue via the neighboring effect, lead ing to irreversible ER stresses and impaired cell protection for destroying the entire solid tumor.	[128]
MPM@P NGs	Polymer nanogels	Cisplatin	Guided chemotherapy/CDT of orthotopic glioma and enhanced CDT	[129]
TAB004-Gem-cisPt-MSN	Heterozygous MSN NPs	Gem/ cisPt	Gemcitabine-cisplatin combination for improved therapeutic outcome in pancreatic cancer by redox-sensitive NPs.	[130]
Fc-Mn NSs	Nanomedicine	–	Engineering Seed-like Fe-Mn Nanomedicine with Ultrasmall Structure and ATP-Responsive Function for Tumor Metabolic and Redox Subversion.	[131]
PLGA-SS-PEG NPs	Copolymers	DTX/ TUBB3	Combining DTX with TUBB3 siRNA in redox-responsive NPs to reduce anticancer drug resistance.	[132]
SRNs	Copolymers	DOX/ IMDQs	The reductive-sensitive and SIL can dually respond to the acidic and high reductive TME for the induction of a tumor ICD effect.	[133]
A-G/NT	Nanodevice	–	The system can be lighted up only when meeting the “dual keys” (overex pressed ATP and GSH in mitochondria) simultaneously.	[134]
G&B@MOF	Nanocarrier	GOx/3-BrPA	Stimuli-responsive cancer nanomedicines inhibit glycolysis and impair redox homeostasis.	[135]
MSN-SP	MSNs	DOX	Overexpressed GSH can be employed to initiate the self-immolation of dif ferent redox-responsive structures.	[136]
CDCs/DOX@ CCM	Carbon Dot Nanoclusters	DOX	Redox-sensitive NPs realizing efficient targeted tumor fluorescence imaging and chemotherapy.	[137]
VC@Lipo	Nanocarrier	VOx/Ce6	Tumor Microenvironment-Responsive Nanocarrier Based on VOx Nano zyme Amplify Oxidative Stress for Tumor Therapy.	[138]

Table 26.3 Representative examples of redox-responsive nanocarriers used for tumor theranostics in vitro and in vivo.—cont'd

Nanoplatforms	Types of NPs	Cargos	Therapeutic strategies and efficacy	Refs.
PolyHCPT NPs	Heterozygous NPs	HCPT	Targeted siRNA delivery and synergistic liver cancer therapy	[139]
NP-RGD (Arginine-glycine-aspartic)	Heterozygous NPs	1-RGD	Redox-sensitive PSs nano-assemblies can enhance delivery and controlled release of PSs.	[140]
Ce6@RMOF	MOFs	Ce6	Ferroptosis machinery contributes to the apoptotic photodynamic anti-tumor therapy.	[141]
MS@MnO2 NPs	Heterozygous NPs	–	Glutathione depletion and to enhance chemodynamic therapy.	[142]
Micelles R4 and R5	Micelles	Curcumin	SILs micellar drug delivery platform for anticancer therapy.	[143]

sorafenib dose was 400 mg, ranging from 200 to 800 mg. In the 770 mg/m^2 cohort, one patient achieved a partial response, and three patients experienced stable disease lasting 8 weeks or longer. In the 550 mg/m^2 cohort, three patients demonstrated stable disease. The combination of PR-104 and sorafenib was poorly tolerated in patients with advanced HCC, likely due to impaired clearance of PR-104A in this group. Thrombocytopenia, primarily, and neutropenia were the most significant toxicities, leading to discontinuation of the study. Therefore, PR-104 combined with sorafenib was unlikely to be a viable treatment option for development in this context. A separate study combining PR-104 and docetaxel for NSCLC was halted prematurely after an interim analysis suggested a low probability of achieving significant results (NCT00862134).

TPZ, is a benzotriazine di-N-oxide compound. Once activated under hypoxic conditions by reductases, TPZ can inhibit topoisomerase I and DNA replication [151]. Several clinical trials studied the efficacy and safety of this drug alone or in combination with chemotherapy and radiotherapy. In phase III clinical trial, the efficacy of TPZ in combination with cisplatin chemoradiotherapy compared tocisplatin chemoradiotherapy alone [152]. Patients were randomized to receive either weekly cisplatin at 40 mg/m^2 or a combination of TPZ and cisplatin during chemoradiotherapy. Initially, TPZ was administered at 290 mg/m^2 and cisplatin at 75 mg/m^2 on specific days. However, following a safety review, the doses were adjusted to 220 mg/m^2 for TPZ and 60 mg/m^2 for cisplatin on those same days. All patients underwent external-beam pelvic radiation therapy (RT) at a total dose of 41.4–45 Gy, delivered in 23–25 fractions of 1.8 Gy each, without the use of intensity-modulated RT. After completing external radiation, patients received brachytherapy, with doses tailored to either low-dose-rate or high-dose-rate protocols. Progression-free survival was assessed in 387 of the 402 patients randomly assigned over 36 months, with enrollment concluding in September 2009. After a median follow-up of 28.3 months, Progression-free survival and overall survival were similar between the two groups. The 3-year progression-free survival rates were 63.0% for the TPZ/CIS/RT arm and 64.4% for the cisplatin/RT arm (log-rank $P = 0.7869$). Similarly, the 3-year overall survival rates were 70.5% and 70.6%, respectively (log-rank $P = 0.8333$). A scheduled interim safety review prompted a dose reduction in the TPZ/cisplatin arm, improving tolerability in both treatment groups. The TPZ/cisplatin

chemoradiotherapy regimen did not demonstrate superiority over cisplatin chemoradiotherapy in terms of PFS or OS. However, definitive conclusions were limited due to an insufficient number of progression or death events. The TPZ/cisplatin regimen was well tolerated when administered at a reduced initial dose.

3.3 Enzyme-responsive nanocarriers

The enzyme-responsive component of enzyme-responsive nanocarriers can be located in the core, on the surface, or act as a linker or ligand. Drug release is triggered by enzymatic interactions, leading to structural alterations such as disintegration, deformation, or bond cleavage [153]. These nanosystems are sensitive to hydrolases, proteases, lipase, glycosidase, and oxidoreductases [154]. One or more of these enzymes are overexpressed in the affected tissues.

Using enzymes as a trigger offers several advantages, as they typically catalyze chemical reactions under gentle conditions, such as low temperatures, neutral pH, and buffered aqueous environments—conditions where many traditional chemical reactions are ineffective [155]. Additionally, enzymes possess remarkable substrate specificity, enabling them to catalyze highly selective and complex biochemical reactions [156].

Although considerable progress has been achieved, many obstacles still need to be addressed before a clinically viable formulation can be developed. A comprehensive understanding of disease-related enzymes is essential, as enzyme activity can vary significantly across different diseases, patients, and even disease stages [156,157]. Special attention should be given to the design of enzyme-specific linkages and substrates, as many enzyme families and subtypes may share similar cleavage sites [158].

Genexol-PM, a PTX-loaded polymer micelle composed of monomethoxy poly(ethylene glycol)-poly(D,L-lactide) (mPEG-PDLLA), was approved by the EMA and South Korean authorities in 2007 for treating breast, ovarian, lung, and pancreatic cancers [159]. These micelles are sensitive to esterase, an enzyme overexpressed in tumor cells, and their drug release mechanism is triggered by the cleavage of PDLLA ester bonds, allowing for targeted delivery in cancer tissues [160]. Genexol-PM size is 23.91 ± 0.41 nm, with a surface charge of -8.1 ± 3.1 mV [161].

In a Phase II clinical trial (NCT01426126) [162], the safety and efficacy of Genexol-PM were evaluated in patients with advanced urothelial carcinoma who had not responded to previous treatment with gemcitabine and cisplatin. Patients received 240 mg/m^2 of Genexol-PM intravenously over a 3-h infusion every 3 weeks. They found that Genexol-PM produced an objective response rate of 21% and a disease control rate of 65%. Both of these rates surpassed those seen with second-line treatments like CrEL-based PTX, docetaxel, ifosfamide, or vinflunine, following platinum-based regimens. The adverse effects of Genexol were notably lower compared to Cremophor-based PTX. In particular, they observed grade 3/4 neutropenia in only 3% of patients, and none experienced febrile neutropenia, highlighting reduced rates of hematologic toxicities. In conclusion, the findings indicated that Genexol-PM was well tolerated and showed promising antitumor activity, supporting further exploration as a second-line treatment for patients with urothelial carcinoma who did not respond to previous gemcitabine-cisplatin therapy.

In another phase II clinical trial, the efficacy of genexol-PM and cisplatin was evaluated in the chemotherapy of locally advanced HNSCC patients [163]. Although the role of induction chemotherapy in locally advanced HNSCC had been debated, some research indicated that a sequential

approach with induction chemotherapy can offer a survival advantage. However, the standard regimen of docetaxel, cisplatin, and 5-fluorouracil (TPF), while effective, was associated with significant toxicity. As a result, some patients were unable to proceed with the planned definitive concurrent chemoradiotherapy due to adverse effects experienced during induction chemotherapy, highlighting the need for a less toxic alternative.

Patients were treated with Genexol-PM (230 mg/m^2) in combination with cisplatin (60 mg/m^2) every 3 weeks as part of their induction chemotherapy (first-line treatment of cancer with a chemotherapeutic drug before surgery or radiotherapy). Fifty-two patients enrolled in the study. They received Genexol-PM (230 mg/m^2) and cisplatin (60 mg/m^2) every 3 weeks. Following three cycles of induction therapy, patients received either concurrent chemoradiotherapy with weekly cisplatin (30 mg/m^2) or surgical intervention as definitive treatment. The main goal was to assess the overall response rate after the induction chemotherapy.

The results indicated that while the overall response rate of Genexol-PM/cisplatin (55.8%) was slightly lower than that of TPF (64%–80%), the 3-year progression-free survival (the time from random assignment in a clinical trial to disease progression or death from any cause.) and overall survival (54.3% and 71.3%, respectively) align closely with those reported in previous studies. Furthermore, the toxicity profiles of Genexol-PM/cisplatin were notably favorable, demonstrating fewer grade 3 or 4 adverse events compared to TPF. In conclusion, Induction chemotherapy with Genexol-PM and cisplatin showed a modest tumor response and was associated with tolerable toxicity levels in patients with locally advanced HNSCC.

In a phase III trial [164], researchers compared the efficacy of PTX with Genexol-PM in patients diagnosed with NSCLC. A total of 448 patients with stage IIIB to IV NSCLC participated in the study. Participants were randomly assigned to receive six cycles of treatment every 3 weeks: either Genexol-PM (230 mg/m^2) combined with cisplatin (70 mg/m^2; n = 300) or solvent-based PTX (175 mg/m^2) with cisplatin (70 mg/m^2; n = 148). The dose of Genexol-PM was increased to 300 mg/m^2 starting from the second cycle, as no toxic effects were observed after the first cycle, allowing for the dose escalation. The study aimed to evaluate the objective response rate and progression-free survival based on assessments from independent review committees.

Patients in the Genexol-PM group showed a notable rise in objective response rates compared to those receiving solvent-based PTX (50% vs. 26%; rate ratio of 1.91; $P < .0001$). Additionally, the mPFS) was significantly longer in the Genexol-PM group than in the solvent-based PTX group (6.4 vs. 5.3 months; hazard ratio of 0.63; $P = .0001$). However, there was no significant difference in median overall survival between the two groups. Furthermore, the incidence of treatment-related serious adverse events was significantly lower in the Genexol-PM group (9% compared to 18%; $P = .0090$) than in the solvent-based PTX group.

Some other clinical trials have investigated Genexol-PM as a candidate for the treatment of metastatic and advanced breast cancer (NCT02064829, NCT02064829, NCT00876486, NCT01169870, NCT00912639, NCT01784120), HCC (NCT03008512), advanced ovarian cancer (NCT01276548, NCT00886717, NCT00877253, NCT05300828), pancreatic cancer (NCT02739633, NCT00111904, NCT00882973), advanced head and neck cancer (NCT01689194), and NSCLC (NCT01770795, NCT01023347).

LiPlaCis is a cisplatin-loaded liposomal formulation that targets solid tumor cells by being sensitive to the overexpressed secretory phospholipase A2 enzyme [159]. This liposome is composed of 1,2-distearoyl-sn-glycero-3-phosphocholine, 1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)]

(sodium salt) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] [165].

Some clinical trials are evaluating the safety and efficacy of LiPlaCis. In a clinical trial (NCT01861496) [142], 12 patients with metastatic breast cancer received 75 mg of LiPlaCis intravenously on days 1 and 8 of every 3-week cycle. Two patients did not complete a full cycle due to early death, which was deemed unrelated to LiPlaCis. Objective response rate (the percentage of individuals in a study or treatment group who exhibit a partial or complete response to the treatment within a specified timeframe) and progression-free survival were used to measure effectiveness, comparing outcomes with the most recent prior treatment of the patients. The objective response rate in the intention-to-treat group (an approach that includes all participants randomized in a trial, regardless of whether they completed it, in the analysis) was 17%. Of the 10 patients evaluated, 6 had a high drug response predictor(DRP) score and 4 had a moderate score. LiPlaCis was the eighth treatment option for advanced disease. Two patients had partial remissions, five had stable disease, and three had progressive disease. The 6 patients with the highest DRP scores, who were platin-naïve, all showed clinical benefit, with a mPFS of 25 weeks, compared to 17 weeks with their previous treatment. Three of these patients had better responses than with earlier treatments, and the objective response rate was 40%. In contrast, two patients with moderate DRP scores had shorter progression-free survival. LiPlaCis was generally well tolerated, with 4 grade 3 and 2 grade 4 side effects related to the drug over 43 treatment cycles. The results demonstrated that LiPlaCis was effective in treating patients with metastatic breast cancer.

4. Innovations in nanocarrier design

Part of the recent innovations in nanocarrier technology have focused on enhancing the functionality of existing commercial formulations. This section examines two primary areas of development: (1) the modification of non-responsive nanocarriers to incorporate stimuli-responsive properties, and (2) the refinement of stimuli-responsive nanocarriers already in clinical trials or on the market. These advancements aim to improve the efficacy, specificity, and safety profiles of nanocarrier-based therapies. In the following discussion, each example or set of examples will be introduced by the name of the commercial nanoformulation, providing a clear structure for exploring these innovations.

Ambisome. AmBisome is a liposomal formulation of amphotericin B, consisting of unilamellar vesicles approximately 100 nm in diameter. It improves drug delivery and reduces toxicity compared to conventional amphotericin B. While AmBisome, as a commercially available liposomal formulation, is effective in treating fungal infections, it does not possess the characteristics of stimuli-responsive nanocarriers that react to specific stimuli to release their drug payload. Instead, it functions through a more traditional drug delivery mechanism that focuses on enhancing the pharmacokinetics and reducing toxicity of amphotericin B. In a study by Saputra et al., the performance of AmBisome was enhanced by making it protease-responsive [166]. They decorated the liposomes with a lipid-modified chitin-binding domain (LysM) linked to a thrombin-cleavable connector. This design allowed the formulation to remain stable in the bloodstream but release LysM in response to thrombin at infection sites. The released LysM binds to chitin in fungal cell walls, enhancing the antifungal activity of AmBisome. The modified formulation showed uniform size distribution and stability over 4 weeks at 4°C, and it demonstrated improved antifungal effectiveness against Candida albicans in the presence of thrombin compared to unmodified AmBisome. This innovative approach aimed to

improve targeted delivery and efficacy in antifungal therapy, offering potential advantages over traditional formulations.

Feraheme. Ferumoxytol (Feraheme) is an FDA-approved USPIO NP used for treating iron deficiency anemia in adults with chronic kidney disease. However, safety concerns have arisen, prompting increased scrutiny. In March 2015, the FDA issued a boxed warning—their strongest warning—due to reports of serious hypersensitivity reactions, including anaphylaxis, associated with its use. Reports indicated instances of severe reactions, including fatalities. These safety issues underscore the need for developing safer contrast agents for angiography with improved performance. One innovative approach involves loading red blood cells (RBCs) with ferucarbotran NPs to create long-circulating magnetic contrast agents. In a notable study, RBC-encapsulated ferucarbotran demonstrated significantly extended circulation time, remaining in the bloodstream for approximately 14 days, compared to free NPs that cleared within about 1 hour [167]. This substantial improvement in retention and biocompatibility suggests that these RBC-based constructs could be promising tools for advanced imaging techniques, such as MRI and magnetic particle imaging.

NanoTherm. SPIO NPs have been FDA-approved for imaging and therapy (e.g., NanoTherm), but not yet for drug delivery. A novel approach combined hyperthermia, a feature that already exists in Nanotherm, and pH-triggered drug release in a single platform using SPIO NPs encapsulated in a dual pH- and temperature-responsive poly(N-vinylcaprolactam-co-acrylic acid) copolymer [168]. This system responded to multiple stimuli (pH and temperature), providing controlled drug release, and enabling localized magnetic hyperthermia. The magnetic core consisted of flower-like magnetite NPs (16.4 nm). The nanocarrier demonstrated a high encapsulation efficiency of over 96% for DOX at neutral pH. Under acidic and heated conditions, the carrier released its drug payload rapidly and almost completely, while maintaining minimal release under normal physiological conditions. These systems provide a crucial advantage in oncology by precisely targeting cancer cells while preserving healthy tissues, potentially improving efficacy while reducing side effects.

In another example, an injectable hydrogel system (MNPs@NO-Gel) was developed for enhanced cancer treatment, combining mild magnetic hyperthermia therapy (MHT) with nitric oxide (NO) release [169]. This system consisted of thermosensitive poly(ethylene glycol)-polypeptide copolymers modified with NO donors, encapsulating ferrimagnetic $Zn_{0.5}Fe_{2.5}O_4$ magnetic NPs. The hydrogel underwent a sol-gel transition upon heating and demonstrated the ability to perform multiple MHT sessions after a single administration due to the uniform distribution and strong immobilization of MNPs within the gel matrix. The MNPs@NO-Gel system maintained its integrity in vivo for over a month, allowing for sustained NO release accelerated by MHT. The continuous NO release inhibited MHT-induced autophagic flux in tumor cells, enhancing the efficacy of mild hyperthermia (41–46°C) while minimizing damage to surrounding tissues. Notably, the $Zn_{0.5}Fe_{2.5}O_4$ MNPs in NO-Gel exhibit a significantly higher intrinsic loss power value (8.44 nH m^2/kg) compared to commercial NanoTherm (0.74–0.99 nH m^2/kg) under safe magnetic field conditions for human MHT. This improved performance, combined with the synergistic effects of NO release and mild hyperthermia, resulted in complete elimination of CT-26 colon tumors without severe side effects, demonstrating the potential of this system for more effective and less invasive cancer treatment.

NC-6300. NC-6300 is a NP formulation of epirubicin that utilizes a pH-sensitive linker to conjugate epirubicin to a block-copolymer. This pH-sensitive hydrazine linkage enables the selective release of epirubicin in the acidic environment of tumor tissue and late endosomes in tumor cells. The pH-responsiveness allows NC-6300 to deliver epirubicin more efficiently to tumor tissue than to normal

tissue, taking advantage of the EPR effect. Recent advancements have further improved NC-6300's capabilities. One development is the creation of immunomicelles by conjugating monoclonal antibodies (mAbs) to NC-6300 [170,171]. Specifically, anti-tissue factor (TF) F(ab')2 fragments resulted in faster and greater intracellular uptake of epirubicin in TF-overexpressing cancer cells and superior antitumor activity in xenograft models [171]. Furthermore, studies comparing different antibody-conjugated NC-6300 formulations have demonstrated that the choice of targeting antibody significantly impacts therapeutic outcomes, with anti-TF-NC-6300 showing improved antitumor activity and more uniform distribution in TF-positive tumors [170]. Another innovation involves combining NC-6300 with trigger-pulsed high-intensity focused ultrasound (TP-HIFU), leveraging NC-6300's sonosensitizer properties to promote ROS generation while protecting epirubicin from ROS-induced degradation [172]. This approach harnesses the unique effects of HIFU, the anticancer properties of epirubicin, and increased production of ROS to effectively damage cancer cell. These advancements highlight NC-6300's potential as a versatile platform for developing more effective and targeted cancer therapies, combining the benefits of NP drug delivery with active targeting and external stimuli responsiveness.

Opaxio. Opaxio, also known as PTX poliglumex, is a NP formulation of the chemotherapy drug PTX designed to enhance drug delivery and efficacy while minimizing side effects. It utilizes a polymer called polyglutamic acid (PGA) to create a conjugate that improves the solubility of PTX and enables targeted delivery to tumor tissues by exploiting the EPR effect. Recent advancements have further improved Opaxio's effectiveness with the development of a new conjugate, PGA-PTX-E-[c(RGDfK)$_2$] [173]. The proposed formulation combined PTX with a cyclic RGD peptide that specifically targets the αvβ3 integrin, which is overexpressed in tumor endothelial and epithelial cells. This dual targeting strategy, passive targeting through the EPR effect and active targeting via integrin binding, made it particularly valuable for well-vascularized tumors that may exhibit poor vascular permeability. Importantly, the PGA component of this conjugate is enzymatically responsive, allowing for the release of PTX in response to cathepsin B that is overexpressed in solid tumors. The PGA-PTX-E-[c(RGDfK)$_2$] conjugate demonstrated improved anti-tumor activity by inhibiting cancer cell growth and blocking endothelial cell migration and tube formation. In orthotopic studies in mice, this targeted approach resulted in preferential tumor accumulation, enhanced anti-tumor efficacy, and significantly reduced toxicity compared to free PTX. These advancements position Opaxio as a more effective and safer option for cancer treatment.

Previous studies have explored the synthesis of a modified poly(L-γ-glutamyl-glutamine)-PTX nanoconjugate (PGG-PTX), which builds upon the existing poly(L-glutamic acid)-PTX conjugate (PGA-PTX) [174]. The modification involved adding an additional glutamic acid to each glutamic side chain in the polymer backbone. This alteration significantly enhanced the water solubility and dissolution rate of PGG-PTX compared to PGA-PTX, facilitating its self-assembly into NPs. In vitro cell toxicity assays revealed that both PGA-PTX and PGG-PTX exhibit similar toxicity profiles, with both being less toxic than free PTX. However, in vivo studies in mice demonstrated a notable difference in toxicity between the two conjugates. PGG-PTX showed lower toxicity compared to PGA-PTX, with a MTD that was 2.2-fold higher than the MTD of 160 mg PTX/kg reported for PGA-PTX. In a more recent study by Wang et al., the contrast agent, SPIO NP, was encapsulated into the drug-loading polymer, PGGA-PTX to construct a diagnostic and treatment system (Fig. 26.4A) [175]. The incorporation of SPIO NPs into pGGA-PTX resulted in a uniform distribution within the core (Fig. 26.4B, lower right image), leading to a slight increase in particle size compared to pGGA-PTX NPs alone. In vitro cellular experiments and in vivo studies (Fig. 26.4C and D) further revealed that pGGA-PTX/SPIO NP were more therapeutically effective than pGGA-PTX while making it possible to track drug distribution inside the body.

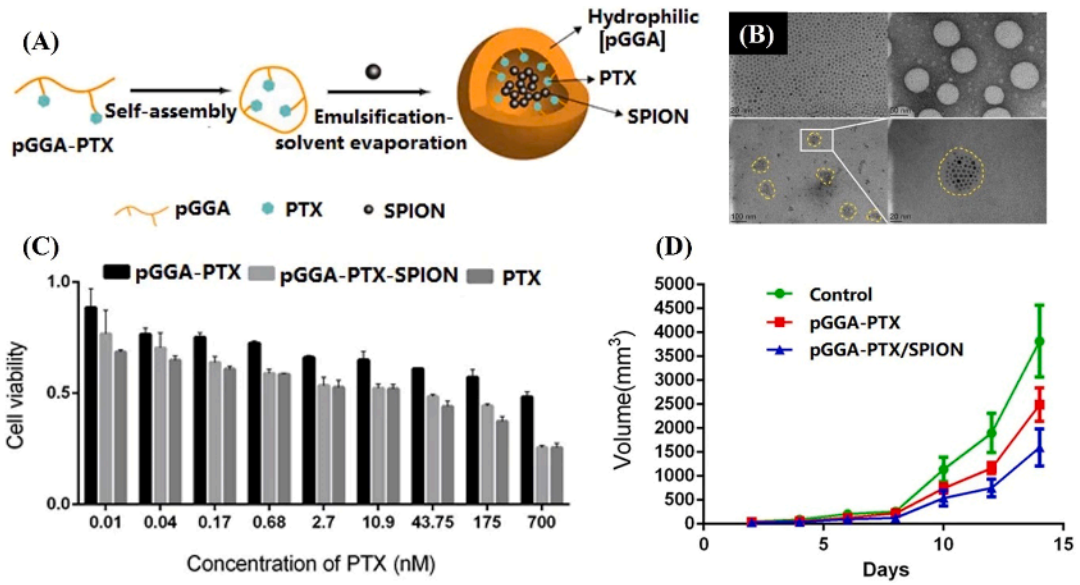


FIGURE 26.4

(A) Schematic representation of SPION encapsulation into self-assembled pGGA-PTX NPs, (B) morphology of the hybrid NPs observed via TEM, (C, D) comparison of the therapeutic efficacy of pGGA-PTX-SPION to SPION-free NPs and free PTX in vitro and in vivo [175].

5. Challenges and future directions

Recent advancements in synthetic chemistry and fabrication techniques have greatly enhanced the use of stimuli-responsive materials for targeting various diseases. However, despite numerous successes, the clinical translation of these nanomedicines remains limited.

One of the reasons is minimal differences between healthy and diseased tissue can result in premature or unintended drug release, thus creating formulations with high specificity requires a deeper understanding of target tissue physiology and disease markers [15]. Another challenge is that most nanoformulations depend on the EPR effect, which limits active, tissue-specific drug accumulation and release [176]. Biosafety is another concern in developing stimuli-responsive nanocarriers. Although numerous internal and external stimuli-responsive nanoformulations are safe in vitro and in vivo, the mechanisms within the human body differ and can sometimes lead to systemic or local toxicity [15]. Additionally, efforts to enhance scalability, stability, and cost-efficiency, particularly through scalable systems, will be crucial for broader clinical adoption [24,55,177].

Incorporating endogenous stimuli into nanoformulations increases complexity, presenting potential obstacles to regulatory approval. The variability in endogenous stimuli among patients can limit efficacy [178]. Future research should prioritize developing bioanalytical tools to map biomarkers more precisely, enabling improved patient-personalized treatments [159]. pH-responsive NPs show considerable promise for drug delivery, with the ionizable lipid-based drug Onpattro, featuring a pH-responsive mechanism, standing out as a rare success. However, a key challenge for these systems is

that the acidity within affected tissues, particularly tumors, is often inconsistent, which can lead to inaccurate drug release. Additionally, similar acidity levels at inflammation sites raise the risk of off-target effects [179].

Enzyme-responsive NPs have been extensively examined for cancer treatment. One obstacle to applying these NPs in clinical studies is the considerable complexity of the tumor microenvironment, which features varying enzyme activity across different cancer types and stages. This variability complicates the creation of a universal enzyme-responsive delivery platform for anti-tumor therapies. Additionally, overlapping substrate preferences among enzymes challenge the specificity of drug release mechanisms. For example, matrix metalloproteinases, which consist of over 20 similar proteinases, can exhibit overlapping substrate preferences, leading to difficulties in designing enzyme-specific substrates [153]. LiPlaCis, a phospholipase A2 enzyme-responsive liposomal cisplatin, has been in phase III clinical trials but struggles with issues like variable biomarker expression among patients [159].

As mentioned earlier, none of the redox-responsive nanocarriers have been successfully translated to clinical use. One reason for this is their low therapeutic effectiveness and high costs. While redox nanodrugs generally have longer-lasting effects and fewer side effects than traditional drugs, their overall therapeutic efficacy is currently insufficient for large-scale clinical use. Additionally, the design costs associated with their complex structures, particularly multifunctional nanoplatforms, are prohibitively high [121]. Another reason is that the effectiveness of reduction-responsive systems is influenced by varying levels of intracellular glutathione and abnormal redox potentials across different cancer types and stages. A thorough understanding of the mechanisms regulating redox signaling is essential for developing personalized and effective polymer delivery agents. Furthermore, while numerous reduction-responsive polymers have been identified, their activation may differ among cell types, complicating their application in various therapeutic contexts, including non-cancer therapies such as antiviral treatments or vaccines [177].

Exogenous controlled stimuli-responsive systems, such as ThermoDox and NanoTherm, have seen greater success in clinical settings, due to their ability to be more efficiently controlled and monitored. However, identifying an external stimulus that can penetrate tissues deeply and target specific locations remains challenging [18].

One limitation of using magnetic NPs in clinical applications is their inability to remain at the target site once the external stimulus is removed, which impedes their use in prolonged or chronic drug delivery. Another challenge in applying these NPs in clinical settings is the difficulty of interpreting the T2 signal in MRI imaging [55]. One of the challenges in using US-responsive nanocarriers is managing the heat generated by acoustic vibrations, which requires further exploration and control to prevent potential damage to healthy tissues [180]. A key challenge in designing thermoresponsive nanocarriers is selecting materials that are both safe and responsive to temperatures close to the body's physiological range [9].

Although stimuli-responsive nanocarriers are generally safe and offer certain advantages, further development is needed to meet clinical requirements. As mentioned earlier, one issue is achieving sufficient accumulation of NPs in the target organ. Future research could focus on employing ligands that interact with specific receptors on the surface of target cells or tissues, as well as incorporating multi-stimuli-responsive nanocarriers, which would enable more precise responses to environmental changes and facilitate more efficient drug release. A second area of research could involve more extensive in vivo and in silico studies of endogenous nanocarriers to better understand their

mechanisms of action and physiological environments of normal and diseased cells to identify more effective delivery vehicles, particularly for redox-responsive nanoformulations. A third research focus could be identifying safe, biodegradable materials with efficient scale-up methods to improve the safety and pharmacokinetics of stimuli-responsive nanocarriers. This approach could facilitate the advancement of more stimuli-responsive NPs into clinical trials.

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Innovative Nanocarriers for Stimulated Drug Delivery

A Comprehensive Guide to Stimuli-responsive Nanoparticles

Edited by
Prashant Kesharwani

Innovative Nanocarriers for Stimulated Drug Delivery: A Comprehensive Guide to Stimuli-responsive Nanoparticles summarizes recent advances and achievements in nanocarriers responsive to external or internal stimuli. The book presents different stimuli-sensitive strategies and their applications in drug delivery, therapy, and theranostics. Sections provide fundamental knowledge on the experiential and experimental aspects of stimuli-responsive nanocarriers; discuss external-responsive nanocarriers for biomedical applications, such as ultrasound, thermal, magnetic, light, and electric field-response systems; and describe internal stimuli-responsive nanocarriers for biomedical applications, including pH, hypoxia, redox potential, enzyme, glucose, and microRNA-responsive nanocarriers.

Additionally, the book covers dual- and multimodal-responsive nanocarriers and the therapeutic aspects of stimuli-responsive nanocarriers, such as metallic and non-metallic stimuli-responsive nanocarriers, in the treatment of various cancer and noncancerous diseases. Finally, recent advances and regulatory aspects of stimuli-responsive nanocarriers, including clinical translation, challenges, biological toxicity, safety issues, clinical trials, and future perspectives, are covered.

Key Features

- Covers all aspects of stimuli-responsive nanocarriers for targeted drug delivery
- Includes external, internal, and dual/multimodal stimuli-response-driven nanoparticles
- Contains a dedicated therapeutic application section, mapping the translation from research to practice

About the Editor

Dr. Prashant Kesharwani, PhD, is a leading researcher and academic in the field of nanomedicine, with extensive expertise in targeted drug delivery systems, dendrimer-based therapeutics, polymeric nanocarriers, and nanotechnology-enabled pharmaceutical formulations. He earned his PhD in Pharmaceutics and completed his postdoctoral research in the United States, where he gained advanced experience in nanocarrier design and translational biomedical applications. Dr. Kesharwani has received several prestigious international recognitions, including the USERN Laureate 2023 in Biological Sciences, awarded through a highly competitive global selection process. He has secured multiple competitive research grants from national funding agencies and has been consistently listed among the World's Top 2% Scientists (2020-2025) by Stanford University, USA. He has authored over 400 research articles and edited more than 30 books, with a cumulative impact factor exceeding 9,000. His work has received over 36,000 citations, with an h-index of 103 and an i10-index of 476 (as of April 2026), placing him among the highly cited researchers in his field. Dr. Kesharwani currently serves as an Assistant Professor in the Department of Pharmaceutical Sciences at Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar, Madhya Pradesh, India. He leads an active research group focused on the development of use-inspired nanomaterials for the treatment of cancer, inflammatory disorders, and infectious diseases.

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