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Teaser Here, we present a systematic approach to design different programmable physical-stimuli-responsive nanotherapeutics intended for controlled and targeted delivery of various therapeutic agents.

Design strategies for physical-stimuli-responsive programmable nanotherapeutics

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Nanomaterials that respond to externally applied physical stimuli such as temperature, light, ultrasound, magnetic field and electric field have shown great potential for controlled and targeted delivery of therapeutic agents. However, the body of literature on programming these stimuli-responsive nanomaterials to attain the desired level of pharmacologic responses is still fragmented and has not been systematically reviewed. The purpose of this review is to summarize and synthesize the literature on various design strategies for simple and sophisticated programmable physical-stimuli-responsive nanotherapeutics.

Introduction

The ever-increasing prevalence of cancer, metabolic disorders and neurodegenerative diseases, as well as the quest for efficient treatments of these and other diseases, has intensified the need for new, alternative and novel drug delivery systems that can release loaded drugs at the target site on-demand. Among the various novel drug delivery approaches investigated, nanotechnology has increasingly been playing important parts for the much-needed targeted drug delivery. Nanomaterials including polymeric [1], lipidic [2], inorganic [3] and inorganic–organic hybrid nanoparticles [4]; liposomes [5,6]; nanocrystals [7]; micelles [8]; microemulsions [9]; polymersomes [10]; dendrimers [11]; nanogels [12]; nanofibers [13]; nanowires [14]; nanoscaffolds [15]; nanopatterned surfaces [16]; nanorods [17]; nanocomposites [18]; nanofluidic devices [19]; carbon nanotubes [20]; nanosheets [21]; and nanomembranes [22] have been developed and evaluated for controlled drug delivery. These nanocarriers can be designed to assume variety of bulk and surface chemistry, sizes, shapes and architectures, for improved drug release, targeting and blood circulation time. For instance, positively charged surfaces generally enhance nanoparticle cellular uptake [23–25]. PEGylation [the process of attaching polyethylene glycol (PEG) chains] of nanocarriers induces steric repulsion of blood opsonins and significantly increases the circulation time of nanomaterials [26]. The size of nanomaterials affects the biodistribution and cellular uptake of the nanomaterials. In general, it is postulated that nanomaterials with sizes 10–100 nm can easily be taken up by cells via endocytosis. However, larger nanomaterials can also enter cells at slower rates through different endocytosis pathways [27]. For example, Oh *et al.* [28] showed that layered double hydroxide nanoparticles were taken up by human osteosarcoma (MNNG/HOS) cells in the order of 50 > 100/200 > 350 nm, where 50–200 nm

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nanoparticles were selectively internalized by clathrin-mediated endocytosis. Nanomaterial sizes >150 nm have a much greater chance of being entrapped in the liver and spleen, and nanomaterials with sizes <5 nm are highly likely to be filtered out by the kidneys [29,30]. Ascribed to their enhanced permeability and retention into various tumors, nanoparticles with sizes in the range 100–200 nm have shown great tumor-targeting potentials. Nevertheless, the desired level of drug targeting and release is yet to be achieved using traditional nanoformulations and, despite decades of efforts, only a few nanoformulations have reached the market [30,31]. There is an unmet need to program nanomaterials with more-appropriate structures and properties for effective therapeutic effects.

Stimuli-responsive nanomaterials can take advantage of the specific microenvironmental changes in some disease conditions such as ischemia, inflammatory diseases, infections and tumors, which have served as the basis for designing most of the chemical-stimuli-responsive nanomaterials. Alternatively, they can be designed to respond to various externally applied physical stimuli such as temperature, light, ultrasound, magnetic field, electric field and X-ray. Generally, unlike the internal stimuli, external triggers are easier to control and are associated with less variability. The choice of a specific stimuli-responsive nanocarrier can be made based on several factors like the intended application, the target site, the cost of treatment and the safety concerns. In addition, there have been many attempts to enhance the programmability of various stimuli-responsive nanomaterials for improved therapeutic effects. For instance, functionalization of the surfaces of nanomaterials using specific ligands and targeting agents such as antibodies, peptides, nucleotide aptamers and other small molecules could significantly improve drug targeting. Another possibility is introduction of linkers or groups that are responsive to different exogenous or endogenous stimuli, which possibly render the nanoparticles responsive to multiple stimuli and provide improved platforms for advanced programmability. In this review, the design strategies for simple and sophisticated programmable physical-stimuli-responsive nanotherapeutics are systematically discussed.

Thermoresponsive nanomaterials

Thermoresponsive nanomaterials are a class of 'smart' materials that undergo phase transition in response to temperature change. The temperature at which the phase transition occurs is called the critical solution temperature (CST). If thermoresponsive materials change from a hydrophilic and highly swollen state to a hydrophobic and collapsed state at CST when temperature is increased, the CST is called a lower CST (LCST). If thermoresponsive materials change from a hydrophobic and collapsed state to a hydrophilic and highly swollen state at CST when temperature is increased then the CST is called an upper CST (UCST). The thermoresponsive materials that have been investigated for biomedical applications usually have a LCST. Through tailoring their chemistry, LCST, architecture and targeting moiety, thermoresponsive nanomaterials can be programmed for different biomedical applications. The strategies for the programming are discussed below.

Programming with different basic chemistry that is thermoresponsive

Various types of thermoresponsive polymers have been used to design thermoresponsive nanomaterials. One type of thermoresponsive polymers is the poly(*N*-substituted acrylamide)s: including poly(*N*-isopropylacrylamide) (PNIPAAM) and poly(*N*,*N*-diethylacrylamide). PNIPAAM is the first and most investigated thermoresponsive polymer and has a LCST of 32 °C, which is close to the physiological temperature of 37 °C. The LCST of PNIPAAM is not dependent on its molecular weight, concentration or other environmental conditions [32,33]. Unlike PNIPAAM, the LCST of poly(*N*,*N*-diethylacrylamide) depends on the tacticity of the polymer [34], which limits its use. The second type of thermoresponsive polymers is the poly(*N*-vinyl-alkylamide)s, such as poly(*N*-vinylcaprolactam) and poly(*N*-vinylisobutyramide) polymers that have LCSTs of 30–50 °C [35]. Poly(*N*-vinylcaprolactam) was well-tolerated by human intestinal Caco-2 and bronchial Calu-3 cell lines but it is less investigated than PNIPAAM as a thermoresponsive polymer [36]. It exhibits a 'classical' Flory–Huggins thermoresponsive phase behavior in water with LCST decreasing with increasing polymer chain length and concentration [36]. It is used to form thermoresponsive nanogels for controlled drug delivery or for polymer surface grafting. For example, chitosan was grafted by *N*-vinylcaprolactam and crosslinked by sodium tripolyphosphate to form chitosan-*g*-poly(*N*-vinylcaprolactam) nanoparticles [35]. The nanoparticles released 5% and 40% of the loaded 5-fluorouracil over 3 days below and above its LCST, respectively. The third type of thermoresponsive polymers is the block copolymers of poly(ethylene oxide) and poly(propylene oxide) called Pluronics®. They have LCSTs between 20 and 85 °C, which can be tailored by the lengths of the hydrophilic poly(ethylene oxide) and the hydrophobic poly(propylene oxide) segments and their ratios. They are amphiphilic polymers approved by the FDA for use as food additives and pharmaceutical ingredients [37]. Owing to their amphiphilic nature, they are commonly used to form thermoresponsive vesicles or surface grafting agents [37,38]. Poly(oligo ethylene glycol methacrylate)s with an oligo ethylene glycol grafted to a poly(methacrylate) backbone are the fourth type of thermoresponsive polymers. Their LCSTs can be tuned from 22 to 90 °C by varying the length and density of the oligo ethylene glycol graft. The higher and longer the oligo ethylene glycol density and chain length, the higher is their LCST [33,39]. For example, Tian *et al.* [40] fabricated doxorubicin-loaded dual thermo- and redox-responsive nanogels using poly(oligo ethylene glycol methacrylate) and 2-(2-methoxyethoxy) ethyl methacrylate using the disulfide-containing crosslinker *N*,*N*′-bis(acryloyl)cystamine. When the mass ratio of poly(oligo ethylene glycol methacrylate) and 2-(2-methoxyethoxy) ethyl methacrylate was varied from 0:100 to 15:85, their LCST changed from 25.7 to 42.8 °C. Poly(*N*-alkyloxazolines) (polyoxazolines) made of a pseudo-polypeptide backbone and alkyl side-chains are the fifth type of thermoresponsive polymers. Polyoxazolines have a broad water solubility and reactivity depending on the alkyl chain length, and thus a tunable LCST [41–43]. They were reported to have low immunogenicity [44], biodegradability [45] and good penetration through porcine gastric mucosa [46]. It is worth pointing out that poly(2-isopropyl-2-oxazoline) is a structural isomer of PNIPAAM with a LCST close to the physiological temperature [41,42]. Polyoxazolines are commonly used as nanostructure surface-grafting agents [41–43]. For example, Kurzhals *et al.* [42] grafted the surfaces of magnetic nanoparticles using poly(2-isopropyloxazoline) (LCST in cell culture medium = 32.5 °C) and poly(2-ethyloxazoline) (LCST in cell culture medium = 37 °C) to form core–shell magnetic nanoparticles. The permeability of poly(2-isopropyloxazoline)-grafted nanoparticles was about fourfold greater

than the permeability of poly(2-ethyloxazoline)-grafted nanoparticles in HeLa cells at 37 °C. The difference is attributed to the hydrophobicity of the former, with LCST below 37 °C.

Thermoresponsive polymers are not only made of the synthetic polymers discussed above but also polypeptides or lipids. Elastin-like polypeptides composed of multiple repeating pentapeptide units of Val-Pro-Gly-Xaa-Gly (Xaa is any amino acid except proline) exhibit a sharp transition temperature within 2–3 °C [47–49]. Their LCSTs can be tuned by internal factors such as amino acid composition and polymer molecular weight and external factors such as ionic strength and concentration. The more-hydrophobic the amino acid and the higher the molecular weight the lower the LCST [49]. Elastin-like polypeptides can be used to form composite nanoparticles and vesicular nanostructures [47–49]. For example, Bessa *et al.* [50] prepared bone morphogenetic protein (BMP)-2 and BMP-14-loaded nanoparticles by thermoresponsive self-assembly of the elastin-like polypeptide $(VPAVG)_{220}$ (transition temperature = 33 °C) at 37 °C. Following an initial burst release for 24 h, the nanoparticles slowly released the loaded cytokines for 14 days *in vitro* at 37 °C. The synthetic *N*-substituted linear homopolypeptoids like poly(*N*-C_3 glycine)s and the random copolypetoids like poly(*N*-methylglycine)–poly(*N*-butylglycine) are another type of thermoresponsive polypeptides with LCSTs 27–71 °C depending on the type and degree of monomer substitution [51]. For example, Kurzhals *et al.* [51] grafted magnetic nanoparticles using poly(*N*-methylglycine)–poly(*N*-butylglycine) polypeptoid with different percentages of *N*-methylglycine and *N*-butylglycine and the aggregation temperature of the nanoparticles increased from 33 to 58 °C when the percentage of *N*-methylglycine increased from 61 to 73%. Poly(*N*-substituted asparagines) are the third type of biodegradable thermoresponsive polypeptides with LCSTs between 28 and 78 °C [30,52]. They are amphiphilic and biodegradable. Liposomes made of dipalmitoyl phosphocholine or myristoyl stearoyl phosphatidylcholine have thermoresponsive properties with UCST (note: not LCST) between 40 and 45 °C [6,53]. Above the UCSTs, the liposomes undergo gel-to-sol transition and the lipid bilayer will be transformed from a solid state to a fully liquid state rendering the membrane highly permeable for the loaded drugs [53]. Thermoresponsive liposomes are among the pioneering stimuli-responsive nanocarriers of which few have advanced to clinical trial stages [5,6]. For example, the doxorubicin-loaded thermoresponsive liposome ThermoDox® has reached a Phase III clinical trial for the treatment of various solid tumors and it enabled a 25-times greater concentration of the drug in cancerous tissues as compared with intravenous doxorubicin [6,53].

Programming the LCST for thermal targeting and release

The LCST is a unique property of thermoresponsive nanomaterials that can be utilized to localize drugs at a target site [11,54]. The thermoresponsive nanomaterials made of different thermoresponsive polymers with different chemistries have different LCSTs that are higher or lower than body temperature (37 °C). Thermoresponsive nanocarriers with LCSTs lower than 37 °C can be used to increase drug retention time and permeability across biological barriers owing to their sol-to-gel phase transition at 37 °C. For example, thermoresponsive self-assembled poloxamer 407 nanogels were shown to adhere on the corneal surface and increase the permeability of muscone across the cornea 3.4-fold [55]. The hydrophilic poly(ethylene oxide) segments and hydrophobic poly(propylene oxide) segments of Pluronics® and d-α-tocopheryl PEG succinate self-assembled into micelles or vesicles at 50 °C and could cross the blood–brain barrier and enhance the permeability of the small molecular-model drug Rho123 in Sprague–Dawley (SD) rats after intravenous administration [56]. Pluronics® are known efflux protein inhibitors and the mixed micelles containing Pluronic® F127 and Plasdone™ S630 increased the oral bioavailability of biochanin A2 16-fold in SD rats compared with the free drug [57].

If the LCST is designed to be slightly higher than 37 °C, the nanomaterials are dispersible in physiological fluid and can circulate in the body at body temperature. However, if the disease site (target site) is locally heated up to 40–42 °C by ultrasound, near-infrared (NIR) light [58], magnetic field [58,59], radiofrequency [6] or other techniques, the thermoresponsive nanomaterials circulating in the blood become hydrophobic and are easily taken up by the surrounding cells and tissue so that thermally targeted drug delivery can be achieved. For example, doxorubicin [60], 17-(allylamino)-17-demethoxygeldanamycin [61] and 5-fluorouracil [35] were loaded into cationic thermosensitive liposomes, core–shell composite thermoresponsive nanoparticles and chitosan-*g*-poly(*N*-vinylcaprolactam) thermoresponsive nanoparticles, respectively. The nanocarriers improved the cellular uptake of the drugs in different tumor cell lines upon hyperthermia and were more cytotoxic than the free drugs alone. Furthermore, when gold nanorods that can absorb NIR light at ~800 nm to generate heat or inorganic nanoparticles such as magnetic nanoparticles that can convert an external alternating magnetic field into heat [58,59] can be imbedded within the core of such thermoresponsive nanoparticles, drug release can be turned 'ON' or 'OFF' by applying and removing NIR or the magnetic field, respectively, to induce 'on-demand drug release'. Such smart nanocarriers, loaded with different drugs such as doxorubicin [59,62], bupivacaine [63], vascular endothelial growth factor [64] or curcumin [65] have been reported. In addition, unlike externally applied direct thermal stimulation, which heats the entire area of operation, utilizing internal heat sources can provide highly localized and remotely controlled drug release [66].

The desired LCST can be obtained by incorporating other components into basic thermoresponsive polymers through copolymerization, conjugation and grafting [63,64,67]. In general, hydrophilic components increase the LCST, and hydrophobic components decrease the LCST [68]. For example, the LCST of NIPAAM nanogels increased from 32 to 37, 42 or 46 °C upon copolymerizing with 51% *N*-isopropylmethacrylamide and 6% acrylamide, 58% *N*-isopropylmethacrylamide and 7% acrylamide or 55% *N*-isopropylmethacrylamide and 11% acrylamide, respectively [63]. Similarly, addition of 20% of the lipophilic monomer poly(*N*-alkylacrylamide) *N*-tert-butylacrylamide lowered the LCST of NIPAAM to 20 °C, whereas incorporation of the hydrophilic monomer poly(*N*-alkylacrylamide) acrylamide increased the LCST to 42.1 °C [67]. Adsorption of superparamagnetic iron oxide nanoparticles (SPIONs) on the PNIPAAM chain increased the LCST from 32 to 52 °C, depending on the amount of SPIONs added [64]. Vesicular nanostructures can also be rendered thermoresponsive for controlled drug release and diagnosis purposes using bubble-generating agents. For example,

ammonium bicarbonate – a CO_2 bubble-generating agent – was incorporated into thermoresponsive liposomes. When heated to a little above 40 °C, CO_2 bubbles were generated, which created permeable defects on the liposomes and enhanced drug release was obtained (Fig. 1a) [69]. In addition, the generated CO_2 bubbles are hyperechogenic and can be used as an ultrasound contrast agent in elucidating the status of the carriers and providing real-time diagnostic images [69]. The potential of using therapeutic gases such as nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S) in such bubble-generating carrier systems for the treatment of tumors has also been assessed [70].

Programming with different architecture

The size, shape and porosity of thermoresponsive nanomaterials also affect the targeting and therapeutic efficiency of the drug-loaded nanomaterials [12,68]. Many of the thermoresponsive polymers developed have been deployed to form composite nanoparticles including crosslinked nanogels. When thermoresponsive block copolymers such as Pluronics® are used, micelles can be formed [55]. Furthermore, thermoresponsive block copolymers can self-assemble into thermoresponsive supramolecular nanostructures with different intraparticle morphologies like lamella and gyroid, which allow different drug release mechanisms. For example, 1-anilinonaphthalene-8-sulfonic-acid-loaded nanoparticles of the triblock polymer polystyrene-PNIPAAM-polystyrene were prepared in three different morphological architectures: polystyrene spheres in PNIPAAM matrix, polystyrene gyroids in PNIPAAM matrix and polystyrene–PNIPAAM lamellar structure. Dye release from the gyroidal nanoparticles (15.7% at 25 °C; 8.1% at 45 °C in 3.6 h) was higher than the sphere-forming nanoparticles (10.6% at 25 °C; 4.3% at 45 °C in 3.6 h) [32]. Micellar aggregates can also be crosslinked to give thermodynamically stable vesicular systems with thermoresponsive cores [24]. Thermoresponsive liposomes are also special type of vesicles comprising hydrophobic lipid bilayers and an aqueous core [71].

Programming with additional functional groups

To make thermoresponsive nanomaterials more functionable, charges, cell-binding ligands and biodegradable crosslinkers have been added to the nanomaterials. Charged nanoparticles can increase drug loading and sustain the release of oppositely charged drugs. For example, incorporation of 20 mole% of the negatively charged acrylic acid to PNIPAAM nanogels significantly increased the loading capacity [72] and sustained the release of the positively charged local anesthetic bupivacaine owing to ionic interactions and increased the duration of action of the drug by more than threefold [23]. Conversely, 2-aminoethyl methacrylamide hydrochloride rendered thermoresponsive nanoparticles cationic and improved the encapsulation efficiency, prolonging the release of the negatively charged proteins insulin, BSA and β-galactosidase [24]. Du *et al.* [25] designed special pH-responsive charge conversional thermoresponsive nanogels that transformed from negatively charged into positively charged in the slightly acidic tumor extracellular environment. The charge conversion significantly enhanced nanogel cellular uptake and doxorubicin release from the nanogels to improve the cytotoxic effect of the drug. The surfaces of thermoresponsive nanoparticles can also be modified by cell-binding ligands such as antibodies, peptides, aptamers or small molecules, which can enhance cell targeting and nanocarrier cellular uptake by endocytosis. For instance, folate receptors are overexpressed in a wide variety of tumor cells and folic acid has been widely used as a tumor-targeting ligand by conjugating it to thermoresponsive nanoparticles [59,73]. In another example, surface modification of composite and hybrid core–shell thermoresponsive nanoparticles by integrin β4 increased the accumulation of the nanoparticles on the surfaces of squamous head and neck carcinoma cells, on which A9 antigen was overexpressed [59].

When nanoparticles are biodegradable, they can achieve sustained drug delivery. Crosslinkers that degrade or hydrolyze in response to different endogenous stimuli such as acidic pH {e.g., 2,2-dimethacroyloxy-1-ethoxypropane [24,74], HEMA-lactate-dextran [75–78], poly(l-lactic acid) [79]}, redox potential {e.g., bis(2-methacryloyloxyethyl) disulfide [80] and disulfide-containing crosslinker *N*,*N*′-bis(acryloyl) cystamine [40]} or enzymes (e.g., dextran-methacrylate [81]) have been introduced to thermoresponsive nanoparticles. PEGylation can help to increase circulation time and improve treatment effectiveness of nanotherapeutics. For example, PEGylation of PNIPAAM-*co*-polymethacrylate thermoresponsive nanogels significantly decreased the uptake of the nanogels by THP-1 human acute monocyte cells

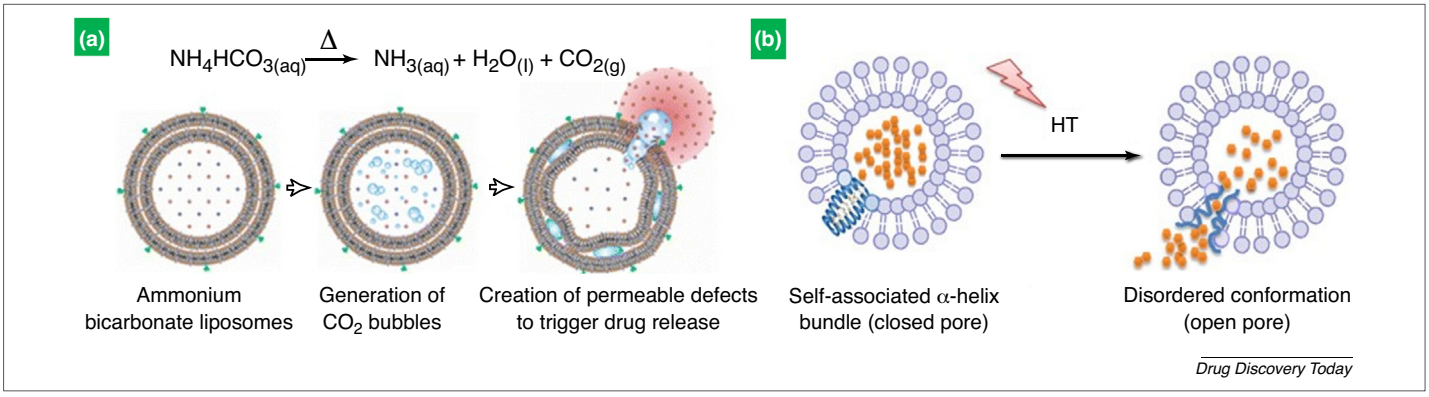

FIGURE 1

Schematic representation of (a) thermoresponsive bubble-generating liposomes, designed by adding bubble generating agents, and (b) liposome–peptide hybrid thermoresponsive vesicles, designed by adding a thermoresponsive amphiphilic leucine zipper peptide into thermoresponsive liposomes and their response to hyperthermia (HT). Reproduced, with permission, from Refs [69,71].

(macrophages) *in vitro* [82]. Hybrids of different thermoresponsive polymers and/or polypeptides into a nanomaterial system can have synergetic effects on the temperature-responsiveness and consequently better therapeutic effects of the nanosystem. For example, a hybrid nanosystem containing the thermoresponsive amphiphilic leucine zipper peptide and thermoresponsive liposomes (Fig. 1b), which have a phase transition temperature 42 °C, prolonged the blood circulation time of the loaded doxorubicin, leading to a threefold accumulation of the drug in the heated tumor site in SW480-tumor-bearing mice compared with lysolipid-modified thermoresponsive liposomes [71,83].

Light-responsive nanomaterials

Light-responsive (photoresponsive) nanomaterials are a class of smart materials that undergo chemical and/or physical changes in response to light stimuli. Light in long UV 200–400 nm and NIR 650–900 nm (wavelength range that is minimally absorbed by skin and tissue) has been utilized as attractive exogenous stimuli for biomedical applications owing to the minimally invasive nature and possibility to be applied with high spatial and temporal precision [84,85]. Drug release from light-responsive nanomaterials can be regulated via adjustments of the chemistry of photosensitive or photocleavable compounds, light wavelength and intensity, and duration of exposure [86]. The strategies for designing these parameters to program light-responsive nanomaterials for desired therapeutic effects are discussed below.

Programming with different basic chemistry that is light responsive

Photosensitive compounds that are commonly used for designing light-responsive nanomaterials are azobenzene, stilbene, spiropyran, dithienylethene, diazonaphthoquinone and pheophorbide A; these undergo reversible or irreversible photoisomerization upon exposure to light (Fig. 2). They are usually doped or covalently bound to various nanostructures. Azobenzene and stilbene undergo reversible *trans–cis* isomerization when exposed to 300–380 nm, where the *cis* isomers have much higher dipole moments than the respective *trans* isomers. Patnaik *et al*. [87] conjugated the hydrophobic azobenzene to the hydrophilic dextran and then obtained self-assembled micelles. These micelles could dissociate and rapidly release the loaded acetylsalicylic acid and rhodamine upon UV irradiation owing to the photoisomerization of the hydrophobic *trans*-azobenzene into the hydrophilic *cis*-azobenzene. Spiropyran is neutral and can isomerize to charged merocyanine. Dithienylethene can undergo a reversible transition from the ring-open isomer to ring-closed isomer. Diazonaphthoquinone undergoes irreversible photoinduced Wolff rearrangement when exposed to UV light [88]. Pheophorbide A is a photosensitizer that, upon exposure to longer excitation wavelengths, generates reactive oxygen species (mainly singlet oxygen) that can rupture endosomes and lysosomes to induce photochemical internalization. Photochemical internalization is a process by which macromolecules and other compounds that are entrapped in endocytic vesicles formed after endocytosis are released to the cytosol by light [89]. Pheophorbide-A-labeled polyethylenimine nanoparticles enhanced the cellular uptake of FITC-labeled ovalbumin by murine dendritic cells by ~2.8-fold and, after irradiation of the cells by a 670 nm laser, a more diffused pattern of the protein was observed in the cytoplasm indicating protein release from the endocytic vesicles to the cytoplasm [89].

The commonly used photocleavable groups include pyrene, *o*-nitrobenzyl, coumarin and thymine (Fig. 2). Pyrene undergoes photosolvolysis in the presence of water or other protonic solvents. The *o*-nitrobenzyl group is sensitive to far-UV light and undergoes photolysis or intramolecular rearrangement even in the absence of water and can also be activated by NIR light through two-photon absorption [88,90]. Azagarsamy *et al*. [91] used hydroxyethyl acrylate and *o*-nitrobenzyl-containing crosslinker to synthesize photodegradable nanogels. When the nanogel was irradiated with 365 nm UV light, it degraded to release the loaded protein alkaline phosphatase. Huu *et al*. [90] prepared nintedanib-loaded, light-responsive nanoparticles using a preformed polymer that contains *o*-nitrobenzyl groups. The nanoparticles remained stable for 10 weeks post-intravitreal injection but rapidly released nintedanib when exposed to 365 nm light to suppress the choroidal neovascularization in Brown Norway rats. Coumarin has a more efficient two-photon absorption of NIR light than *o*-nitrobenzyl derivatives [88]. Thymine photodimerizes upon irradiation above 270 nm and reverts to its monomeric form when irradiated below 270 nm [92]. He *et al*. [93] grafted thymine derivatives on the surfaces of mesoporous silica nanoparticles as gatekeepers. When the nanoparticles were irradiated with 240 nm UV light, thymine was cleaved to open the gate and then the loaded model compound tris(bipyridine)ruthenium(II) dichloride was released. Afterwards, the gate could be closed by applying 365 nm UV light to induce photodimerization of thymine.

Some metals or metallic oxides like TiO_2, ZnO, CuO and Au have also been utilized to prepare light-responsive nanomaterials. For example, Wang *et al*. [94] fabricated paclitaxel-loaded porous TiO_2 nanoparticles and grafted their surfaces using polyethylenimine by amide linkage to close the pores. The nanoparticles were further modified by folic acid for tumor targeting. The cumulative amount of paclitaxel release from the nanoparticles after 3 h was 3.2%. However, upon UV irradiation of the nanoparticles for 5 min, 10 min and 15 min, the polyethylenimine molecules on the surface were cleaved by the free radicals ($OH^{\bullet}$ and $O_2^{\bullet}$) generated by TiO_2 and released 20.1%, 37.2% and 73.4% of the paclitaxel over 3 h, respectively. Nanoparticles made of gold in rod, shell or hollow sphere shapes, as well as carbon nanotubes, can absorb NIR light and generate heat for photothermally targeted drug delivery [26]. This technology has also been mentioned above, and can be used to deliver drugs in deep tissues because NIR can penetrate through 10 cm with minimal absorption or scattering by water and tissues [26,95–97]. Doxorubicin-loaded hollow gold nanospheres were administered intravenously to mice bearing Hey tumors and irradiation of the tumor area 24 h after injection using 808 nm NIR laser light resulted in rapid release and distribution of the doxorubicin in the treated area [96].

Programming with additional functional groups

The programmability of light-responsive nanomaterials can be enhanced by attaching additional functional groups such as folic acid [73], antibodies [34], aptamer [98], PEG [73] and thermoresponsive materials [85,98] for targeted and efficient drug delivery. Xiao *et al*. [85] developed interesting light-responsive nanocarriers based on complementary DNA strands that contained sequential

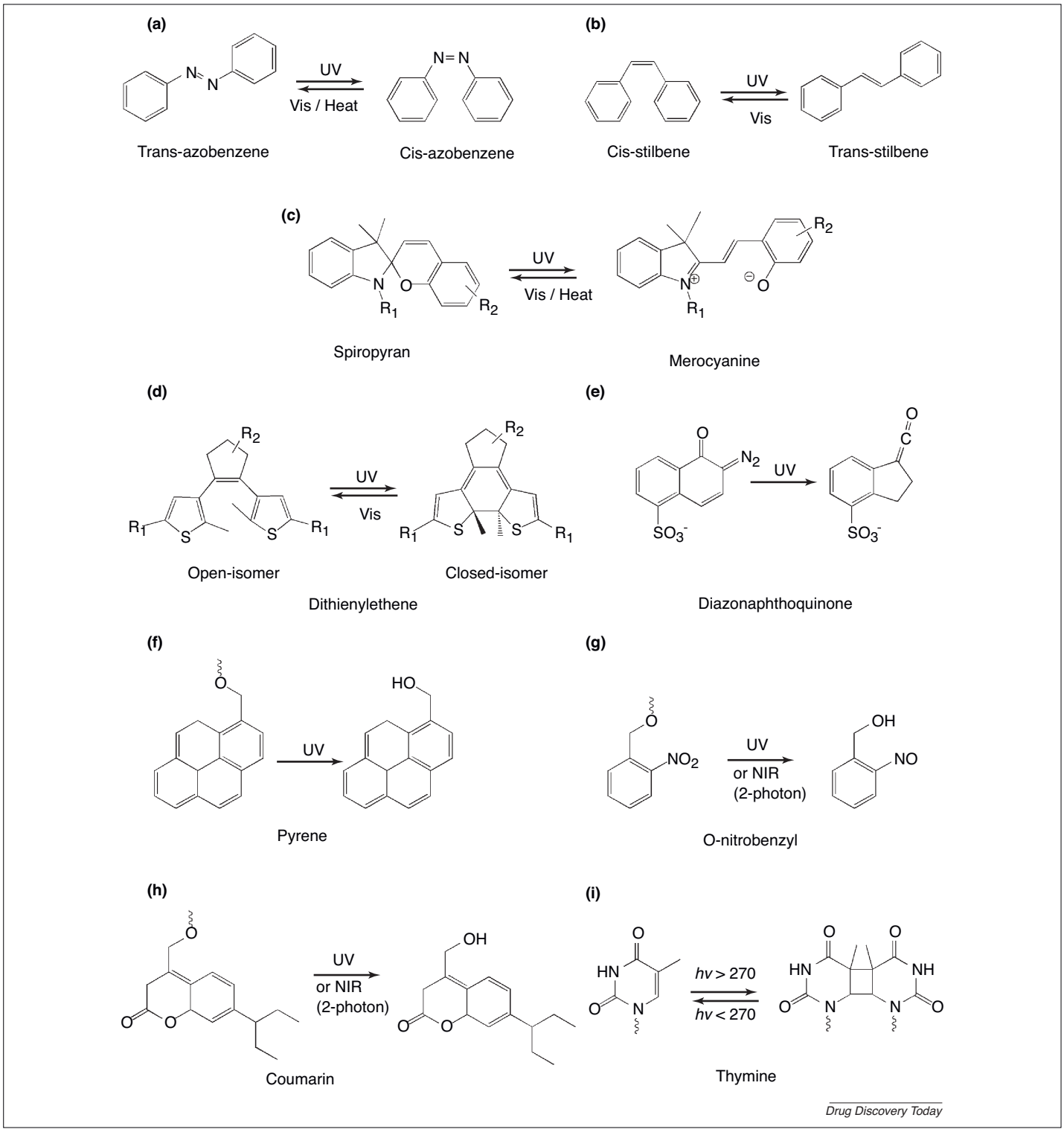

FIGURE 2

Commonly used photosensitive (a–e) and photocleavable (f–i) compounds and functionalities used for the preparation of light-responsive nanomaterials and their reactions to light.

CG base pairs to provide a loading platform for doxorubicin (Fig. 3). One end of one of the DNA strands (capturing strand) was thiolated and attached to gold nanorods, whereas the opposite end of the other complementary DNA strand (targeting strand) was conjugated with folic acid ligand for cell-specific targeting. Upon 808 nm NIR irradiation, the gold nanorods served as NIR light-to-heat transducers and the heat generated by the gold nanorods dehybridized the DNA strands to release the loaded doxorubicin in a BALB/c nude mice xenograft tumor site. Furthermore, the nanoparticles were PEGylated to improve their blood

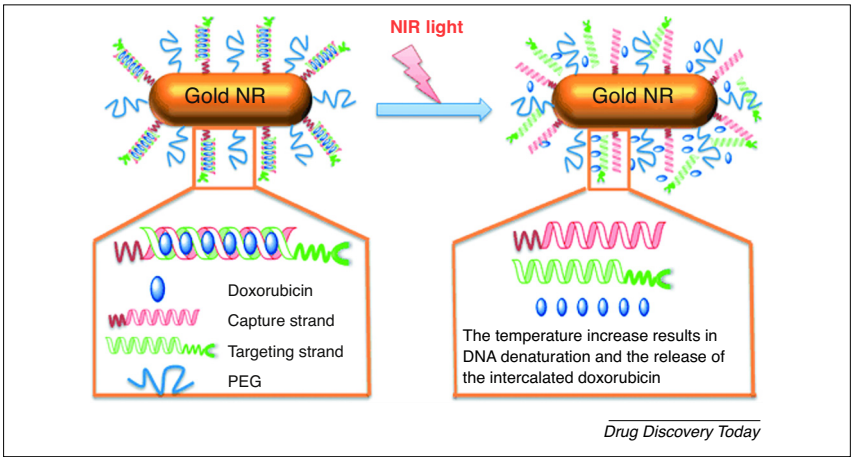

FIGURE 3

Doxorubicin-loaded and folic-acid-modified DNA nanoaggregates that are attached to gold nanorods (gold NR) to form near-infrared (NIR)-responsive nanotherapeutics. Upon NIR exposure, the gold NR generate heat that dehybridizes the DNA aggregates and releases the loaded doxorubicin. Reproduced, with permission, from Ref. [85].

circulation half-life. The folic-acid-targeted nanoparticles showed greater cytotoxicity than the nontargeted nanoparticles in human nasopharyngeal epidermoid carcinoma cell lines (34.37 ± 3.03 versus 56.37 ± 0.69 cell viability). In cancerous mouse models, induced by injection of human nasopharyngeal epidermoid carcinoma cells, the relative tumor volume growth after 14 days of administration of the targeted nanoparticles was 35% less than the nontargeted nanoparticles owing to targeted photothermal ablation. Doxorubicin loading decreased tumor growth rate by a further 28%. In another study, Lee *et al.* [99] conjugated herceptin, an antihuman epidermal growth factor receptor 2 (HER2) antibody, to poly(lactic-*co*-glycolic acid) (PLGA)-gold half-shell nanoparticles, to have dual receptor binding and NIR irradiation effects and to increase the accumulation of the nanoparticles. This technology allowed slow release of doxorubicin at breast cancer cells in mice. When the mice were treated with doxorubicin alone or the targeted nanoparticles without NIR, the tumor grew continuously, but at a slower rate than the control groups. When they were treated with the nontargeted nanoparticles or targeted nanoparticles without doxorubicin and irradiated with NIR for 10 min, tumor growth was reduced by 75% and 65% in 10 and 18 days, respectively, and afterwards the tumor started to grow rapidly. Treatment with the targeted doxorubicin-loaded nanoparticles followed by 10 min NIR irradiation resulted in complete tumor destruction within 7 days with no tumor recurrence.

To further enhance drug delivery at the targeted site, ammonium-bicarbonate-loaded bubble-generating and mucin-1 aptamer surface-modified thermoresponsive liposomes were used together with gold nanocages [98]. Upon irradiation, the gold nanocages converted the NIR into localized heat and decomposed the loaded ammonium bicarbonate to generate CO_2 bubbles, which created permeable defects on the lipid membrane and rapidly triggered doxorubicin release (Fig. 4). The mucin-1 aptamer that was hybridized on the surfaces of the thermoresponsive liposomes not only functioned for drug targeting but also acted as a molecular beacon signaling the optimal timing of photothermal heating. Administration of the loaded liposomal systems in tumorigenic rat models reduced the relative tumor volume to ~25% and ~60% over 12 days when administered with and without NIR, respectively. Administration of free doxorubicin did not significantly reduce the tumor volume. Drug release from UV/visible light-responsive nanomaterials can also be modulated in deep tissues by introduction of upconversion luminescent materials such as lanthanide ions, ytterbium and erbium, which convert low energy NIR light to higher energy radiation UV/visible light via multiple absorption or energy transfer. For example, Liang *et al.* [73] fabricated folic-acid-functionalized, doxorubicin-loaded, hollow mesoporous multifunctional upconversion luminescent ytterbium- and erbium-codoped sodium yttrium fluoride nanoparticles. The nanosystem showed more cytotoxicity in folate-receptor-positive KB cells owing to increased nanoparticle uptake by receptor-mediated endocytosis in comparison to the folate-receptor-negative A549 cells, and the nanoparticles converted the 980 nm NIR light to three lower wavelength emission peaks at 521, 541 and 656 nm, which can be used for cell imaging.

Ultrasound-responsive nanomaterials

Ultrasound-responsive nanomaterials are a class of smart materials that undergo chemical and physical changes in response to ultrasound stimulus. Ultrasound, especially high-intensity focused ultrasound, has been utilized as a promising exogenous stimulus for biomedical applications owing to its noninvasiveness, ease of accessibility, cost effectiveness, lack of ionizing radiation residues, controllable spatiotemporal effect and high patient acceptability [100–102]. In this section, the design strategies for programmable ultrasound-responsive nanomaterials having desired therapeutic effects are discussed.

Programming with different basic chemistry that is ultrasound responsive

Ultrasound-responsive nanomaterials can be designed by introduction of ultrasound-labile moieties – called mechanophores – to polymeric nanoparticles. Tetrahydropyranyl is the most commonly used ultrasound-labile compound and is usually conjugated to methacrylic monomer via an ester bond for synthesizing ultrasound-responsive polymers. Upon insonation, the hydrophobic

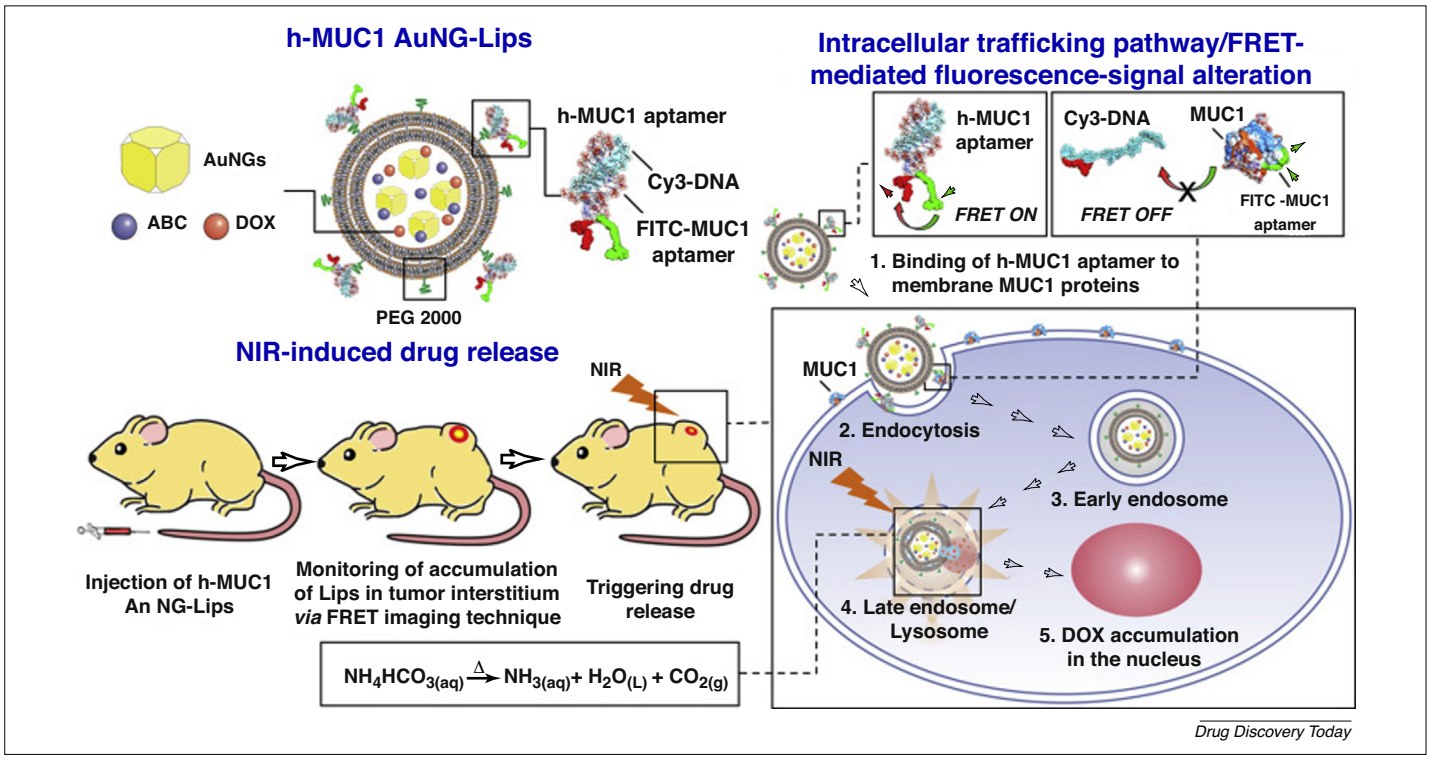

FIGURE 4

Selective endocytosis of mucin-1 aptamer and PEG 2000 modified and gold nanocages (AuNG), ammonium bicarbonate (ABC) and doxorubicin (Dox) loaded bubble-generating thermoresponsive liposomes (Lips) by cancerous cells. Upon near-infrared (NIR) exposure, the AuNGs convert the NIR to heat, which heats the ABC and generates bubbles that disrupt the liposome to release the Dox at the target site. Reproduced, with permission, from Ref. [98].

tetrahydropyranyl group is cleaved from the polymer and leaves the hydrophilic acidic group on the polymer (Fig. 5) [8,103]. The transition of the polymer from the hydrophobic to the hydrophilic state upon ultrasound stimulus can be used for controlling drug delivery. For example, Paris *et al.* [103] grafted the surface of mesoporous silica nanoparticles with 2-tetrahydropyranyl methacrylate copolymerized with a thermoresponsive monomer 2-(2-methoxyethoxy)ethyl methacrylate to obtain a polymeric gatekeeper that released the loaded model dye fluorescein in response to ultrasound stimulus. Xuan *et al.* [8] copolymerized a small amount of 2-tetrahydropyranyl methacrylate with an amphiphilic diblock copolymer comprising poly(ethylene oxide) and poly(2-(2-methoxyethoxy)ethyl methacrylate), which formed

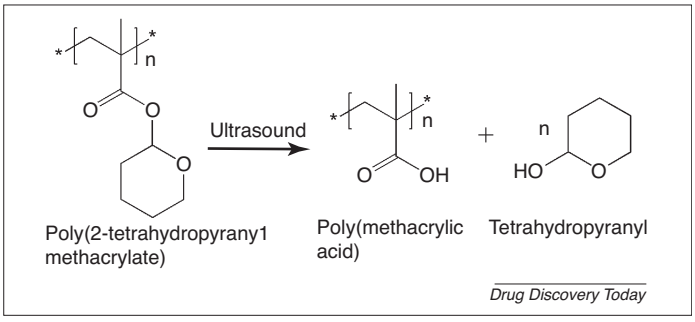

FIGURE 5

Cleavage of 2-hydroxytetrahydropyranyl group to from poly(2-tetrahydropyranyl methacrylate) by the action of ultrasound.

micelles at 25 °C. The micelles dissociated upon insonation owing to the cleavage of the tetrahydropyranyl group and subsequently released the loaded model hydrophobic compound Nile red.

Ultrasound-created strong acoustic cavitation can also disrupt several drug-loaded lipidic or polymeric nanoaggregates such as liposomes [104], Pluronic® micelles [105], nanobubbles [106] and nanodroplets [101] for ultrasound trigger drug release at the target site. Marin *et al.* [105] showed that continuous wave and pulsed 20 kHz ultrasound significantly enhanced the uptake of doxorubicin from Pluronic® micelles by HL-60 cells owing to the disruption of the Pluronic® micelles as well as perturbation of the cell membrane by the action of the ultrasound. Xin *et al.* [104] wrapped PLGA nanoparticles in liposomes and, upon insonation, the liposomes immediately vibrated and broke down to release the PLGA nanoparticles and the loaded mitoxantrone. Encapsulation of the drug increased its half-life 6.7-fold in adult SD rats, which again decreased to 1.7-fold upon insonation. Yildirim *et al.* [102] showed that ultrasound could even disrupt solid inelastic polymeric nanoparticles made by 3,4-dihydro-2H-pyran-*co*-2-((tetrahydro-2H-pyran-2-yl)oxy)ethyl methacrylate-*co*-2-(dimethylamino) ethyl methacrylate copolymer. Apart from its vesicular nanocarrier destabilizing effect, the mechanical cavitation applied to the tissue by ultrasound could also enhance nanoparticle extravasation across blood capillaries and penetration across cell membranes [100,107].

Furthermore, ultrasound-responsive nanomaterials can also be designed by incorporating drugs into various ultrasound contrast agents [107,108]. Ultrasound-induced hyperthermia

can also be used to generate gas bubbles for vascular occlusion and ablation of cancer cells [109]. For example, Wang *et al.* [109] incorporated doxorubicin into perfluorocarbon nanodroplets, which remain stable in the blood stream. Upon ultrasound insonation, ultrasound-induced hyperthermia caused the perfluorocarbon droplets to undergo an instant phase transition into gas bubbles, a phenomenon described as acoustic droplet vaporization effect, which resulted in a 12.5 ± 5.6% decrease in human acute lymphoblastic leukemia cell viability *in vitro* after 6 h of incubation [109].

Programming with additional functional groups

Drug release from ultrasound-responsive nanoparticles can be well controlled by the action of the ultrasound. The biodistribution and targeting of ultrasound-responsive nanoparticles can, however, be enhanced through the introduction of active ligands such as antibodies, peptides or aptamers to the nanoparticles. For example, Wang *et al.* [109] designed sgc8c aptamer-conjugated, doxorubicin-loaded acoustic droplets consisting of liquid perfluoropentane core and lipid shell for tumor theranostic purposes (Fig. 6a). High-intensity focused ultrasound insonation of the aptamer-conjugated droplets resulted in 56.8% decrease in cell viability *in vitro*, which was 4.5-fold higher than that of the nonconjugated analogs. Recently, anticancer monoclonal antibody 9E5-conjugated phase-change nanodroplets that contained a perfluorocarbon liquid core (a mixture of perfluoropentane and perfluorohexane) and a phospholipid shell were designed for intracellular vaporization and drug release (Fig. 6b). The conjugated antibody bound to epiregulin receptors, which are overexpressed on human colonic adenocarcinoma cell line DLD1 and caused 97.8 ± 0.5% accumulation of the nanoparticles into the DLD1 cells, which was significantly higher than the 1.4 ± 0.3% accumulation of the nanodroplets without the antibody. Furthermore, upon insonation, intracellular vaporization generated by the perfluorocarbon liquid in the nanodroplets killed 57% of the targeted DLD1 cells [110]. In a different approach, placental mesenchymal stem cells were used as cell-targeting vectors for the ultrasound-responsive nanoparticles into tumor cells. The ultrasound-responsive nanoparticles were prepared by grafting porous silica nanoparticles using the ultrasound-responsive copolymer, poly(2-(2methoxyethoxy)ethyl methacrylate-*co*-2-tetrahydropyranyl methacrylate) as a gatekeeper. The ultrasound-responsive nanoparticles were loaded with doxorubicin and were coated with polyethylenimine to enhance their permeation into the mesenchymal stem cells. The ultrasound-responsive nanoparticle-loaded mesenchymal stem cells were then co-cultured with *N*-nitroso-*N*-methylurea-induced tumor cells obtained from SD female rats. Stem cell migration did not significantly change as a result of nanoparticle loading, and insonation of the stem cells decreased tumor cell viability by ~60% owing to doxorubicin release by insonation [108]. In another strategy, magnetic nanoparticles were introduced into an ultrasound-responsive protein–polymer nanodroplet core to achieve trio magnetic-field-, receptor- and ultrasound-mediated targeted drug delivery and a 40% increase of the cancer cell killing effect of paclitaxel was obtained [101].

Magnetic-field-responsive nanomaterials

Magnetic-field-responsive (magnetic) nanomaterials are a class of smart materials that respond to magnetic field stimuli and have emerged as attractive nanotherapeutics for diagnostic and therapeutic applications [111]. Generally, a magnetic field frequency below 400 Hz is hardly absorbed by the body and can be remotely directed to the desired tissue [112]. Magnetic nanoparticles are easy to synthesize, are biocompatible and can be remotely controlled via magnetic fields. When exposed to an alternating magnetic field, they can generate local hyperthermia, which can be used to increase blood vessel permeability, induce drug release or kill cancerous cells [113]. In this section, the design strategies for programming magnetic-field-responsive nanomaterials for desired therapeutic effects are discussed.

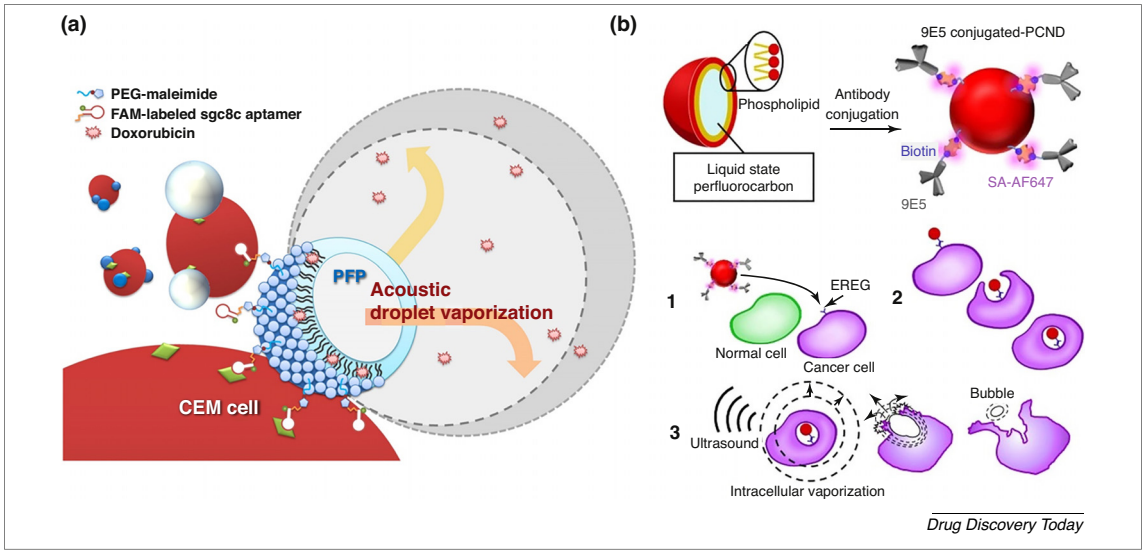

FIGURE 6

(a) Aptamer- and (b) antibody-conjugated ultrasound-responsive nanodroplets designed for tumor-targeted therapy and their interaction with cancerous cells and their subseqent degradation by ultrasound. Reproduced, with permission, from Refs [109,110].

Programming with different basic chemistry that is magnetic-field responsive

Generally, magnetic-field-responsive nanomaterials are core–shell systems containing magnetite (Fe_3O_4) or maghemite (Fe_2O_3) in the core [3]. Various materials such as polymers, mesoporous silica, squalenoyl-gemcitabine [83] and lipids have been used to form the shell of the magnetic-field-responsive nanomaterials [114]. SPIONs are the predominantly studied magnetic-field-responsive nanomaterials because they can be guided to the target site without retaining any residual magnetism, which is attributed to quantum effects at the nanometer scale. SPIONs coated with polyethylenimine have been used for gene transfection and DNA vaccine delivery (magnetofection). Polyethylenimine is positively charged and can interact with the negatively charged sugar phosphate backbone of the nucleic acid to form a stable complex. It also provides a proton sponge effect to the nanoparticles, which enables release of the nanoparticles from endolysosomes into cytoplasm. Prijic *et al.* [112] loaded a cytokine interleukin 12A encoded plasmid DNA in polyethylenimine and acrylic-acid-coated SPIONs. The nanoparticles stimulated an immune response and delayed tumor growth in murine mammary-adenocarcinoma-transfected female BALB/c mice by 0.6 ± 0.5 and 7.8 ± 1.3 days without or in the presence of a Nd-Fe-B generated magnetic field, respectively. The free plasmid and gene electrotransfer delayed tumor growth by -0.3 ± 0.00 and 6.6 ± 1.1 days, respectively, showing that gene magnetofection is as effective as gene electrotransfer. Furthermore, Park *et al.* [115] reported that, when 3,4-dihydroxy-l-phenylalanine-conjugated, branched polyethylenimine was coated on SPIONs, the SPIONs formed clusters and showed better magnetoresponsive properties than individual magnetite nanoparticles, and efficiently delivered siRNA into cancer cells.

Magnetic nanoparticles can also be designed to generate localized hyperthermia and control drug release from thermoresponsive and lipid nanomaterials [116,117]. For example, alternating magnetic-field-induced localized hyperthermia caused DNA dehybridization and released the loaded model compound fluorescein on-demand from mesoporous silica nanoparticles that were designed by using complementary DNA strands as gatekeepers (Fig. 7) [118]. In another study, SPIONs and ethosuximide were loaded in thermoresponsive Pluronic® F127 micelles, which were stabilized by poly(vinyl acetate) (Pluronic® F127:poly vinyl acetate 3:2). The LCST of the nanocarrier was ~38 °C and, when a magnetic field of 2.5 kA/m at a frequency of 44.2 kHz was applied, heat was rapidly generated, which broke the H-bonds between the PVA and F127 to irreversibly deform and rupture the micelle-like structure and trigger drug release [116]. Katagiri *et al.* [117] designed hybrid thermoresponsive liposomes loaded with pyra-

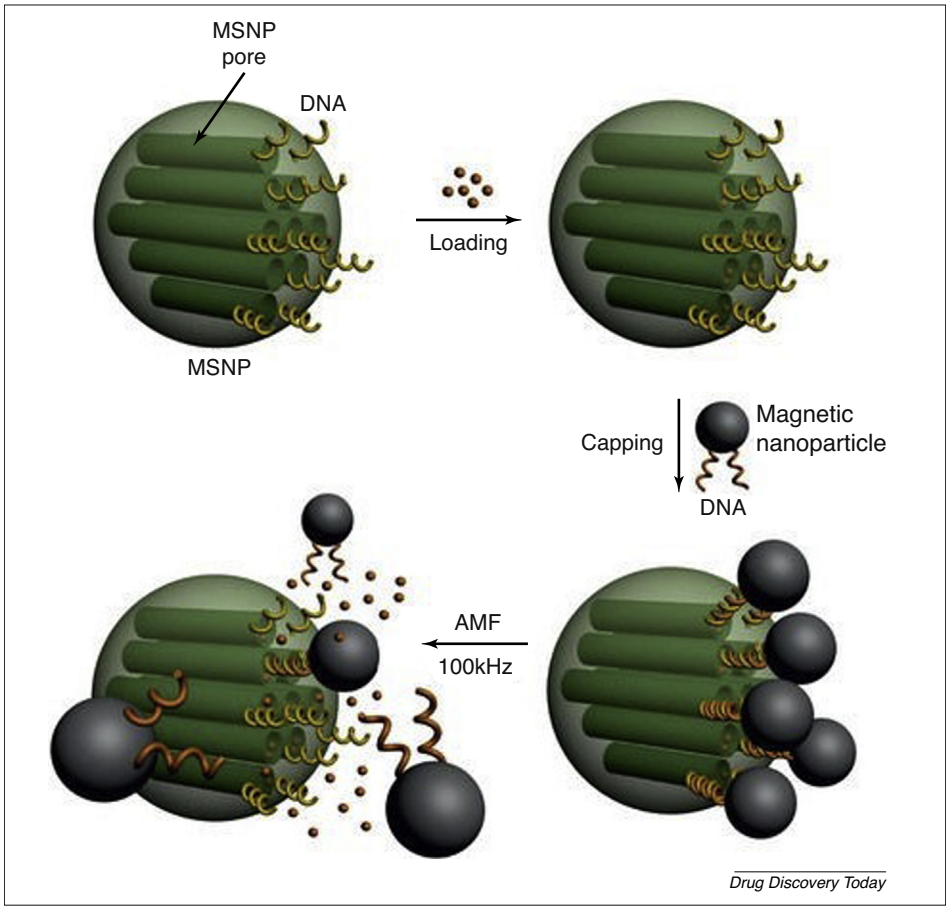

FIGURE 7

DNA-modified drug-loaded mesoporous silica nanoparticles (MSNP) that are hybridized with magnetic nanoparticles as gatekeepers. Upon exposure to an alternating magnetic field, the nanoparticles generated hyperthermia, caused DNA dehybridization, pore opening and on-demand drug release from the mesoporous silica nanoparticles. Reproduced, with permission from Ref. [122] permission, from Ref. [118].

nine dye and iron oxide nanoparticles using phosphatidylcholine, PEG-modified phosphatidylethanolamine and a thermosensitive block copolymer of (2-ethoxy)ethoxyethyl vinyl ether and octadecyl vinyl ether at a molar ratio of 84:7:4. The phase transition temperature of the hybrid liposome was ~35 °C. At 10 °C and 30 °C the liposomes released negligible amounts of the loaded pyranine over 10 min. However, at 45 °C the magnetic-nanoparticle-loaded and -unloaded hybrid liposomes released >95% and 70% of the dye in 1 min, respectively. Similarly, when the nanoparticles were exposed to an alternating magnetic field (360 kHz and 234 Oe) for ~60 min the hybrid liposomes that contained no iron oxide nanoparticles released negligible amounts of the dye, whereas the iron-oxide-loaded nanoparticles released >80% of the dye *in vitro*.

Besides their application in drug delivery and gene therapy discussed above, SPIONs can be used to localize micelles at target tissues and induce drug release. Qin *et al*. [119] encapsulated SPIONs in ferrogel-based Pluronic® F127 micelles, along with the lipophilic drug indomethacin, to form injectable ferrogels. Upon magnetic field application, the indomethacin release half-life decreased from 3195 to 1500 min *in vitro*. This was attributed to the tendency of the SPIONs to orient and approach each other by the action of the externally applied magnetic field, which squeezed the hydrophobic core and pumped the drug out. In addition, SPIONs can be used as cores to form layer-by-layer assembled magnetic nanoformulations. Jayant *et al*. [120] were successful in depositing nelfinavir and rimcazole dihydrochloride layer-by-layer on SPIONs with the help of dextran sulfate sodium polyelectrolytes. The assembled nanocarriers were able to cross an *in vitro* blood–brain barrier model with the aid of magnetic force and released the loaded drugs for over 8 days.

Programming with additional functional groups

Magnetic fields localize magnetic nanoparticles within a certain area of the body, and additional cell-targeting ligands and other stimuli-responsive materials like aptamers can be added to the surface of magnetic nanoparticles to achieve better targeting. For example, Wang *et al*. [121] conjugated A10 RNA aptamer, which binds to the extracellular domain of the prostate-specific membrane antigen, to thermally crosslinked SPIONs for prostate cancer therapy and imaging. The A10 RNA aptamer contained a CG sequence in which doxorubicin was encapsulated. Unlike the nonconjugated SPIONs, the aptamer-conjugated nanoparticles were taken up by prostate-specific-membrane antigen-expressing prostate cancer cells *in vitro*. In addition, the aptamer-conjugated nanoparticles were not taken up by non-prostate-specific-membrane antigen-expressing prostate cancer cells.

Electric-field-responsive nanomaterials

Electric-field-responsive (electroresponsive) nanomaterials are a class of smart materials that respond to weak electric field to attain pulsed or controlled diagnostic and therapeutic effects [111]. An electrical stimulus is relatively easy to generate, control and remotely apply without the need for sophisticated instruments, which makes electroresponsive nanocarriers very attractive drug delivery systems. Drug release from electroresponsive nanomaterials can be regulated via adjustments of the chemistry of electroerodible or electroconductive materials, and electric voltage, current and exposure duration. In this section, the strategies for designing these parameters to program electroresponsive nanomaterials for desired therapeutic effects are discussed.

Programming with different basic chemistry that is electroresponsive

Electroresponsive nanomaterials can be designed by using the common electroerodible or electroconductive materials such as polypyrrole, multiwalled carbon nanotubes, polyelectrolytes, montmorillonite, ferrocene or tetraaniline [123,124]. Samanta *et al*. [123] designed fluorescein-, piroxicam- and insulin-loaded electroresponsive nanoparticles using polypyrrole. Fluorescein release from the nanoparticles increased linearly when the applied current increased from 0 to −300 μA, the duration of exposure increased from 0 to 75 s and the applied voltage increased from 0 to −1 V, and in each case dye release increased by at least 50%. Besides this, the release of piroxicam and insulin from the nanoparticles increased linearly from ~1.5 to ~2.2 and 7.0 μg/ml when the number of pulses increased from 0 to 3 (−100 μA for 25 s) and 2 (−1 V for 4 min), respectively. Ying *et al*. [125] synthesized phenytoin-sodium-loaded electroresponsive nanogels using sodium 4-vinylbenzene sulfonate based polyelectrolyte that swelled from 102.3 ± 16.8 to 388.0 ± 20.4 nm when exposed to 500 μA for 1 min. Phenytoin sodium release from the nanogels also increased from 34.6% to 60.8% and 87.3% upon exposure to a 100 and 200 μA current for 4 h, respectively. Yan *et al*. [126] reported interesting electroresponsive self-assembled micellar nanostructures based on an amphiphilic block copolymer comprising two end-functionalized polymers, PEG-ferrocene and polystyrene-β-cyclodextrin (Fig. 8). The amphiphilic block copolymer was formed by inclusion of the hydrophobic ferrocene on the hydrophilic end of the PEG to the β-cyclodextrin cavity of the hydrophobic styrene polymer, which spontaneously self-assembled into micelle-like vesicles. Upon application of an external electric field, the ferrocene became hydrophilic and left the β-cyclodextrin cavity to reversibly disassemble the micelle-like vesicle and release the encapsulated model compound rhodamine B. Rhodamine B release was highly dependent on the applied voltage and it took ~32, 120 and 450 min to release the loaded compound at +4, +2 and +1 V, respectively, and in the absence of electric stimuli <25% of the loaded dye was released in 600 min.

Electroresponsive nanoparticles can be good candidates for the treatment of epilepsy. Epilepsy is characterized by recurrent, abrupt and unpredicted seizures. Patients take prophylactic doses of antiepileptic drugs, and the prolonged use of higher doses of these drugs is associated with severe side effects. To avoid this, the epileptic seizure can be utilized as an internal stimulus to induce on-demand drug release from electroresponsive nanoparticles. Consequently, Wang *et al*. [127] synthesized phenytoin-sodium-loaded electroresponsive nanogels using 2-(dimethylamino)ethyl methacrylate, styrene and the electroresponsive monomer 4-vinylbenzene sulfonate and the crosslinker *N*,*N*′-methylenebisacrylamide, which released the loaded drug in a sustained manner. Interestingly, pentylenetetrazole-induced epileptic seizure in rats triggered rapid drug release and increased the concentration of phenytoin sodium in the rat hippocampus by ~150%. Electroresponsive nanocarriers have also been extensively investigated in the areas of transdermal drug delivery. Iontophoresis, which uses

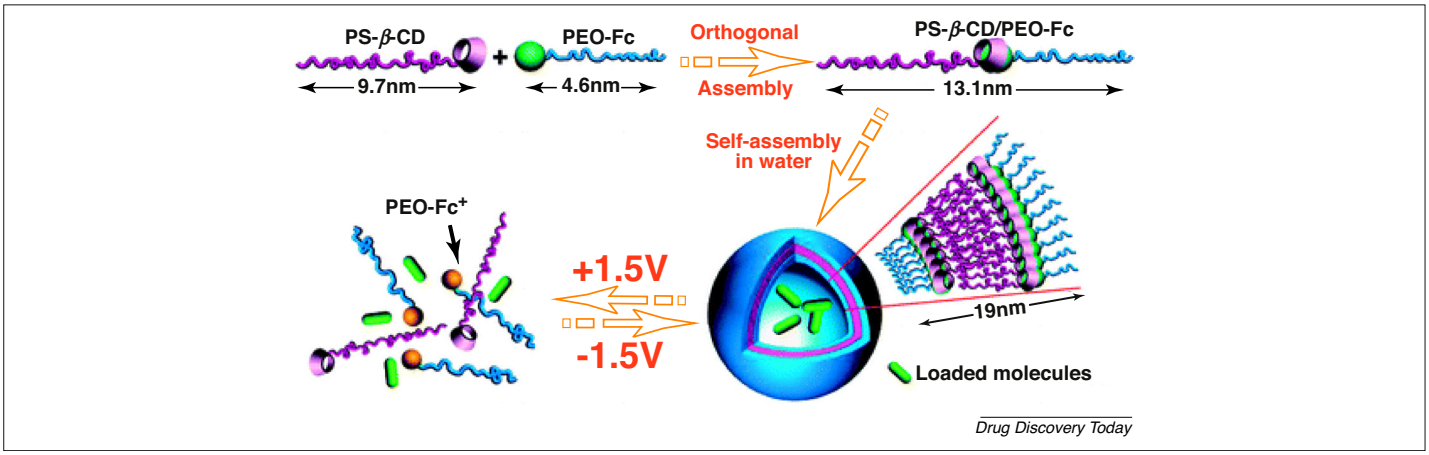

FIGURE 8

A schematic representation of the formation of electroresponsive, drug-loaded, micelle-like vesicles by self-assembly of an electroresponsive amphiphilic molecule that was formed by inclusion complexation of the hydrophobic ferrocene (Fs) group attached to the hydrophilic polyethyleneoxide moiety (PEO-Fs) with the β-CD group attached to the hydrophobic polystyrene moiety (PS-β-CD). Upon exposure to electric stimuli, the Fs became hydrophilic and left the β-CD group to disrupt the vesicle and release the loaded cargo on-demand. Reproduced, with permission, from Ref. [125].

very low voltages to enhance the penetration of charged compounds across the skin, has been employed to enhance drug penetration from various electroresponsive nanocarriers across the skin and sclera. Electroporation, which uses relatively high transmembrane voltage to cause the formation of pores in cell membranes, has also been utilized to enhance the permeability of drugs and various nanocarriers across biological membranes. For example, PEG-coated silica nanoparticles, which were rendered positively charged (+4.06 mV) and negatively charged (−5.51 mV) by surface adsorption of 5-propylsulfonyloxyimino-5H-thiophen-2-ylidene-(2-methylphenyl)acetonitrile and poly(4-methyl-2-pentyne), respectively, were investigated as gene transporters. The nanoparticles were labeled by covalent conjugation of the fluorescent dye rhodamine-B-isothiocyanate and the negatively charged pEGFP-N1 was loaded on the nanoparticles. The negatively charged nanoparticles significantly enhanced gene transfection in HeLa cells when combined with electroporation [128]. In a similar study, electroporation enhanced the permeability of antisense-oligonucleotide-loaded transferrin-decorated liposomes across leukemia cells [129].

Programming with additional functional groups

Surface modification of electroresponsive nanocarriers with different active ligands has been utilized to enhance drug targeting to the target tissue. For example, Ying *et al.* [125] modified the surfaces of phenytoin-sodium-loaded electroresponsive nanogels using brain-targeting angiopep-2 peptide, a ligand of the low-density lipoprotein-receptor-related protein, to improve the blood–brain barrier penetration of the nanogels for the treatment of epilepsy. In comparison to the free drug, the concentration of phenytoin sodium in the brain from the nonmodified and surface-modified nanogels increased by 1.49- and 1.97-fold, respectively, *in vivo* in rats. Another method that can enhance the programmability of electroresponsive nanomaterials is to combine electro-stimuli nanoparticles with other stimuli-systems. Ge *et al.* [130] dispersed daunorubicin-loaded polypyrrole nanoparticles in the thermoresponsive and biodegradable PLGA-PEG-PLGA polymer to form an injectable, conductive hydrogel. The hydrogel was injected into the dorsal sites of FVB adult mice and, upon application of 1.5 V/cm for 40 s, pulsatile drug release was attained.

Concluding remarks and future perspectives

Physical-stimuli-responsive nanomaterials are smart materials that can control drug release in response to physical stimuli including temperature, light, ultrasound, magnetic field and electric field. Many strategies have been explored to program them to have multiple functionality, lower degree of variability and high precession to address the unmet need of on-demand and targeted drug delivery over the past few decades. These strategies can be divided into three categories: the chemistry including the basic/core chemistry and the chemistry of surface targeting ligands (antibody, peptides and aptamers, etc.), the architecture of the nanomaterials, and the parameters of the physical stimuli such as type, intensity and duration, among others. These strategies can be utilized to control the interactions of the nanomaterials with drugs, and thus drug loading and release efficiency. Uptake of the nanotherapeutics by cells and tissues, and the permeability of the nanotherapeutics across biological barriers, which indicates targeting effect, can also be manipulated via these strategies [34]. However, there are several major hurdles that need to be overcome to successfully translate these physical-stimuli-responsive nanomaterials into clinical practice. The first challenge is to avoid uncontrolled accumulation and/or cellular uptake of these nanomaterials by non-target tissues [131,132]. The off-target accumulation and uptake mainly occurs owing to nonspecific adsorption of proteins on nanomaterial surfaces (forming a protein corona) in the biological milieu. Thus, protein adsorption often causes protein denaturation that leads to a signaling cascade, resulting in either nanomaterial aggregation and/or phagocytosis via activated macrophages [132]. Because the protein adsorption is nonspecific, it can also happen to nanomaterial-targeting moieties. Consequently, the protein adsorption negatively causes more nanomaterials to reach organs involved in clearance (like the kidney, liver and spleen) rather than the target sites [131]. The second challenge that these stimuli-responsive nanomaterials

share with conventional nanotherapeutics is the lack of efficient clearance of the nanotherapeutics from the body once they have accomplished their mission. Most nanotherapeutics have sizes beyond the renal threshold and cannot be removed from the body via the kidneys, and thus if they are not biodegradable they tend to accumulate in the body. Even for some biodegradable nanomaterials, their degraded fragments might be sequestered in lysosomal compartments to cause toxicity and side effects [131]. The third challenge is that in most cases targeting moieties conjugated on the nanomaterials are actually not specific to the target sites, because the receptors for the targeting moieties are expressed not only at the target sites but also in other organs. For example, folate receptor is overexpressed in a large number of malignancies but it is also expressed to a moderate-to-high level in normal organs including small intestine, placenta and kidneys. In addition, the overexpressed folate receptor is also inhomogeneously distributed on malignant cells, resulting in nonuniform accumulation of the nanotherapeutics in the target tissue. Furthermore, some targeting moieties like antibodies and peptides could lose their activity during conjugation with the nanomaterials and might not induce the intended tissue-targeting effect. Targeting ligands on the surface of the nanocarriers could also alter nanomaterial surface characteristics like the charge and hydrophobicity and lead to increased opsonization, aggregation and clearance of the nanomaterials by the mononuclear phagocyte system. The fourth challenge is that some of the physical stimuli might not be fully tolerated by the body and their use and controlling could incur additional cost. For example, UV light cannot penetrate into tissues deeper than ~10 mm owing to its absorption by endogenous chromophores such as oxy- and deoxy-hemoglobin, lipids and water; and prolonged UV irradiation can be cytotoxic [95]. Therefore, UV-responsive nanotherapeutics should be restricted to the eye, skin and other mucosal surfaces, be doped by upconversion luminescent materials or be used along with NIR [95–97,133]. The cavitation caused by ultrasound stimulus could enhance vessel permeability of cancer cells to cause metastatic dissemination. Electrical stimuli also have low tissue penetration and can possibly cause tissue damage, and thus limit the clinical application of electroresponsive nanoparticles despite the nanoparticle flexibility and low-cost advantages. A magnetic field stimulus is costly owing to its complexity and need of special set-up for adequate focusing and deep penetration into the disease area with sufficient strength. Thermoresponsive materials need longer duration to undergo phase transition that results in burst drug release, and precise temperature control at the target site without causing tissue damage is a challenge [134]. Owing to these challenges, restricted numbers of physical-stimuli-responsive nanotherapeutics have been advanced to the level of clinical studies. Therefore, for physical-stimuli-responsive nanotherapeutics to be developed into intelligent drug delivery systems to treat human diseases, continuous design improvements, more *in vivo* toxicology and efficacy evaluations, and robust stability and production scale-up studies on these nanomaterials are required in the future.

Acknowledgment

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