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Multi-Stimuli-Responsive Polymer Particles, Films, and Hydrogels for Drug Delivery

Xiao Fu,1 Leticia Hosta-Rigau,2,* Rona Chandrawati,3,* and Jiwei Cui1,*

Stimuli-responsive polymer materials are powerful tools in drug delivery and tissue engineering. Because of the large variations in physiological conditions between normal microenvironments and diseased sites, polymer materials with single responsiveness may not achieve the desired goals in a complex physiological microenvironment. Instead, polymer materials responsive to multiple physical or chemical stimuli are highly desired for biomedical applications (e.g., drug delivery). In this review, we highlight recent studies in multi-stimuli-responsive materials with a specific emphasis on polymer particles, films, and hydrogels. The synthetic strategies employed to produce these responsive materials are described. Applications in drug delivery are highlighted, followed by a discussion of the current research focus and future trends.

INTRODUCTION

Stimuli-responsive polymer materials have been widely investigated in the field of bio-nano research for biomedical applications (e.g., drug delivery and tissue engineering).1–4 Understanding the biological microenvironments is essential for the design of polymer materials that can selectively respond to biological stimuli at the organ level, under pathological conditions, and in different intracellular compartments. The Langer and Gu groups have recently summarized the most typical biological stimuli in the body.5 For example, pH values, enzyme concentrations, redox species, and glucose levels vary significantly in the microenvironments of different body parts and, therefore, can be exploited as pristine biological triggers to achieve controlled release of therapeutic molecules from drug carriers. Hence, bridging materials science and biology has advanced the design of polymer materials for biomedical applications.6,7

Recent reviews have focused on the assembly of polymer materials (e.g., polymer particles, films, and hydrogels) that are responsive to a single trigger.8–11 However, polymer materials with single responsiveness, in some cases, cannot achieve the desired goals in a complex physiological microenvironment. For example, a series of physiological and pathological barriers (e.g., mononuclear phagocyte system [MPS], non-specific distribution, cell internalization, endosome escape, multi-drug resistance) exist in organs, tissues, and cells, which leads to low drug-delivery efficiency.12–15 Therefore, polymer materials with rationally designed properties, including multi-stimuli responsiveness, are highly desired for biomedical applications. Here, we focus on the design, assembly, and applications of polymer particles, films, and hydrogels that are responsive to multiple stimuli, including biological and physical triggers (e.g., pH, reduction, enzyme, temperature, diol moieties, reactive oxygen species, ionic strength, shear stress, and light). We first provide an

The Bigger Picture

Responsive polymer materials are designed to alter their properties upon exposure to physical, chemical, and biological stimuli. They have served as powerful tools in drug delivery and tissue engineering. Through the integration of functional groups, these materials are engineered to “sense” the surrounding physiological environments and enable on-demand release of encapsulated therapeutic cargos into highly specific targets. However, the physiological environment is complex, and polymer materials with single responsiveness, in some cases, cannot achieve the desired goals in drug-delivery systems. The exploitation of polymer materials responsive to multiple stimuli promises new innovations in the design of functional materials for drug delivery. We provide an overview of the recent design and applications of multi-stimuli-responsive polymer particles, films, and hydrogels.
introduction to the nanoscale polymer particles mostly with dual-stimuli responsiveness and their application in drug delivery and further introduce the multi-stimuli-responsive polymer films engineered by the layer-by-layer (LbL) technique and surface-initiated polymerization method, as well as free-standing polymer films. Lastly, hydrogels with dual- and triple-stimuli responsiveness are reviewed. Our intention is to provide an overview and insight accessible to researchers (e.g., chemists, physicists, biologists, and medical scientists) active in different research fields with a special focus on exploring new directions and opportunities for the application of multi-stimuli-responsive polymer materials.

MULTI-STIMULI-RESPONSIVE POLYMER PARTICLES

For polymer particles to reach the desired sites of action, they have to go through several transport steps with multiple biological barriers, including circulation in the bloodstream, cell binding, cell internalization, and intracellular delivery. Although the enhanced permeability and retention (EPR) effect can improve the accumulation of particles in tumor tissues, insufficient cell uptake and intracellular processing can limit the therapeutic efficacy. Positively charged particles facilitate cell uptake because of the electrostatic interaction with the negatively charged cell membrane, which promotes the subsequent internalization. However, positively charged particles could induce rapid clearance by the MPS, instability with opsonins, and serum inhibition. Meanwhile, neutrally charged and highly hydrated particles can decrease non-specific interactions and improve circulation time. Therefore, it is essential to design a system that is neutrally charged to minimize the non-specific interactions with tissues and cells while being able to convert to a positive charge at targeted sites to help the cell uptake (e.g., tumor). It is well known that pH varies significantly in different body parts from tissue to cell level. For example, the pH of plasma is around 7.4, decreases to lower than 7.0 at tumor sites, and experiences a further decrease to lower than 5.0 in late endosomes and lysosomes after cellular uptake. Accordingly, particles responsive to pH have been engineered via surface modification with charge converters, which can revert the surface charge to be positive at low pH or can strip outer layers to expose positively charged inner layers at low pH. To circumvent biological barriers and trigger the drug release more efficiently, we require multi-responsive polymer particles with a hierarchical targeting strategy for the development of the next generation of nanomedicines (Figure 1).

Multi-Stage pH Stimuli

Multi-stage pH responsiveness indicates that the particles are engineered with different components, which have different sensitivity to pH changes. The Wang group reported the first example of dual pH-responsive polymer-drug particles where the conjugates were synthesized by conjugating doxorubicin (DOX) and 2,3-dimethylmaleic anhydride (D MMA) onto the copolymer of poly(ethylene glycol)-b-poly(allyl ethylene phosphate) (PEG-b-PAEP). The assembled polymer-drug particles could respond to the extracellular tumor pH by stripping D MMA to convert the surface charge, which promoted the drug accumulation at tumor sites and facilitated the cell internalization (Figure 2A). Furthermore, the free DOX was released in the intracellular subcompartments (e.g., endosomes and lysosomes) by cleaving the hydrazone bonds between the polymers and the drugs. Similarly, transactivator of transcription (TAT) peptides were conjugated onto the surface of polymer particles (i.e., polyethyleneimine-modified poly(β-L-malic acid), PEI-PMLA) and protected by pH-stripped PEGylation (i.e., PEG-D MMA). When the pH decreased at the tumor sites (pH < 7), PEG-D MMA was stripped to reverse the
particle surface charge from negative to positive, and TAT was exposed to enhance the cell internalization, followed by drug release at endo/lysosomal pH below 6. These particles showed improved inhibition of the tumor growth with negligible systemic toxicity. This design leads to more effective cell uptake and enhanced endo/lysosomal escape for improved drug delivery.

**pH and Reduction Stimuli**

The concentration of reducing species (e.g., glutathione [GSH]) in intracellular subcompartments (e.g., lysosomes and cytoplasm) is approximately 1,000-fold higher than that in the extracellular fluids. Therefore, the combination of pH and reduction responses into one carrier is also helpful to circumvent biological barriers for improved drug delivery.

The LbL assembly has been widely used to fabricate responsive polymer films with tunable architectures and properties.\textsuperscript{24} This technique simply involves the alternating deposition of different interacting materials onto substrates, and the driving forces for LbL film formation range from electrostatic interactions to hydrogen bonding, charge-transfer interactions, host-guest complexes, and coordination bonding.\textsuperscript{25,26} Advantages of this technique include the possibility of all-aqueous processing, operationally simple control over the thickness of the resulting films through the number of deposition cycles, and the possibility of creating stratified films.\textsuperscript{27,28} Capsules with a hollow structure can be obtained when the LbL films are deposited on templated particles followed by the template removal, which has shown promising applications in drug delivery.\textsuperscript{29,30}
The assembly of poly(2-diisopropylaminoethyl methacrylate) (PDPA, pK_a ~ 6.4) into multiple layers endows the capsules with pH-responsive properties given that the capsules swell at pH < 6.4 because of protonation of PDPA followed by a charge-shifting transition from a hydrophobic to a hydrophilic state. Both the size (swelling at pH < 6.4 and shrinking at pH > 6.4) and the surface charge of the capsules are reversible. Crosslinking of PDPA multi-layers based on a disulfide bond, which is one of the most used strategies to prepare reduction-sensitive carriers, renders degradable capsules in the presence of GSH. The degradation of the swelled capsules is much easier than that of the shrunk capsules. Thus, the synergistic effects
of pH together with reduction allow for rapid and effective degradation and cargo release at extremely low GSH microenvironments (down to 0.01 mM). In addition, intracellular degradation of the PDPA capsules can be tuned by varying crosslinking density through adjusting the quantity of crosslinkers.32

Dual pH- and reduction-sensitive drug-delivery systems have also been designed by self-assembly strategy. The Chen group reported a shell-stacked nanoparticle system via the formation of poly(L-lysine) (PLL)-based nanogels crosslinked by disulfide bonds followed by electrostatic adsorption of DMMA-modified PEG-b-PLL.33 The size of the obtained nanoparticles reduced from 145 nm to 40 nm and the surface charge reversed from −7.4 to 8.2 mV at acidic tumor tissues, which is due to the PEG-b-PLL release after cleaving the DMMA groups. The reduced size and positively charged surface favor the tumor accumulation and penetration, as well as the cell uptake. The disulfide crosslinking maintains the stability of the particles and prevents undesired premature drug release before the release of the stacked PEG-PLL shells, which accelerates the cleavage of disulfide bonds and intracellular drug release after cell uptake. Similar responsiveness was engineered via self-assembly of preformed polymers. Pluronic P123 was firstly conjugated with PEI via disulfide bonds and then modified with DMMA, which was used to assemble dual-responsive polymer particles.34 Acidic pH can cleave the DMMA groups to reduce the particle size and convert the surface charge from negative to positive, which helps the cell association and uptake as well as the subsequent endosome escape. The reduction microenvironment in the cytoplasm can strip PEI from particles and expose the dexamethasone-conjugated Pluronic P123, which can dilate the nuclear pores and facilitate the entry of DOX-loaded Pluronic P123 particles into the cell nuclei to improve the drug-delivery efficacy. In addition to the design of pH-stripped layers on polymer particles, Dai et al. reported self-assembled polymer micelles with a triblock copolymer composed of 2-(diisopropylamino)ethylamine-grafted poly(L-aspartic acid), 2-mercaptoethylamine-grafted poly(L-aspartic acid), and monomethoxy PEG.35 The micelles comprise a pH-responsive hydrated core, a disulfide crosslinked interlayer, and a PEG corona, which were used to load anti-cancer drugs (i.e., DOX), tie up the core against expansion at neutral pH, and reduce non-specific interactions, respectively. The interlayer crosslinked polymer micelles were stable and drug leakage was avoided in a neutral pH environment during blood circulation. After intracellular internalization, the micelles were disassembled by swelling the hydrated core at low pH and cleaving the disulfide bond crosslinked interlayers in endosomes or lysosomes, which induced the burst release of the loaded drugs.

Instead of reducing the disulfide bonds to release the encapsulated cargo, drugs can also be released by cleaving the ester bonds used to conjugate drugs onto polymers in the presence of reducing reagents. For example, PEGylated clustered nanoparticles were fabricated by polycaprolactone (PCL) and PEG-b-PCL together with a cis-platin (Pt) prodrug-conjugated poly(amidoamine)-graft-polycaprolactone (PAMAM-g-PCL), whereby PAMAM and PCL were conjugated with 2-propionic-3-methylmaleic anhydride (CDM) (Figure 2B).22 At physiological pH, the PEGylated nanoparticles with a size of around 100 nm could circulate for a long time and enhance tumor accumulation based on the EPR effect. At pH < 7 at tumor sites, the clustered nanoparticles disassembled by cleaving the CDM groups and released small PAMAM/Pt prodrug conjugates. The released PAMAM/Pt prodrugs with an average size of 5 nm and increased surface charge are highly capable of penetrating tumors to reach cancer cells that are far away from the blood vessels. After cell internalization, the prodrug conjugates can be rapidly cleaved to release Pt, thus killing cancer cells and restricting tumor growth.
**pH and Diol Stimuli**

One-step assembly of metal-phenolic networks (MPNs) has been recently proved to be a versatile method for drug-carrier engineering, owing to their pH responsiveness and negligible cytotoxicity. Different polyphenols and metal ions can be used as components for capsule assembly. The difference of binding affinity between polyphenols (e.g., tannic acid [TA]) and metal ions induces various pH-disassembly profiles. MPN capsules composed of higher-valence metal ions are typically more stable under acidic pH (e.g., Zr<sup>4+</sup> > Al<sup>3+</sup> > Cu<sup>2+</sup>), although Fe<sup>3+</sup> results in one of the most stable capsules probably because of its high binding affinity with polyphenols. In addition to metal ions, polyphenol can also coordinate with boronic acid to form boronate-phenolic networks (BPNs). The Caruso group has reported the assembly of BPN capsules by using TA and benzene-1,4-diboronic acid, which are responsive to acidic pH and also in the presence of exogenous competing cis-diols (e.g., glucose) (Figure 3A). These capsules are stable at physiological pH in vivo and can be potentially used for insulin delivery by glucose activation and anti-cancer drug delivery by acidic pH stimulus.

**pH and Light Stimuli**

Photothermal therapy (PTT) and photodynamic therapy (PDT) based on light irradiation have been widely used for tumor therapies, whereby the photothermal or photodynamic agents are incorporated into the drug carriers. However, these therapeutics are still limited by rapid renal clearance and non-specific tissue distribution. Very recently, the Wang group designed a transformable polymer nanoparticle system that can minimize the interaction with the MPS and has a long circulation time (Figure 3B). When the nanoparticles accumulated in the tumor sites based on the EPR effect, sheddable modifications (i.e., DMMA) on the nanoparticles were stripped in the trigger of acidic pH and then TAT peptides (i.e., YGRKKRRQRRRC-NH<sub>2</sub>) were exposed, which improved cell association and internalization. Near-infrared (NIR) light irradiation of the encapsulated IR-780 iodide promoted the DOX release loaded in the nanoparticles, thus killing the tumor cells.

**pH and Temperature Stimuli**

To introduce temperature responsiveness into polymer particles, polymers with a lower critical solution temperature (LCST) are typically used as building blocks. PNIPAM is a classical temperature-responsive material used in drug-delivery systems. PNIPAM undergoes a phase transition from a hydrophobic to a hydrophilic state when the external temperature is below the LCST of about 32°C in aqueous solution. The reason for the phase transition is mainly the formation of hydrogen bonds between its amide groups and water molecules below the LCST. When the surrounding temperature is above the LCST, the hydrogen bonds between PNIPAM and water are disrupted, and PNIPAM collapses into a globule state, making the gel network become aggregated and hydrophobic. Chiang et al. reported pH- and temperature-responsive polymer particles partially comprising poly(acrylic acid) (PAA) and PNIPAM. The drug (i.e., DOX) can be loaded below LCST because of the swelling of the polymer particles and is released slowly above the LCST. When the microenvironment pH is decreased, the extensive disruption of carboxyl groups and DOX resulting from the reduced ionization of AA residues enables the rapid release of DOX. Similarly, poly(β-aminoester) dendrimers with pH and temperature responsiveness can load drugs below LCST and allow the fast drug release in acidic intracellular subcompartments (e.g., endosomes or lysosomes). In addition, multi-stimuli-responsive poly(vinylcaprolactam) (PVCL)-based particles were reported via a precipitation polymerization method. The temperature sensitivity of the particles can be tuned by varying the pH and the doped content of methacrylic...
Figure 3. Examples of Polymer Particles that Are Responsive to pH and Diols or pH and Light

(A) The formation of films on particulate template and dual-responsive degradation of the particles in the presence of acidic pH and/or diols (left). The chemistry between tannic acid (TA) and benzene-1,4-diboronic acid (BDBA) in response to pH and/or diols for film assembly and disassembly (right). Reprinted with permission from Guo et al. Copyright 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

(B) IR-780 iodide and DOX are encapsulated in TAT-modified polymer nanoparticles composed of poly(ethylene glycol)-block-poly(2-hexoxy-2-oxo-1,3,2-dioxaphospholane), which are responsive to pH and light for cancer therapy. Reprinted with permission from Li et al. Copyright 2017 American Chemical Society.
acid (MA), whereby the LCST increases when the MA content or the pH of the solution is increased. The introduction of MA and the disulfide-bonded crosslinker \(N,N'\)-bis(acryloyl)cystamine endow the PVCL-based particles with pH and reduction sensitivities, which cause the particles to shrink at acid pH followed by their degradation in the presence of the GSH reduction reagent.

Other Stimuli

The multi-responsiveness, as discussed above, is mostly based on single particles. However, for specific applications it is required to either engineer preformed particle aggregates followed by the release of single particles, or to form assemblies from single particles with biological triggers that disassemble when the microenvironment is changed. In the case of drug targeting to obstructed blood vessels, micrometer-sized poly(lactic-co-glycolic acid) (PLGA) particle aggregates were fabricated with multiple PLGA nanoparticles, which are stable in aqueous solution because of hydrophobic interactions between the nanoparticles (Figure 4A).\(^46\) However, these microscale PLGA particles can break into individual nanoparticles when exposed to a high-shear-stress microenvironment (e.g., vascular narrowing caused by thrombosis) (Figure 4B). The released nanoparticles coated with thrombolytic drugs (i.e., tissue plasminogen activator) can effectively bind and dissolve the blood clot to restore normal flow dynamics. The biocompatible PLGA can be degraded by hydrolysis of the ester bonds in the body.

As opposed to particle sizes that change from microscale aggregates to nanoscale particles, in vivo triggered assembly from small particles to big aggregates has also been recently investigated to improve cancer therapy. The Chen group reported the endogenous phosphatase-triggered assembly of nanofibers with micelles
composed of NapFFKYp peptide and indocyanine green (ICG) at the tumor site followed by PTT triggered by NIR radiation. In addition, the doped ICG served both as a fluorescent and a photoacoustic imaging probe. Although the in situ formation of nanofibers could avoid the uptake in MPS-rich organs (e.g., liver and spleen) and simultaneously improve tumor accumulation and retention, the tumor penetration and cellular uptake of the formed nanofibers were impaired, which limits the strategy for the delivery of therapeutics without photothermal or photodynamic properties. To circumvent this scientific issue, a deoxygenation of polyvinylpyrrolidone-modified Mg$_2$Si nanoparticles was recently introduced for cancer starvation therapy. The Mg$_2$Si nanoparticles released SiH$_4$ in the acidic tumor microenvironment, which efficiently reacted with oxygen dissolved in tissue and bound with hemoglobin to form SiO$_2$ microsheets. Co-registration of multi-wavelength photoacoustic and B-mode ultrasonic measurements and positron emission tomography and computed tomography assessment indicated efficient deoxygenation around the tumor via intratumoral injection of Mg$_2$Si nanoparticles. Magnetic resonance imaging confirmed that the formed microsheets significantly inhibited the tumor vasopermeability, which also induced intratumoral hypoxia by blocking the supply of blood oxygen. As a result, Mg$_2$Si acts as an efficient inhibitor of tumor growth with no detectable toxicity in the main organ tissues. Moreover, the SiO$_2$ microsheets are degradable in tumor tissues while maintaining the severe hypoxia.

MULTI-STIMULI-RESPONSIVE POLYMER FILMS

Polymer films that simultaneously respond to multiple stimuli have attracted increasing interest among researchers from various fields, especially in the bio-related area (e.g., drug delivery). Various types of stimuli, including chemical stimuli (pH, ionic strength, reduction and oxidation, or type of salt) and physical stimuli (such as temperature or light), have been employed to trigger responsiveness of those polymer films. Multi-stimuli-responsive films have been mainly fabricated by the LbL technique or by using polymer brushes. To date, LbL films have been designed to respond to a number of stimuli, including pH, temperature, or electrochemical potential, which are promising for diverse biomedical applications. In a different approach, polymer brushes with one end anchored to the surface of substrates through physical adsorption or covalent chemical attachment have also been employed to create functional films. The appealing features of polymer brushes are their high degree of synthetic flexibility as well as their mechanical and chemical robustness. By integrating different reactive moieties into individual polymer chains, often taking advantage of controlled and living polymerization techniques, the films composed of polymer brushes can be responsive to various stimuli.

LbL Films

A very common approach to create multi-responsive films is by combining pH and temperature responsiveness. Zhuk et al. reported LbL films with pH, temperature, and salt-concentration responsiveness. The films were assembled by employing temperature- and ion-responsive PNIPAM and montmorillonite clay nanosheets. Clay nanoparticles were introduced onto the LbL films to enhance their mechanical robustness. PNIPAM undergoes a phase transition from a hydrophobic to a hydrophilic state when the external temperature is below the LCST of about 32°C in aqueous solution. Additionally, the different type of ions and their concentration in solution may significantly influence the LCST of PNIPAM. In particular, the addition of a simple salt generally results in a decrease of PNIPAM’s solubility, thus decreasing the LCST because of the “salting out” effect. In the case of certain thiocyanates or quaternary ammonium salts, the opposite is observed and an
increase in the salt concentration results in an increase of PNIPAM’s solubility, thus increasing the LCST because of the “salting in” effect. Importantly, it has also been demonstrated that the anions of a given salt influence the LCST to a greater extent than the cations and can be ranked according to their position on the Hoffmeister series. The pH responsiveness of the system was achieved by embedding poly(methacrylic acid) (PMA) in the LbL films. The PNIPAM/clay/PNIPAM/PMA films’ response to pH, temperature, and salt concentration was assessed by monitoring the changes in the films’ swelling ratio and permeability of fluorescein-labeled dextran molecules with various molecular weights. The results demonstrated that at a temperature higher than the LCST of PNIPAM, the PNIPAM/clay/PNIPAM/PMA films deswelled as a result of the PNIPAM collapse into a globular state, which generated a decrease in permeability to the fluorescein-labeled dextran molecules. This effect could be reversed by decreasing the temperature to values lower than the LCST of PNIPAM. The pH responsiveness was assessed by lowering the pH to acidic conditions, which resulted in the deswelling of the films because of the hydrogen bonding between PNIPAM and the protonated PMA. This deswelling completely obstructed the diffusion of the fluorescein-labeled dextran.

Responsive polymer conjugates based on host-guest chemistry and PEGylated nanoparticles have been assembled to fabricate pH-, light-, and ionic-strength-responsive films (Figure 5A). In particular, α-cyclodextrins (α-CDs) labeled with rhodamine B (RhB) were used as a model drug (α-CD-RhB) and conjugated to the copolymer poly[2-(4-phenylazophenoxy)ethyl acrylate-co-acrylic acid] (PEAPE) modified with trans-azobenzenes via host-guest interaction between the α-CDs and the trans-azo moieties. The resulting α-CD-RhB-loaded PEAPE...
(PEAPE@α-CD-RhB) was able to interact with PEGylated NPs via hydrogen bonding at acidic conditions to form LbL multi-layered films (Figure 5A). Because of the light-sensitive nature of the azobenzene groups, the obtained films were able to release α-CD-RhB in a controlled manner after irradiation with UV light. Figures 5Bi and 5Bii demonstrate that 98 wt % of α-CD-RhB is released within 100 min from the 20-bilayer film under UV irradiation, while the 10-bilayer film can release ca. 99% drugs within 60 min. Additionally, the release of α-CD-RhB from the films could also be tuned by the ionic strength of the solution, as shown by immersing the multi-layer film in NaCl solutions at different concentrations (Figure 5Biii). The results demonstrate that more α-CD-RhB is released in the presence of NaCl, which can be attributed to the dissociation of the host-guest interaction between the PEAPE polymer and the α-CD-RhB. Furthermore, the films could be rapidly disassembled to simultaneously release nanoparticles upon exposure to physiological conditions because of the pH-erasable characteristics of the hydrogen-bonding multi-layers (Figure 5Biv). The PEAPE@α-CD-RhB films responsive to light, pH value, and ionic strength show potential to serve as multi-functional coatings containing nanocarriers for drug-delivery applications.

In contrast to the introduction of responsive polymers to engineer responsive films, the pH and temperature responsiveness of films can be controlled by the equilibrium of dynamic bonds formed in LbL films. For example, LbL films were crosslinked by Schiff base dynamic bonds created between the aldehyde groups of partially oxidized dextran (PO-Dex) and amino groups of chitosan (Chi). The swelling degree of the resulting multi-layers could be tuned by pH and temperature but also by molecules such as L-lysine and pyridoxal, since these stimuli shifted the equilibrium of the Schiff base reaction that changed the effective crosslinking density of the film and, hence, the swelling degree. The pH responsivity at different pH values (pH 1–9) was attributed to the pH-dependent protonation of the free amino groups on Chi chains as well as to the equilibrium of the formation and breakage of the Schiff base bonds between the layers. The responsiveness of PO-Dex/Chi films to temperature was examined by heating the film, which revealed a swelling of the film upon heating from 10°C to 40°C. The temperature responsiveness was also attributed to a shift on the equilibrium of the Schiff base reaction since neither Dex nor Chi are thermosensitive. Heating the films resulted in the breakage of the Schiff base reaction, which induces a decreased crosslinking density and, therefore, an increased swelling degree. The PO-Dex/Chi films are also sensitive to molecules containing amino or aldehyde groups, such as L-lysine and pyridoxal, respectively. As a result, upon introduction of L-lysine or pyridoxal, the films swelled by 50% and 20% for L-lysine and pyridoxal, respectively. These results can be attributed to the competition of the amino groups of L-lysine with Chi for binding with the aldehyde groups of PO-Dex and the competition of the aldehyde groups of pyridoxal, which compete with PO-Dex to bind with the amino groups of Chi.

**Polymer Brushes**

In addition to responsive LbL films, polymer brushes have been used for the controlled release of encapsulated molecules in response to pH, light, and temperature stimuli. Surface-initiated atom transfer radical polymerization was employed to grow sequentially a first block serving as an inner reservoir for cargo loading and a second block acting as a stimulus-responsive outer layer controlling the closing or opening of the brush in water (i.e., the outer chain is collapsed or swelled). Diblock copolymer brushes based on PNIPAM were employed to achieve thermosensitivity. For encapsulation and release of both hydrophobic and hydrophilic compounds, polymers of polystyrene-b-PNIPAM (PS-b-PNIPAM) with a hydrophobic inner...
layer and poly(N,N'-dimethylacrylamide)-b-PNIPAM (PDMA-b-PNIPAM) with a hydrophilic inner layer were synthesized. As model dyes, hydrophobic Nile red and hydrophilic 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt were encapsulated within PS-b-PNIPAM and PDMA-b-PNIPAM films, respectively. The thermosensitive release of the dyes was demonstrated by the release kinetics obtained at 20°C and 40°C, respectively. At 20°C, below the LCST of PNIPAM, the PNIPAM blocks of the top layer will swell, thus opening the channels within the brush as a result of the PNIPAM’s solubility followed by the release of the dyes. In contrast, at 40°C, which is above the LCST of PNIPAM, the top layer will collapse because of the loss of hydrophilicity, thus contracting the polymer chains and constraining the release of the loaded dyes. The results demonstrated that for NR, although the diffusion rate was very slow owing to its hydrophobic nature, the maximum NR release was observed at 20°C with a much faster release than at 40°C. As expected, for PTS at 20°C, the soluble PNIPAM top layer led to a faster release of the dye than the collapsed PNIPAM top layer at 40°C. The hydrophilic nature of PTS made its overall release much faster than that of NR. For the tuning of dye release kinetics with light, a diblock copolymer brush of PS-b-poly(4,5-dimethoxy-2-nitrobenzyl methacrylate) (PS-b-PNBA) was designed. The PNBA segment of the polymer is converted into hydrophilic PMA by cleaving the o-nitrobenzyl groups upon exposing the hydrophobic PNBA groups to UV light. This conversion, in turn, induces a transition from a collapsed to swelled state of the top layer. The resulting PS-b-PMA is pH sensitive, and a reversible transition between collapsed chains (with protonated acid groups, insoluble in water) and swelled chains (with deprotonated acid groups, soluble in water) can be triggered by a pH change. All in all, diblock copolymer brushes can be used as stimuli-sensitive release systems for three different stimuli.

Free-Standing Films
Free-standing films with multi-responsiveness can also be fabricated by the LbL method. Light was first used as a stimulus to detach the film from the support. Redox stimuli were then employed to release a model drug from the free-standing film. Furthermore, the film could be completely dissolved by increasing the pH (Figure 6A). To obtain such a design, a bilayer of photodegradable polymer (P1) and poly(styrene sulfonate) (PSS) was first deposited onto a substrate (Figure 6A), followed by the deposition of a redox/pH dual-responsive polycation (P2) and PSS. The photodegradable polymer P1 was prepared from the methylated nitrobenzyl (NB) methacrylate monomer (MNBA) (Figure 6Bi) and N,N-dimethylaminomethylmethacrylate (DMAEMA). The NB esters on the copolymer are photochemically degradable (Figure 6Bii, step 1). P2 is a triblock copolymer prepared from DMAEMA, coumarinyl methacrylate (CMA), and a methacrylate with RhB conjugated via a disulfide bond (RhoSSMA) (Figures 6Bi and 6Bii). The P1 layer can be cleaved from the substrate upon irradiation, and the remaining P2/PSS film can be peeled off from the substrate to form a free-standing film. As a demonstration of the redox responsiveness of the films, the films were incubated in a DTT solution and the release of RhB via the cleavage of disulfide bonds was monitored by fluorescence spectroscopy (Figure 6Bii, step 2). In addition, the P2/PSS films can be dissolved within seconds after incubation in a solution at pH >9.3. Under basic conditions, the ammonium groups of P2 deprotonate, thus converting into neutral amines, which results in the loss of electrostatic interactions between the P2/PSS bilayer and the subsequent film dissolution (Figure 6Bii, step 3). The free-standing films were able to release covalently bound and physically trapped cargoes upon three different stimuli, which will be advantageous in drug-delivery applications.
Figure 6. Free-Standing Films with Responsiveness toward Light, Redox Stimuli, and pH

(A) Schematic representation of multi-layer film composed of negatively charged polystyrene sulfonate (PSS), the photodegradable polymer P1, and the redox/pH dual-responsive polycation P2.

(B) (i) Monomers employed for P1 and P2 synthesis. (ii) Responsive behavior of the polycations employed in the assembly of the triple-responsive multi-layered films.

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MULTI-STIMULI-RESPONSIVE HYDROGELS

Hydrogels are crosslinked, hydrophilic polymer networks that are capable of absorbing large amounts of water or biological fluids. 3D hydrogels are attractive materials owing to their resemblance to natural soft tissues, their ability to encapsulate cells and bioactive molecules, and their high permeability for oxygen and nutrients. In general, hydrogels can be prepared from either natural polymers (e.g., alginate, gelatin, or hyaluronic acid) or synthetic polymers (e.g., PEG or PAA). Stimuli-responsive hydrogels particularly have been widely reported to tune drug release rates for drug delivery.

Dual-Stimuli-Responsive Hydrogels

Because of the large variations in pH between the normal physiological environments and the diseased sites, the majority of multi-stimuli-responsive hydrogels reported to date are based on pH responsiveness. Hydrogels are designed to swell, shrink, or dissociate in response to pH changes, and encapsulated drugs are locally delivered through diffusion or degradation of the biomaterials.

Fu and coworkers developed PAA-PEG hydrogels that swelled or shrunk reversibly in response to pH and Ca²⁺. Since PAA has a pKₐ value of about 4.5, at pH < pKa the carboxylic groups of PAA were protonated and interacted with the PEG chains via hydrogen bonding. On the other hand, at pH > pKa (pH 5–10) the hydrogen bonding between PAA and PEG chains was disrupted, causing a repulsion between the polymer chains within the hydrogel, which led to an increase in the swelling ratio. The swelling ratio could also be controlled by different levels of Ca²⁺ ions, which interacted electrostatically with PAA. The hydrogels exhibited a lower swelling ratio and a higher storage modulus at Ca²⁺ concentrations above 10 mM. Since the concentration of Ca²⁺ plays an important role in the function and organization of articular cartilage, this hydrogel system has a potential in drug-delivery systems for treatment of osteoarthritis in the knee joint.

In situ copolymerization of neutral monomers, acrylamide (AAm), and cationic monomers, methyl chloride quaternized N,N-dimethylamino ethylacrylate (DMAEA-Q) has been used to fabricate pH- and ion-responsive hydrogels. The hydrogels were crosslinked using self-assembled Pluronic F127 diacrylate (F127DA) micelles. The charged DMAEA-Q endowed the hydrogels with reversible responsiveness to both pH (pH 3–11) and ionic strength changes (50–200 mM). As an alternative to copolymerization methods, pH- and ion-responsive hydrogels can also be obtained via β-CD-ferrocene host-guest supramolecular interactions. Three hydrogel building blocks were employed by tuning the presence of the host and guest molecules: (1) responsive host (RH) hydrogels that contained β-CD and methacrylic acid (MAA) units, (2) non-responsive host (NRH) hydrogels that only contained β-CD host units, and (3) non-responsive guest (NRG) hydrogels that only contained ferrocene guest units (Figure 7A). Because of the presence of responsive MAA units (pKₐ 4.3), RH hydrogels exhibited a tunable swelling ratio upon variation of pH (pH 2–12) and ionic strength (0–100 mM) (Figures 7B and 7C). Annealing the three building blocks together (RH hydrogel, red; NRH hydrogel, blue; NRG hydrogel, brown), structures of complex geometries were assembled with reversible shape-changing behavior in response to various pH and ionic strength (Figure 7D). By varying the host-guest interactions and the hydrogel building blocks, this approach could be further extended to responsive systems to meet the need of specific drug-delivery applications.
In addition to pH, temperature is one of the most frequently studied stimuli to engineer responsive materials, in particular for drug-delivery research. For example, β-CD-based hydrogels with dual pH and temperature sensitivities were fabricated via a copolymerization method. Specifically, β-CD was modified with MAA,

Figure 7. pH- and Ion-Responsive Hydrogels formed via β-Cyclodextrin-Ferrocene Host-Guest Supramolecular Interactions
(A) Chemical structures of responsive host (RH) hydrogel building block that contains β-cyclodextrin (β-CD) and methacrylic acid (MAA) units, non-responsive host (NRH) hydrogel building block that contains only β-CD host units, and non-responsive guest (NRG) hydrogel building block that contains only ferrocene guest units.
(B and C) Swelling ratio of RH hydrogels upon variation of (B) pH and (C) ionic strength.
(D) Complex multi-stimuli-responsive hydrogels are assembled by annealing RH (red), NRH (blue), and NRG (brown) hydrogels together. Reversible shape changing behavior in response to various pH and ionic strength is shown. Scale bars, 5 mm.
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followed by copolymerization with MAA (pH-responsive units) and N,N-methylene diacrylamide (MBA, temperature-responsive units). Atorvastatin, a cholesterol-lowering drug, was encapsulated in the hydrogel via physical adsorption and through the atorvastatin/β-CD inclusion complex, which increased the drug-loading efficacy. Ninety percent of the encapsulated atorvastatin was released at pH 8.0 as controlled by both diffusion and hydrogel swelling, in comparison with a drug release of only 4.5% at pH 2.7. Increasing the temperature from 25°C to 42°C led to an increase in the swelling ratio of the gels, enhancing the drug release. The temperature-sensitive swelling of the hydrogel was due to the MBA units because polymers of acrylamide and its derivatives are temperature responsive. Mocanu et al. reported responsive pullulan-based hydrogels containing carboxymethyl and Jeffamine groups (combination of polyethylene oxide [PEO] and polypropylene oxide [PPO]), which are pH and temperature sensitive, respectively. Recently, molecules covalently modified with several oligo(ethylene glycol) moieties, such as PEO and PPO, have been reported to show thermoresponsive properties that originate from the hydrophobic/hydrophilic balance of the different oligo(ethylene glycol) components. In particular, pullulan containing Jeff M-600 (PPO/PEO 9:1, LCST = 59°C) and Jeff M-2005 (PPO/PEO 29:6, LCST = 25°C) were employed. The pH-sensitive properties of the hydrogels are caused by the ionizable carboxylic units, which determine the swelling and shrinkage of the hydrogels resulting from the electrostatic interactions as a function of pH and the temperature-responsive-ness results from the Jeffamine groups. Whereas the hydrogels containing the Jeffamine M-2005 shrunk at 40°C and 60°C because of the low LCST of Jeffamine M-2005, M-600 Jeff hydrogels swelled at 40°C and started shrinking at around 60°C, close to the LCST of Jeffamine M-600. Two proteins of different molecular weight and isoelectric point (pI), namely lysozyme (14 kDa, pI 11) and BSA (67 kDa, pI 5), were encapsulated in the gels, and size-dependent diffusion of proteins in response to changes in pH (pH 1.2–7.2) and temperature (20°C–40°C) was shown. The functionality of the released proteins was preserved within the gels as confirmed by their enzymatic activity.

Similar to polymer particles, reduction-responsive hydrogels can be designed based on the formation of disulfide bonds. To design pH- and reduction-responsive hydrogels, lipoic acid (Lipo) has been used to functionalize PLL. The PLL-Lipo hydrogels can be formed in water through ethylene oxide sterilization, which is possibly due to the cleavage of disulfide bonds during the sterilization and reformation of the intramolecular and intermolecular disulfide bonds in aqueous solution. The as-prepared hydrogel swelled at low pH as a result of the protonation of amino groups and subsequent charge repulsion, and disassembled in the presence of GSH. In addition, reduction- and temperature-responsive hydrogels have been used for site-specific drug delivery. Specifically, poly(ether-urethane) solution underwent a sol-to-gel phase transition with increasing temperature at physiological conditions (pH 7.4), which is favorable for direct drug loading to the polymer solution and direct injection without any surgical procedures. The disulfide bonds in the hydrogels can be degraded by GSH at pH 7.4, inducing the drug release.

Most of the studies to date on multi-stimuli-responsive hydrogels required steps to chemically incorporate different responsive units into polymer building blocks. Klymenko et al. reported a different approach for the formation of multi-responsive hydrogels by simply mixing solutions of two different triblock copolymers, each responding to a different stimulus, i.e., a PAA-based pH-responsive amphiphilic triblock copolymer and a PEO-based UV-responsive amphiphilic triblock copolymer. The polymer properties were preserved, and an advantage of this technique is that it
offers the fabrication of multi-stimuli-responsive hydrogels in a more straightforward way as an alternative to having to synthesize new types of block copolymers. By employing a combinatorial approach, a range of stimuli-responsive ingredients can be incorporated, leading to the synthesis of new hydrogel systems that combine all the functional elements contained in the building block design.

### Triple-Stimuli-Responsive Hydrogels

With the progress and success in the design of dual-responsive hydrogels, there is an increasing amount of reports on hydrogels responding to more than two stimuli. Fu and coworkers further developed two-stimuli hydrogels to three-stimuli (i.e., pH, ion, and temperature) responsive hydrogels via in situ copolymerization of NIPAM (temperature-responsive units) and acryloyloxyethyltrimethyl ammonium chloride (DAC) (pH- and ionic-responsive units). The temperature responsiveness of the gels was tuned by varying the NIPAM/DAC molar ratio. An increase in DAC content or a decrease in NIPAM content enhanced the hydrophilicity of the gels, resulting in a shift of the LCST to a higher temperature (in a range of 32°C–41°C); as a result, a higher temperature was needed to deswell the gels. The presence of positively charged DAC rendered the gels responding to pH (pH 3–11) and ionic strength (0–100 mM NaCl).

As enzyme dysregulation is associated with many pathological disorders, their altered expression level is exploited as a trigger to achieve local, controlled release of biomolecules from biomaterials. pH-, ion-, and protease-responsive poly(L-glutamic acid-co-L-lysine) hydrogels were prepared by mixing poly(L-glutamic acid-co-L-lysine) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride followed by 5 min of gelation. Since the pI of the copolymer is 6, the carboxylic acid and amine groups were protonated (accumulation of positive charges) and deprotonated (accumulation of negative charges) at pH < 6 and > 6, respectively. Therefore, the pH changes led to an increase in the swelling ratio of the hydrogels caused by charge repulsion in both cases. The hydrogels were also responsive to changes in ionic strength (10–1,000 mM NaCl) at a constant pH (7.4). In low ionic strength buffer, the carboxylic acid and amine groups interacted electrostatically, leading to the shrinkage of hydrogels. An increasing amount of NaCl screened these functional groups (NH₃⁺ and COO⁻ groups were screened by Cl⁻ and Na⁺, respectively), hence the hydrogels were more relaxed and swollen. For investigation of the enzyme responsiveness, two drugs with different charges, namely DOX (anti-cancer drug, positively charged) and diclofenac sodium (anti-inflammatory drug, negatively charged), were encapsulated in the gels, and the release behaviors of the drugs were investigated in response to trypsin (expressed in various carcinomas and inflammations). Complete enzymatic degradation of hydrogels and drug release was observed over 6 hr in the presence of 50 U/mL trypsin at physiological conditions. Substrates within hydrogels can be altered with the aim of increasing or decreasing accessibility to the enzyme. This can greatly affect the kinetics of the enzyme-catalyzed reactions, i.e., degradation or morphological transformation of hydrogels, and provide more precise control of drug delivery.

Feng and coworkers developed pH-, temperature-, and light-responsive hydrogels through the coassembly of L-phenylalanine-based amphiphiles and azobenzene derivatives (Figure 8A). The phenylalanine groups provided the pH response, and the amide bonds in the phenylalanine-based amphiphiles exhibited thermosensitivity. Small-molecule amphiphiles bound together through non-covalent interactions have recently been demonstrated to be an effective way to attain thermal sensitivity. The azobenzene groups, on the other hand, have a unique light-induced trans-cis isomerization property that made the gel light responsive. Upon
photoirradiation with UV light of 365 nm, the trans-azobenzene units were converted to their cis configurations, which leads to gel disassembly.

(B) Photographs of the reversible gel-sol transition triggered by multiple stimuli: temperature, host-guest interaction, pH, and photoirradiation. α-CD, α-cyclodextrin.

(C) (i) Schematic illustration of release of encapsulated cells within hydrogels after UV irradiation. (ii) The density of mouse embryonic fibroblast cells (NIH 3T3) encapsulated in the hydrogels before and after 30 min of UV irradiation at different culture times. (iii) Photographs of the hydrogel-cell construct after different times of UV exposure.

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Reversible gel-sol transitions were achieved as follows (Figure 8B): (1) dissolution was triggered with an increasing temperature and gelation was achieved upon cooling; (2) hydrogels could be dissolved in the presence of α-CD and recovered by adding adamantane into the solution; (3) hydrogels could be dissolved and reformed at pH above and below 10, respectively; and (4) upon UV (365 nm) irradiation, gels collapsed and the reversible formation of the hydrogel was achieved by visible light (450–490 nm) irradiation. Mouse embryonic fibroblast cells (NIH 3T3) were seeded in the hydrogel with an increased cell density observed over 7 days. The hydrogels were then exposed to UV light for 30 min, and a complete gel-to-solution phase transition was achieved that led to the release of the cells entrapped in the hydrogel (Figure 8C). The possibility to release cells under UV light without the use of additional factors such as enzymes offers a biologically friendly system for controlling cell encapsulation and release from responsive biomaterials.

Reversible chemical and intermolecular interactions enable the development of self-healing materials. Hydrogels that have the ability to self-heal from an event of damage have the potential to restore their original properties and prolong their lifetime. Wang et al. developed self-healing pH-, redox-, and ion-responsive Chi hydrogels using ferrocene groups as the responsive units (Figure 9A). Ferrocene is an attractive compound for the design of supramolecular hydrogels because of its unique sandwich structure, low toxicity, hydrophobicity, and redox properties. The hydrophobic aggregation of ferrocene groups on the Chi chain acted as reversible crosslinking points. When the gel was cut in half, the ferrocene groups reaggregated into hydrophobic microdomains and allowed the gel to bond back together. The gelation behavior of the ferrocene-modified Chi system could be tuned at pH 4 and at pH above 6.5 (Figure 9B), and in the presence of an oxidant (NaClO) or a reductant (GSH) (Figure 9C). At pH > 6.5, the amine groups of Chi backbone were deprotonated and the polymer formed strong intramolecular and intermolecular hydrogen bonds, which led to Chi insolubility. In the presence of NaClO, ferrocene was oxidized to ferrocene cation and the π-π stacking of ferrocene groups was disrupted, which led to gel disassembly. The gels also demonstrated different phenomena toward a range of ions (Cd^{2+}, Cr^{3+}, Pb^{2+}, and Cu^{2+}) (Figure 8D). These ions changed the surrounding ion environment of ferrocenyl moieties and disrupted the π-π stacking of ferrocene groups, causing gel-to-sol transition.

An impressive achievement was reported by DeForest and colleagues, who designed 17 distinct stimuli-responsive crosslinkers (“logic gates”) and fabricated a number of PEG-based hydrogels that responded to specific cues based on a simple
principle of Boolean logic, “YES,” “AND,” or “OR” (Figure 10A). Each of these crosslinkers is uniquely sensitive to the combination of three environmental triggers (light, enzyme, reductant). For example, hydrogels can be instructed to open gates and release their cargo in response to light OR reductant, light AND enzyme, and light AND enzyme AND reductant. Controlled release of DOX in response to disease-associated cues was demonstrated. HeLa cells were incubated in supernatants of DOX-containing hydrogels crosslinked with enzyme AND reductant gates. In the absence of treatment, or in the presence of enzyme matrix metalloproteinase 8 (MMP-8) or GSH alone, the hydrogel remained intact and normal cell proliferation was observed. In stark contrast, the presence of both MMP-8 and GSH resulted in complete cell eradication (Figure 10B). The crosslinkers within the hydrogels can be easily switched during the synthesis process, allowing the design of many different types of hydrogels with greater specificity for organs, tissues, or disease states.

CONCLUSIONS AND PERSPECTIVES

In this review, we have highlighted the most recent advances in multi-stimuli-responsive materials (i.e., particles, films, and hydrogels), which can go through physical or chemical changes via two or more environmental stimuli. Responses of polymer particles, films, and hydrogels translated from the activities of different stimuli in biomedical applications were discussed. Despite diverse strategies and increasing efforts as shown by the growing number of scientific publications, several barriers to clinical translation remain. The first challenge relies on obtaining...
multi-stimuli-responsive polymer materials that exhibit a sensitive and durable response in vivo. Many of the reports have been evaluated in vitro and in small animals. Although the designed platforms successfully respond to disease-associated hallmarks, these markers are rarely unique to a single diseased location, which may lead to suboptimal selectivity in the highly complex in vivo milieu. Furthermore, ideally the polymer materials will need to be removed from the body once they have delivered their drug payloads. The clinical usefulness of the responsive materials will be limited if the polymers persist in tissues for a long time after administration. Polymer size has dramatic effects on their transport throughout the body in vivo. Renal filtration is the primary mechanism for elimination of polymers from the body. In general, polymers and the degraded compositions should be less than ~40 kDa to be filtered by the kidney. Polymers that are too large to be excreted will be retained in the body for an extended period of time, which, depending on their long-term toxicity and immunogenicity, may limit their possible clinical applications. In addition to size, polymer chemistry, molecular conformation, and flexibility also play a significant role. Although advances in polymer synthesis have made it possible to design materials with tunable biological, chemical, and pharmacological properties, it is difficult to overcome the biological barriers (e.g., MPS clearance) without incorporating other rational design of the material physicochemical properties. Therefore, in order to have translational prospects, these parameters need to be taken into account for the translation of responsive materials in vivo.

Figure 10. Hydrogels Designed with Distinct Stimuli-Responsive Crosslinkers (“Logic Gates”)

(A) Chemical crosslinkers to enable hydrogels to respond to specific cues based on a simple principle of Boolean logic “YES,” “AND,” or “OR.” (i) The YES-gate crosslinker contains a single stimuli-responsive unit (red). (ii) The OR-gate crosslinker contains two different stimuli-responsive units (red and blue) connected in series. The presence of either relevant input cleaves the crosslinker to allow hydrogels to release their encapsulated cargo. (iii) The AND-gate crosslinker contains two different stimuli-responsive units (red and blue) connected in parallel. The presence of a single input alone does not fully sever the crosslinker.

(B) DOX-containing hydrogels crosslinked with enzyme AND reductant gates. In the absence of treatment (N), or in the presence of GSH (R) or enzyme matrix metalloproteinase-8 (MMP-8, E) alone, the hydrogel remained intact and normal cell proliferation was observed. The presence of both GSH and MMP-8 (RE) resulted in the release of DOX from the hydrogels and eradication of HeLa cells.

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Next, although the biomaterials can be engineered to respond to multiple stimuli, an overcomplicated design with responsiveness to multiple triggers may not be feasible when translating it from the bench to a real-world scenario. In general, complicated synthesis steps with multiple mechanisms are involved in the engineering of multi-stimuli-responsive polymer materials and it may be difficult to scale up for commercialization. Finally, with respect to the stimuli responsiveness, external stimuli (e.g., light) enable manual regulation in a controlled way at the site of action. In contrast, materials’ response to endogenous stimuli may suffer from inconsistent biological parameters because the levels of environmental cues may differ within individuals and over the course of treatment. Therefore, this will require engineering of stimuli-responsive materials that can dynamically change to achieve precision-controlled drug delivery. To overcome these challenges, it would be more feasible to have a better understanding of biologically responsive mechanisms and reconsider the biomedical requirements when designing the materials. Advances in molecular diagnostics in identifying molecular markers of disease (including genomics and proteomics) may lead to the discovery of novel internal stimuli in the future.

Bionanotechnology is having a growing impact on the ongoing development of next-generation polymer materials with multi-stimuli responsiveness. Therefore, with the collaborative efforts of chemists, physicists, biologists, and medical scientists, bridging bio-nano science and medical requirements can revolutionize the design of multi-responsive polymer materials for biomedical applications, such as drug delivery and tissue engineering.

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AUTHOR CONTRIBUTIONS

L.H.-R., R.C., and J.C. proposed the topic of the review. X.F., L.H.-R., R.C., and J.C. investigated the literature and wrote and revised the manuscript.

REFERENCES AND NOTES
