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Programming Stimuli-Responsive Behavior into Biomaterials

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Abstract

Stimuli-responsive materials undergo triggered changes when presented with specific environmental cues. These dynamic systems can leverage biological signals found locally within the body as well as exogenous cues administered with spatiotemporal control, providing powerful opportunities in next-generation diagnostics and personalized medicine. Here, we review the synthetic and strategic advances used to impart diverse responsiveness to a wide variety of biomaterials. Categorizing systems on the basis of material type, number of inputs, and response mechanism, we examine past and ongoing efforts toward endowing biomaterials with customizable sensitivity. We draw an analogy to computer science, whereby a stimuli-responsive biomaterial transduces a set of inputs into a functional output as governed by a user-specified logical operator. We discuss Boolean and non-Boolean operations, as well as the various chemical and physical modes of signal transduction. Finally, we examine current limitations and promising directions in the ongoing development of programmable stimuli-responsive biomaterials.

Contents

1. INTRODUCTION

The past several decades have seen substantial progress in our ability to understand, design, and synthesize materials that interact with living systems to guide biological processes in a predictable and controlled manner. Through advances in synthetic chemistry, monomer availability, and enhanced processing techniques, the biomaterials community has evolved beyond its initial reliance on commercial precursors to develop novel systems exhibiting application-specific properties. This transition has permitted a shift from static to dynamic materials that respond on demand to specific inputs, forming the basis of the stimuli-responsive biomaterials field (1–4). Such stimuli sensitivity can be encoded into various types of materials, including physically assembled nanoparticles, supramolecular hydrogels, and covalent polymer networks.

Hydrogel: cross-linked polymer network with a high water content

Stimuli-responsive moiety: a chemical functional group that undergoes a predictable change to a physical or chemical stimulus

Drug delivery: refers to the strategies and technologies used to transport a pharmaceutical within biological systems to safely achieve its desired therapeutic effect

Synthetic flexibility has yielded dynamic biomaterials that respond to a diverse set of stimuli (5). These constructs can be characterized by their environmental triggers and the sources from which they are presented. Bioresponsive systems react to intrinsic cues provided by the physiological environment, whereas externally responsive systems react to extrinsically administered cues (**Figure 1*a***). Bioresponsive systems are most commonly triggered by enzymes, pH, redox conditions, or hydrolysis. Materials that autonomously respond to cues characteristic of specific tissues or diseases (e.g., acidic conditions in the stomach, reductive environment of tumors) can enrich bioresponsive behavior at bodily locations that are encoded into the material design. Alternatively, materials that respond to light, ultrasound, temperature, or magnetic fields, stimuli typically absent from living systems, can be remotely triggered in four dimensions with spatial and temporal control.

Although stimuli-responsive biomaterials have found utility in biosensing, three-dimensional (3D) cell culture, tissue regeneration, and other biomedical applications, their primary niche remains in targeted drug delivery (6). So-called smart carrier vehicles that are designed to degrade, swell, or dissociate from a therapeutic in response to a given stimulus can be used to confine drug release to specific cells, tissues, or organs within the body. Such site-specific therapeutic delivery can reduce or eliminate adverse effects arising from off-target distribution, enhancing the efficacy of conventional drugs (e.g., chemotherapies) while potentially rescuing the use of

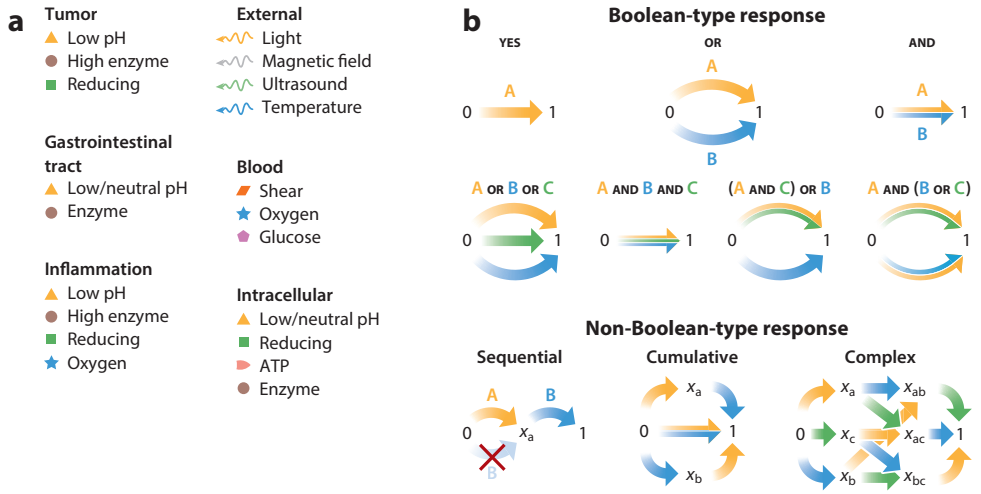

Figure 1

(*a*) An overview of the locations of commonly used biological and external triggers. (*b*) A schematic representation of recurring stimuli-responsive modalities.

otherwise-flawed compounds with systemic toxicity, poor solubility, or untenable pharmacokinetics. Building on the early success of materials responsive to a single stimulus for enriching local delivery, recent efforts have focused on the development of materials that can respond to more than one cue (7–9). Compared with singly responsive systems, those that respond to either of two inputs are sensitive to a broader set of environments, while those that require two inputs to invoke a response can further increase delivery specificity.

As the field moves toward materials that are sensitive to different input combinations, we have elected to describe the manner in which multiple inputs are transduced into a functional output by using the Boolean logical operators of computer science (**Figure 1*b***). When a single input induces a material response, this is denoted as a YES gate; when either of two inputs elicits a complete material response, this is denoted as an OR gate; when two different inputs are both required to invoke a material response, this represents an AND gate (10). To depict stimuli-responsive modalities that are not fully Boolean, we also introduce the concepts of sequential and cumulative response. A sequential response requires order-specific presentation of inputs to elicit a functional change. A cumulative response exhibits differential responses to each relevant cue, where complete response is triggered by the combination of both relevant cues. We find that describing stimuli responsiveness through these programmable operations serves as a useful framework to highlight the current and future efforts of our community.

This review seeks to summarize our understanding of stimuli-responsive materials in a systematic and structured framework. First, we discuss biomedically relevant chemical and physical signals used as material triggers along with the corresponding chemistries and the processes by which they actuate material properties. Second, we describe the mechanisms and strategies that combine different functionalities into multiresponsive systems, organized by the class of material and mode of response. Third, we summarize the available tool kit, highlighting the most successful strategies and providing design considerations for tailoring biomaterial response. Finally, we critically examine the successes and shortcomings of current efforts and speculate on future opportunities for programming stimuli sensitivity into synthetic biomaterials.

YES gate: a Boolean logical function in which the input is returned as an output

OR gate: a Boolean logical function in which either of two inputs triggers an output

AND gate: a Boolean logical function in which two specific inputs are required to trigger an output

Sequential response: material response in which order-specific presentation of inputs elicits a functional change

Cumulative response: material response in which several cues elicit differential triggering; complete response requires all relevant inputs

2. SINGLE-INPUT-RESPONSIVE BIOMATERIALS

Tissue engineering: the use of cells, materials, and biological factors to repair or replace damaged tissues

The simplest dynamic biomaterials are engineered to undergo response to a single cue, which can be biologically presented or externally administered. When such a material exhibits a binary response, it acts as a YES gate. Other systems may exhibit a proportional response wherein the magnitude of the input determines the magnitude of the output state. In this section, we describe these diverse input classes and the chemistries most commonly employed to translate response (**Figure 2**).

2.1. Bioresponsive Material Inputs

Systems that exploit chemical signals presented heterogeneously by biology can be exploited to control where material response occurs in a biologically dictated manner. Here, we discuss both the strategies and the specific chemistries utilized to make materials sensitive to water, pH, enzymes, redox condition, small molecules, and shear forces.

2.1.1. Hydrolysis. Polymers that undergo hydrolysis, or water-induced degradation, are some of the most fundamental and well-studied classes of stimuli-responsive materials. As water constitutes 70–80% of most tissues, hydrolytic materials in biological environments spontaneously exhibit dynamic behavior. A key limitation of hydrolytically degradable biomaterials is that the presence of water in nearly all tissues precludes usage for site-specific delivery. Hydrolyzable materials provide a useful platform for applications ranging from bioresorbable medical implants and cell scaffolds to temporal control over therapeutic delivery.

Hydrolytically sensitive materials contain functional groups that covalently cleave upon reaction with water. Esters are the most commonly utilized moiety, cleaving to form an acid and an alcohol. Other well-explored water-labile groups include carbonates and ureas.

The ester-rich poly(lactic-*co*-glycolic acid) (PLGA) is one of the best-characterized stimuli-responsive materials and is both biocompatible and approved by the US Food and Drug Administration for use in a variety of medical applications. Key characteristics of PLGA, including its degradation rate and mechanical properties, may be tuned by varying its copolymer ratio, stereochemistry, molecular weight, and morphology (11–13). As the polymer degradation rate dictates the release of its encapsulated payload, the ability to tune PLGA degradation time from a few weeks to several months enables user-defined control over the rate of therapeutic release or implant degradation (13, 14).

The ester-containing poly(ε-caprolactone) (PCL) is another popular hydrolytic polymer used in biomaterials. Owing to its increased hydrophobicity relative to PLGA, PCL exhibits an extended in vivo degradation time, persisting as long as several years. PCL is more elastic and pliable than PLGA, but is also mechanically weaker. Despite these limitations, PCL is often utilized as the basis for tissue engineering both hard and soft tissues (15).

Hydrolytically degradable systems have additional factors that must be considered when designing translational materials. The release rate of the encapsulated payloads is governed by the simultaneous processes of material degradation and molecular diffusion. Furthermore, degradation can take place homogeneously throughout the material (i.e., bulk degradation) or from the surface inward; the latter mechanism dominates in hydrophobic materials through which water poorly penetrates. Practically, both surface and bulk erosion occur simultaneously but in varying proportions (12, 16).

2.1.2. pH. Physiological environments contain significant heterogeneities in local pH values. Compared with normal extracellular environments, which have a pH of 7.4, endosomes and

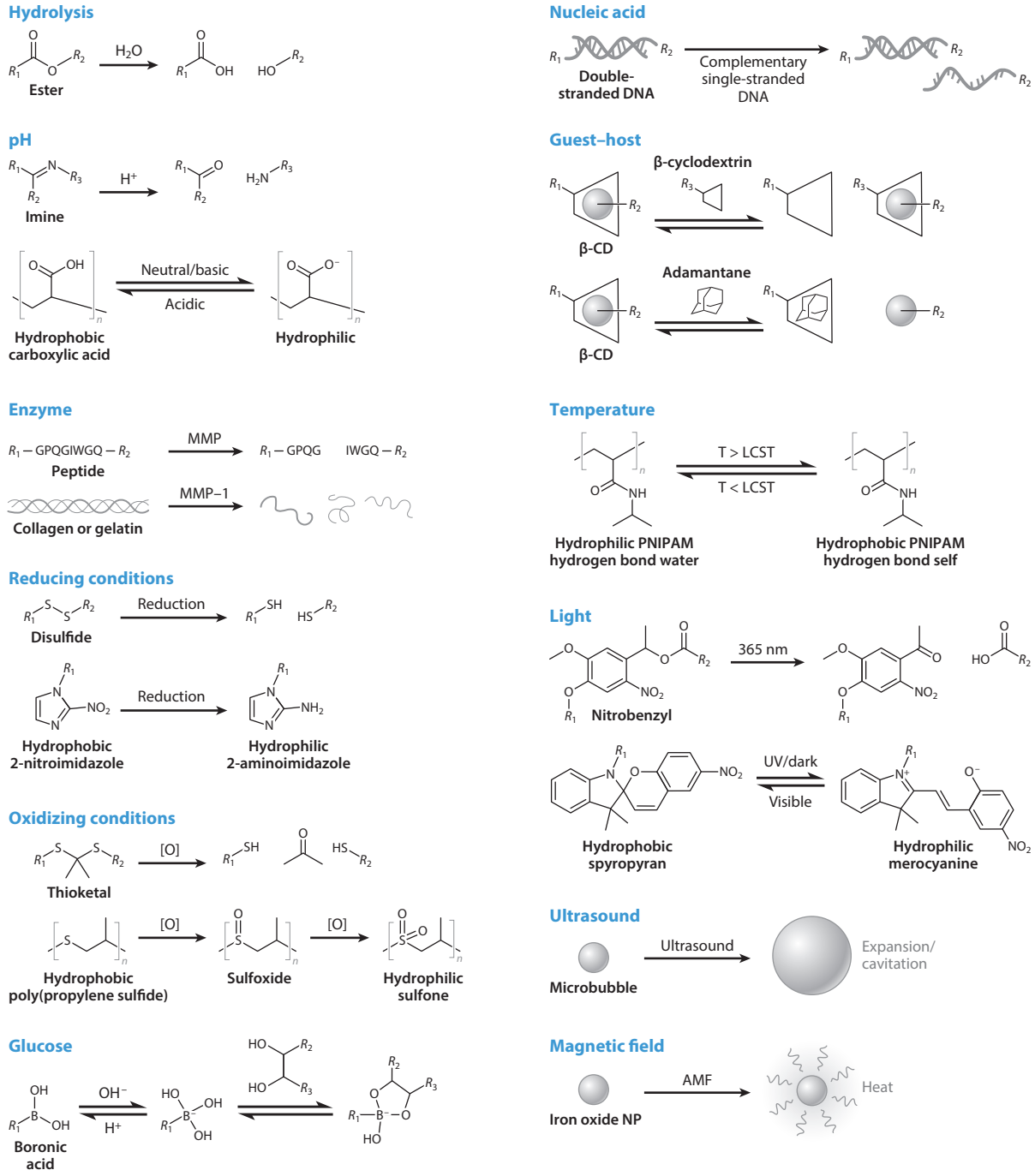

Figure 2

A summary of common stimuli-sensitive chemical functionalities employed in biomaterials. Abbreviations: AMF, alternating magnetic field; β-CD, β-cyclodextrin; dsDNA, double-stranded DNA; LCST, lower critical solution temperature; MMP, matrix metalloproteinase; PNIPAM, poly(*N*-isopropylacrylamide); NP, nanoparticle; ssDNA, single-stranded DNA; UV, ultraviolet.

lysosomes have an acidic environment, with a pH of 5–6.5. Similarly, the extracellular tumor microenvironment is more acidic than healthy tissue (pH = 6.5–7.2), a property also associated with inflammation and chronic wounds (17). As such, pH-responsive therapeutic carriers have been employed to achieve tumor- or intracellular-specific delivery (18).

pK_a: the median pH at which a functional group becomes ionized

pH-responsive behavior can be imparted to materials through the incorporation of acid-labile bonds within the carrier (e.g., hydrazones, imines, acetals, ketals). Triggering bond cleavage can induce carrier breakdown or degradation. This is demonstrated in a microgel platform in which three benzacetal linkers, each with different acid sensitivities, can be used to tune the degradation kinetics of cell-loaded microgels over the pH range of 4.5–7.4 (19). Alternatively, acid-labile bonds can link a therapeutic with a carrier to enable targeted release (20). Finally, pH-cleavable linkers can anchor a protective stealth layer on the surface of a nanoparticle that, upon exposure to acid, is shed to enable drug-carrier uptake (21).

pH-responsive materials may also undergo a dynamic response to their environment through a protonation/deprotonation mechanism. The charge state of ionizable functional groups (e.g., carboxylic acids, amines) incorporated within the material can be altered upon exposure to relevant acidic or basic environments. Materials may be tuned by matching the functional group's pK_a to the pH of the targeted microenvironment. pH-induced charge alteration can yield either swelling or breakdown of the carrier. For example, a poly(methacrylic acid-graft-ethylene glycol) (PMAA-g-PEG) hydrogel formulated for oral insulin delivery remained protonated and collapsed in the stomach, protecting its payload; upon reaching the intestines, the neutral pH induced deprotonation of the carboxylic acids, triggering material swelling and subsequently insulin delivery (22). Changes in pH may also be used to alter polymer charge so as to disrupt electrostatic carrier–drug binding, for example, between cationic doxorubicin (DOX) and a pH-dependent anionic polymeric nanoparticle (23). Another novel application exploiting pH-triggered ionization was the development of pH (low) insertion peptide technologies. These cell-penetrating peptides become activated in acidic environments to facilitate cellular internalization of cargo (24).

2.1.3. Enzymes. Similar to pH, enzymes are heterogeneously distributed within the biological environment. Owing to their specificity in both location and function, enzymes have been used as triggers in targeted drug delivery and tissue engineering platforms. Otherwise stable functional groups may be incorporated into biomaterials, where enzymatic degradation induces bond cleavage. Common enzymatic classes exploited in stimuli-responsive biomaterials are proteases, lipidases, and oxidoreductases (25, 26), which respectively cleave proteins, cleave lipids, and catalyze electron transfer.

The most common class of enzyme used to trigger biomaterial responses are proteases, in particular matrix metalloproteinases (MMPs) (27). MMPs, a family of proteases that degrade the extracellular matrix (ECM), are associated with a host of diseases, including tumor metastasis and invasion. MMPs recognize specific amino acid sequences and cleave an amide bond of the oligopeptide substrate. Nagase & Fields (28) examined the kinetics of many MMPs on a wide variety of peptide sequences, providing a blueprint for tuning biomaterial kinetic response by choosing the substrate sequence. MMP-labile oligopeptides have been incorporated within hydrogels to create bioactive synthetic materials that permit cell-mediated ECM remodeling for tissue engineering applications (29), as well as to deliver a therapeutic MMP inhibitor to tumors (30). In another approach, MMP-labile peptides enabled enzyme-triggered cell-penetrating peptide activation of nanocarriers for tumor-targeted magnetic resonance imaging contrast agents (31).

Beyond MMPs, many other proteases have served as inputs for stimuli-responsive biomaterials, including cathepsins, phospholipidases, thrombin, and azoreductase. Another mechanism by

which enzymes can induce stimuli-responsive behavior is by linking a therapeutic to a material via a stimuli-labile bond. In one demonstration, heparin bound to a PEG hydrogel through a thrombin-labile peptide was cleaved in response to thrombin; the released heparin subsequently inactivated thrombin to induce an autoregulated anticoagulation cascade (32). In another system, azobenzene was incorporated between hydrophilic and hydrophobic blocks of an amphiphilic polymer. This polymer spontaneously formed micelles that enzymatically degraded upon exposure to azoreductase found in the colon (33).

Hydrophilic/(hydrophobic): refers to a polar (nonpolar) domain of a molecule that favorably (unfavorably) associates with water

Micelles: in aqueous environments, amphiphilic molecules can aggregate into these particles with a polar surface and a nonpolar interior

2.1.4. Redox conditions. Differences in redox potential characterize several important microenvironmental targets for drug delivery. Glutathione (GSH) and its corresponding oxidized species, glutathione disulfide, represent the most common redox pair in biology. The concentration of GSH in intracellular environments (0.5–10 mM) is approximately 1,000-fold greater than in extracellular environments (2–20 μM) (34). Similarly, the GSH concentration in mouse tumors is at least four times higher than in healthy tissues (35). The high levels of reactive oxygen species (ROS) in sites of inflammation and tissue injury (36) indicate that they may be targets for delivery from oxidation-responsive materials.

The disulfide bond, ubiquitous in biology, is also the redox-sensitive chemical functionality most commonly exploited by biomaterials. Disulfide bonds cleave in the presence of reducing conditions to yield two free thiols. As with other stimuli-labile linkers, disulfide bonds may be incorporated into materials in several ways to improve tumor-specific delivery or aid endosomal escape for intracellular delivery. Disulfide bonds have been used to achieve redox-mediated carrier breakdown and drug release through incorporation into hydrogel cross-linkers (37) and between the two regions of an amphiphilic block–copolymer micelle (38). Drugs have also been linked to dendrimers through a disulfide bond to achieve intracellular release (39). Reduction of hydrophobic 2-nitroimidazoles to hydrophilic 2-aminoimidazoles has also been exploited to increase the rate of DOX release (40).

Oxidative-labile linkers, including thioketals, thioethers, arylborinic esters, and aryloxylates, can be triggered by ROS such as H_2O_2, superoxide, or hydroxyl radicals. For example, oral delivery of a therapeutic agent targeted sites of intestinal inflammation by complexation with polymeric nanoparticles containing thioketals. In a mouse model, the oral formulation of small interfering RNA (siRNA) was able to reach the inflamed colon tissue to protect against ulcerative colitis (41). In another system, amphiphilic block copolymers between the hydrophilic PEG and hydrophobic poly(propylene sulfide) (PPS) formed polymersome vesicles. Upon exposure to H_2O_2, the sulfides of PPS domain oxidized into sulfoxides, converting the PPS domain from hydrophobic to hydrophilic to disrupt the carrier and release its cargo (42).

2.1.5. Small molecules. Significant advances have been made in creating bioresponsive materials sensitive to many small molecules, including glucose, adenosine triphosphate (ATP), and nucleic acids.

2.1.5.1. Glucose. Diabetes is characterized by the inability of the body to properly regulate its blood glucose levels. Managing diabetes is a burdensome and cyclic process involving the self-monitoring of glucose levels and intravenous injections of exogenous insulin. To ease this process and improve patient compliance, glucose-responsive biomaterials have been engineered to both autoregulate insulin release and enable oral delivery.

A glucose-responsive hydrogel was engineered to be cross-linked through dynamic covalent bonding between phenyl-boronic acid (PBA) and poly(vinyl alcohol). Glucose has affinity for PBA; its presence interrupts the hydrogel network, triggering the release of encapsulated

proteins (43). An alternative methodology for enabling glucose-responsive delivery employed the enzyme glucose oxidase (GOx), which catalyzes the conversion of glucose to gluconic acid. Here, both insulin and GOx-loaded nanocapsules were loaded into chitosan microgels that swell in an acidic environment. These particles convert glucose to gluconic acid, lowering the local pH to enable gel swelling and insulin delivery in a glucose concentration–dependent manner (44).

Mesoporous silica nanoparticle (MSNP): an amorphous silica nanoparticle with pores of 2 to 50 nm, which are often uniform in size and orientation

Four-dimensional (4D): refers to control in both time and 3D space

2.1.5.2. ATP. ATP is a ubiquitous biomolecule that facilitates the energy transfer necessary to carry out cellular functions. Intracellular ATP concentration (1–10 mM) is approximately 1,000-fold greater than in the extracellular environment (<5 μM) (45), making ATP an attractive candidate for intracellular-targeted drug delivery.

ATP has been used as a competitive binding partner to trigger material response. In one case, DOX was loaded into an ATP-binding DNA aptamer; ATP–aptamer binding induced a DNA conformational change, selectively releasing DOX (46). In another system, PBA–siRNA complexes have been engineered such that ATP binds to the PBA, triggering intracellular siRNA delivery (47). Other ATP-responsive materials have repurposed ATP's native biological function to drive reactions within a dynamic material. In one system, ATP hydrolysis provides the energy needed to induce a conformational change in protein-based tubular nanocarriers, resulting in therapeutic payload release (48).

2.1.5.3. Nucleic acids. Nucleic acids (i.e., RNA, DNA) remain relatively underexplored as triggers for stimuli-responsive biomaterials. Given their highly specific binding mechanism, nucleic acids may provide a route to targeted delivery. In one system, a gold nanoparticle assembly was linked together with single-stranded DNA (ssDNA). Hybridization with complementary ssDNA chains altered nanoparticle configuration. These changes in the nanoparticle cluster structure hide or expose folic acid moieties to influence cell–particle interactions (49). A mesoporous silica nanoparticle (MSNP) loaded with a DOX payload was engineered such that its pores were capped with a DNA hybrid complex responsive specifically to a microRNA that is upregulated in cancers. Exposure of the MSNP to the tumor environment, and subsequent binding of miR-21, removed the pore gatekeeper and triggered drug release (50).

2.1.6. Shear forces. Partially obstructed blood vessels and healthy capillaries are sites of high shear stresses that can be leveraged to enable targeted delivery. For example, microaggregates of nanoparticles were engineered to break up under pathophysiological shear forces. By coating the aggregates with a therapeutic, researchers were able to dissolve blood clots in an otherwise-fatal embolism mouse model (51). Another mechanism for targeting obstructed blood vessels employs platelet-like nanoparticles with an enhanced ability to adhere under high shear stress to target damaged sites (52).

2.2. Externally Responsive Biomaterial Inputs

Systems whose properties can be exogenously regulated enable user-defined four-dimensional (4D) control of biomaterial response. Here, we describe synthetic approaches and the specific chemistries utilized to make materials sensitive to temperature, light, ultrasound, and magnetic fields.

2.2.1. Temperature. Thermoresponsive polymers are one of the oldest and most widely explored classes of stimuli-responsive materials (53). As the temperature of a human body is

relatively homogeneous, thermoresponsive biomaterials can be selectively modulated when heated upon the application of an external field (e.g., light, magnetic field, ultrasound).

A unique aspect of these materials is their ability to undergo a phase transition at a distinct temperature—typically a lower critical solution temperature (LCST). Below their LCST, polymers are hydrophilic and water soluble; above their LCST, polymers become hydrophobic and water insoluble, exhibiting gel-like behavior (54). The critical solution temperature is determined by the polymer concentration and identity.

Lower critical solution temperature (LCST): temperature below which biomaterial components are fully miscible with their surrounding solvent

The first and most widely utilized thermoresponsive polymer is poly(*N*-isopropylacrylamide) (PNIPAM). The LCST of PNIPAM, 32°C, is near physiological temperature and may be further tuned through the incorporation of a copolymer (55). PNIPAM has been explored as an injectable hydrogel, owing to its liquid-like behavior at room temperature and rapid gelation in the body (56). PNIPAM also exhibits temperature-dependent swelling, which has been exploited to culture cell sheets for tissue engineering, whereby cells can be lifted from PNIPAM surfaces following cooling of the system from 37°C to 20°C. This approach preserves cell–cell junctions that would be destroyed by proteolytic surface removal, enabling cell sheets to remain intact for tissue assembly (57).

PNIPAM has some limitations, including potential cytotoxicity of the residual monomer and phase-transition hysteresis. To circumvent these drawbacks, a vast number of other thermoresponsive polymers have been developed (54). Some promising alternatives are elastin-like polypeptides (ELPs), biopolymers with an LCST that can be varied from at least 30°C to 80°C by tuning the sequence and length (58). The inherent biocompatibility of ELPs positions them as a useful platform for tissue engineering (59).

2.2.2. Light. Light, particularly near-infrared (IR) wavelengths, can penetrate human tissue to modulate subcutaneous biomaterials (60). Photosensitive biomaterials often incorporate photoscissile moieties that covalently cleave upon exposure to specific wavelengths of light. *Ortho*-nitrobenzyl ester (*o*NB) is frequently used, owing to its ease of synthesis and susceptibility to cytocompatible near-ultraviolet (UV) light. In one example, vitronectin was attached to a human mesenchymal stem cell–laden PEG hydrogel via an *o*NB-containing linker (61). Multiphoton lithography enabled precise control over vitronectin presentation, affording 4D control over cell fate. Coumarin represents another popular photolabile functional group, degrading under UV light as well as multiphoton activation in the near-IR region (62).

Photoresponsive materials have also been engineered by exploiting functional groups that undergo a conformational change in response to light. Azobenzene undergoes a reversible *trans*-to-*cis* conformational change under UV exposure, and a *cis*-to-*trans* reversion in the dark or in the presence of visible light, a phenomenon that has been leveraged to generate macroscopic material responses. For example, azobenzene-containing block copolymer–based vesicles have been engineered such that the photoisomerization of azobenzene disrupts the amphiphilic character of the drug carrier, triggering payload release (63). Spiropyran (SP), a hydrophobic closed-ring functional group, converts to the hydrophilic open-ring merocyanine (MC) group upon exposure to UV light or the dark. MC reverts to its SP state upon exposure to visible light. SPs have been exploited to enable photoresponsive delivery of a payload from nanocapsules (64).

2.2.3. Ultrasound. Ultrasound is energy in the form of acoustic waves and may be controlled with high precision and tissue penetration. Focused ultrasound can induce hyperthermia and/or cavitation to locally disrupt nanocarriers with spatiotemporal control (65). Ultrasound has been demonstrated to induce cavitation in DOX-loaded micelles and nanodroplets, thereby enhancing drug transport into both the cell and the nucleus (66). Microbubbles ranging from 1 to

10 μm in diameter are composed of a gas core with a stabilizing shell (e.g., polymer, protein, lipid). Microbubbles are particularly attractive as ultrasound-sensitive materials, owing to their cavitation susceptibility. In one system, PLA-shell microbubbles loaded with DOX were used to treat a rat tumor model, with ultrasound focused on the cancerous tissue. The microbubbles underwent destructive cavitation at the tumor site, and the polymer shards enhanced DOX uptake (67).

2.2.4. Magnetic fields. Similar to ultrasound, magnetic fields are an attractive option for facilitating triggered drug release as they can be spatiotemporally presented to living organisms. Magnetically responsive materials may be engineered such that an applied magnetic field can guide carrier accumulation or induce disruption, leading to payload delivery. Nanoparticles consisting of a magnetic iron oxide core and a polymer shell can be manipulated by magnetic fields to guide their distribution and accumulation in vivo. The polymeric shell can be engineered to carry genes or drugs, enabling noninvasive magnetic targeting (68). An alternating magnetic field (AMF) can be applied to induce heating in a magnetic nanoparticle, which in turn can trigger payload delivery. In one example, MSNPs were capped with a lipid bilayer and loaded with iron oxide nanoparticles and a model therapeutic. The application of an AMF heated the particle, increasing the porosity of the lipid bilayer to permit payload delivery (69).

3. TWO-INPUT-RESPONSIVE BIOMATERIALS

Although single-stimulus-responsive biomaterials have been successfully created for many inputs and exploited for a variety of applications, investigators have devoted increasing efforts to developing systems that respond to more than one cue. The use of additional inputs enables the use of materials with a greater range of response types, as well as improved specificity. By simply incorporating different combinations of stimuli-responsive moieties within a single biomaterial, one can create a nearly limitless number of material systems to undergo a wide variety of responses (i.e., swelling, degradation, cargo release) via several modalities (i.e., OR gate, AND gate, cumulative triggering, sequential triggering). Here, we categorize two-input platforms on the basis of their material type, focusing on nanoparticles that are physically assembled or based on mesoporous silica, supramolecular hydrogels, and covalent networks.

3.1. Physically Assembled Nanoparticles

Physically assembled nanoparticles are stabilized by intermolecular forces that arise from the amphiphilic nature of the constituent polymers. In aqueous biological environments, polymers self-assemble such that the hydrophilic domains are on the surface of the nanoparticle and the hydrophobic domains are buried in the micellar core. Similar phenomena can be exploited to create responsive bilayer structures (e.g., polymersomes, vesicles), which can be further cross-linked to improve stability. Here, we characterize dually sensitive materials on the basis of their response mechanism, including whether the stimuli change the polymer polarity, induce bond cleavage, or act through a combination of these mechanisms (**Figure 3*a***).

3.1.1. Dual regulation of hydrophobic/hydrophilic polarity. In physically assembled systems, stimuli-responsive groups may be incorporated within the constituent polymers such that a signal triggers the conversion of one domain from hydrophobic to hydrophilic, or vice versa. This change disrupts the supramolecular interactions that provide the nanostructure, inducing carrier breakdown and/or swelling, and subsequent payload release.

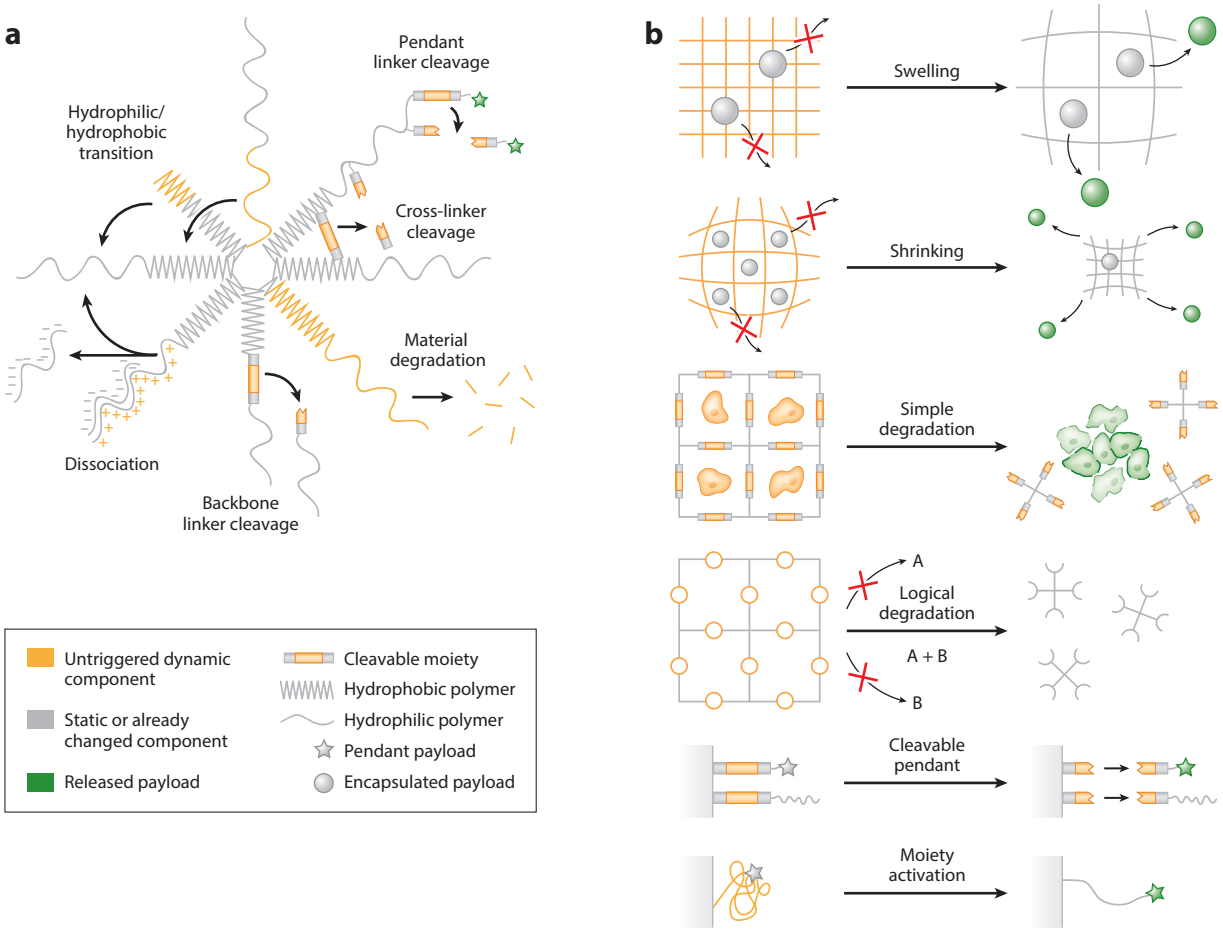

Figure 3

An overview of the mechanisms and strategies that enable functional material responses from (*a*) physically assembled systems and (*b*) covalent networks.

One example of such a material arises from diblock copolymers forming "schizophrenic" micelles that undergo stimuli-triggered inversion. Each polymer block is responsive to a separate stimulus capable of independently controlling its polarity. The polymer self-assembles into micelles when blocks are oppositely charged, but becomes a unimer when its blocks display the same polarity. Poly(acrylic acid) (PAA) was incorporated into the block copolymer PAA-*b*-PNIPAM, which exhibits such behavior: Thermoresponsive PNIPAM is hydrophobic at 37°C and hydrophilic at 25°C, whereas pH-responsive PAA is deprotonated/hydrophilic at pH < 4 and protonated/hydrophobic at a neutral pH. This system can reversibly transition between a PNIPAM-core micelle (37°C, pH 7), a unimer (25°C, pH 7), and a PAA-core micelle (25°C, pH 3) (70). In a similar approach, the Fe^{3+}-responsive poly[sodium 2-(acrylamido)-2-methylpropanesulfonate] (PAMPS) was incorporated into the block copolymer PAMPS-*b*-PNIPAM. This system can reversibly convert between a PNIPAM-core micelle (45°C, no Fe^{3+}), a unimer (25°C, no Fe^{3+}), and a PAMPS-core micelle (25°C, Fe^{3+}) (71).

The two stimuli-responsive groups can also exist within the same block copolymer domain. A block copolymer synthesized from hydrophilic PEG and hydrophobic poly[2-(diethylamino)ethyl

methacrylate-*co*-(2,2,2-trifluoro)ethyl methacrylate] (DEA-*co*-FMA) was self-assembled into nanovesicles. CO_2/DEA binding introduced a positive charge, leading to a vesicle–micelle transition and particle shrinking, while O_2/FMA binding increased segment hydrophilicity to yield vesicle swelling (72). Finally, a block copolymer containing a hydrophilic PEG segment and a hydrophobic poly[(2-tetrahydrofuranyloxy)ethyl methacrylate-*co*-DEA] segment responded to DEA-protonating acid OR structure-disrupting ultrasound, either of which induces vesicle shrinkage and drug release (73).

3.1.2. Dual regulation via cleavable bonds. Cleavable bonds also provide a stimuli-responsive handle for biomaterial systems. Bond cleavage can be used to unlink a drug from the material, cleave two polymer domains, degrade a polymer, or irreversibly switch domain polarity. For example, micelles were created from ABA block copolymers containing hydrophilic PEG domains flanking a hydrophobic region containing both disulfide and *o*NB moieties; exposure to either reducing conditions OR light severed the hydrophobic domain, leading to material breakdown and payload delivery in a Boolean manner (74). In another system, micelles were formed from hydrophobic DOX linked to a hydrophilic PEG via a disulfide bond. The hydrophobic core was stabilized by Cu^{2+}, which dimerizes DOX at neutral pH but not under acidic conditions. These nanoparticles exhibited a cumulative release profile in which a reductant or an acidic environment elicited partial delivery, but maximal release occurred in the combined environment (75). In yet another system, a hydrophobic polymer containing a thioether solubility switch and an acid-labile ketal group was formulated into a sequentially triggered nanoparticle. This hydrophobic polymer resists acid-catalyzed hydrolytic degradation until the thioether is oxidized into a thioketal, permitting material degradation only after H_2O_2 and acid are sequentially presented (76).

In other systems, stimuli-labile cross-links are used to stabilize micelles. Hydrophilic polyethylenimine modified with hydrophobic *o*NB groups self-assembled into micelles and were further stabilized with disulfide-containing cross-links. Particles persisted until both cross-links were reduced and *o*NB was photoremoved (77). Similarly, the hydrophilic biopolymer chitosan was modified with *o*NB side chains, self-assembled into micelles, and subsequently cross-linked with species containing acid-labile imine bonds. A hydrophobic anticancer drug, camptothecin, was released upon nanoparticle breakdown cumulatively triggered by exposure to both reducing and acidic conditions (78). In another demonstration, micelles containing DOX bound to PEG-*b*-PMAA via an acid-labile hydrazone bond were cross-linked with a disulfide-containing species; maximal drug release was achieved upon exposure to both acid and reductant (79).

Multicomponent materials, in which each portion is sensitive to a different stimulus, have also been created. For instance, payload-containing nanocapsules synthesized from the pH-responsive polyaniline polymer were electrostatically modified with gold nanoparticles whose surfaces were functionalized with a second payload through a reductive-labile gold–sulfur bond. Delivery of each of the two payloads was independently triggered by basic pH (12.4) and reductant (80).

3.1.3. Dual regulation via both cleavable bond and physical mechanism. The two primary mechanisms to enable stimuli-sensing behavior—reversing domain polarity and bond cleavage—can also be combined to generate multistimuli-responsive materials. For example, PEG and cyclic RGD side chains were grafted to a poly(β-amino ester) (BAE) backbone via disulfide bonds, and subsequently self-assembled into micelles with a PEG corona and a BAE core. DOX payload release could be triggered by two independent mechanisms: Disulfide reduction severs the link between hydrophobic and hydrophilic domains, and acidic environments protonate the basic BAE residues and polarizing the core (81). In another example, block copolymers synthesized from PEG and PNIPAM domains were linked through an enzyme-labile peptide sequence;

micelles assembled upon heating above the LCST, but were disrupted upon proteolytic cleavage of the PEG corona (82). In a recent example, hydrogels were formed by cross-linking thermoresponsive liposomes with polyacrylamide chains through DNA susceptible to enzymatic cleavage. Liposome contents were released upon heating, while hydrogels were degraded by the restriction enzyme EcoRI (83).

3.2. Mesoporous Silica Nanoparticles

Many stimuli-responsive biomaterials have been created by decorating MSNPs with an environmentally responsive polymer layer. MSNPs have pores, ranging from 2 to 30 nm, into which drugs can be loaded. Polymers grafted to the particle surface serve as selective gatekeepers, permitting therapeutic release only upon an environmentally triggered change to the polymer physics or chemistry (84).

Significant efforts have been dedicated to the creation of MSNP drug delivery platforms that release their payload in response to multiple different stimuli. Those sensitive to both temperature and pH have been engineered with surface-grafted PNIPAM-*co*-PMAA, where polymer-shell collapse above the LCST or at low pH cumulatively triggers the delivery of a small-molecule payload (85, 86). In another system, PAA attached to the surface of an MSNP via a disulfide bond afforded payload delivery in the presence of GSH or an acidic environment (pH 5) (87). Alternatively, OR-gated cargo release was achieved under acidic or reducing conditions for dextran grafted to MSNPs via an acid-labile imine bond and cross-linked with reducible disulfide bonds (88).

Other MSNP drug delivery platforms have been developed in which sequential exposure to two different cues triggers payload delivery. In one example, PCL was polymerized in the core of DOX-loaded MSNPs, which were then coated with PAA. The PAA disassociated from the particle in an acidic environment (pH 5.5), enabling PCL to be degraded by esterases or through hydrolysis (89). In an example exploiting stepwise pH responsiveness, a surface-grafted PEG stealth layer can be shed under mildly acidic conditions (pH 6.5) characteristic of the tumor microenvironment, presenting a positive charge to facilitate cellular uptake; the more acidic intracellular environment (pH 5) cleaves a boronate ester bond to remove the polymer gatekeeper and trigger payload delivery (90). Another system, sequentially responsive to pH and light, was created by attaching a cucurbit[6]uril nanovalve to the surface of the MSNP through either an acid- or base-labile manner, wherein the MSNP pores remain capped until the relevant pH is achieved. Through further functionalization of the pores with an azobenzene-based nanoimpeller that undergoes a photoreversible *cis–trans* isomerization, a light-induced wagging motion is required to actuate payload release (91).

3.3. Supramolecular Hydrogels

Hydrogelators, which are typically small molecules or polymers, are amphiphiles that spontaneously self-assemble into fiber-like structures and, subsequently, bulk hydrogels on the basis of their hydrophobic/hydrophilic intermolecular interactions. Hydrogelators can be readily engineered such that their structure and subsequent hydrogel properties respond to environmental signals. To date, supramolecular hydrogel systems have yielded some of the most complex responses programmed into multistimuli-responsive biomaterials. Here, we describe and characterize systems formed through physical associations, covalent bonds, or guest–host interactions.

3.3.1. Physically responsive supramolecular gels. Supramolecular gels are stabilized through physical interactions that can undergo stimuli-triggered rearrangement to reversibly respond to

Guest–host: the association between two or more molecules (or ions), with unique structural relationships through noncovalent forces

Supramolecular gel: a hydrogel with noncovalent cross-links, typically from the aggregation of amphiphilic molecules

Interpenetrating polymer networks (IPNs): two or more interlocking polymer networks, interlaced but not covalently bound

their environment. In one system, a supramolecular hydrogelator was built with a hydrophilic ion-sensitive phosphate head group and a hydrophobic tail containing a photoresponsive olefinic bond. Introduction of cationic species such as Ca^{2+} and H^+ ions strengthened the resultant hydrogel network, while the trans-to-cis UV-induced photoisomerization event destroyed supramolecular hydrogel assemblies; a variety of logical behaviors were achieved with different initial conditions and inputs (92). Another hydrogelator system was created with amphiphilic phenylalanine and photoresponsive azobenzene moieties; deviations from neutral pH OR photoswitching of azobenzene isomerization yielded reversible gel degradation (93).

Supramolecular gels may also employ guest–host chemistry, in which two counterreactive functional groups form strong noncovalent intermolecular bonds material components, which give rise to a unique class of shear-thinning injectable hydrogels. In one system, cadmium sulfide quantum dots were functionalized with β-cyclodextrin (β-CD) and mixed with a thermoresponsive block copolymer end-functionalized with azobenzene [azo-(PDMA-*b*-PNIPAM)]. The azobenzene forms noncovalent bonds with the β-CD quantum dots to yield a hybrid inclusion complex with a quantum dot core and PNIPAM corona. Upon heating above the LCST, the PNIPAM chains aggregate to form a supramolecular hydrogel, which degrades upon cooling or in the presence of competitive binders to the guest–host system (i.e., adamantane, α-cyclodextrin) (94).

3.3.2. Covalently responsive supramolecular gels. Supramolecular hydrogels may also exhibit irreversible phase transitions when the gelator undergoes covalent modification in response to external signals. Ikeda et al. (95) have developed stimuli-sensitive amphiphilic gelators in which the hydrophobic head group was cleaved upon exposure to oxidative conditions. Encapsulation of oxidases that produce hydrogen peroxidase in response to a variety of analytes (i.e., glucose, sarcosine, choline, uric acid) underwent irreversible degradation triggered by small molecules. Encapsulation of multiple enzymes within a single gel permitted formation of OR- and AND-gated materials. In one example, materials containing choline oxidase and a hydrolase that converts acetylcholine to choline generated degradation-inducing H_2O_2 in the presence of either acetylcholine or choline. Alternatively, materials containing both redox- and electron-responsive nanofibers, respectively containing nitroreductase and glucose oxidase, required inputs of both nicotinamide adenine dinucleotide and glucose to induce complete material degradation. In another approach, an ABC triblock copolymer platform was created in which the A block was either oxidative-sensitive PPS or hydrolytic PCL or PLGA, B was hydrophilic poly(*N*,*N*-dimethylacrylamide) (PDMA), and C was the thermoresponsive PNIPAM. At room temperature, the particle formed micelles, which assembled into supramolecular hydrogels at 37°C. These gels degrade upon cooling or upon exposure to either oxidative or hydrolytic conditions on the basis of the A-block composition (96).

3.4. Covalently Assembled Systems

Stimuli-responsive biomaterials have also been created through covalent interactions. Since these materials are generally more stable than physical assemblies and may be formulated into a wider range of sizes and shapes, these systems may be preferable over physically assembled systems for certain applications. Here, we discuss both single and interpenetrating polymer networks (IPNs), as well as covalently assembled nanoparticles (**Figure 3*b***).

3.4.1. Single covalent polymer networks. Multistimuli-responsive hydrogels have been engineered by incorporating multiple stimuli-labile moieties within a single polymer network. For example, gels formed from the ECM protein hyaluronic acid cross-linked via disulfide bonds

underwent an OR-gated gel-sol transition upon exposure to either hyaluronidase or reducing conditions (97). Additionally, PEG–PAMAM hydrogel constructs incorporating both acetal and disulfide bonds underwent degradation in the presence of acidic or reducing conditions to deliver DOX (98). Another step-growth PEG network was engineered with peptide-based cross-linkers that contained the photolabile *o*NB group and an MMP-labile peptide sequence, permitting degradation through user-directed laser light or cell-secreted enzymes for the creation of custom endothelialized vasculature (99).

Step-growth polymer networks: nearly ideal polymeric networks synthesized through one-to-one addition of functionalized macromers

3.4.2. Interpenetrating polymer networks. IPNs are composed of two independent polymer networks that are interlaced at the molecular scale. If these networks are orthogonally labile, both environmental signals are required for full material degradation. In the case of an interpenetrating network between gelatin and dextran, exposure to both α-chymotrypsin and dextranase is required to release encapsulated lipid microspheres that serve as drug reservoirs (100).

IPNs may also be exploited to control material swelling in response to multiple environmental stimuli. An IPN between pH-responsive PMAA and thermoresponsive PNIPAM polymers exhibited tunable swelling. At low pH (<5.5), the PMAA acid side chains are protonated, inducing network collapse; at higher pH values, the PMAA side chains become charged, and the gel swells due to electrostatic repulsion. The PNIPAM network was significantly swollen below its LCST (32°C) and collapsed above this temperature (101). A similar IPN of PAA and PNIPAM has been formulated as hollow-core nanogels; these materials can be loaded with cargo in their swollen state at room temperature, shrunk at 37°C, and then reswollen upon acidic treatment for pH-targeted drug delivery (102).

3.4.3. Covalent nanoparticle carrier systems. Several novel nanoparticle carriers have been developed such that material response is triggered through exposure to multiple external stimuli. In one creative example, the small protein capsid of the adenoassociated virus is decorated with small peptide-based locks that inhibit transportation across the cell membrane. These locks are removed upon treatment with MMP-7 and MMP-9, enabling AND-gated gene delivery and cell transduction (103). Similarly, DNA nanocapsules have been engineered to contain two DNA-based clasps and locks, such that exposure to both relevant aptamer keys unlocked the carriers to trigger AND-gated delivery of antibody fragments (104).

4. MULTISTIMULI-RESPONSIVE BIOMATERIALS

While biomaterials sensitive to two unique environmental cues can exhibit complex environmentally defined responses, further specificity and control can be gained by building sensitivity to additional stimuli. With each additional stimulus, an exponential increase in the number of possible material states gives rise to systems that exhibit even more complex responses. Despite this immense potential, materials engineered to respond to three or more environmental signals remain relatively underexplored. In this section, we describe some exciting examples of multistimuli-responsive biomaterials based on physically assembled nanoparticles, supramolecular hydrogels, and covalent networks.

4.1. Physically Assembled Nanoparticles

Amphiphilic polymer systems can self-assemble into supramolecular nanostructures including micelles and polymersomes. As discussed in Sections 3.1 and 3.2, stimuli-responsive functionalities can be used to control the swelling, degradation, or payload release from such nanoparticles.

Multi-input materials in this category build on this existing framework by incorporating three or more stimuli-responsive moieties.

4.1.1. Three-input-responsive nanoparticles. The most common multistimuli-sensitive nanoparticles can incorporate information from three different environmental inputs. To date, the majority of these systems combine thermal responsiveness about an LCST with two other stimuli. In one example, an amphiphilic copolymer was created from disulfide-linked PNIPAM and tetrahydropyran (THP)-protected 2-hydroxyethyl methacrylate (HEMA) blocks (105). Acidic conditions removed the THP, converting the HEMA domain from hydrophobic to hydrophilic; raising the temperature above the LCST converted the PNIPAM domain from hydrophilic to hydrophobic; reducing conditions severed the disulfide linkage between the two domains. In each case, the encapsulated guest molecule was released from the micelle, effectively creating a double OR gate. In another example, multistimuli-sensitive star-polymer aggregates were built from PEG, PCL, PAA, and PNIPAM (106). These micelles are destabilized above their LCST (37°C), leading to DOX release. Reducing conditions removed the PAA–DOX chains, while acidic conditions disrupted the DOX–PAA electrostatic interaction; both stimuli cumulatively triggered DOX release, though maximal release was achieved in the combined environment. In a final example, micelles were created from amphiphilic copolymer poly(NIPAM-*co*-SP) (107). Micellar disruption occurred above their LCST (35°C) as PNIPAM was converted from hydrophilic to hydrophobic. Micelles could also degrade when the hydrophobic SP was converted to the hydrophilic MC group in the presence of light or the hydrophilic MCH^+ group in the presence of acid; maximal payload release occurred when the materials were treated with both light and acid.

Triply responsive nanoparticles that do not rely on an LCST transition have also been created. For example, a polyanionic polymer containing charged head groups attached to its backbone via disulfide bonds, upon complexation with a cationic surfactant, formed a micelle-like assembly (108). Particles disassembled in the presence of a reductant OR acidic conditions OR high-ionic-strength environments. In another example, a DNA-intercalating agent was tethered to the surface of an MSNP and capped with double-stranded DNA (109). The pore-loaded calcein payload was released following reductant-mediated release of the intercalating agent, OR when the DNA was cleaved by a DNase OR converted to ssDNA upon heating. The intercalating agent was released upon exposure to reducing conditions AND either heat OR DNase in a complex Boolean response.

4.1.2. Four- and five-input-responsive nanoparticles. Several investigators have created nanoparticles that respond to more than three environmental stimuli. In one example, the triblock copolymer SP-*b*-NIPAM-*b*-AMPS self-assembled into micelles with an SP core, a PNIPAM shell, and an AMPS corona (110). Upon exposure to UV light, SP solubility increases, causing particle expansion, which is reversed upon exposure to visible light. Heating the particles increases the hydrophobicity of the PNIPAM shell, shrinking the particle. Fe^{3+} ions bind to the sulfonate groups on the AMPS, decreasing its solubility and shrinking the particle. Exposure to acid increases the hydrophilicity of the SP group, swelling the particle. In another example, the diblock copolymer poly(2-nitrobenzyl methacrylate)-SS-poly(dimethylaminoethyl methacrylate) (PNBM-SS-PDMAEMA) self-assembles into micelles (111). The hydrophobic PNBM core irreversibly becomes less hydrophobic upon UV cleavage of the *o*NB groups, and the disulfide linker is cleaved in reducing conditions. The PDMAEMA corona is responsive to both temperature and pH; at elevated temperature and pH the micelle shrinks, while in acidic conditions (pH = 3) the micelle swells. Combinations of acid

and UV or acid and dithiothreitol are able to fully degrade the micelles to enable Nile red (NR) delivery. Finally, quintuple-stimuli-responsive micelles have been formed from the block copolymer poly (2-methacryloyloxyethyl ferrocenecarboxylate)-(5-propargylether-2-nitrobenzyl bromoisobutyrate)-poly(dimethylaminoethyl methacrylate) (PMAEFe-*o*NB-PDMAEMA) with *N,N′-bis*(bromoacetyl) cystamine (BBAC) cross-linking of the PDMAEMA shell (112). The ferrocene-containing core swelled in the presence of H_2O_2-triggered oxidation of Fe^{2+} to Fe^{3+}, releasing much of the NR payload. UV-induced *o*NB photodegradation separated the two domains and induced micelle breakdown if the BBAC cross-linker was also cleaved with reducing conditions. The PDMAEMA corona is sensitive to temperature and pH, causing micelle swelling in acidic conditions and shrinking when heated above the LCST.

4.2. Supramolecular Polymer Networks

The framework used for creating two-input-responsive supramolecular hydrogels (Section 3.3) can be extended to generate systems responsive to three or more inputs simply by incorporating additional stimuli-responsive functionalities within the hydrogelators. In an example of such a supramolecular material triply sensitive to light, temperature, and pH, microgels formed from PNIPAM modified with SP were formed that collapse above the polymer's LCST (113). SP is highly hydrophobic after visible light exposure, and reversibly converts to a less hydrophobic MC species in the dark state or upon UV exposure. Visible light exposure increases the gel hydrophobicity, thus decreasing the LCST and leading to gel collapse. Residual amine groups were partially ionized at a neutral pH, but deprotonated in basic conditions (pH = 10), leading to gel shrinkage. In another example, in which the material is sensitive to temperature, pH, and glucose, P(NIPAM-*co*-5-methacrylamido-1,2-benzoxaborole) (MAAmBO) was synthesized with different ratios of boronic acid side chains, which dimerize with glucose (114). The PNIPAM component of these polymers imparts an LCST to the system, yielding temperature responsiveness. Glucose binds to the MAAmBO chains, increasing the hydrophilicity of the system and their LCST. P(NIPAM-*st*-MAAmBO) can be mixed with a glycopolymer to form a cross-linked gel in basic conditions (pH = 13); these gels are glucose sensitive because competitive binding induces gel degradation.

Guest–host chemistries have also been used to create triple-stimuli-responsive supramolecular gels. One study used a two-polymer system consisting of (*a*) a β-CD dimer linked via disulfide bonds and (*b*) PNIPAM with azobenzene side chains (115). Reducing conditions severed the cross-linkers and resulted in gel degradation. UV light induced an azobenzene isomerization that inhibited its guest–host binding to β-CD; this process was reversed upon isomerization induced by visible light. Finally, the gels were hydrated and swollen below their LCST and underwent a volume phase transition into a collapsed state above their LCST.

A quadruple-stimuli-responsive supramolecular gelator was engineered with a naphthalene-based salicylideneaniline and a sorbitol moiety (116). This molecule can interact with four unique environmental stimuli—Cu^{2+}, light, pH, and temperature—which influence the presence and properties of its supramolecular assemblies. Cu^{2+} ions complexed with two naphthalene moieties to enable supramolecular fiber assembly. Similarly, the phenol group can be deprotonated in basic conditions, and the salicylideneaniline group reversibly tautomerizes under UV light (reversed by visible light), which can influence system assembly. Finally, these materials exhibit thermosensitive behavior. Ultimately, these different stimuli can have complex cooperative or competitive combined effects on whether hydrogels form; several logic-gated systems were defined by specifying the initial conditions and variable inputs.

4.3. Covalent Polymer Networks

On the basis of the frameworks presented in Section 3.4, efforts have been made to generate covalent polymer networks sensitive to three or more environmental inputs through the inclusion of additional stimuli-responsive functional groups within a material. In one example, a cross-linked zwitterionic hydrogel network comprising glutamic acid and lysine residues was found to deliver DOX in response to three inputs (117). This hydrogel is enzymatically digested by trypsin. Furthermore, this hydrogel swells as the pH deviates from 6 or the ionic strength of its buffer increases, owing to the change of charge states of its ionizable side chains. Cross-linked copolymers of NIPAM with phenylalanine and/or valine containing embedded Fe_3O_4 nanoparticles also exhibited triple-stimuli-responsive delivery (118). DOX binds electrostatically to the carboxylic acid side chains of the hydrogel network, and this binding is disrupted at elevated temperature and sensitive to pH. Application of an external AMF induces heating, also stimulating DOX release. In another example, stimuli-triggered expansile nanogels were formulated by cross-linking a copolymer of PNIPAM/PEG/PDA (119). Under reducing conditions, acidic conditions, and elevated temperature, these particles expanded in volume up to 1,000-fold. The copolymer P(DMAEMA-*co*-AAPBA) was cross-linked into a hydrogel network (120). This system exhibited pH- and temperature-responsive swelling, owing to its PDMAEMA domain. The boronic acid component of AAPBA binds with glucose to modify the thermoresponsive behavior of the system. At physiological pH and temperature, environmental glucose induced hydrogel swelling to increase the rate of encapsulated bovine serum albumin release.

Almost every extant triple-stimuli-responsive hydrogel system undergoes responses primarily via physical mechanisms, resulting in complex behaviors when multiple stimuli are present. More predicable behaviors can be engineered by incorporating only stimuli-labile groups within hydrogel cross-linkers, enabling a true off/on material degradation in response to multiple cues. For example, step-growth polymer networks were polymerized from four-arm PEG macromers such that each cross-link contained a photolabile *o*NB group, a redox-responsive arylthiol-based thioether succinimide, and a hydrolytically sensitive ester (121). This double-OR-gated system was responsive to light, reducing conditions, or water, with degradation rates spanning three orders of magnitude, depending on the mode of cleavage. Our research group has developed a modular framework for imparting hydrogels with degradability toward multiple stimuli, governed by Boolean operators (122). This is achieved by arranging different stimuli-labile groups within synthetic cross-linkers with a well-defined architecture and connectivity. Two stimuli-labile groups arranged in series form an OR gate, while two stimuli-labile groups in parallel form an AND gate. This concept was extended to three-input systems described through combinations of two logical operators; photo-, reductive-, and enzyme-labile groups were used to generate all 17 possible YES/OR/AND Boolean logical PEG hydrogels. While the complexity of these cross-linkers is practically limited by synthetic capabilities, this framework could theoretically enable logical circuits with an arbitrary number of inputs.

5. LOOKING FORWARD

This review has highlighted the diverse synthetic and strategic advances used to create a growing number of stimuli-responsive biomaterials. Categorizing systems on the basis of the number of inputs, material type, and response mechanism, we have examined how functional moieties that undergo predictable chemical or physical changes toward a specific stimulus can be combined to create multisensitive constructs. Engineering of materials responsive to biologically presented and externally administered signals (see the sidebar) presents tremendous opportunities to exploit these systems for applications in drug delivery, biosensing, and regenerative medicine.

DESIGN CRITERIA FOR CREATING STIMULI-RESPONSIVE BIOMATERIALS

1. Consider the application. How would the application benefit from having a stimuli-responsive material? How should the material behave in its normal and triggered states?
2. Identify relevant stimuli. What are the relevant biological signals? Would responsiveness to externally presented cues be useful? Strive to minimize stimuli while maximizing selectivity.
3. Determine the material type. Micelles and nanoparticles are candidates for systemically circulating materials. Supramolecular hydrogels are suitable for injectable materials; covalent polymer networks are ideal for tissue engineering.
4. Select a responsive profile. If using multiple inputs, consider how the material should respond to each individual cue. For drug delivery applications, selectivity is best achieved with AND or sequential profiles.
5. Define the response scheme. How should the material interact with the environment and its cargo? Will it link to, bind with, or encapsulate cells and/or a therapeutic?
6. Identify responsive chemical functionalities. Examine the catalog of stimuli-responsive chemical functionalities to identify application-appropriate triggers.
7. Design the material. Use the chosen material class and potential stimuli-responsive chemistries to identify a strategic blueprint.
8. Characterize the system. How does the material behave toward different combinations or magnitudes of signals? What is the temporal response profile? How would it translate to a complex in vivo environment?

Although a near-infinite number of stimuli-sensitive materials may be created using solely existing chemistries, the development of new stimuli-responsive functional groups and strategies will broaden the utility and improve the specificity of these systems. Materials that sense and respond to critical aspects of the cellular transcriptome or proteome may enable cell-selective responses in vivo. Systems that can be more precisely controlled by externally applied forces will provide finer 4D material modulation within the body. While the majority of materials used for stimuli-triggered delivery have been designed to carry small molecules, the rapid development of gene-editing technologies provides significant motivation to expand the scope of potential therapeutic cargos.

Beyond simple degradation or swelling, greater attention should be paid to creating materials that exhibit other useful changes in their physical, biochemical, or optical properties in response to external stimuli. Multiregioned heterogeneous materials, in which each component responds differently to presented stimuli, could be combined in many creative ways to yield complex responsive systems. Finally, expanded operations (e.g., NOT gates) and cascade reactions will permit a greater range of programmable behaviors, enhancing our ability to precisely dictate material behavior in dynamically heterogeneous biological environments.

Beyond creating individual and specific materials, there is a need to establish modular approaches that enable the rational design of different multistimuli-responsive systems from a common framework. Such approaches could enable biomaterials to exhibit user-programmable responsiveness to arbitrary input combinations, thereby permitting creation of tailored systems for specific applications and with a lower cost of material development.

Perhaps most importantly, our community must endeavor to translate multistimuli-responsive materials out of the lab and into clinical applications. To date, relatively few systems have demonstrated successful in vivo outcomes, and even fewer have been clinically translated. While the regulatory approval process is partly responsible for the gap between technological development and clinical translation, the community must collectively focus on achieving this goal. Despite tremendous advances within this field, significant efforts continue to be wasted on the development of

novel materials that neither expand stimuli-responsive material technologies nor are practically useful. These include systems that exhibit responsiveness to signals that are neither medically nor physiologically relevant, as well as overdesigned materials that respond to more inputs than they are capable of integrating.

Building on a rich past and fueled by recent chemical, mechanistic, and strategic advances, diverse responsiveness to combinations of stimuli can now be readily encoded into an ever-increasing variety of biomaterials. These user-programmable materials will prove invaluable in the quest to probe and direct fundamental biological processes, as well as in establishing next-generation technologies for personalized medicine.

DISCLOSURE STATEMENT

The authors have filed for a patent on technology discussed in this review (PCT/US2017/053034). The authors are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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