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Perspective

Stimuli-Responsive Polymers at the Interface with Biology

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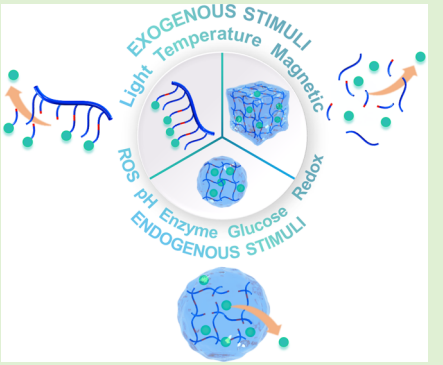
ABSTRACT: There has been growing interest in polymeric systems that break down or undergo property changes in response to stimuli. Such polymers can play important roles in biological systems, where they can be used to control the release of therapeutics, modulate imaging signals, actuate movement, or direct the growth of cells. In this Perspective, after discussing the most important stimuli relevant to biological applications, we will present a selection of recent exciting developments. The growing importance of stimuli-responsive polysaccharides will be discussed, followed by a variety of stimuli-responsive polymeric systems for the delivery of small molecule drugs and nucleic acids. Switchable polymers for the emerging area of therapeutic response measurement in theranostics will be described. Then, the diverse functions that can be achieved using hydrogels cross-linked covalently, as well as by various dynamic approaches will be presented. Finally, we will discuss some of the challenges and future perspectives for the field.



INTRODUCTION

Over the past several decades, polymers have been of growing importance in a wide range of biomedical applications. For instance, poly(ethylene glycol) (PEG) is widely used as a pharmaceutical excipient and to control the stability and biodistribution of proteins and nanoparticles[1] while biodegradable polymers such as poly(glycolic acid) and poly(lactic acid) are used in adsorbable sutures.[2] Stable or slowly degrading polymer systems are ideal for some applications, but in other cases, it is desirable to have materials that change properties or break down on demand. For example, the selective release of drugs in specific biochemical environments can enhance their therapeutic efficacy while reducing their side effects.[3]

There are different approaches by which stimuli-responsiveness can be incorporated into polymeric systems. In one approach, a specific cargo of interest, such as a drug or imaging agent, can be conjugated to a polymer by a linkage that is cleaved in a stimuli-responsive manner, thereby releasing the cargo (Figure 1a). In another approach, the stimulus can induce a solubility change through a change in the protonation state of an ionizable group, cleavage of pendent groups from the polymer, or the thermodynamically driven release of bound water. Such changes can result in the swelling, contraction, or dissolution of polymer assemblies or hydrogels (Figure 1b). A third strategy can involve the construction of the platform using stimuli-responsive linkages in the polymer backbone. When the stimulus is applied, the system disintegrates (Figure 1c).

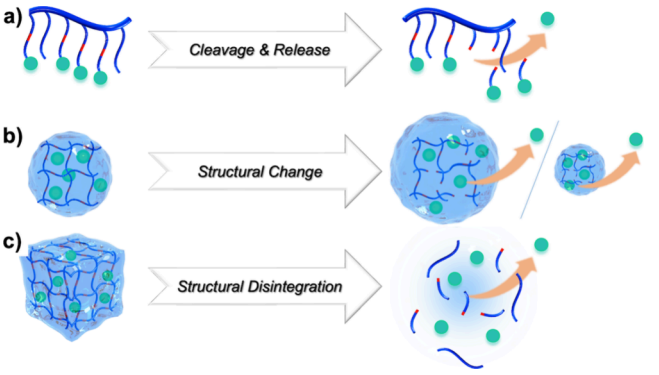


Figure 1. Examples of stimuli-responsiveness incorporated into polymeric systems: (a) Cargo can be conjugated to a polymer by a linkage that is cleaved in a stimuli-responsive manner. (b) Stimulus induces a solubility change that results in the swelling or contraction. (c) Platform is constructed using stimuli-responsive linkages. When the stimulus is applied, the system disintegrates.

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In this Perspective, we provide an overview of the key stimuli that are relevant to biological systems followed by a selection of research topics where we have seen substantial interest over the past several years and envision exciting prospects for future research. These areas include polysaccharide-based materials, polymers, and self-assembled carriers for drug and nucleic acid delivery, the measurement of therapeutic release in theranostics, and hydrogels. We highlight a selection of exciting recent work in these areas to inspire future research innovations.

RELEVANT STIMULI

pH change is one of the most widely explored stimuli due to the inherent pH differences that exist normally throughout the human body and which are often associated with pathological conditions. Most healthy tissues and blood have a pH of 7.4, although the stomach typically has a pH of 1.5−3.5 and the pH of the intestines is about 8. In addition, while the pH in the cytosomes of cells is about 7, the pH within endosomes and lysosomes can range from 4.5−6.5.[4] Furthermore, the environment of solid tumors and inflamed tissues can be mildly acidic.[5,6] One simple approach to exploit pH variations to stimulate property changes in polymer systems is to incorporate ionizable groups. For example, carboxylic acids typically have pK_a values of 4.5−5, while amines and their derivatives can have pK_a values ranging from 5−10 depending on their structures (e.g., aromatic versus aliphatic). Changes in the protonation state of polymers can impart substantial changes in properties such as solubility and swelling. pH changes can also be exploited to stimulate bond cleavage. The most commonly used pH-sensitive linkages include acetals, imines, hydrazones, phosphoramidates, vinyl ethers, and certain amides. These linkages can be incorporated into polymer backbones to enable pH-mediated degradation or can be used as pendent groups to conjugate cargo to polymers.

Redox changes are also of great interest due to the significant changes in redox balance that occur intrinsically within cells and tissues when they deviate from their normal state. A cascade of events can lead to variations in reductant and oxidant levels, such as glutathione (GSH) and reactive oxygen species (ROS), within cells experiencing stress or in a disease state. Oxidative stress is not only observed in cancer cells but also in inflammatory and neurodegenerative diseases, including atherosclerosis, arthritis, Parkinson's, and Alzheimer's.[7] The most widely exploited redox-sensitive linkages are disulfides, dithioacetals, and boronic esters.[8,9] While the disulfide linkage is cleaved upon exposure to elevated amounts of GSH, the latter two undergo decomposition in an environment with high levels of H_2O_2 and hydroxyl radicals. Chalcogen ethers can also undergo oxidation, resulting in changes in polymer hydrophilicity or polymer degradation.[8]

Enzymes can also serve as intrinsic stimuli. For example, cathepsin B, which cleaves GFLP peptide spacers, is overexpressed in many cancers and can be exploited to selectively release therapeutics in cancer tissue.[10] Matrix metalloproteinases (MMPs) are involved in tissue remodeling and their dysregulation is associated with musculoskeletal disease, cancer, and cardiovascular disease, allowing MMP-cleavable peptides to be used in stimuli-responsive polymeric systems for biomedical applications.[11] Other enzymes of interest include alkaline phosphatase (ALP), oxidoreductases, and azoreductases which are associated with certain tumors.[12] In addition, specific small molecules can also be used as stimuli. A notable example is glucose, where the elevated concentrations associated with diabetes can be used to induce changes to polymer systems.[13] One approach involves the incorporation of glucose oxidase (GOx), which converts glucose to gluconic acid and H_2O_2, and then the resulting changes in redox status and pH can be used as stimuli. Alternatively, phenylboronic acids can be incorporated such that binding of the *cis*-1,2 or 1,3 diols of the sugar results in the formation of boronic esters, thereby increasing the anionic charge and hydrophilicity of the system.

Externally applied stimuli are also of great interest as they can often be applied with high spatiotemporal control. For example, light has been widely used as a stimulus, particularly in conjunction with the photolabile *o*-nitrobenzyl moiety.[14] While useful for proof-of-concept studies with model systems and for ex vivo applications, ultraviolet light cannot be applied in vivo due to poor tissue penetration and potential tissue damage. These limitations can be mitigated to some extent by the use of two-photon processes and chromophores responsive to near-infrared (NIR) light, but still only a few millimeters of tissue penetration is feasible.[15] Therefore, other forms of electromagnetic radiation are gaining attention. X-rays or γ-rays exhibit excellent tissue penetration and can induce the radiolysis of water, producing highly reactive ROS as well as free electrons, enabling bond cleavage.[16] Ultrasound irradiation can induce the disruption of microbubbles and cleavage of mechanochemically responsive bonds such as disulfides and cycloaddition-based mechanophores in polymer systems.[17,18]

Heat is an attractive stimulus that lies at the interface of intrinsic and extrinsic stimuli. Upon the injection of a polymeric system into the body, the temperature changes from room temperature to 37 °C. Alternatively, a selected location of the body can be heated above 37 °C using externally controlled devices. Most biomedical applications of polymers that involve heat as a stimulus involve polymers exhibiting a lower critical solution temperature (LCST).[19,20] These polymers are soluble at lower temperatures but undergo a coil-to-globule transition and precipitate above their LCST due to the entropically favorable release of bound solvent. Such temperature-driven changes can be used to collapse polymer assemblies, induce gelation, or stimulate volume changes in hydrogels. External magnetic fields are often used in conjunction with thermoresponsive polymers, and are particularly attractive due to their compatibility with biological systems. After embedding magnetic or superparamagnetic nanoparticles into polymer systems, the application of high frequency magnetic fields results in localized heating of the material, actuating changes in the thermoresponsive polymers, while minimizing heating to the surrounding tissues to mitigate potential tissue damage.[21]

POLYSACCHARIDES

Polysaccharides are a diverse set of natural molecules, with various forms of poly(glucose), including cellulose and starch, or modified forms of poly(glucose), such as chitin, as the most abundant classes. As abundant sources of carbon-based materials, polysaccharides are biosynthesized across the biological kingdoms in amounts that exceed annual production of synthetic polymers by several orders of magnitude. While traditionally used as static structures, stimuli-responsive variants of polysaccharides are beginning to make inroads in different applications. In addition to their abundance and widespread availability, their typically minimal toxicity makes polysaccharides important natural building blocks for future stimuli-responsive materials.

Hydrogels, described in further detail later in this perspective, are particularly promising stimuli-responsive materials because

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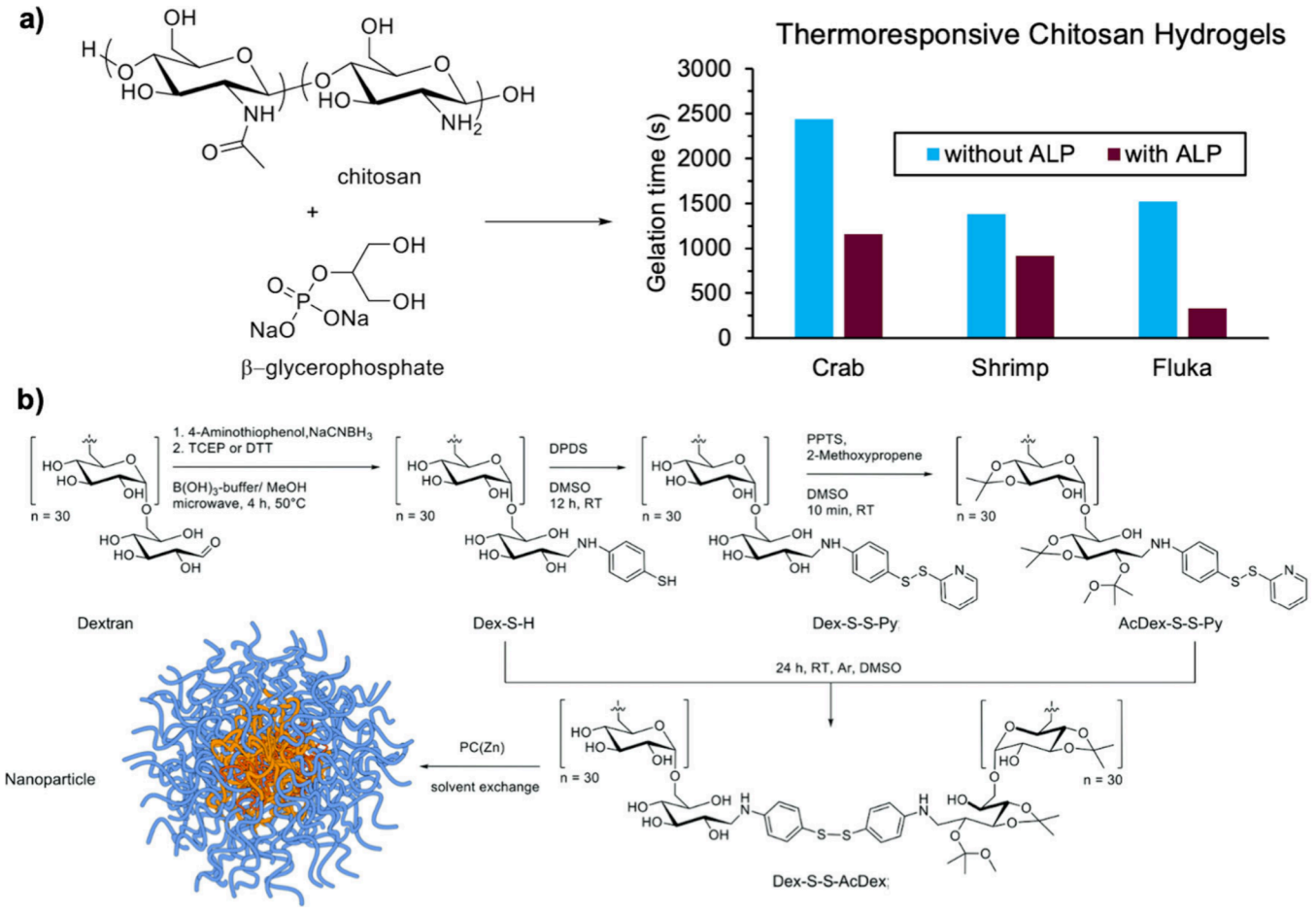

Figure 2. Polysaccharide-based stimuli-responsive polymers: (a) Chitosan from three different sources forms a thermoresponsive gel with *β*-glycerophosphate, with gelation accelerated by ALP. Adapted with permission from ref 25. Copyright 2013 Elsevier. (b) Synthesis of doubly responsive block copolymer micelles based on polysaccharides. Adapted from ref 26. Copyright 2019 Royal Society of Chemistry, permission conveyed through Copyright Clearance Center, Inc. Abbreviations: TCEP = tris(2-carboxyethyl)phosphine; DTT = dithiothreitol; DPDS = dipyridyl disulfide; DMSO = dimethyl sulfoxide; PPTS = pyridinium *p*-toluenesulfonate; and PC(Zn) = phthalocyanine zinc.

changes at the molecular level (e.g., protonation/deprotonation, hydration/dehydration, or *cis*–*trans* isomerism) translate to changes in conformation or solubility, which lead to macroscopic changes in swelling behavior, rheological properties, or cross-link density, among others. Polysaccharides can be decorated with various functional groups or side-chain polymers to add stimuli-responsiveness.[22] Alternatively, some polysaccharides naturally respond to stimuli, in particular pH and temperature. For example, chitosan, derived from the partial deacetylation of natural chitin, contains an amine group with a pK_a near 7, allowing it to swell or deswell in response to pH changes.[23] As demonstrated by Zhou, Chen, and co-workers, some chitosan hydrogels can also undergo a solution-to-gel transition near body temperature,[24] similar to the well-known and thoroughly studied thermoresponsive polymer poly(*N*-isopropylacrylamide) (PNIPAM). However, unlike PNIPAM, which has an all-carbon backbone, thermoresponsive chitosan-based hydrogels are biodegradable. As shown by Douglas and co-workers, chitosan in combination with *β*-glycerophosphate forms a material that gels above 37 °C over the course of several minutes, with rate acceleration upon adding ALP (Figure 2a).[25] Recent efforts focusing on drug delivery and tissue engineering from stimuli-responsive polysaccharide-based hydrogels highlight the versatility of this class of polymers either as inherently stimuli-responsive materials or as degradable backbones that can be functionalized with stimuli-responsive groups.

Through side chain modifications and end-group exchange reactions typically used in synthetic polymers, complex polysaccharide-based materials can now be constructed. For example, Wich and co-workers used a disulfide exchange reaction between two dextran blocks, one with side-chain pH-responsive acetal protecting groups and one without, to make a doubly responsive block copolymer (Figure 1b).[26] The block copolymer self-assembled into micelles, and upon loading with a Zn-based photosensitizer, the micelles released their payloads under either reductive or acidic conditions. Key to this work was the chain-end modification of dextran, and these types of regiospecific polysaccharide modification reactions that target only one chain end are critical for moving the field forward.[27,28] In another example of stimuli-responsive polysaccharides, Akiyoshi and co-workers grafted poly(propylene oxide) onto a pullulan backbone.[29] Upon heating from a chilled solution, the molecularly dissolved graft polymers assembled into vesicles, driven by dehydration of the poly(propylene oxide) block. A critical control study in this work on a structurally similar linear diblock copolymer, which formed poorly defined aggregates upon heating, highlighted the importance of the graft structure.

Current challenges with polysaccharides often stem from their natural origin. Unlike for most synthetic stimuli-responsive

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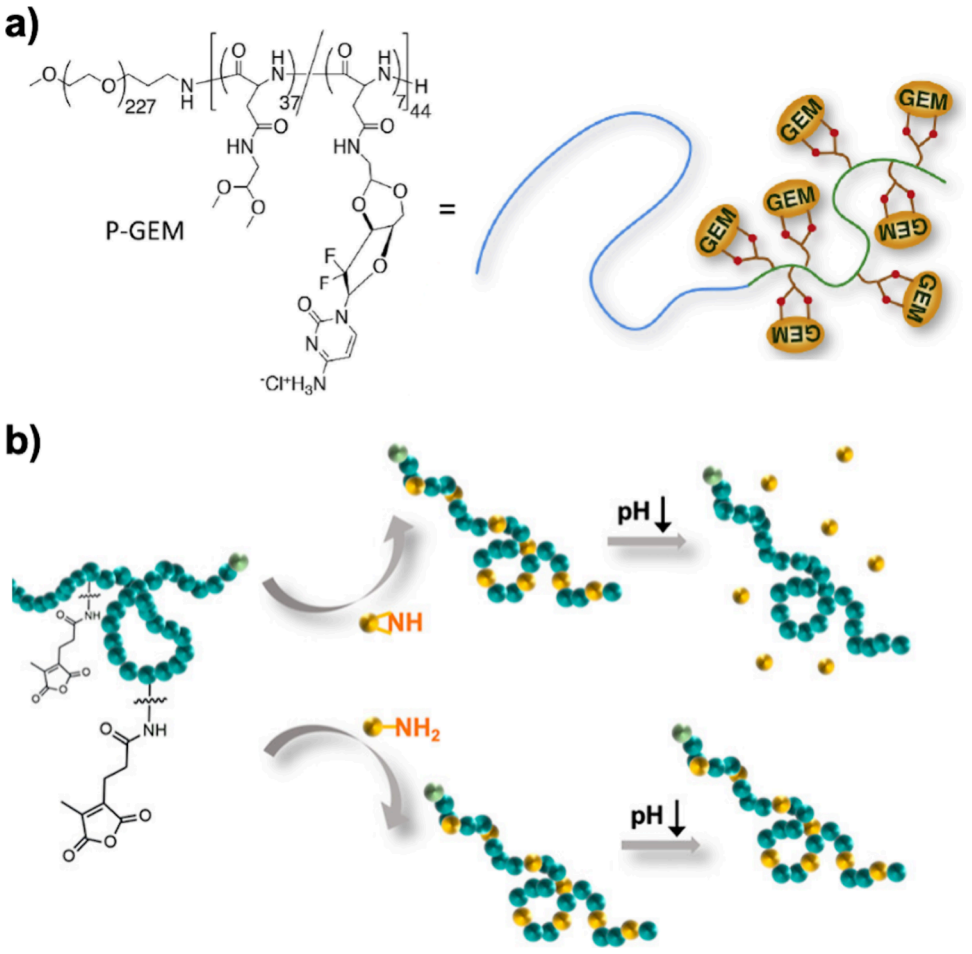

Figure 3. Acid-responsive polymer therapeutics: (a) Self-assembled nanoparticles with anticancer drug gemcitabine conjugated to the polymer via an acetal linker. Adapted with permission from ref 42. Copyright 2020 Elsevier. (b) 2-Propionic-3-methylmaleic anhydride-containing polymers allow conjugation of secondary amine-containing drugs and their controlled release in acidic environment. Adapted from ref 44. Copyright 2023 American Chemical Society

polymers, consistent control over molecular weight and molecular weight distribution can be problematic. Although a narrow molar mass dispersity is not required for all applications, and in fact broad dispersity values can be advantageous in some scenarios, robust and reliable fractionation methods to isolate polysaccharide fractions with minimal batch-to-batch variability are needed. Another challenge in synthesizing and characterizing stimuli-responsive polysaccharides is their solubility. Some polysaccharide derivatives are soluble in organic solvents, but additives, heat, and extended dissolution times may be required, and aggregation can still occur due to extensive hydrogen bonding. Similar problems can occur for water-soluble polysaccharides. These solubility limitations often limit reaction conversion, including side chain modification reactions and chain-end substitutions. Finally, low reactivity of polysaccharide functional groups is a problem that has existed since the field began. Reactions that proceed smoothly on small molecules and other synthetic polymers often do not reach full conversion in polysaccharides. Continued experimental development of synthetic methods is required to achieve clean and quantitative reactions on polysaccharides to add stimuli-responsive groups. Despite these drawbacks, we envision continued exciting progress in achieving functional, stimuli-responsive polysaccharides during the coming years.

POLYMERS AND SELF-ASSEMBLED CARRIERS

Polymers and Polymer Assemblies for Drug Delivery.

Stimuli-responsive drug delivery systems have emerged as an important class of biomaterials aimed at enhancing the pharmacological potential of small molecule drugs by increasing their water-solubility, blood circulation times, or improving their specificity for pathological processes, such as cancer.[30] Polymeric systems are proposed to be passively targeted to cancerous tumors via the enhanced permeation and retention (EPR) effect, which arises from the leaky vasculature and a poorly developed lymphatic system serving tumors.[31] Passive targeting of inflamed tissues is thought to occur through the extravasation through leaky vasculature and subsequent inflammatory cell-mediated sequestration (ELVIS) effect.[32] Stimuli-responsive polymers can be used to enable drug release in a specific environment or to promote biodegradation of the polymeric carrier, facilitating its excretion. Essentially all the biologically relevant stimuli discussed above can be used to actuate drug release from delivery systems.

pH-responsive systems are the most extensively studied class of stimuli-responsive drug delivery vehicles, targeting the acidic environment of tumors and inflamed tissues, as well as the endolysosomal system.[33] Anticancer therapeutics based on doxorubicin (Dox) conjugated to polymer carriers via hydrazone bonds have been extensively investigated due to

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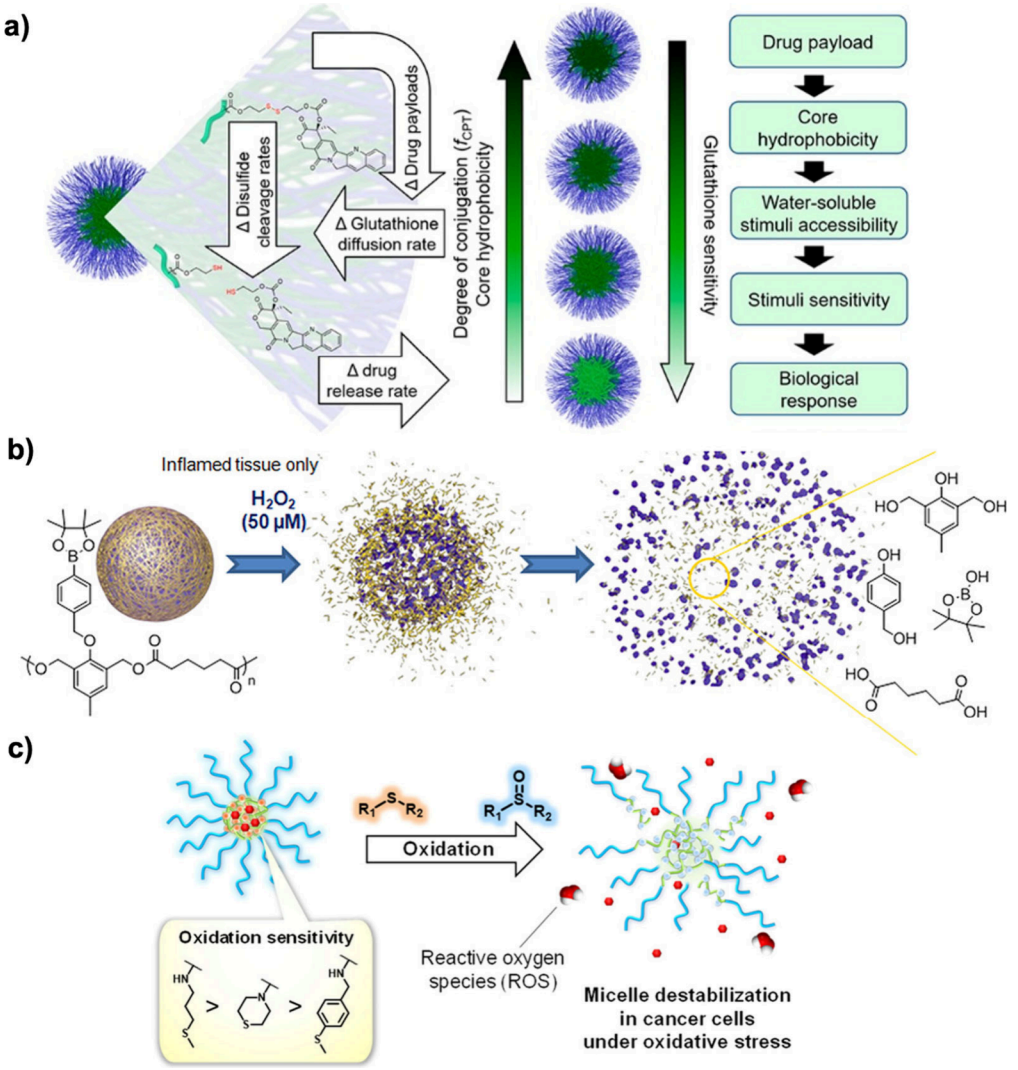

Figure 4. Examples of redox-responsive polymer systems: (a) Micellar aggregates from a disulfide-linked polymer drug conjugate. Adapted from ref 54. Copyright 2020 American Chemical Society. (b) ROS-sensitive delivery vehicle with self-immolative linkers in the polymer backbone. Adapted from ref 56. Copyright 2012 American Chemical Society. (c) Disassembly of a micellar aggregate by disruption of hydrophilic−hydrophobic balance upon oxidative transformation of side chain residues. Adapted from ref 57. Copyright 2022 American Chemical Society.

their straightforward conjugation protocol and nearly ideal drug release profile: negligible release at pH 7.4 during blood circulation and rapid drug release in a mildly acidic environment.[34] Several polymer-Dox conjugates have entered clinical trials; however, these trials were mostly discontinued due to toxicity or low therapeutic efficiency. One key issue was insufficient accumulation in the tumors of human patients.[35] While tumor accumulation typically increases at higher molar mass,[36] due to the lack of degradability of the poly(*N*-[2-hydroxypropyl]methacrylamide) (PHPMA) backbone, which was employed in this work, and its broad mass distribution resulting from conventional free radical polymerization, the molar mass had to be limited to 28 kg/mol to ensure complete renal excretion of the polymer.[35] More recent efforts have focused on exploiting more controlled polymerization approaches and examining various architectures, from self-assembled micelles to bottlebrushes or star polymers and nanogels.

A recent interesting study by Etrych and co-workers compared the in vivo anticancer efficacy of Dox conjugated by hydrazone bonds to different high molar mass PHPMA carriers, revealing superior tumor growth suppression for starlike polymer conjugates compared to self-assembled micellar analogs (linear PHPMA containing hydrophobic cholesterol units distributed along the polymer chain).[37] On the other hand, the micellar conjugates showed lower systemic toxicity and better inhibition of multidrug resistance.[38] Water-soluble PHPMA was used as a carrier polymer due to its excellent biomedical properties and the possibility of straightforward functional group introduction by statistical copolymerization, as compared to PEG for example.[39]

Acid-cleavable acetal linkers have also been used to conjugate hydroxyl-containing drugs to polymers, to induce stimuli-responsive degradation of the polymer carrier backbone, or to enable an increase in hydrophilicity, resulting in disassembly of polymer micelles.[40] Acetal-based polymer-drug conjugates are accessible through acid-catalyzed conjugation of a vinyl ether and a hydroxyl in anhydrous conditions. For example, Sun, Zhai, and co-workers conjugated paclitaxel to self-assembled PEG-*b*-poly(aliphatic polycarbonate) nanoparticles, resulting in minimal drug release at pH 7.4 and rapid release in a slightly acidic environment.[41] The polymer carrier is then excreted upon

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biodegradation of the polycarbonate-based micelle core. Acetal linkers can also be used for the conjugation of diol-containing drugs such as gemcitabine (GEM), as shown by Nishiyama and co-workers, who conjugated GEM to a PEG-poly(amino acid) diblock copolymer via a cyclic acetal (Figure 3a).[42] Despite the relatively slow GEM release, the polymer-GEM conjugate significantly outperformed free GEM in terms of anticancer efficacy in vivo and suppressed its main side effects (i.e., myelosuppression and gastrointestinal toxicity). Acetal-based backbone-degradable polymers can also provide tools for the development of advanced delivery systems with tailorable biodegradation.[40] While most of the acetal-containing polymers are synthesized from fossil fuel-based feedstocks, Schlaad and co-workers recently developed a new class of polyacetals by cationic ring-opening polymerization (ROP) of biomass-derived levoglucosenyl methyl ether.[43] While not yet used for the construction of delivery systems, such biobased polyacetals might enable the development of advanced biomaterials while also considering the growing importance of sustainable chemistry.

Amines can also be used as reactive groups for the conjugation of drugs to polymers by pH-responsive linkages. For example, Nuhn and co-workers developed a novel strategy to conjugate imidazoquinoline immune-stimulatory drugs containing primary or secondary amine groups with 2-propionic-3-methyl-maleic anhydride groups of polymer carriers (poly(2-propionic-3-methyl maleic anhydride methacrylamide) homopolymer, Figure 3b).[44] While the primary amine-containing drug conjugate was not cleavable, secondary amine-containing compounds showed rapid release at pH 5.5 while remaining intact at neutral pH. This strategy is quite universal and can be used for the synthesis of acid-releasable conjugates of secondary amine-containing drugs, while the irreversible conjugation of primary amines can be used for the attachment of tracing labels or targeting compounds.

Polymerization-induced self-assembly (PISA) has emerged as an attractive strategy for nanoparticle synthesis, merging polymerization and self-assembly into one step.[45] The PISA method produces well-defined nanoparticles at high concentrations that are not achievable by other methods such as nanoprecipitation. Furthermore, in addition to spherical micelles, wormlike micelles, polymersomes,[46] more exotic morphologies are often observed, such as jellyfish-like micelles,[47] yolk/shell particles,[48] framboidal vesicles,[49] or lamellae.[50] Despite numerous reports on PISA in recent years, reports on PISA-based drug delivery systems are still relatively sparse. Hong and co-workers recently reported the synthesis of self-assembled vesicles by PISA chain extension of a hydrophilic PEG block with a combination of (diisopropylamino)ethyl methacrylate and benzyl methacrylate in ethanol.[51] The vesicles were loaded with rhodamine B as a model drug, which underwent accelerated release at acidic pH, as the amine-containing block became more hydrophilic upon protonation. Given the current popularity of PISA, a surge in the number of reports on PISA-based DDS might be expected in the near future.

There has also been increasing interest in leveraging the redox-responsiveness of polymeric materials to enable the controlled release of therapeutic agents. Most commonly, redox-active chemical bonds such as disulfides have been used as linkages in attaching drug molecules to polymeric carriers.[52,53] Depending on the hydrophilic nature of the polymeric backbone, the hydrophobicity of the drug, and the amount of drug attachment, the polymer-drug conjugates can behave as drug-appended single polymeric constructs or can self-assemble to form nanosized aggregates. The formation of aggregates such as micelles can also be engineered using a block copolymer where the drug-containing second block forms a drug-loaded micellar core. These nanosized carriers with deeply embedded drugs have the advantage of limiting premature release before accumulation at the disease site, and with appropriate targeting, off-site drug distribution may be reduced. Once the hydrophobic drugs are released inside the cell, they could maintain their solubility either through protonation in the acidic compartments or by interactions with the proteins within the cellular environment and eventually get transported and released at the intracellular compartment where they can induce cytotoxicity

Although the concept of redox-responsive polymer-drug conjugates seems straightforward, many factors need consideration, as recently reported by Wooley, Zhang, and co-workers (Figure 4a).[54] Poly(ethylene glycol)-*b*-poly(glucose carbonate) diblock copolymers obtained using organo-catalyzed ROP were conjugated with hydrophobic drug molecules on the sugar-containing block. It was observed that the conjugation of varying amounts of the hydrophobic drugs provides micelles with varying core hydrophobicities, which also affects their stimuli-responsive release. Thus, each drug-polymeric carrier combination may require individual case-by-case optimization. In addition, early approaches where a thiol-containing therapeutic agent was conjugated to a polymer using the thiol−disulfide exchange reaction were not straightforward, as most drug molecules do not possess a thiol group, hence requiring additional steps to modify the drug. As a solution, self-immolative linkers can be used, enabling the use of drug molecules that do not contain a thiol group. Instead, an amine group (which is present in numerous drugs) can be conjugated as a prodrug and released in the GSH-rich cytoplasm.[55]

The use of drug delivery vehicles containing redox-responsive linkages in the polymeric platform has been another area of intense research. In contrast to the approach involving conjugates, drugs can be loaded through physical encapsulation without the need for modifying the active drug molecule. For instance, Oh and co-workers used diblock copolymers with redox-sensitive junction points to create redox-responsive micellar aggregates.[58] In this case, after cleavage and loss of the hydrophilic coronas from the micelles, the polylactide cores precipitated, as colloidal stability was lost. Another method involves using redox-sensitive linkages for interchain cross-linking of copolymers to produce nanosized carriers. For example, disulfide-linked nanogels have been employed by Thayumanavan and co-workers as well as Maynard and co-workers to deliver not only anticancer drugs but also biologics such as proteins and oligonucleotides.[59,60]

ROS-sensitive polymeric materials are also an attractive platform for targeted therapy.[61] Particularly, thioketal-based linkages, which are sensitive to ROS species such as H_2O_2, are gaining increased attention. Since the seminal demonstration by Murthy and co-workers of ROS-sensitive thioketal polymers for gene delivery in 2010,[62] this linker has been used for delivering conventional drug molecules either through conjugation to polymers or by using polymeric carriers stabilized with these stimuli-sensitive linkages. While most studies rely on elevated levels of ROS in diseased cells, innovative approaches as reported by Wang, Yang, and co-workers have broadened the scope of this approach.[63] A polyphosphoester copolymer

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containing hydrophilic PEG-based side chains and multiple copies of the anticancer drug Dox conjugated to the polymer backbone through a thioketal linkage was obtained using ROP of cyclic phosphoesters. Nanoparticles encapsulating the photodynamic agent Ce6 were obtained through the self-assembly of these polymers in aqueous media. The authors demonstrated that through the incorporation of the photodynamic agent, the singlet oxygen generated locally by red light stimulation triggered the disassembly of the carrier to release the encapsulated drug.

Boronic ester linkages have been explored for the development of ROS-sensitive delivery vehicles. As early as 2011, Fréchet and co-workers formulated oxidation-sensitive dextran microparticles for vaccine delivery using aryl boronic esters appended to dextran through carbonate linkages, which underwent degradation upon exposure to pathophysiological levels of H_2O_2.[64] Subsequent studies have used boronic esters to either link drugs or fabricate delivery vehicles. An innovative design by Almutairi and co-workers employed this chemistry in fabricating nanoparticles where the polymer chains degraded in an oxidative environment to release the encapsulated drugs (Figure 4b).[56] An alternating copolymer obtained through condensation polymerization of adipoyl chloride and an aromatic monomer bearing a pinacol-protected boronic ester group was utilized to prepare the particles. Exposure of these particles to H_2O_2 resulted in the oxidative removal of the aryl boronic ester group, which led to the deprotection of the phenol group, resulting in a quinone-methide rearrangement mediated polymer degradation. Lastly, an approach gaining attention involves oxidative environments leading to chemical modifications instead of bond cleavages, resulting in a change in the hydrophobic−hydrophilic balance, destabilizing the micellar carriers to release the encapsulated drug (Figure 4c).[57] In this work, Hasegawa and co-workers demonstrated that the stability of the micellar structure could be modulated by tuning the structure of the thioether groups in a diblock copolymer-derived carrier. Amphiphilic diblock copolymers composed of a hydrophilic poly(*N*-acryloyl morpholine) block and a second block consisting of thioether groups were synthesized using reversible addition−fragmentation chain-transfer (RAFT) polymerization. Upon oxidation of the thioethers to sulfoxides, the hydrophobic block became hydrophilic, leading to micelle disintegration. Furthermore, Dox-loaded micelles prepared from copolymers bearing the thiomorpholine group showed higher toxicity in HepG2 cells than in normal cells (HUVECs). These results suggest that there is much more that can be achieved by fine-tuning and optimizing the molecular structure around these stimuli-sensitive linkages.

While ROS can be intrinsically elevated in certain pathological conditions, it is also possible to artificially trigger elevated levels of ROS inside the body using ionizing radiation to induce the radiolysis of water, a key mechanism involved in cancer radiotherapy. This approach enables stimuli-responsive polymers not only to respond to naturally occurring ROS but also to be triggered by an external stimulus, providing a desired response at a target location and time. A key advantage is that high energy ionizing radiation can penetrate tissues, breaking through the depth limitations of conventional photochemical systems.[65,66] The first radiation-responsive polymeric materials were pioneered by Xu and co-workers with diselenide and ditelluride linkages that were susceptible to oxidation under radiation.[67,68] While early work required extremely large radiation doses (500 Gy),[69] optimization of the chemistry led to systems that could be activated by clinically relevant doses (2 Gy).[67] The inherent redox chemistry of selenium was also harnessed to generate immune stimulating seleninic acid, creating a synergistic combination of immune, chemo, and radiation therapy for cancer treatment.[68] While there have been limited other examples of radiation responsive switchable polymers in the literature, reports on organic small molecule prodrugs have recently emerged.[16,70,71] These examples all use previously known ROS-activated chemistries and highlight that many of the ROS-responsive polymers described above are likely to respond to radiation-induced ROS as well. This opens new opportunities for existing systems for use in combination therapy of cancer, where radiation is already used as part of the treatment regime and the generated ROS is an untapped chemical potential.

Thermoresponsive polymers are frequently used in the design of drug delivery systems, as they allow in situ self-assembly of nanoparticles upon administration and heating to body temperature.[72] Temperature change is rarely used to trigger drug release due to the minor temperature variations accessible naturally within the body. However, the phase transition in LCST polymers can be achieved using magnetic hyperthermia. For example, Alem and co-workers prepared superparamagnetic iron oxide nanoparticles coated with a thermoresponsive shell composed of 2-(2-methoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate loaded with Dox.[73] In a high-frequency alternating magnetic field, the nanoparticles were heated, resulting in the collapse of the thermoresponsive polymer shell and Dox release.

Enzyme-responsive polymeric carriers are particularly attractive for biomedical applications due to the variation in their expression depending on the in vivo location and disease state. Some of the earliest polymer-drug conjugates, reported by Duncan, Kopeček, and co-workers were composed of PHPMA with Dox or other anticancer drugs conjugated by a tetrapeptide that could be cleaved by the lysosomal protease cathepsin B.[74−76] In recent years, more advanced and multifunctional designs have been introduced. For example, Pu and co-workers introduced nanoparticle sensors aimed at the detection of cancer as well as liver allograft rejection.[77] The nanoparticles were composed of polymers containing NIR fluorophores bonded covalently to self-immolative linkers that were caged with peptides sensitive to either cathepsin B or granzyme B, associated with tumor progression and allograft rejection, respectively. β-Cyclodextrins were introduced as pendent groups on the dye molecules to impart amphiphilicity, allowing the polymers to self-assemble into ∼180 nm particles. The dyes were nonfluorescent when conjugated to the self-immolative linker, but upon cleavage of the peptide cages by the protease, self-immolative elimination occurred and NIR fluorescence was activated, enabling the detection of cancer and allograft rejection through noninvasive imaging. Furthermore, the dye-cyclodextrin conjugate was excreted into the urine, enabling urinalysis. Christman, Gianneschi, and co-workers used ring-opening metathesis polymerization to prepare copolymers of phosphoramidates and norbornenes with MMP-cleavable peptides.[78] These amphiphilic copolymers self-assembled into 30 nm particles, but in response to peptide cleavage by MMPs, they assembled to form micrometer-sized aggregates, allowing them to selectively accumulate at the site of postmyocardial infraction with elevated MMP expression in a rat model.

In the design of enzyme-responsive systems there are considerations that are different from those related to small

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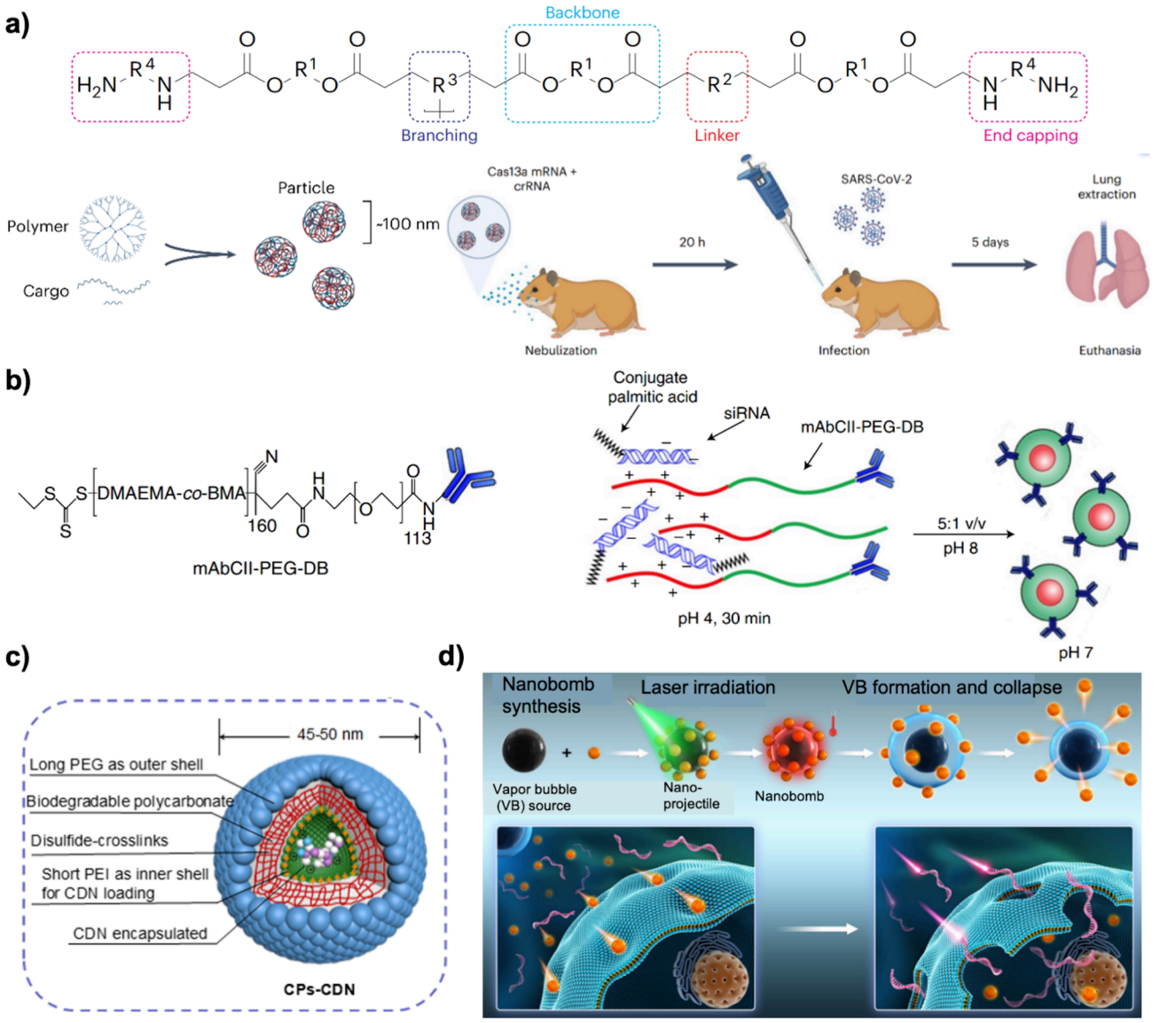

Figure 5. Stimuli-responsive nanosystems for nucleic acid delivery: (a) Screening of ionizable dendritic polymers for mRNA delivery via nebulization. Adapted from ref 92. Copyright 2023 Springer Nature BV, permission conveyed through Copyright Clearance Center, Inc. (b) MMP-13 siRNA loading via ionizable polymer forming nanoparticles for targeted osteoarthritis therapy. Adapted from ref 93. Copyright 2021 Springer Nature BV, permission conveyed through Copyright Clearance Center, Inc. (c) Disulfide-cross-linked polymersomes with stably loaded CDNs that can be released by intracellular reducing conditions. Reprinted with permission under a Creative Commons CC BY-NC-ND 4.0 license from ref 95. Copyright 2022 Elsevier. (d) Light triggered vapor bubble expansion and the resulting nanoprojectile penetrating the cell membrane, leading to the cell entry of nucleic acids in the medium. Adapted with permission under a Creative Commons CC BY 4.0 license from ref 96. Copyright 2022 Nature Publishing Group.

molecule stimuli such as protons and ROS, which can readily diffuse in and out of polymer assemblies. For example, Amir and co-workers studied the enzyme-induced disassembly of nanocarriers composed of highly precise linear−dendritic amphiphiles.[79] On the basis of observations across multiple studies,[80,81] the group postulated that enzymes have limited access to the hydrophobic cores of polymer micelles and that enzymatic activation occurs in the unimer state of the unimer-micelle equilibrium. This phenomenon may explain why limited enzymatic degradation is sometimes observed for polymer assemblies. In addition, the charge state of the enzyme can play an important role in its accessibility to the nanoassembly core, as demonstrated by Wooley and co-workers, when they compared two classes of core−shell assemblies with the same hydrolyzable poly(DL-lactide) cores but either anionic (poly(acrylic acid)) or cationic (poly(acrylamidoethylamine)) shells.[82] Hydrolysis of the core with the positively charged enzyme proteinase K occurred preferentially for the anionic assemblies, while with the negatively charged enzyme porcine liver esterase it occurred more rapidly for the cationic assemblies. These results indicate that charge of both the polymer system and enzyme should be taken into account in the design of enzyme-responsive materials.

Overall, stimuli-responsive polymeric systems offer promising platforms for drug delivery, and the examples mentioned above highlight innovative approaches that can enhance their efficacy. Rather than focusing solely on the specific linkage, it is essential to consider the entire package: the molecular and macromolecular structural details, as well as the nanosized constructs derived from them. Another consideration is that despite their often more straightforward synthesis, the main drawback of

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systems with noncovalently incorporated drugs is the potential for premature drug leaching (so-called burst release) from the nanoformulation.[83] Because of these considerations, in vivo experiments involving individual constructs are still needed to select candidates with high potential for translation from the lab to the clinic.

Polymer Nanosystems for Nucleic Acid Delivery. In recent years, nucleic acid-based drugs, including small interfering RNA (siRNA), mRNA (mRNA), double stranded RNA (dsRNA), microRNA (miRNA), antisense oligonucleotides (ASO), oligodeoxynucleotides (ODN), and nucleic acid–based constitutional dynamic networks have emerged as highly promising drugs for the treatment of various diseases such as infection, cancer, autoimmunity, inflammation, and genetic disorders. Their high potential can be attributed to their high specificity, low cytotoxicity, low off-target effects and low drug resistance. For example, siRNA can down-regulate specific target mRNAs in a sequence-specific manner, showing prospects in treating diseases that were previously undruggable or impossible to target using antibodies or small-molecule drugs. mRNA holds immense potential for direct control over protein production in specific cells. Since siRNA and mRNA exert their functions in the cytosol, endocytosis and endosomal escape are of utmost importance. Oligonucleotides like cyclic dinuclide (CDN) and CpG ODN as well as dsRNA poly(I:C) are major agonists for stimulation of STING, TLR9, or TLR3 pathways, respectively, in antigen-presenting cells (APCs).[84−86] CpG and poly(I:C) have receptors in the inner membranes of endosomes of APCs, and CDNs bind to the STING proteins anchored in the endoplasmic reticulum of APCs to promote innate immunity.[87−89] Therefore, to maximize the effect of nucleic acid drugs, it is crucial to load them with high efficiency, protect them from nuclease degradation, and deliver/release them into target cells, which are typically either tumor cells or APCs. Taking advantage of intracellular cues, pH-, reduction-, and enzyme-responsive nanosystems have been investigated to achieve this.

pH-responsive nanosystems with ionizable moieties have been widely explored for nucleic acid delivery, as they can complex negatively charged nucleic acids and assist in endosomal escape, where a reduction in pH occurs. Lipid nanoparticles with ionizable lipids as a key component have been of particular interest due to their successful application in the mRNA Covid-19 vaccines.[90] However, ionizable polymers have also been increasingly investigated in recent years. For example, for inhalable mRNA delivery with the goal of mucosal vaccination against SARS-CoV-2, Suh, Saltzman, and co-workers optimized biodegradable poly(amine-*co*-ester) polyplexes by screening blends that combined the benefits of PEG stabilization and end group-mediated enhanced endosomal escape,[91] while poly(*β*-amino-thio-ester)s were optimized in terms of one or more of four components (backbone, linker component, branching component, and end-capping structure) (Figure 5a).[92] Substantial recent effort has also been invested in preventing tissue damage in inflammatory diseases through the delivery of nucleic acid drugs. Duvall and co-workers showed that poly(2-(dimethylamino)ethyl methacrylate) with ionizable tertiary amines formed polyplexes with MMP-13 siRNA, and further functionalization with a type II collagen-targeting antibody enabled the use of a local injection to reduce MMP-13 expression and protect cartilage in post-traumatic osteoarthritis (Figure 5b).[93] In addition, Li and co-workers reported a fluorinated polyamidoamine dendrimer formed polyplexes with miR-23b, which showed high transfection efficiency in inflamed synoviocytes and reduced inflammation.[94] The nanoparticles based on these polymers generally displayed high drug loading, cellular uptake, endosomal escape, and transfection efficiency.

The reductive environment in the cytosol is a very important cue to stimulate siRNA and mRNA release after endosomal escape. Recent research has focused on reduction-responsive cross-linked nanoparticles that facilitate long circulation of the nanosystem followed by efficient nucleic acid release in cytosol. Zhou and co-workers designed alendronate-modified reduction-responsive cross-linked cationic nanoparticles for controlled loading and release of Dcstamp siRNA, which displayed bone-targeted delivery of siRNA and good transfection, promoting osteogenesis and reduced bone resorption.[97] Meng and co-workers developed disulfide-cross-linked biodegradable polymersomes with a protonatable inner shell made of spermine or low molar mass branched poly(ethylenimine), which showed efficient loading and protection of siRNA, mRNA, proteins, and oligonucleotides as well as high efficiency in tumor therapy and immunotherapy.[95] The CDN STING agonist ADU-S100 was loaded into these polymersomes and delivered to dendritic cells to promote innate immunity (Figure 5c). In combination with radiotherapy, the polymersomes substantially improved anti-melanoma efficacy. Furthermore, CpG ODN as a TLR9 agonist was formulated with polymersomes, and intravenous or intranasal administration enhanced T cell immunity and prolonged survival of mice bearing orthotopic glioblastoma.[98]

Enzyme-responsive polymers and short peptide linkers have also been applied to build nanoparticles for nucleic acid delivery. Cai and co-workers reported MMP-9 responsive self-assembled gelatin/silk fibroin composite particles (GSC) that encapsulated siPD-L1 and an interleukin 12 plasmid (pIL-12) with high efficiency.[99] Upon inhalation, GSC displayed good transmucosal penetration and lung retention as well as high targeting efficiency. Short peptides cleavable by enzymes such as peptidase and cathepsin were applied to link CDNs to lipid or polymer nanoparticles. Artzi and co-workers conjugated CDNs to a poly(*β*-amino ester) (PBAE) via a cathepsin-sensitive linker and further complexed them with arginine oligopeptide-functionalized PBAE.[84] Intravenous administration resulted in tumor accumulation, CDN release in the endosome and cytosol, and stimulated activation of the STING pathway and T cell immunity.

Facing the bottleneck of intracellular delivery of nucleic acids, Braeckmans and co-workers took a completely different path by developing light-triggered nanobombs that consisted of a photothermal core nanoparticle coated with smaller nanoparticles that were loaded with mRNA or pDNA and acted as "nanoprojectiles" (Figure 5d).[96] Upon irradiation with 561 nm laser light, the core was rapidly heated, forming vapor bubbles that drove the nanoprojectiles across the membranes of nearby cells, bringing mRNA or pDNA in the medium into the cells. The transfection efficacies of mRNA were 5.5−7.6 times that of traditional method in several cell lines. Such methods open new paths to the intracellular delivery of nucleic acids.

Overall, the efficient intracellular delivery of nucleic acid-based biopharmaceutics using stimuli-responsive nanosystems is an attractive strategy to maximize their function. Due to the inherent advantages of nucleic acid drugs, it would be beneficial for more future efforts to be directed to the design of new nucleotide sequences and their delivery to treat a wide range of conditions such as cancer, infection, chronic inflammatory diseases, and genetic disorders. In addition, combination therapy using nucleic acids with other drugs such as small

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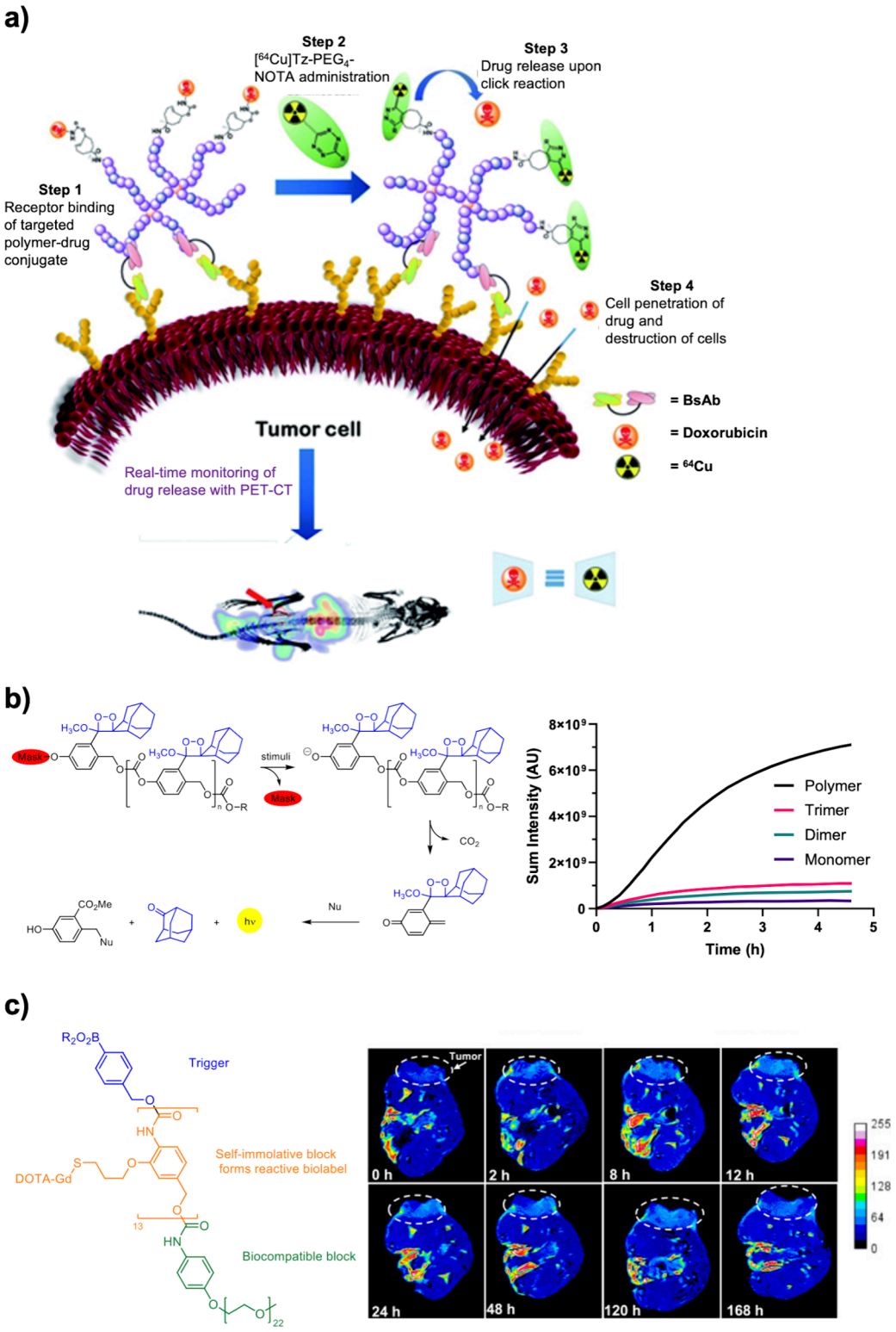

Figure 6. Highlights of recent innovations in switchable polymers for theranostic applications: (a) Bioorthogonal click-to-release chemistry allows for quantification of drug release from a hyperbranched polymer in vivo, using PET-CT imaging. Adapted with permission under a Creative Commons CC BY 3.0 license from ref 104. Copyright 2020 Royal Society of Chemistry. (b) Self-immolative polymers can greatly amplify detected imaging signal over small molecule systems, in vitro through chemiluminescence.[106] (c) Self-immolative polymers can amplify ^{1}H MRI signal in vivo but also label biomolecules of interest in situ. Adapted with permission from ref 107. Copyright 2020 Elsevier.

molecules, proteins, and immunostimulants along with optimized administration methods should be emphasized, as combination approaches may yield synergistic therapeutic efficacy via their targeting of multiple biological pathways. In addition to the proper modification of the nucleotide sequence, to further improve the efficacy, researchers should seek to enhance the stability of formulations during circulation and before reaching the final target, improve the accumulation in

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target organs (e.g., lung, tumor, or lymph nodes) and in target cells (e.g., tumor cells or APCs), and trigger nucleic acid release inside cells. Furthermore, to guide the design of effective nanoparticles, it is also of great importance to study nanoparticle structure and interactions with tissues or cells as well as the dynamic distribution of particles across organs and cell types.

MEASUREMENT OF THERAPEUTIC RESPONSE IN THERANOSTICS

Stimuli-responsive polymers have been extensively used to develop imaging agents to measure the biochemical and physiological changes of tissues. This principle has helped to shape the field of theranostics, which aims to detect, monitor, stage, and treat a disease in one construct.[100] Moving beyond measuring physiological changes, there is also a critical need to directly measure changes in the theranostic material to understand how efficient switchable chemistries are in vivo. This strategy requires synchronizing the therapeutic process to modulation of the signal intensity in the imaging modality.

In a recent example by Li, Cheng, Zhou, and co-workers, hypoxia-sensitive nanoassemblies were synthesized by PISA.[101] A hypoxia-sensitive fluorogenic linker between the blocks was created by coupling azobenzene to a NIR emitting BODIPY fluorophore. In the self-assembled state, the BODIPY demonstrated reduced emission due to aggregation associated with the hydrophobic environment. However, cleavage of the azobenzene unit by the enzyme nitroreductase led to disassembly of the block copolymer nanoparticles, release of encapsulated Dox, and increased BODIPY fluorescence upon solubilization of the hydrophilic blocks. This system relied on a ratiometric relationship between fluorescence switch-on and drug release, but there is a growing interest in developing quantifiable systems, enabling researchers to "count" the number of therapeutics released at the target site. Such quantification requires the design of linkers where the release of payload is directly coupled to switching of an imaging signal. In an example reported by Göstl, Herrmann, and co-workers, mechanochemical and redox-responsive polymers were synthesized using a linker with a disulfide and both a drug and fluorophore coupled through carbonate linkages.[102] Cleavage of the disulfide by ultrasound or reducing agents triggered a reductive scission of the carbonate by the free thiol, resulting in simultaneous release of the drug and fluorophore. Release was quantified by fluorescence spectroscopy and monitored semi-quantitatively by flow cytometry.

Stimuli-responsive polymers can also be used to gate the therapeutic response of a theranostic, to help improve target tissue specificity, and reduce side effects. Bioorthogonal chemistry has become a powerful tool in this endeavor, allowing for synthetic chemical reactions to occur inside the body, with limited side-reactions. Thurecht and co-workers recently translated the bioorthogonal click-to-release strategy, exploiting the reaction between trans-cyclooctene and tetrazine,[103] to polymer theranostics, enabling the use of positron emission tomography to visualize and quantify drug release from a hyperbranched polymer (Figure 6a).[104] This approach uses the advantages of nuclear imaging, including deep tissue penetration and absolute quantification in a target tissue, while overcoming its biggest challenge: modulation of the nuclear imaging signal. The authors demonstrated a pretargeting of the tumor with a drug-conjugated hyperbranched polymer, where drug release was triggered and quantified by injection of a radiolabeled tetrazine small molecule. This example constituted a revolution in the field, moving to quantifiable drug release in vivo. It also introduced a method to directly couple tumor pretargeting and drug release, with tumor pretargeting by polymers already demonstrating improvements in target specificity and reducing drug side-effects.[105]

Bioorthogonal chemistry and stimuli-responsive polymers have been used in synergy to gate therapeutic responses other than just drug release. In one example, Yuan, Wang, and co-workers self-assembled polymer nanoparticles for photodynamic therapy, with a pH-sensitive hydrophobic core of 2-azepane methacrylate and a tetrazine methacrylate.[108] Upon accumulation in acidic tumor tissue, the pH-sensitive nanoparticle disassembled, revealing the reactive tetrazines from the core. A second polymer with a vinyl ether-caged hemicyanine NIR fluorophore and photosensitizer was subsequently injected. When this polymer coaccumulated in the tumor tissue, the released tetrazine units from the first polymer reacted with the vinyl ether, uncaging the photoactive molecule, switching on both fluorescence and photodynamic therapy. The synergistic advantages of this approach were demonstrated in vivo, including enhanced specificity of the fluorescence signal to the tumor tissue, as well as enhanced therapeutic PDT response.

Photoacoustic imaging (PAI, alternatively multispectral optoacoustic tomography) is an emerging technique that helps to bridge the gap between preclinical and clinical imaging. It has been used by Liu and co-workers to guide in vivo combination sono-immunotherapy of cancer using pH-sensitive polymer nanoassemblies.[109] Upon uptake into the tumor tissue, the acidic environment triggered disassembly of large nanoassemblies into unimolecular micelles, which revealed an immune-stimulating anti-PD-L1 peptide and a croconic acid sonosensitizer. Combination therapy of the nanoassemblies with ultrasound led to effective T-cell recruitment and activation, demonstrating immunotherapy as another key therapeutic approach in switchable polymer theranostics.

While optical imaging techniques are easily amenable for developing switchable imaging agents to follow therapeutic response, they suffer from low sensitivity in vivo. This is particularly true where one chemical event leads to the generation of one imaging molecule. As demonstrated in early work by Shabat, McGrath, and co-workers, amplification of this signal can be achieved by using self-immolative polymers or dendrimers, where one biologically triggered reaction can lead to many more downstream chemical reactions.[110−112] More recently, this amplification has been demonstrated by Shabat and co-workers through the development of self-immolative polycarbonates, where depolymerization led to chemically induced electron-exchange luminescence of each monomer unit.[106] The authors showed that a significant amplification of signal occurred from the self-immolative polymers, compared to analogous small molecule systems (Figure 6b). A similar phenomenon was serendipitously discovered by Li, Park, and co-workers in self-immolative polyurethanes, with ROS sensitive boronic ester end-caps.[113] Upon ROS triggered depolymerization, energy was transferred to encapsulated chlorin e6 chromophores, leading to chemiluminescent emission of far-red light detectable in mouse models of ROS-related diseases. Similarly, He and co-workers demonstrated hypoxia-induced fluorescence switch-on of self-immolative polymers, though not yet with fluorophores suitable for use as theranostics in vivo.[114] Signal amplification can overcome some of the limitations of optical imaging in vivo, making further exploration of self-immolative polymers of significant interest, although the

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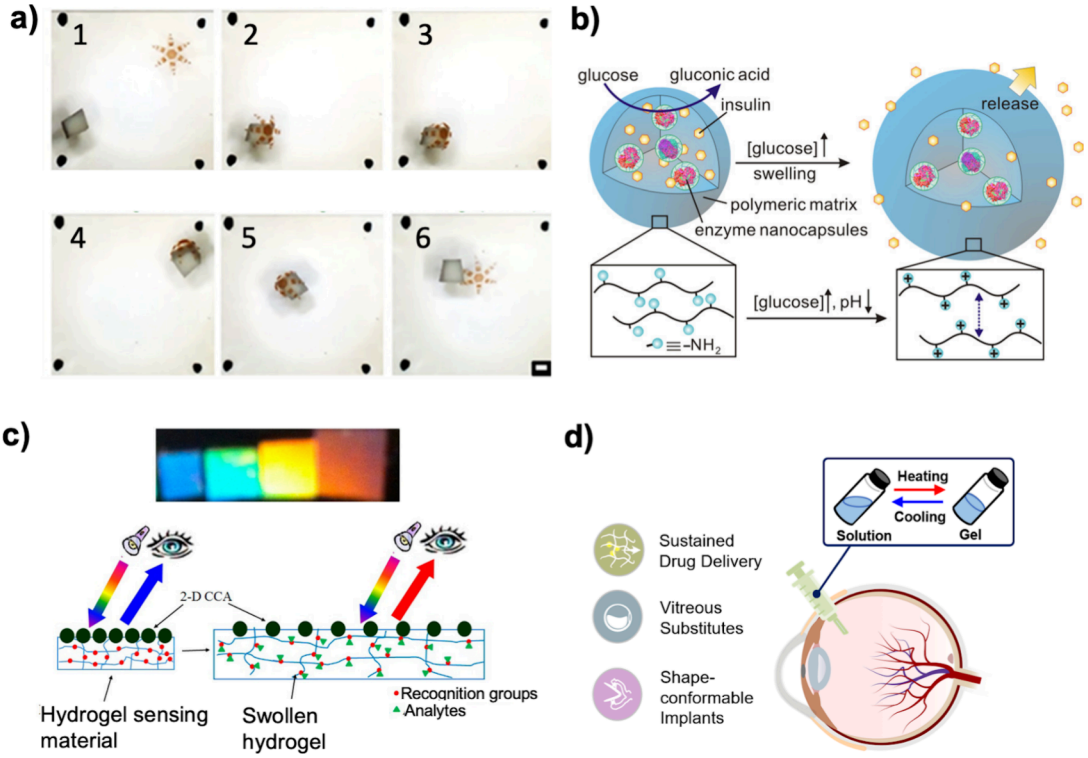

Figure 7. Applications of stimuli-responsive hydrogels: (a) Actuation and shape-morphing polymers in thermally actuated and magnetically positioned untethered microgrippers. 1: Gripper moved with a magnetic field. 2: Gripper closes on cooling. 3: Gripper securely grips soft cargo (PICK). 4: Gripper + soft cargo moved with a magnetic field. 5: Gripper opens on heating. 6: Gripper releases soft cargo. Adapted from ref 117. Copyright 2019 American Chemical Society. (b) Smart Drug Delivery uses the change in pH arising from glucose oxidase in the presence of glucose, causing swelling, releasing insulin. Adapted from ref 118. Copyright 2013 American Chemical Society. (c) Sensing using 2D photonic crystals. Readouts of sensors are based on colorimetric observations arising from increased or decreased spacings in the presence of an analyte. Adapted from ref 119. Copyright 2015 American Chemical Society. (d) In-situ gelation of thermal-responsive polymers and its utility in minimal-invasive applications for the eye. Adapted with permission under a Creative Commons CC BY-NC 3.0 license from ref 120. Copyright 2023 Royal Society of Chemistry.

technique may still be limited for clinical translation due to issues associated with the tissue penetration of light.

Magnetic resonance imaging (MRI) is a clinically relevant imaging modality, and it is relatively straightforward to use switchable polymers to control signal intensity by modulating their relaxation parameters. Hu, Liu, and co-workers prepared self-immolative polyurethane-polyethylene glycol block copolymers as dual $^{1}H/^{19}F$ MRI imaging agents.[107] The polyurethane monomer had a reactive vinyl ether that allowed for simple conjugation of either fluorinated side chains or gadolinium chelates. When fluorinated block copolymers were self-assembled into micelles, the restricted mobility and strong spin−spin coupling of fluorinated units led to broadening and suppression of the ^{19}F MRI signal. Self-immolation was triggered by reaction of ROS with a boronic ester end-cap and depolymerization through a 1,6-elimination mechanism that led to the formation of azaquinone methide (AQM) adducts of the monomers. Release of the fluorinated and highly reactive AQM units from the micelle core led to attachment to thiol-containing biomolecules and a significant increase in ^{19}F MRI signal. Attachment of the AQM adducts to macromolecular biomolecules also led to long-term retention of the imaging signal in vivo, which was demonstrated in ^{1}H MRI, using the gadolinium-functionalized polymers (Figure 6c). While self-immolation can clearly amplify the imaging signal, they can also be used to generate reactive intermediates in situ, for in situ labeling of biomolecules of interest, offering new opportunities for developing smart theranostics.

HYDROGELS

Hydrogels offer covalently or physically cross-linked hydrophilic networks that can be composed of >99% water. As a biomedical material, hydrogels tend to be favored due to their chemical (i.e., mostly water) and mechanical properties being similar to biological tissues. These gels provide a way of spatially defining the mechanical and chemical environment of encapsulated payloads such as drugs and cells. Furthermore, the chemical customizability of synthetic polymers and biomacromolecules not only allows well-defined interactions within the hydrogels but with the environment as well. By carefully selecting the chemical characteristics of the monomers and functional groups, it is possible to design hydrogels that are stimuli-responsive, undergoing changes in their physical or chemical properties in response to environmental changes. Such hydrogels have been used widely in applications including actuation and shape morphing, smart drug delivery, sensing, fabrication (i.e., *in situ* gelation), and cell culture.

Covalent Hydrogels. Shape-morphable hydrogels provide avenues toward the creation of actuators for soft robotics and medical devices. To create shape-morphable structures, 4D printing leverages stimuli-induced changes in hydrogel swellability. By spatially defining the swelling mismatch and structures, Magdassi, Long, and co-workers showed that these hydrogels can undergo complex and predetermined motions.[115] Using the thermoresponsive volumetric changes of PNIPAM incorporated within a hydrogel matrix, Spinks and co-workers demonstrated the potential of hydrogels to create dynamic and

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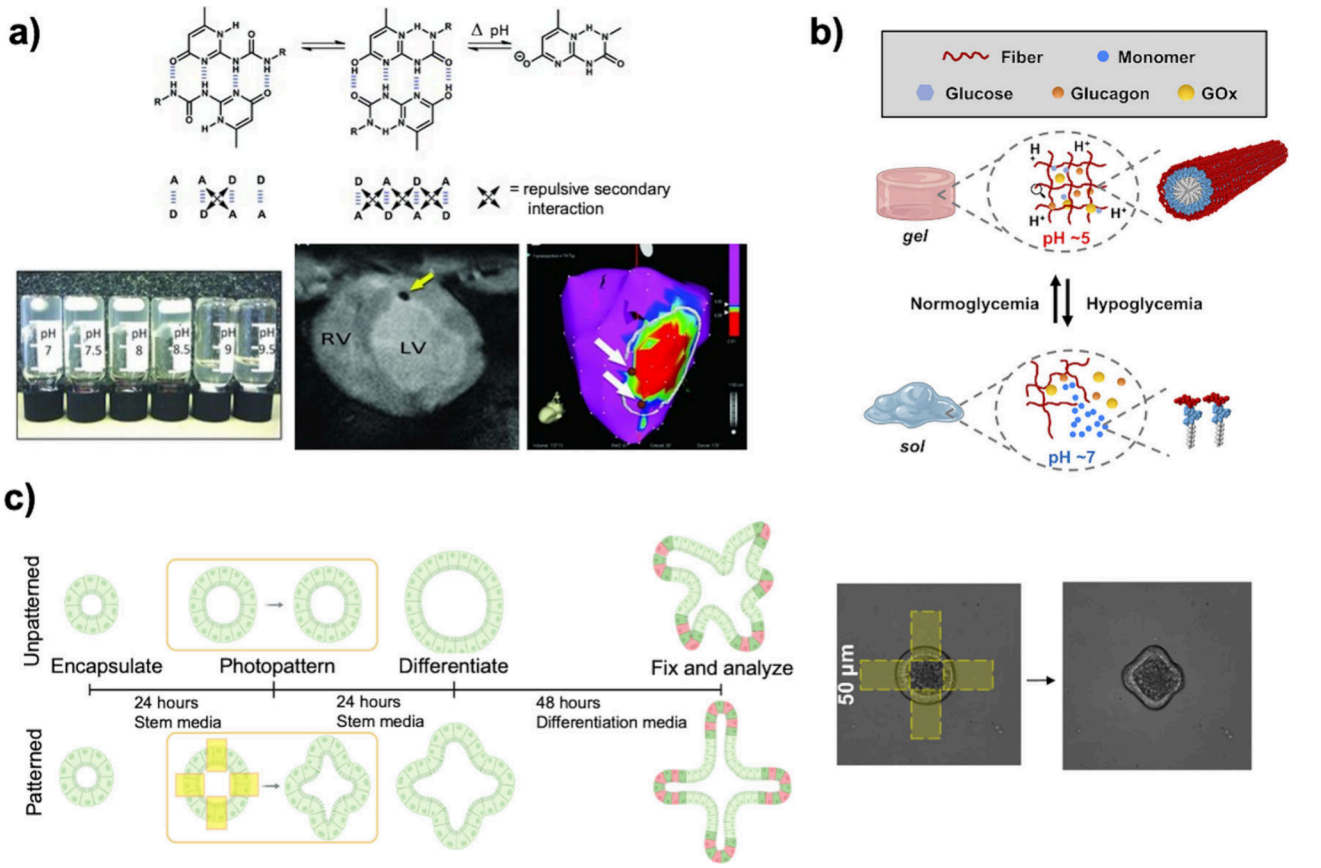

Figure 8. Stimuli-responsive dynamic hydrogels: (a) UPy-based supramolecular hydrogel that responds to pH enabling catheter delivery and in situ gelation with growth factors in a pig heart. Adapted with permission from ref 130. Copyright 2014 John Wiley and Sons. (b) Transient peptide hydrogel that releases glucagon in response to changes in glucose concentration through pH changes afforded by GOx. Adapted from ref 132. Copyright 2021 American Chemical Society. (c) Dynamic photoinduced exchange in an allyl sulfide cross-linked hydrogel directs formation in intestinal organoids. Adapted with permission under a Creative Commons CC BY 3.0 license from ref 133. Copyright 2023 American Association for the Advancement of Science.

mechanically robust smart valves.[116] Gracias and co-workers introduced a mechanism where biodegradable, thermally and magnetically dual-responsive, untethered grippers were made from a magnetic iron oxide-hydrogel composite (Figure 7a).[117] To achieve biodegradability and gripper actuation, the matrix was fabricated using photolithography with thermoresponsive high-swelling poly(oligoethylene glycol methyl ether methacrylate-bis(2-methacryloyl)oxyethyl disulfide) and a low-swelling poly(acrylamide-*N*,*N*′-bis(acyloyl)cystamine). Thermal stimuli actuated the grippers, while their magnetic properties allowed for their manipulation and positioning (e.g., lateral movements). This pioneering work marked the first of its kind, moving the use of biodegradable untethered microgrippers closer to practical application as a less invasive device for biomedical intervention.[117]

Stimuli-responsive hydrogels facilitate dynamic interactions with both their encapsulated payload and the surrounding environment, making them particularly suited for adaptive drug delivery and sensors. For example, Corrigan and co-workers showed that controlling the temperature-responsive swelling of PNIPAM hydrogels enabled pulsatile drug release.[121] Recent advances in glucose-responsive insulin delivery for diabetic patients have used the synergy between GOx and pH-responsive hydrogels (Figure 7b). Anderson and co-workers immobilized GOx in a pH-responsive cationic gel loaded with insulin.[118] Under hyperglycemic conditions, gluconic acid was produced enzymatically by GOx, leading to protonation and swelling of the pH-responsive cationic microgels. Under normoglycaemic conditions, the swelling reversed, and the microgel shrank, halting insulin release. By optimizing the formulation, these microgels could serve as a self-regulating glucose release system.

The chemical versatility of hydrogels also serves as a powerful toolkit for designing biosensors for point-of-care testing. The three-dimensional matrix increases surface area, immobilizes recognition motifs such as enzymes and aptamers, and provides a native and stabilizing aqueous environment for detection motifs and analytes. Asher and co-workers made use of the properties of a hydrogel-embedded 2D photonic crystal to transduce stimuli-responsive volumetric changes in the hydrogels into colorimetric changes (Figure 7c).[119] To achieve analyte-specificity for Pb^{2+}, they incorporated crown ether 4-acryloylamidobenzo-18-crown-6 into an acrylamide hydrogel. In the presence of bound Pb^{2+}, a change in Donnan potential within the hydrogel caused swelling and a corresponding red-shift in the photonic spectrum. With a similar mechanism, by incorporating phenylboronic acid, the hydrogel shrank in the presence of glucose due to the enhanced cross-linking caused by interactions between two phenylboronic acids and one glucose molecule, leading to a red to blue color change.[119] Taking advantage of signal transduction (e.g., through enzymatic cascades, and embedded photonic crystals), stimuli-responsive hydrogels can serve as a versatile, chemically tailorable, and

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modular platform to achieve analyte-specific drug delivery (e.g., glucose-triggered) and sensing (e.g., Pb^{2+} triggered).[119] Furthermore, the broadly applicable approach of molecular imprinting and availability of recognition molecules such as aptamers and biomolecules hold promise for expanding the range of utility in developing hydrogel-based sensors.[122]

Hydrogels Based on Noncovalent Interactions or Dynamic Covalent Bonds. While traditional covalently cross-linked hydrogels are damaged after passage through a narrow-gauge needle, injectable hydrogels do not suffer from the same issue. In situ forming implants offer a minimally invasive option for administering drug depots and tissue engineering scaffolds. Using the sol−gel transitions in stimuli-responsive polymers, body temperature as well as physiological pH and ions can be used to induce the formation of hydrogels postinjection (Figure 7d).[123] Lingam, Loh, Su, and co-workers reported injectable thermoresponsive hydrogels (thermogels), which gelled upon being warmed to 37 °C, as vitreous endotamponades, thereby applying positive pressure through the exertion of a swelling counter-force on the retina to assist in the reapposition of the detached retina.[124] Additionally, a polyurethane thermogel facilitated the formation of a substance that resembled the natural vitreous. Relying on the formation of thermally induced micellar cross-links, these thermogels have also been widely used as depots for sustained drug delivery.[120,125−127] In the promising work by Su, Loh, and co-workers, the sustained release of the anti-VEGF drug, aflibercept, for up to two months was demonstrated.[127] Such drug depots could vastly improve patient compliance by minimizing the uncomfortable, yet necessary, intravitreal injections for patients with posterior eye segment proliferative diseases such as age-related macular disease and diabetic retinopathy.

Beyond thermoresponsive or ionic hydrogels, dynamic hydrogels based on noncovalent or dynamic covalent bonds are widely applied in the biomedical field due to their inherent stimuli-responsive character. One strategy to achieve these soft materials involves a supramolecular approach, incorporating specific and reversible noncovalent interactions (e.g., hydrogen bonds, $\pi-\pi$, hydrophobic interactions) into molecular recognition units on covalent polymers for cross-linking, or into small molecule monomers that self-assemble to yield fibrillar structures, with both forming gel phase materials above a critical concentration.[128] Another approach to access dynamic hydrogels involves the use of stronger dynamic covalent bonds (e.g., boronates, disulfides, hydrazones) to cross-link covalent polymers.[129]

Even when polymers with LCST properties or polyelectrolytes are not used, temperature and pH of the biological environment are still commonly relied on in dynamic hydrogels to trigger structure and property changes through the bonds between the (macro)molecular units. These changes can include self-assembly, remodeling, and/or degradation of the hydrogels based on the chemistry of the material components. Hydrogen bonds are often included in these supramolecular designs, and when heterocyclic compounds are used that are susceptible to tautomerism, disassembly and reassembly of the materials can be achieved on-command by environmental changes. For example, Dankers and co-workers showed that raising the pH of a supramolecular ureidopyrimidinone (UPy)-based hydrogel to pH 9 resulted in a dramatic reduction in viscosity due to formation of the enolate tautomer (Figure 8a).[130] This rheological change permitted its flow through a one meter long catheter to an infarcted pig heart, where the physiological pH of the tissue restored the keto-form of the tautomer and resulted in a supramolecular hydrogel laden with growth factors at the injection site. Such dynamic hydrogels can also impart adaptive and viscoelastic mechanical character as encountered in native tissues for 3D cell culture. Lutolf and co-workers demonstrated that stress-relaxing hydrogel-containing noncovalent cytosine cross-links can accommodate the growth and morphogenesis of mouse intestinal stem-cell organoid cultures that exert mechanical forces on their matrices, leading to increased symmetry-breaking and Paneth cell formation during crypt budding.[131]

Beyond using dynamic bonds to assemble the hydrogels, orthogonal chemistries can be engineered into the molecules to respond to small molecules or enzymes associated with a disease condition. Numerous examples have shown that enzymatically cleavable or modifiable peptide sequences can be easily introduced at specific locations on self-assembling peptides through solid-phase synthesis methods to form dynamic hydrogels that can release therapeutics or assemble in response to enzymes. For example, Cui and co-workers demonstrated that insertion of a MMP-2 cleavable peptide sequence into the hydrophilic domain of a peptide-based bolaamphiphile promoted enzyme accessibility and permitted controlled chemotherapeutic release from hydrogels by an MDA-MB-231 breast cancer cell line.[134] Additionally, small molecules abundantly present in the intracellular environment, such as GSH or cysteine, or ROS in diseased cells or tissues can also be advantageously used to degrade or form peptide-based self-assemblies using their redox responsiveness to deploy biologically relevant molecules or therapeutics. Through consecutive reactions on a boronic acid-functionalized short peptide, as shown by Weil and co-workers, H_2O_2 in the cytosol of A549 cancer cells led to deprotection, triggering self-assembly of supramolecular fibers and programmed cell death.[135]

Most often, as in the above examples, the peptides respond through a one-way output to a specific stimulus, either through formation or degradation of their self-assembled structures. In contrast, if the peptide structures enable dissipative self-assembly, where a continuous input of a chemical fuel source is required to sustain an out-of-equilibrium state, transient hydrogels can be made that respond to environmental changes of a particular stimulus. Webber and co-workers showed that alterations in glucose concentration modulated peptide self-assembly and released glucagon in a prophylactic manner prior to an insulin overdose in a diabetic mouse model, reducing the extent and duration of hypoglycemia in vivo (Figure 8b).[132] Incorporation of GOx in the hydrogels yielded a pH stimulus that stabilized the assemblies, and when limited glucose was present, physiological buffering resulted in disassembly and release of glucagon.

Light-responsive chemistries offer powerful levers to spatiotemporally control the properties of dynamic hydrogels at several length scales for advanced 3D cell culture applications. Photocages such as 2-nitrobenzyl moieties installed on self-assembling peptides, as demonstrated by Stupp and co-workers, can be used to control their supramolecular polymerization into fibers by photoirradiation, eventually leading to a sol−gel transition with increased focal adhesion formation by NIH 3T3 cells.[136] To further augment the mechanical properties of dynamic hydrogels, that are typically weak, light-responsive chemistries can be introduced to enable cross-linking between chains. The tagging of a squaramide-based monomer with 1,2-

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dithiolane by Kieltyka and co-workers enhanced the mechanics and bioactivity of the fibrous supramolecular hydrogels in space and time post-UV light irradiation displaying dramatic differences in Hs57T cancer cell migration in 3D culture.[137] Because photo-cross-linking strategies are often fast (on the order of seconds) and efficient, they are relied on in various microfabrication methods (e.g., microfluidics, 3D printing) to set polymer shape. Combining favorable shear-thinning properties of supramolecular benzene tricarboxamide fibrous hydrogels and covalent PEG polymers by Baker and co-workers resulted in mechanically tough and printable hydrogels with ATDC5 chondrocytes.[138] While cross-linking is commonly sought after in dynamic hydrogels to increase stability and stiffness, there is growing recognition that various cell behaviors such as spreading, differentiation, and morphogenesis are regulated by the material viscoelasticity. The photopatterning of dynamic photoinduced exchange in reactions in hydrogels cross-linked with allyl sulfides by Anseth and co-workers enabled user-defined, locally tunable viscoelasticities that directed crypt formation of intestinal organoids (Figure 8c).[133]

Overall, the integration of stimuli-responsive properties enables hydrogels and polymers to respond dynamically to their environment, allowing for actuation, self-regulation, and in situ formation. These advancements not only demonstrate adaptability and efficacy but also the potential of stimuli-responsive hydrogels in advancing patient care through innovative, less invasive, and more effective medical interventions. The increasing number of stimuli-responsive functions that can be achieved within dynamic hydrogels and a greater understanding of macromolecular designs needed to reach them provides a rich palette to start engineering biomimetic materials tailored for any biological question. As the field moves forward, the continued inspiration by nature and a greater understanding of biology and its dynamics at several length scales will undoubtably lead to dynamic hydrogels with increasingly complex functions that operate under spatial and temporal control, showcasing the full range of what can be possible starting from simple macromolecular building blocks.

CONCLUSIONS AND FUTURE PERSPECTIVES

Tremendous progress is being made in the development of stimuli-responsive polymeric systems for biomedical applications. For example, while switchable polymers for drug and nucleic acid delivery are relatively more developed compared to other areas, new understandings of the role of molecular structure, molecular architecture, and nanoparticle morphology continue to be achieved. In addition, new materials such as modified polysaccharides and technologies such as PISA are increasingly being incorporated into the field. With accompanying advancements in molecular and diagnostic imaging, the field of theranostics is thriving, offering the potential to diagnose and visualize the delivery of the therapeutic with a single agent. While the above systems tend to exist in solution at the molecular or nanoscale, stimuli-responsive hydrogels have shown exciting capabilities in the growing field of soft robotics, as their properties can mimic aspects of soft tissues, while their stimuli-responsiveness can be exploited for functions such as sensing, in situ gelation upon injection, and directing the growth of cells in 3D culture.

While we highlighted synthetic advancements in the context of polysaccharides, the development of simple and efficient routes to the target polymers will be critical across the whole spectrum of polymer systems described here. Many stimuli-responsive systems are relatively complex, incorporating multiple different elements including cargo, responsive functional groups, linkers, and targeting moieties. The statistical incorporation of these different elements through polymerization can lead to a range of different structures, each of which has different biological properties, which can be problematic. Therefore, research is still needed toward strategies for well-defined polymer synthesis and functionalization. In this regard, the growing power of computational approaches and artificial intelligence to predict the properties and function of different structures should allow potential polymer systems to be prioritized or for existing systems to be more readily adapted for different drugs and applications. Such approaches could be accompanied by self-driving lab technologies to optimize syntheses and prepare well-defined arrays of structures for testing.

In the quest for complex multifunctional polymers, the use of synthetic routes that incorporate the principles of green chemistry should be prioritized. Recently, concerns have been raised regarding the high levels of industrial waste associated with the large scale synthesis of new peptide drugs such as semaglutide and tirzepatide.[139] Polymerization has the advantage of generally being more efficient and scalable than solid-phase peptide synthesis. In addition, given the growing array of starting monomers available from renewable and sustainable starting materials, it should be possible to design new stimuli-responsive systems from such starting materials and to adapt existing approaches to such starting materials.

Switchable polymers can be engineered to deliver a wide range of cargo. While early demonstrations focused mostly on small molecules, a growing number of new therapeutics are biomolecules such as peptides, proteins, and nucleic acids. These molecules often require delivery systems to protect them from degradation and assist them in crossing cell membranes. Furthermore, their high cost incentivizes the use of systems that can enhance their efficiency. It is likely that the next decade will see a growing focus on the development of delivery systems for these therapeutics. However, to achieve efficient delivery, more research is needed to better understand the complex process and role of polymer structure in cell uptake, endosomal escape, intracellular trafficking, and drug release, which are particularly important for these cargos. Increased use of technologies such as organ on a chip[140] would also be valuable to assess biological properties early in the process. Such an approach could enhance translation to the clinic.

Finally, with the growing interest in wearable devices and biosensors as well as regenerative medicine, we anticipate a growing role for stimuli-responsive hydrogels. Through their combination with 3D printing and other state-of-the-art microfabrication approaches, such systems have the potential to perform increasingly complex tasks, such as 3D movement, as well as sensing and responding to their surrounding environment. As stimuli-responsive hydrogels become more advanced in their functions, they increasingly mimic natural biological systems, showing immense promise for therapeutic and regenerative functions in vivo.

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Notes

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