

Polymeric Biomaterials with Engineered Degradation

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ABSTRACT: Polymeric biomaterials are widely used as carriers for cells and therapeutic agents. Until recently, most research has been limited to a relatively narrow number of monomers and chemistries. A fundamental challenge in developing clinically relevant polymeric biomaterials is to independently control their chemical and physical properties across multiple scales in both time and space. Control over a biomaterial's chemical and physical properties is critical to recapitulate the complex cascades of signals and complex microenvironments found in nature. Typically, dynamically responsive biomaterials either degrade in the presence of a stimulus (i.e., water induces hydrolysis) or experience a change in solubility on application of the

stimulus (i.e., temperature induces gelation via lower critical solution temperature LCST). This highlight discusses recent advances in stimulated and controlled degradation of polymeric biomaterials. The goal of this review is to provide a broad overview of physiologically relevant stimuli used to control the degradation of a wide range of polymeric biomaterials with varying architectures and physical forms. © 2013 Wiley Periodicals, Inc. *J. Polym. Sci., Part A: Polym. Chem.* **2013**, *51*, 3531–3566

KEYWORDS: enzymolysis; hydrolysis; oxidation; photolysis; polymeric biomaterials; stimuli-sensitive polymers; degradation; bioengineering

INTRODUCTION Over the past few decades, polymeric biomaterials have revolutionized the medical field and have been used in a wide variety of applications such as controlled drug delivery systems, cell culture supports, scaffolds for tissue engineering, biosensors, actuators, bioseparation devices, reactive coatings, medical imaging, and medical devices. Historically, most research has focused on testing the biocompatibility of polymers developed for other applications and developing or applying new processing techniques to fulfill specific design criteria (i.e., porosity), limiting the number of monomers and chemistries explored. More recently, researchers have shifted toward synthesizing materials specifically for biomedical uses. Many methods for chemically modifying naturally occurring polymers have been reported. Synthetic proteins, glycopolymers, and other biomimetics have been described. Many sophisticated synthetic polymeric and supramolecular systems have also emerged. Through these approaches, polymer chemists have created a niche for designed biomaterials.

As our understanding of developmental biology, wound healing, and disease increases, so must the sophistication of our approach for new polymeric biomaterials. If we look to nature for clues on how to design materials to carry cells and deliver signals, we observe that a finely tuned cascade of mechanical and chemical signals is required to produce and maintain healthy tissue. A fundamental limitation of

many polymeric biomaterials is the lack independent control over their physical and chemical properties across multiple scales in both time and space. Control over both the chemical and physical properties of a biomaterial is critical to recapture the complex cascades of signals and complex microenvironments found in nature. Without sophisticated and well-characterized biomaterials, researchers are extremely limited in the questions they can ask and answer in tissue development and regeneration, or in the control they have over the targeting and release of therapeutic agents.

The most important requirements for biomaterials are biocompatibility and controlled degradability. Biocompatibility prevents toxic effects of materials on the physiological system. Synthetic biomaterials are not typically rejected by the body, although solid scaffolds or devices may be encapsulated in a fibrous capsule after implantation, and soluble polymers or small particles undergo various routes of elimination, sometimes including chemical breakdown. While the biocompatibility is an intrinsic property of the polymer (related to chemical structure), degradation can be directly engineered, chemically tuned, and controlled by various stimuli. Indeed, degradation of polymeric biomaterials can be initiated by an environmental, internal or external stimulus (Fig. 1). For example, hydrolysis is a commonly used controlled degradation mechanism, and the stimulus for

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Andrea M. Kasko graduated from the University of Michigan with a B.S. in Chemistry, Case Western Reserve University with an M.S.E. in Macromolecular Science Engineering (working with Professor Virgil Percec), and the University of Akron with a PhD in Polymer Science (working with Professor Coleen Pugh). After two years of post-doctoral research sponsored by the Howard Hughes Medical Institute working with Professor Kristi Anseth, Andrea joined the Bioengineering Department at UCLA. The overall theme of her research group is the synthesis of new polymeric biomaterials. In recognition of the creative nature of her work, Andrea received the NIH Director's New Innovator Award in 2011.



degradation is environmental (water). Enzymolysis is another popular method that relies on an internal stimulus (enzyme production) to control degradation. For environmental and internal stimuli, the degradation is pre-engineered and cannot be controlled or arrested once the construct is introduced into the body. In contrast to internal triggers, externally controlled stimuli allow user-dictated, on-demand degradation simply by switching the trigger on or off.

There are multiple reasons why biomaterials with controlled degradation are attractive. One of the foremost motivations for designing degradable biomaterials is to allow their safe and non-invasive elimination from the body. If a material naturally degrades within the body into excretable by-products, surgical removal is not required, nor does the material have continued activity after it is no longer therapeutically useful. For example, the persistent presence of non-resorbable rigid implant to repair damaged bone might induce "stress shielding" while a degradable implant would not. In addition to allowing biomaterial elimination from the body, degradation can also be utilized to spatially and/or temporally control the release of therapeutics, imaging agents, proteins, genes or cells, recapitulating natural signaling cascades or biological processes, and reducing side effects. Because there are many variations in patients, pathological conditions and physiological targets, degradation

profiles may need to be uniquely defined for each application. For example, drug release may require rapid degradation of the polymer within few hours, while scaffold degradation may be desirable over several months as new tissue evolves. Degradation can be varied in polymeric biomaterials by controlling the trigger that stimulates the process and the mechanism by which it occurs. Additionally, the architecture and location of the degradable linkage in the polymeric materials can also be varied (Fig. 2). Cleavage of the backbone results in lower molecular weight fragments that generally have increased solubility. In self-immolating systems, a single cleavage event of an end-cap can trigger the entire chain to degrade into small molecules. Side chains can also be cleaved, releasing a small-molecule by-product. Upon cleavage, the solubility of the polymer may also drastically change. In polymer networks, crosslinks can be cleaved, resulting in lower molecular weight polymeric fragments. These approaches can also be combined; for example, crosslinks and backbone chains can be cleaved in polymer networks, or side chains and backbones, or side chains and crosslinks.

Besides the complexity of designing engineered degradable polymeric devices, the researchers are limited by the toxicity of the degraded by-products and have to ensure that the degraded material is still biocompatible until it is completely cleared from the body.

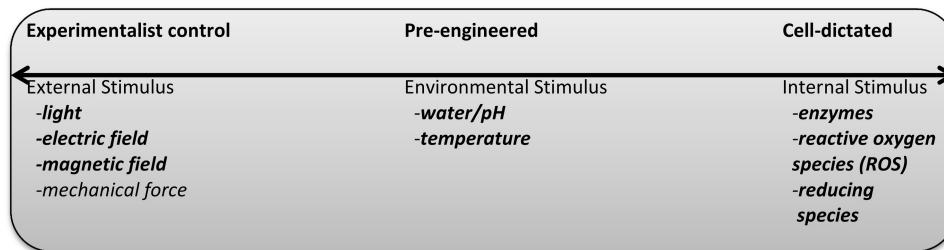


FIGURE 1 Triggers of controlled degradation in polymeric biomaterials.

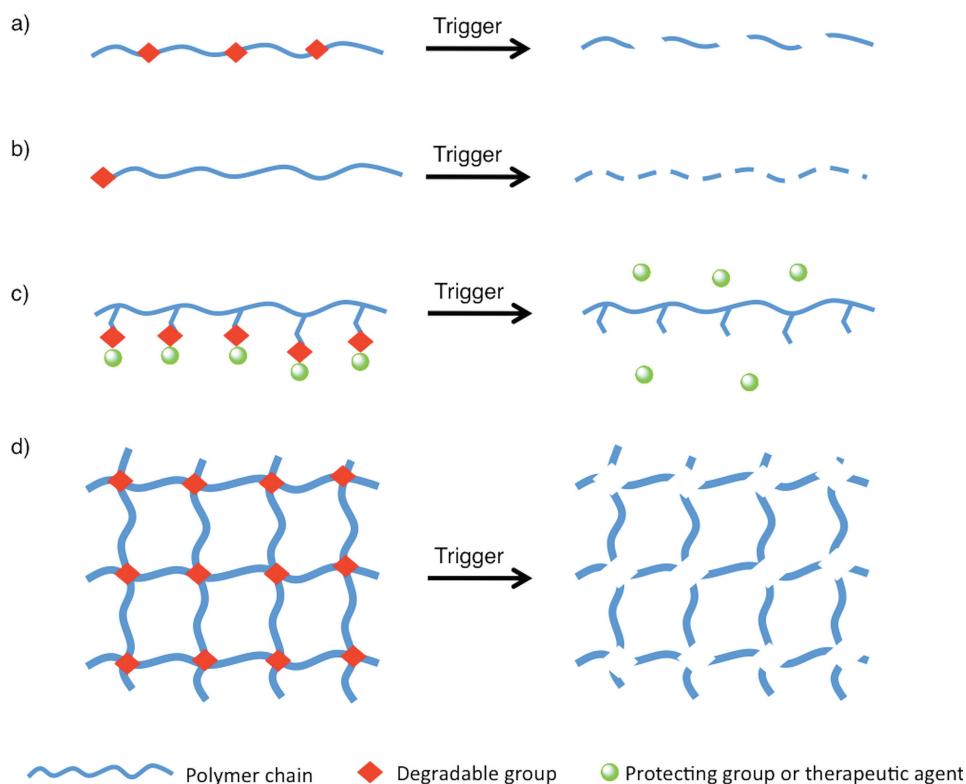


FIGURE 2 Different routes of molecular breakdown depend on the placement of the stimuli-sensitive moiety: (a) backbone cleavage, (b) self-immolation, (c) side-chain cleavage, (d) crosslink cleavage.

The present review aims to highlight recent advances in stimulated and controlled degradation of polymeric biomaterials. Although a large number of reviews have been published on “smart” stimuli-sensitive biomaterials, these reviews tend to cover a single trigger or a specific class of materials. The goal of this review is to give a broad overview of physiologically relevant stimuli used to control the degradation of a wide range of materials with varying architectures and physical forms. First, we present a brief section to describe the different polymer architectures and forms encountered in biomedical applications. Then, we will discuss recent advances in environmental, external and internal stimuli for controlling degradation of polymeric biomaterials. Special attention will be paid to the chemical structures of the polymers and the responses generated by each trigger, as both the polymer and its degradation products must be considered in biomedical applications. The current limitations of these approaches are noted, and future challenges in biomedical applications are indicated.

POLYMER ARCHITECTURES

Various polymer architectures and assemblies have been designed as biomedical materials including micelles, polymersomes, nanoparticles, dendrimers, capsules, and hydrogels (Fig. 3). Since many examples of these architectures will be mentioned in the course of this review, it is worthwhile to first highlight their features.

Polymeric micelles are colloidal particles usually spherical with a size within a range of 10–100 nm. They are based on amphiphilic block copolymers that self-assemble in aqueous solution. Their hydrophobic core allows the encapsulation and protection of hydrophobic molecules that makes the micelles useful as drug carriers. Furthermore, the core is stabilized by a hydrophilic shell that prevents the micelles from the uptake by the reticuloendothelial system (RES) and increases their blood circulation time.¹ The most commonly used hydrophilic corona is poly(ethylene glycol) (PEG) because of its hydrophilicity, biocompatibility, low toxicity, and “stealth” (reduced protein interactions) behavior.

When amphiphilic block copolymers form vesicles, they are called polymersomes, in analogy to liposomes. Polymersomes comprise an aqueous core protected from the aqueous environment by a hydrophobic membrane. Contrary to micelles, they are capable of encapsulating both hydrophilic (in the core) and hydrophobic (into the membrane) molecules.² The size of polymersomes can be tuned ranging from 10 nm to 10 μm.

Polymeric nanoparticles refer to solid colloidal particles ranging in size from 1 to 200 nm for therapeutic applications. They can be prepared by a variety of methods including solvent evaporation, nanoprecipitation, and emulsion. They can be used as drug vehicles by entrapping or covalently attaching various therapeutics.

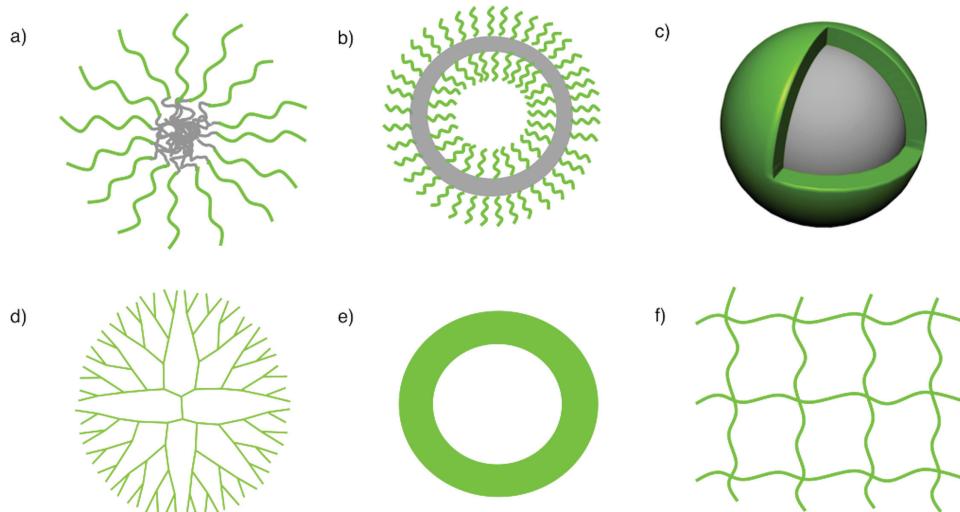


FIGURE 3 Polymer architectures for biomedical applications: (a) micelle, (b) polymersome, (c) solid nanoparticle, (d) dendrimer, (e) capsule, and (f) hydrogel.

The dendrimer term was first introduced by Tomalia in 1985 and originates from “dendron” meaning tree in Greek.³ Indeed, the dendrimers are highly branched polymer structures. Dendrimers have attracted considerable attention over the past decades because of their unique properties including high degree of branching, structural uniformity, multivalency, well-defined architecture, and variable chemical composition. Dendrimers are composed of three distinct parts: (1) a focal core allowing for the encapsulation of active substances, (2) inner blocks with several layers providing a flexible space capable of entrapping small molecules, and (3) a multivalent surface. Two main defined methods have been proposed to prepare dendrimers: divergent and convergent synthesis.⁴ The former strategy is a stepwise, generation-by-generation approach, which assembles the molecule from a multifunctional core toward the periphery. The latter begins ultimately at the surface and works inward. The most common used dendrimers for biological applications are poly(amidoamine) (PAMAM) and poly(propyleneimine) (PPI).

Polymeric capsules have a vesicular structure with a hollow core and a polymeric shell composed of amphiphilic polymers. These capsules can be fabricated by a multitude of different methods, such as layer-by-layer assembly (LbL), emulsion, polymer precipitation by phase separation, and surface-initiated polymerization.⁵ Their main application is exactly what their name implies, the encapsulation of different active substances.

Hydrogels are water-swollen three-dimensional networks made of crosslinked hydrophilic polymeric chains. Hydrogels result either from physical interactions (ionic or hydrogen bonds) or chemical covalent bonds. Hydrogels can be made from both natural (collagen, alginate, chitosan, dextran, gelatin, hyaluronic acid (HA), etc.) and synthetic (PEG, poly(*N*-isopropyl acrylamide), poly(methyl methacrylate) etc.)

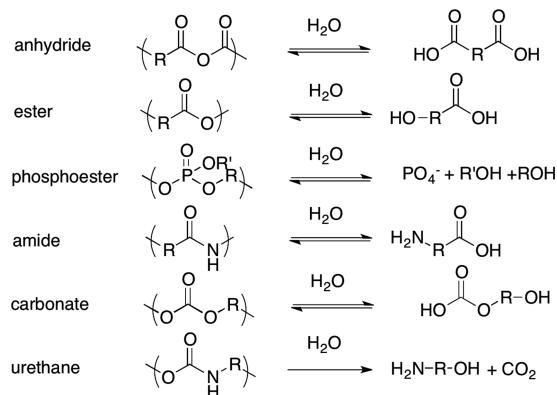
polymers. Hydrogels are attractive biomaterials due to their high water content and moderate mechanical properties, both of which are similar to native tissue. Their chemical and physical properties are also highly tunable, making them desirable candidates to mimic native extracellular matrix (ECM) for tissue regeneration. Hydrogels are also of great interest for encapsulating drugs, proteins, and cells.⁶

ENVIRONMENTAL STIMULI FOR BIOMATERIAL MODULATION

Environmentally responsive biomaterials respond to a specific environmental trigger that stimulates a physical or chemical change in the material. Triggers include water (including pH-specific applications), temperature, and ionic concentration. While water and pH are able to induce bond-breaking reactions under pathological or physiological conditions, ion concentration and temperature are only able to induce changes in solubility. These triggers can cause disassembly of a polymeric biomaterial but not molecular degradation. Changes in ion concentration have been proposed for controlling biomaterial properties but are not routinely used.

Hydrolysis

The most widely used environmental trigger is water, as the human body is roughly 60% water. In hydrolysis, a molecule of water adds to the polymer backbone, causing chain scission. Hydrolytically degradable sutures were first reported in 1967,⁷ and since then hydrolysis has been a popular mechanism for engineering degradable implants and controlled release materials. Several classes of hydrolytically degradable polymers have been reported, including polyanhydrides, polyesters, polyurethanes, polycarbonates, polyphosphoesters, and polyamides (Scheme 1), although not all classes are hydrolytically degradable on a relevant time scale at physiological conditions (neutral pH, 37 °C, no enzyme, or other catalyst). In general, researchers typically use anhydride or ester linkages in designing hydrolytically degradable



SCHEME 1 Hydrolytically degradable linkages used in biomaterials.

materials. Since these systems have been extensively reviewed,^{8,9} we will highlight more recent advances in the field.

Hydrolytic Materials Degradation

Hydrolytically degradable polymeric scaffolds and implants have been used for many decades to support cell and tissue growth. Ideally, the scaffold should degrade at a rate comparable to the production of new extracellular matrix (ECM) by the cells. The rate of new ECM production (and the subsequent lack of need of the synthetic scaffold for support) varies with different tissue types, states of injury or disease, and individual patient factors such as age and overall health. Therefore, a broad range degradation time scales are needed to address many different systems.

Polyesters. The most commonly used linkage in hydrolytically degradable scaffolds is an ester bond. Ester bonds typically degrade over the course of weeks to months in physiological systems, an appropriate time scale for neotissue evolution. Many reports exist for polyester based materials, including both solid polymeric materials as well as hydrogels incorporating these linkages. In general, polyesters are formed either by condensation polymerization of carboxylic acids and alcohols, or by ring-opening polymerizations

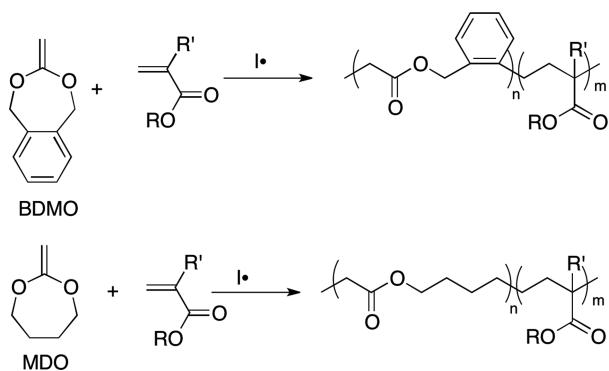
of cyclic lactones. In both cases, the polymeric materials may eventually break down completely into monomers, or, depending on the extent of degradation, into oligomeric chains. Notably, many of the monomers are naturally occurring metabolic by-products (i.e., lactic acid).

Several groups have reported radical ring-opening copolymerization of cyclic ketene acetal monomers such as 5,6-benzo-2-methylene-1,3-dioxepane (BMDO)¹⁰ and 2-methylene-1,3-dioxepane (MDO)¹¹ with traditional vinyl monomers such as acrylate and methacrylate, resulting in the formation of an ester linkage in the backbone (Scheme 2).^{12–16}

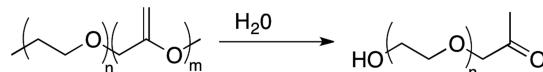
This approach is unique from the traditional polyesters, as the frequency of ester linkages can be controlled by reaction composition and reactivity ratios of the monomers. Furthermore, the polymeric materials do not degrade into monomers, but rather into shorter polymeric chains. In addition to acrylates and methacrylates, MDO monomers have been copolymerized with vinyl acetate, vinyl phosphinic acid, *N*-isopropylacrylamide, and fluoroalkanes.

Polyethers. PEG is a hydrophilic polymer commonly used in biomedical applications. Since the 1970s, PEG has been conjugated to therapeutic proteins and peptides in order to increase their circulation half-life, decrease their toxicity, and increase their aqueous solubility.¹⁷ PEG is also used to passivate surfaces of biomaterials, and, when crosslinked, as a hydrogel for culturing cells and delivering therapeutics. Although PEG is generally considered to be a hydrolytically stable polymer, Lundberg et al. demonstrated that hydrolytically degradable ether linkages could be introduced into the polymer backbone.¹⁸ In this report, epoxide is copolymerized with epichlorohydrin. Upon treatment with potassium *tert*-butoxide, the epichlorohydrin repeat units undergo elimination, resulting in degradable methylene ethylene oxide repeat units (Scheme 3). The heterobifunctional α -hydroxy- ω -keto-poly(ethylene oxide) is formed upon hydrolysis of the methylene ethylene oxide repeat units (Scheme 3). The hydrolysis is rapid under acidic conditions ($\text{pH} = 5$, $n = 91$, $m = 9$, 37°C), where complete degradation occurred within 24 h, but significantly slower at $\text{pH} 7.4$, where degradation of the same sample was not complete after 72 h.

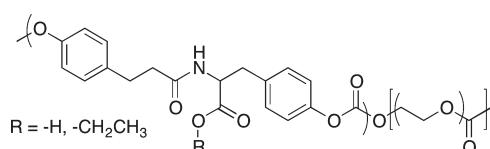
Polycarbonates. While polycarbonates as a class are often used as hydrolytically stable systems, the Kohn group reported a degradable polycarbonate derived from the amino acid tyrosine.^{19,20} Homopolymer poly(desaminotyrosyl-tyrosine)s degrade hydrolytically, but the degradation rate is too slow for most biomedical applications. Copolymerization



SCHEME 2 Radical copolymerization of BMDO and MDO with (meth)acrylate monomers.



SCHEME 3 Hydrolysis of poly(ethylene oxide-co-methylene ethylene oxide) yields α -hydroxy- ω -keto-poly(ethylene oxide).



SCHEME 4 Hydrolytically degradable copolymers of desaminotyrosyl-tyrosine, desaminotyrosyl-tyrosine ethyl ester, and poly(ethylene oxide).

with PEG increases the rate of backbone degradation, presumably due to increased water uptake.^{21,22} More recently, Magno et al. reported that varying the ratio of desaminotyrosyl-tyrosine ($R=-H$) and desaminotyrosyl-tyrosine ethyl ester ($R=-CH_2CH_3$) allows further tuning of the degradation rate (Scheme 4).²³ Notably, these materials exhibit low cytotoxicity and cause little inflammation.

Hydrolytic Drug Release

Hydrolytic degradation of polymeric materials has been used for decades to release therapeutic agents that are either physically entrapped within a polymeric matrix, or covalently bound to the polymer chain through a degradable linkage. For example, therapeutic agents such as dexamethasone²⁴ or statins²⁵ have been tethered into hydrogels through degradable lactide linkages; their sustained release induces the differentiation of mesenchymal stem cells (MSCs) into osteoblasts. If sustained release over several weeks or more is desired, ester linkages are typically used. For faster release, anhydride linkages have been employed. The literature is rife with examples of each of these chemistries, and commercial products exist (i.e., Gliadel®, LupronDepot®). In this review, we wish to highlight a more modern approach, namely, forming degradable polymeric biomaterials using drugs as monomers (polymeric pro-drugs).

The Uhrich group has reported several different polymeric pro-drugs in which the small molecule drugs are connected through anhydride linkages. In the first report, salicylic acid (active form of aspirin) was co-polymerized with sebacic acid to yield a polyanhydride.^{26,27} Upon hydrolysis, salicylic acid and sebacic acid are released (Scheme 5). In addition to aspirin, other bioactive small molecules have been incorporated in the anhydride-based polymeric pro-drugs, including antibiotics,²⁸ antiseptics,²⁹ antioxidants,³⁰ and opioids.³¹

pH Responsive Materials

Although many polymeric biomaterials are hydrolytically degradable, the time scale of degradation at neutral pH may

TABLE 1 pH in Various Biological Tissues³²

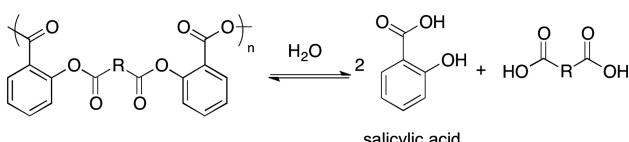
Tissues and Cell Compartments	pH
Blood	7.4
Tumor extracellular environment	6.5–7.2
Early endosome	6.0–6.5
Late endosome	5.0–6.0
Lysosome	4.5–5.0
Colon	7.0–7.5
Intestine	5–8
Stomach	1–3

be too long for specific applications. Since the pH in the body is not constant, but rather varies with location and disease state (Table 1), pH can be exploited as a specific trigger for biomaterials degradation. This may be desirable for controlling the site of drug delivery; for example, a drug carrier may be stable for long periods of time in the blood where the pH is close to neutral, but may degrade rapidly in a tumoral environment.

pH-Sensitive polymers can respond to changes in pH in one of two ways. Polymers can either undergo acid or base catalyzed hydrolysis, in which covalent bonds in the polymeric material are broken. Alternatively, variation in pH can result in a change in the overall charge (ionization) of a polymeric biomaterial, altering its solubility. In this case, the material does not degrade at the molecular level, but physical changes, such as swelling or dissolution, may occur. Here, we will restrict our discussion to catalyzed hydrolysis since molecular degradation occurs.

Various chemical linkages are pH-sensitive including acetal/ketal, orthoester, hydrazone, imine, *cis*-aconityl, and carbamate bonds. The choice of their integration into the polymer structure will depend on the pH of the biological target.

One major area of research for pH responsive materials is the controlled release of a drug at a specific location. Controlled and targeted release is desirable as it may reduce side effects that occur with systemic administration by limiting release of the drug to the diseased tissue. Once the carrier has delivered the therapeutic cargo, its pH-dependent degradation allows for its elimination from the body. A variety of pH-sensitive polymeric architectures and self-assembled structures have been designed including polymer-drug conjugates, micelles [Fig. 4(a)], polyplexes [Fig. 4(b)], polymersomes, dendrimers, and hydrogels. The nature of the acid-labile linker as well as its location in the polymer structure will influence the extent of molecular degradation. Incorporation of the linker into the backbone of the polymer will result in chemical degradation into small residues, whereas the introduction of the linker between the drug and the polymer will result in the drug release without further degradation of the polymer. Since this field has been recently reviewed,³³ we will focus on the most relevant examples



SCHEME 5 Poly(salicylic acid-*co*-sebacic-acid) as a hydrolytically degradable polymeric pro-drug.

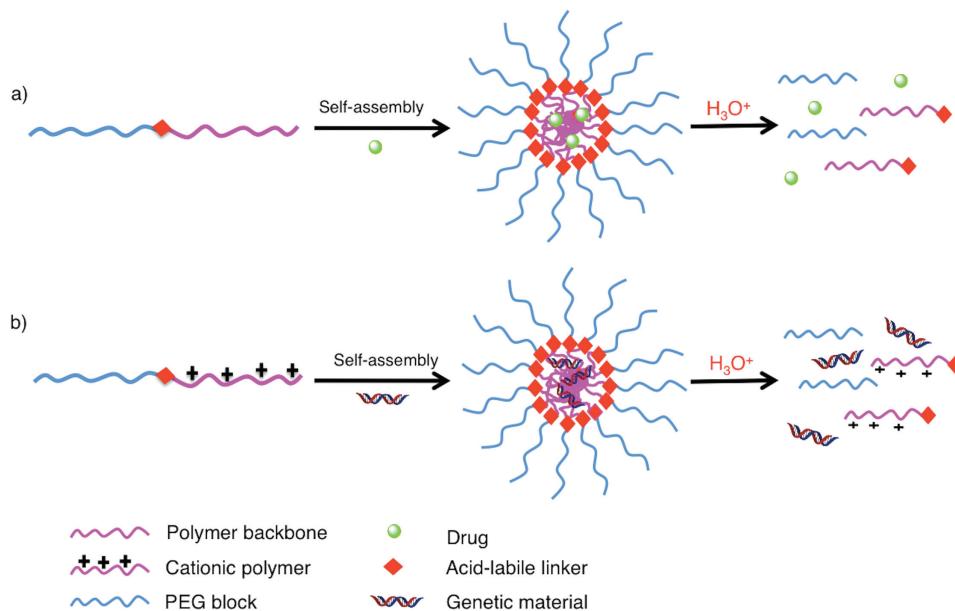


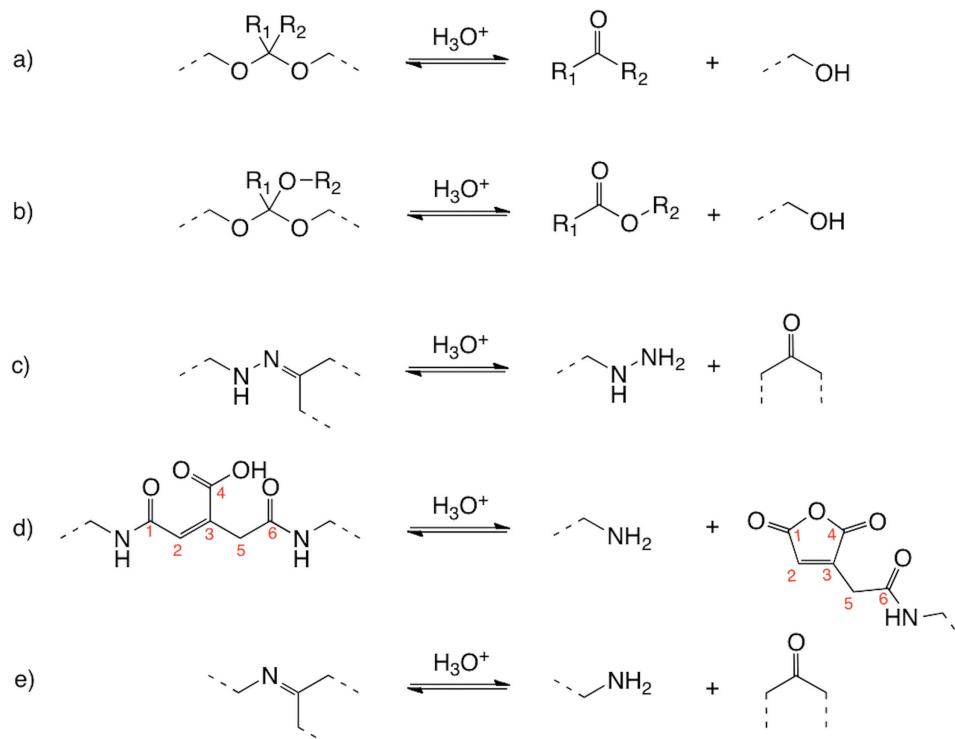
FIGURE 4 Self-assembly of (a) PEGylated polymers into micelles and (b) PEGylated cationic polymers into polyplexes.

showing a chemical degradation of the polymeric material or a conformational switch inducing a disruption of the system.

Various Acid-Labile Bonds

The acid-catalyzed hydrolysis of acetal groups produces an aldehyde (or ketone) and an alcohol [Scheme 6(a)]. The reaction is generally first order with respect to the hydronium

ion making its hydrolysis rate ten times faster with each unit of pH decrease.³⁴ Many groups have reported the insertion of acetal moieties in various systems such as linear polymers, hydrogels, micelles, nanoparticles, and dendrimers.³⁵⁻⁴¹ Orthoester linkers are readily hydrolyzed in mild aqueous acid to form alcohols and esters [Scheme 6(b)]. They have been mainly used as linear polyorthoesters.⁴² Hydrazone



SCHEME 6 Hydrolysis of acid-labile groups under acidic conditions: (a) Acetal, (b) orthoester, (c) hydrazone, (d) *cis*-aconityl, and (e) imine.

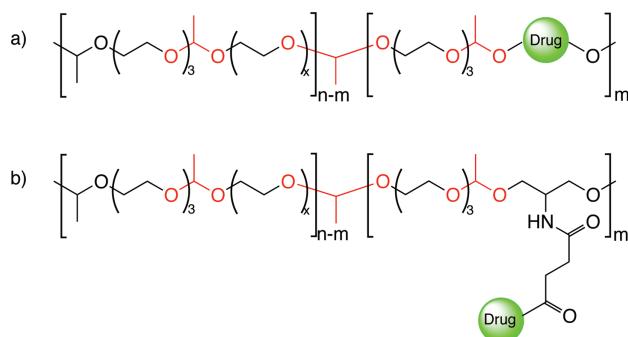


FIGURE 5 Polyacetal-drug conjugates with (a) backbone-incorporated drug or (b) pendant drug.

groups are hydrolyzed into aldehyde or ketone and hydrazine upon acidic conditions [Scheme 6(c)]. They have been extensively exploited as linker between the drug and the polymer in different systems such as polymer-drug conjugates,^{43–46} micelles,^{47–51} and dendrimers,^{52,53} but only a few reports deal with linear polyhydrazones.⁵⁴ The *cis*-aconityl group has a carboxylic acid (C-4) in *cis* position relative to the hydrolytic bond (C-1) and undergoes an assisted intramolecular acid-catalyzed hydrolysis due to the proximity of the pendent carboxylic acid [Scheme 6(d)]. As with hydrazones, the *cis*-aconityl groups have mostly been used as acid-labile linkers between the drug and the polymer in polymer-drug conjugates. The first example of a *cis*-aconityl-linked polymer-drug conjugate was in 1981 for the delivery of daunorubicin, a DNA intercalator used as a chemotherapeutic agent.⁵⁵ Since then, many groups have reported the use of *cis*-aconityl linker to trigger the release of chemotherapeutic agents such as doxorubicin (trade name: Adriamycin).^{56–60} Although the hydrolysis of this linker induces the release of the drug, it does not trigger any chemical degradation or physical disruption of the polymeric carrier. The imine bonds hydrolyze into an aldehyde (or ketone) and an amino group under very weak acidic conditions and are unstable at physiological pH [Scheme 6(e)]. Due to the instability and potential toxicity of these reaction products, imine linkers are rarely exploited as acid-labile linkers for the synthesis of degradable systems. Nevertheless, the imine bond can be stabilized by the presence of π - π conjugation. For example, a benzoic-imine linker was found to be more stable at neutral

pH and exhibits increasing an rate of hydrolysis with decreasing pH.^{61,62}

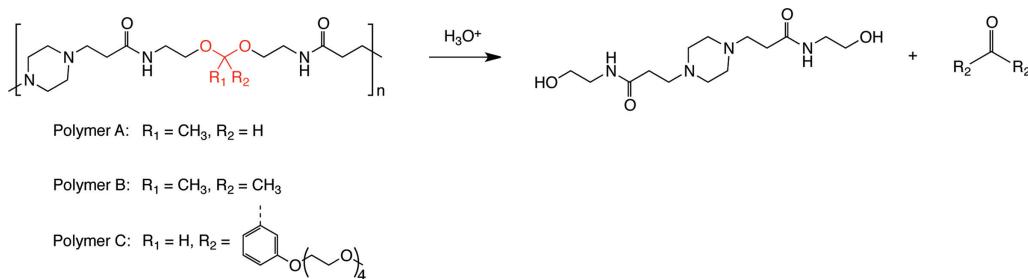
Linear Polymers with Acid-Labile Linkers in the Polymer Backbone

The simplest way to induce a pH-triggered degradation of the polymer is to incorporate the acid-labile linker into the backbone; the most common examples are polyacetals and polyorthoesters.

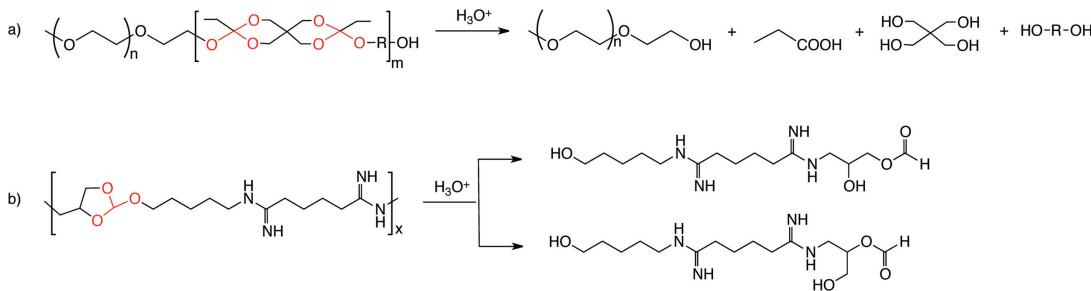
Polyacetals. Linear polyacetals can be prepared by polycondensation of diols with divinyl ethers.⁶³ Duncan and coworkers synthesized various linear polyacetal-drug conjugates, in which the drug is either pendent to³⁵ or directly incorporated within the backbone of the polymer (Fig. 5).³⁶ Degradation of these systems occurs more rapidly at pH 5.5 than at physiological pH (7.4). Release that selectively occurs at lower pH is potentially advantageous as such systems may be used to specifically target tumor cells.

Frechet and coworkers inserted acetal bonds into the backbone of poly(amidoamine)s (PAMAMs). They prepared a variety of pH-sensitive PAMAMs by polyaddition of acid-degradable bis(acrylamide) monomers with piperazine (Scheme 7).⁶⁴ The bis(acrylamide) monomers contained acetaldehyde acetal, dimethyl ketal or substituted benzaldehyde acetal linkages. The benzaldehyde acetal is particularly attractive because its acid sensitivity can be tuned by the introduction of substituents at different positions of the aromatic ring. All polymers degraded faster in acidic environments than at physiological pH and demonstrated a high potential for gene or drug delivery.

Polyorthoesters. Linear polyorthoesters were originally developed in the 1970s and have already been reviewed.^{42,65} Polyorthoesters can be synthesized by transesterification between an orthoester and a diol or by the addition of polyols to diketene acetals. Polyorthoesters are generally highly hydrophobic which can impair the water penetration into the bulk material, and thus their erosion occurs mainly at the surface with a very slow rate. Increasing the hydrophilicity of the polyorthoester by coupling it with PEG chains can accelerate the hydrolysis, leading to a



SCHEME 7 Acid-catalyzed hydrolysis of acetal-containing poly(amidoamine)s.

**SCHEME 8** Acid-catalyzed hydrolysis of (a) PEG-polyorthoester, (b) POEAmid.

complete degradation into monomers in a few hours [Scheme 8(a)].⁴²

Recently, Tang et al. incorporated orthoester groups into the backbone of poly(amidine) to obtain a new class of pH-labile cationic polymers, poly(orthoester amidine) (POEAmid) copolymers, for a gene delivery [Scheme 8(b)].⁶⁶ These copolymers formed polyplexes after condensation of plasmid DNA at neutral pH. Their acid-catalyzed hydrolysis results in dissociation of the polyplexes, releasing the genetic material. However, the polyplexes exhibited a low transfection efficiency compared to poly(ethyleneimine)s (PEIs), likely due to the deactivation of plasmid DNA in the endosome. Therefore, further optimization is needed to orchestrate the endosomal escape.

Acid-Sensitive Linkers in Self-Assembled Systems

As an alternative to inducing breakdown to monomers or release of small molecules, hydrolysis of an acid-sensitive linker can trigger the swelling and/or the disruption of self-assembled systems (micelles, polymersomes, or polyplexes, Fig 3).^{40,67-69} Whereas a simple swelling will induce or enhance diffusion of a drug out of the core of one of these self-assembled systems, disassembly will cause a rapid and instantaneous release of the therapeutic. The linker is generally pendent to the backbone polymer.

Zhong and coworkers recently synthesized acid-sensitive polymersomes made of poly(ethylene glycol)-*b*-poly(2,4,6-trimethoxybenzylidene-1,1,1-tris(hydroxymethyl)ethane methacrylate)-*b*-poly(acrylic acid) (PEG-PTTMA-PAA) triblock copolymers [Fig. 6(a)].⁶⁹

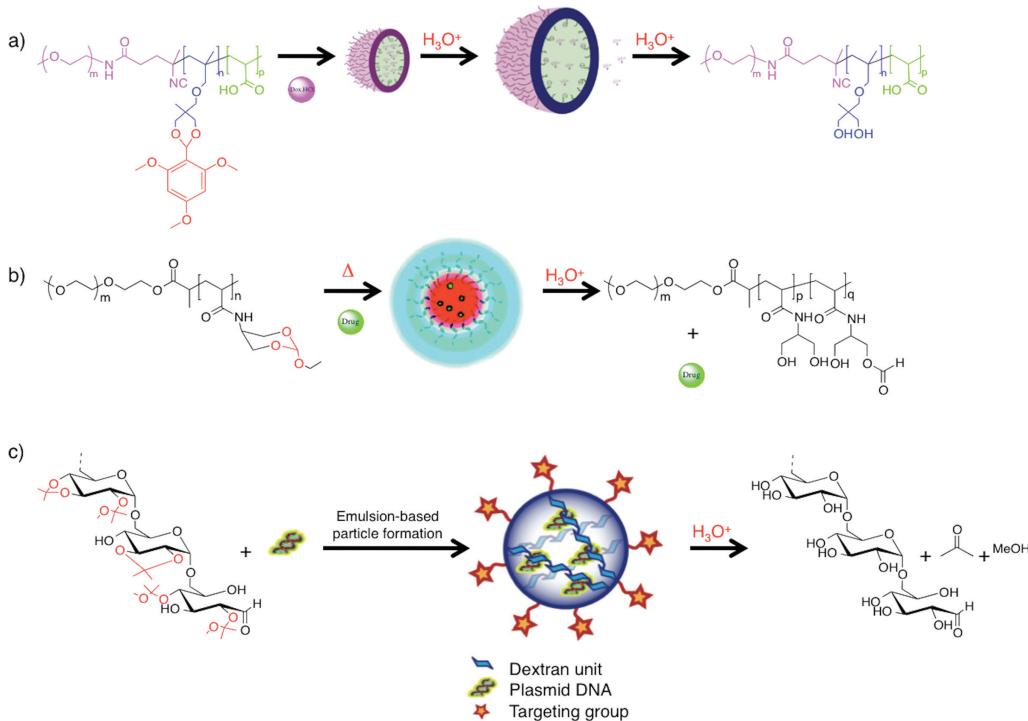


FIGURE 6 Acid-degradable self-assembled systems: (a) PEG-PTTMA-PAA polymersomes with pendent acetal. Adapted from ref. 69. (b) Thermoresponsive micelles based on block copolymer with pendent orthoester. Adapted from ref. 70. (c) Acetal-dextran particles. Adapted from ref. 79. (Reproduced from refs. 69, 70, and 79, with permission from Elsevier, ACS Publications and CSIRO Publishing, respectively.)

The acetal-containing polymersomes are stable at pH 7.4, while at more acidic pH they first swell, and then disassemble into water-soluble unimers as a result of complete acetal hydrolysis. The hydrochloride salt of doxorubicin (DoxHCl) was loaded into the aqueous core of the polymersomes, and its release was triggered by acid catalyzed hydrolysis of the acetal linkages between the blocks. While DoxHCl-loaded polymersomes exhibited similar anti-tumor activity to that of free DoxHCl against HeLa cells, the killing effect was partially due to the DoxHCl released from polymersomes before the cellular uptake. Conjugating targeting ligands to the surface of the polymersomes may allow for better spatial control over release.

In another report, Huang et al. prepared acid-labile thermoresponsive block copolymers composed of a PEG block and a polyacrylamide segment with pendent cyclic orthoester groups [Fig. 6(b)].⁷⁰ Upon heating above their critical aggregation temperatures, the copolymers undergo a phase transition and spontaneously self-assemble into micelles. The acid-catalyzed hydrolysis of the orthoesters on the acrylamide repeat units induces a change of the hydrophilicity balance leading, in a first stage, to the partial dissociation and swelling of the micellar core before a complete disruption of the micelles into soluble polymers. The authors then sequestered Nile red (a model therapeutic) in the micellar core and demonstrated its pH dependent release. Similar examples of thermoresponsive polymers with pendant orthoester groups have been reported in the literature.^{72–74}

Frechet and coworkers have designed another original example of pH-sensitive micelles made of dextran able to switch their solubility at a specific pH. Dextran is a bacterially derived homopolysaccharide of glucose with many advantages, including widespread availability, biocompatibility, hydrosolubility, degradability, and ease of chemical modification. The hydroxyl groups of dextran can be masked with acetals (Ac-Dex) to render it insoluble in water, allowing it to be processed into particles in organic solvent [Fig. 6(c)].⁷⁵ The acetal group hydrolyzes in acidic environments, reversing the solubility of dextran from organic solvent soluble to water soluble. The byproducts of the hydrolysis are dextran, acetone, and methanol. Although the released amounts of methanol were shown to be low enough to be below the recommended limit of daily exposure, a recent report has demonstrated the synthesis of an Ac-Dex analogue producing ethanol as safer degradation by-product.⁷⁶ The Frechet group has demonstrated the high potential of Ac-Dex for the transport of various hydrophobic and hydrophilic molecules^{75,77–79} mainly for immunotherapy, and more recently, for gene delivery.⁷¹

Acid-labile linkers have also been incorporated into polyplexes based on cationic polymers such as PEIs. PEIs are linear or branched cationic polyamines capable of complexing genetic material due to electrostatic interactions. They provide high gene transfer efficiency; however, they also display molecular weight and architecture dependent toxicity.⁸⁰ Whereas PEIs with high molecular weight (22–25 kDa)

exhibit a high transfection efficiency as well a high cytotoxicity, shorter PEIs (800 Da) have negligible toxic effects, but also a reduced efficiency. In order to optimize these carriers, one approach is to develop PEIs with appropriate size and introduce acid-degradable linkages that allow them to break down into smaller fragments with lower toxicity. A variety of linear or branched topologies have been explored with different linkers including acetals^{81,82} and imines.⁸³ In one example, Knorr et al. synthesized acid-degradable polyplexes by polymerization of oligo(ethyleneimine) with two types of acetal crosslinkers [Fig. 7(a)].³⁷ These systems degrade rapidly at low pH ($t_{1/2} = 3$ min at pH 5.5) compared to pH 7.4 ($t_{1/2} \sim$ hours). The acid-sensitive polyplexes exhibited an improved *in vitro* and *in vivo* biocompatibility compared to the acid-stable control polymers while maintaining a similar transfection efficiency.

In a similar approach, Kim et al. incorporated imine cross-linkers into PEIs in order to render them degradable into non-toxic low molecular weight polymers at endosomal pH.⁸³ The acid-degradable PEIs were synthesized by polymerization of low molecular weight PEIs and glutadialdehyde. The degradation kinetics of the polymers were studied by capillary viscosity measurements. The half-lives of the polymers were much shorter ($t_{1/2} = 1\text{--}2$ h) at low pHs than at physiological pH ($t_{1/2} = 118$ h). The acid-labile PEIs exhibited markedly lower cytotoxicity than high molecular weight PEI (25 kDa) while maintaining a comparable transfection capacity.

Acid-sensitive linkers can also be used to link a polymer backbone and grafted side chains. Hydrolysis leads to the fragmentation of the grafted architecture into linear pieces. For instance, Lin et al. developed pH-sensitive comb-like polymers that can enhance the intracellular delivery of therapeutic nucleic acids.⁸⁴ The polymer backbone consists of two blocks. The first block incorporates pH-sensitive ethyl acrylic acid (EAA) and hydrophobic butyl methacrylate (BMA) or hexyl methacrylate (HMA) monomers. The second block is a homopolymer of *N*-acryloxy succinimide (NASI) or β -benzyl L-aspartate *N*-carboxy-anhydride (BLA-NCA) monomers, which are grafted to hydrophobic HMA and cationic trimethyl aminoethyl methacrylate (TMAEMA) copolymers via a hydrazone linker [Fig. 7(b)]. The cationic charges allow the condensation of therapeutic nucleic acids. The pH-sensitive EAA monomers switch from an ionized and hydrophilic conformation at physiologic pH to a hydrophobic and membrane-destabilizing one in response to acidic pH values. This disrupts the endosomal membrane allowing the carrier to enter the cytoplasm. In addition, when exposed to acidic pH the hydrazone linker triggers degradation of the comb-like polymers into small fragments, which can be excreted easily from the body by renal clearance.

PEGylation of Self-Assembled Systems via Acid-Labile Linkers

PEGylation of polyplexes is crucial to prevent their recognition by the RES and prolongs their circulation half-life. However, PEGylation can also impair their internalization into the

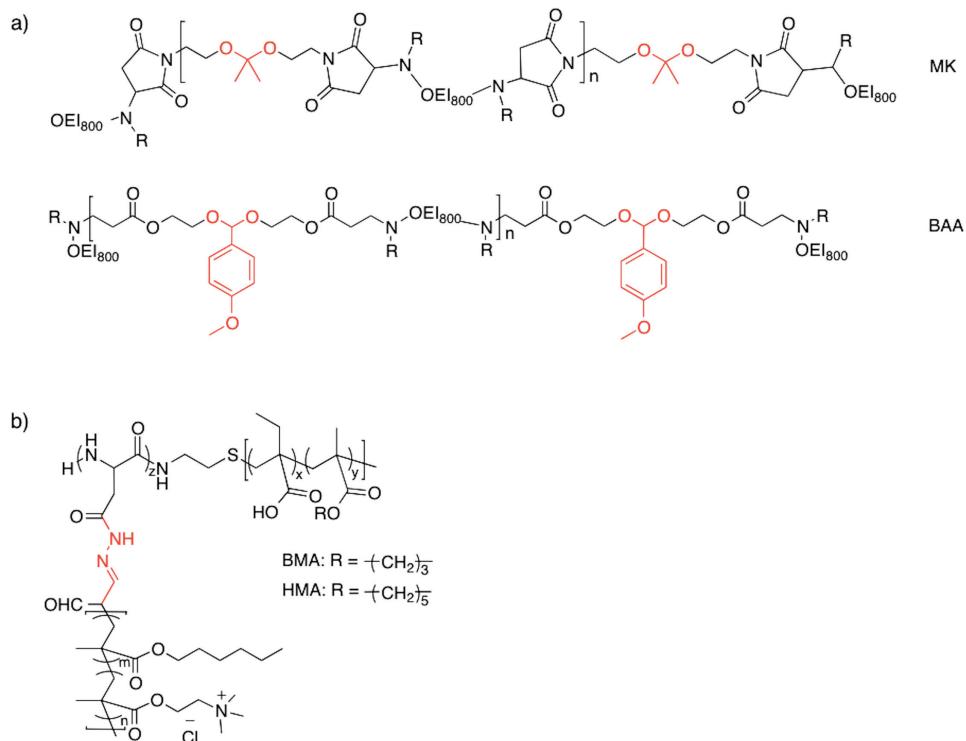


FIGURE 7 (a) Chemical structures of oligo(ethyleneimine)s crosslinked with two types of acetal crosslinkers: 2,2-bis(*N*-maleimidooethoxy) propane (MK) or 1,1-bis-(2-acryloyloxy ethoxy)-[4-methoxy-phenyl]methane (BAA). (b) Chemical structure of amphiphilic comb-like polymer [poly(EAA-*co*-BMA or HMA)-*b*-Asp-*g*-(HMA-*co*-TMAEMA)] where the grafts are connected to the backbone through a hydrazone.

tumor cells and their escape from the endosome, both critical events for efficient gene transfer. To overcome this issue, one strategy consists of incorporating an acid-labile linker between the carrier and the PEG chain. It assures, upon a specific pH, the cleavage of the PEG shell (deshielding) and unmasks the cationic charges at the vehicle surface. If the linker is sensitive at mildly acidic pHs (~ 6.5), the deshielding can happen in the extratumoral environment, which will facilitate the cellular uptake of the carrier. In contrast, if the linker is sensitive at lower pH (~ 5), the cleavage can occur in the endosome, which favors the endosomal escape of the drug or gene. For a better cellular uptake, Hu et al. designed acid-sensitive PEGylated polymeric micelles made of stearic acid modified chitosan (CS) [Fig. 8(a)].⁸⁵ The cleavage of the PEG caused by the hydrolysis of the *cis*-aconity linker was confirmed to be pH-dependent. Doxorubicin was encapsulated into the core of the micelles and interestingly, its release was accelerated by a decrease in pH directly correlated to the PEG removal. That phenomenon suggests that the PEG shell plays a protective role and once it is cleaved, the drug can diffuse more easily from the resulting smaller micelles. In order to enhance the endosomal escape in gene delivery, similar approaches of PEG cleavage have been reported using various types of acid-labile linkers such as hydrazone,⁸⁶ orthoester,⁸⁷ and acetal.⁸⁸

An appropriate choice of the linker could improve both the cellular uptake of the carrier and the endosomal escape of

its cargo (Fig. 9). As previously noted, the benzoic-imine linker exhibits a highly pH-dependent hydrolysis (increasing hydrolysis rate with decreasing pH). Block copolymers of PEG and an aliphatic C18 segment linked through a benzoic-imine group [Fig. 8(b)] self-assemble into micelles at neutral pH [Fig. 6(b)].⁶² At tumor pH (~ 6.5), the copolymers were partially hydrolyzed and the residual octadecyl amines generate positive charges on the micelle surface, enhancing their uptake into tumor cells. In contrast, at endosomal pH (~ 5.0), the benzoic-imine hydrolyzes completely, inducing the complete dissociation of the micelles and increasing their hemolytic activity (membrane disrupting capability) in endosomes (Fig. 9).

Tuning the Hydrolysis Rate

The degradation rate of a linker can be tuned by the judicious choice of the substituents on the linker, which influence the stability of the bond. In demonstration of this concept, Kale and Torchilin have studied the stability of various aliphatic and aromatic aldehyde-derived hydrazone (HZ)-based acid-sensitive poly(ethylene glycol)-phosphatidylethanolamine (PEG-HZ-PE) micelles under neutral and acidic conditions [Fig. 8(c)].⁸⁹ Interestingly, the aromatic aldehyde-derived HZ bond was stable to hydrolysis regardless of pH and therefore cannot be used for the purpose of pH-triggered drug release. In contrast, PEG-HZ-PE conjugates derived from aliphatic aldehyde-based HZ are highly unstable under acidic conditions while relatively stable at physiological pH. In addition, Zhou

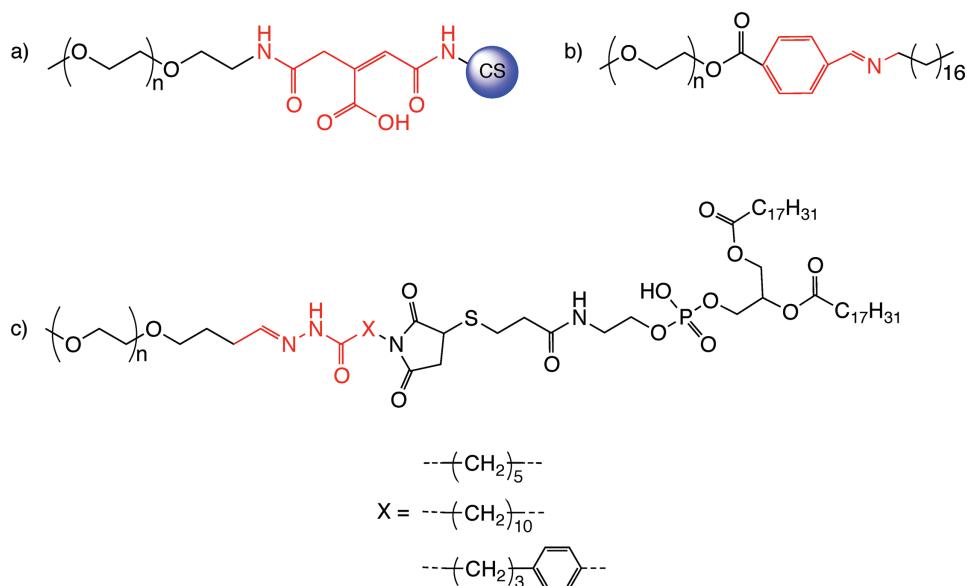


FIGURE 8 Chemical structures of systems PEGylated via acid-sensitive linker: (a) *cis*-aconityl-linked PEG glycolipid conjugates (CS = stearic acid modified chitosan), (b) PEG-b-C18 via an imine, and (c) PEG-HZ-PE via a hydrazone.

et al. recently demonstrated that the HZ content can also influence the degradation rate of the material.⁹⁰ They synthesized a variety of pH-sensitive biodegradable polyurethanes with

different HZ bond contents and investigated their biocompatibility and biodegradability *in vitro* and *in vivo*. The presence of multiple HZ bonds decreases the degree of crystallinity and the extent of microphase segregation of the polymers, allowing faster degradation of the polyurethanes in acidic media compared to the polymers with low HZ content. They also showed the advantage of pH-dependent degradation over a random hydrolysis (faster and controlled degradation with decreasing pH). Finally, intramuscular implantation of the polyurethane films in rats results in efficient *in vivo* degradation with no apparent toxic effect in the surrounding muscle tissues.

Temperature-Responsive Materials

Temperature is another reliable trigger for controlling biomaterial properties because physiological temperature is generally stable (37°C), significantly different from room temperature ($\sim 20^\circ\text{C}$), and yet variations exist within the body. For example, temperatures at the extremities or in a diseased state are often significantly lower than in the core of the body (Table 2). Because physiological temperature is typically not high enough to induce covalent bond degradation, most temperature-responsive materials undergo a physical transformation rather than chemical breakdown.

Thermo-responsive polymers undergo a phase transition upon a variation of the temperature, which might induce a

TABLE 2 Temperature Variation in the Body

Location in Body	Temperature ($^\circ\text{C}$)
Normal core	37
Deviations during disease	20–42.5
Normal skin	28
Skin at extremities	0–45

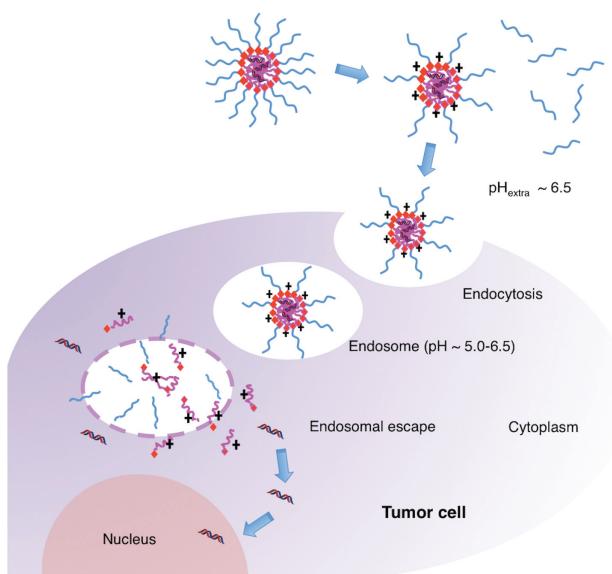


FIGURE 9 Internalization of pH-sensitive polyplexes in tumor cells. The PEG shell is tethered to the cationic vector via an acid-labile linker. In a first stage, the mildly acidic pH in the extratumoral environment induces a partial deshielding of the polyplexes and unmasks some cationic charges. As a result, it increases the electrostatic interactions between the vector and the cell membrane and facilitates the cellular uptake by endocytosis. In a second stage, the drop of the pH in the endosomal compartment allows the complete cleavage of the PEG, and causes the disassembly of the polyplexes and the disruption of the endosomal membrane. The genetic material can escape from the endosome to reach the nucleus.

TABLE 3 Polymers Exhibiting Physiologically Relevant LCSTs

Polymer	Chemical Structure	LCST	Reference
PNIPAM		30–34 °C	94
PDEAM		25–34 °C	95,96
PMVE		34–37 °C	97
PVCL		30–50 °C	98,99

sudden change in the solvation state. Thermosensitive polymers have been mostly applied for gelation⁹¹ and micellization⁹² in controlled drug delivery.^{32,93} Researchers often take advantage of the difference between room temperature and core body temperature to induce gelation. Alternatively, a temperature change can also be induced by heating (hyperthermia) or by cooling (hypothermia). Multiple external heat sources exist and their choice depends on whether the entire body or a small area needs to be heated. Whereas ultrasounds, microwaves, and radiofrequencies can be used for a local heating, immersion of the body in hot (or cold) water and wrapping in hot (or cold) blankets can be utilized for whole-body hyperthermia (or hypothermia). The body can also be prone to internal variation in temperature as observed in some tumor or infected tissues, in which the temperature is slightly more elevated (40 °C) than physiological body temperature (37 °C) because the cells operate at a higher metabolic rate.

The temperature above which the polymer becomes insoluble is called lower critical solution temperature (LCST), whereas the temperature above which the polymer becomes soluble is called upper critical solution temperature (UCST). Indubitably, polymers have to display this on-off solubility switch at a temperature suitable for a biological application and in a narrow temperature range. Thermo-responsiveness can be either swelling/dissolution (in which increasing the temperature increases the solvent quality) or aggregation/collapse (in which increasing the temperature decreases the solvent quality). Since this section is aimed to highlight the degradation upon a temperature trigger, we will not detail the thermosensitive polymeric systems such as hydrogels that reversibly swell and shrink. Here, we will highlight recently reported systems in which a temperature change results a solubility change of the polymer chains. Although this is not true molecular degradation by breaking covalent bonds, such changes in solubility can disrupt self-assembled systems resulting in a supramolecular degradation.

LCST Polymers

The most common LCST polymers used for a biological application are poly(*N*-isopropylacrylamide) (PNIPAM),⁹⁴ poly(*N,N*-diethylacrylamide) (PDEAM),^{95,96} poly(methylvinylether) (PMVE),⁹⁷ and poly(*N*-vinylcaprolactam) (PVCL)^{98,99} because they display a phase transition temperature close to 37 °C (Table 3). Polymers with an LCST between room temperature and physiological temperature can be used as injectable scaffolds or depots of drugs that are released in a controlled manner due to retarded diffusion. On the molecular scale, these polymers can also be used as hydrophilic blocks in amphiphilic block copolymers to form micelles. Upon crossing the LCST, the hydrophilic outer shell of the micelle becomes hydrophobic, shrinking the micelle and potentially releasing entrapped therapeutics. In either example, the LCST polymer/block is soluble at room temperature and phase separation/segregation occurs above the LCST. In addition to relying on the stable temperature of the body to control phase behavior, researchers can also induce local temperature changes to spatially and temporally control biomaterial properties (induced hypothermia or hyperthermia).

PNIPAM, the most extensively investigated thermosensitive polymer, exhibits an LCST of approximately 33 °C in aqueous solution.⁹⁴ At body temperature, the polymer is not soluble and is relatively hydrophobic. In addition to its use as a homopolymer to form injectable gels, NIPAM can be used as one of the hydrophilic blocks of an amphiphilic block copolymer, allowing micellization. Lipophilic drugs can be entrapped in the inner core [Fig. 10(a)]. The stability of the micelles in the physiological environment enables long blood circulation times. Once the micelles arrive at the target site, a decrease in the local temperature below the LCST (induced hypothermia) causes the dissolution of the micelles, releasing the drug [Fig. 10(a)]. Many examples of PNIPAM as the hydrophobic core of micellar structures have been reported.^{92,100,101} Nevertheless, hypothermia is not always feasible and therefore, to overcome this limitation, one strategy is to increase the LCST of PNIPAM above body temperature by copolymerization with hydrophobic monomers.¹⁰² The resulting copolymer becomes soluble at 37 °C and forms the hydrophilic outer shell of micelles [Fig. 10(b)]. Once circulating into malignant tissues in which the temperature has been increased (induced hyperthermia), the local temperature is above the LCST, and the shell of these micelles turns into a hydrophobic and insoluble structure. This enhances the hydrophobic interactions between the micelles and the cells and facilitates their cellular uptake. Thermosensitive micelles with PNIPAM as outer shell have been extensively studied and have been already reviewed.⁹² As temperature increases the PNIPAM chains collapse and the micelles shrink [Fig. 10(b)]; however no degradation of the system occurs.

The temperature responsiveness of polymers can be also internally regulated in response to a change in the biological environment. For example, a change in the LCST of the polymers can be triggered by hydrolysis of the polymer, which might be accelerated with a pH change [Fig. 10(c)].

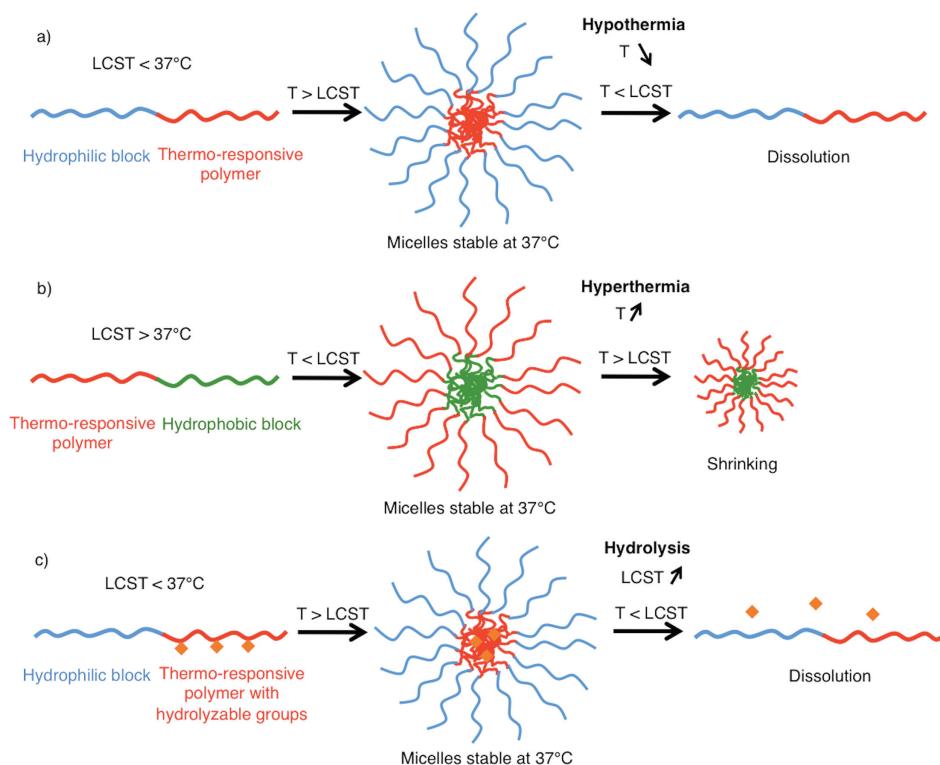


FIGURE 10 Schematic illustration of thermo-responsive micelle formation at body temperature and their destabilization upon a local change (temperature) or intrinsic change (polymer hydrolysis). (a) Micellization with a thermo-responsive polymer as inner core and their dissolution after hypothermia. (b) Micellization with a thermo-responsive polymer as outer shell and their shrinking after hyperthermia. (c) Micellization with a thermo-responsive polymer as inner core and their dissolution after hydrolysis.

Hennink's group has developed a series of thermosensitive diblock copolymers of PEG and poly(NIPAM-*co*-*N*-(2-hydroxypropyl)methacrylamide lactate) [poly(NIPAM-*co*-HPMAM-lactate)] with hydrolytically sensitive side chains [Fig. 11(a)].^{103,104} The initial LCST of those systems is below the body temperature allowing their micellization at 37 °C. Then, during the hydrolysis of the lactate side groups, the LCST of the thermo-responsive polymer steadily increases and finally passes 37 °C, resulting in solubilization of the polymer and the disruption of the particles at body temperature [Fig. 10(c)]. A kinetic study of the degradation revealed that the degradation rate increases with temperature,

alkaline pH, and dielectric constant of the medium.¹⁰³ A similar approach has been reported by Ulbrich and coworkers with hydrolyzable linkers more sensitive in acidic conditions.¹⁰⁵ They synthesized thermo-responsive systems based on copolymers of *N*-isopropylmethacrylamide with a comonomer containing hydrophobic alkyl groups of varying length connected to a polymer backbone through a hydrolytically labile hydrazone [Fig. 11(b)]. Polymer solubility at physiological pH depends on the length of the hydrophobic group and the fraction of hydrazone comonomer; some copolymer samples precipitate at body temperature. Hydrolysis of the hydrazone bond induces an increase of the LCST providing complete dissolution of the copolymer at body temperature. Finally, a model therapeutic radionuclide, ⁶⁴Cu, in the form of its hydrophobic chelate, was efficiently entrapped into the phase-separated polymer until its complete dissolution, demonstrating the use of this system for local radiotherapy.

In those two examples, the degradation is tuned by the cleavage of hydrolytic groups accompanied by a solubilization of the thermo-responsive polymers.

UCST Polymers

In contrast to the LCST systems, the UCST polymers have received much less attention, and so far only one review dealing exclusively with water-soluble UCST polymers was found.¹⁰⁶ Few UCST systems suitable for a biological application have been designed and typical examples are based on

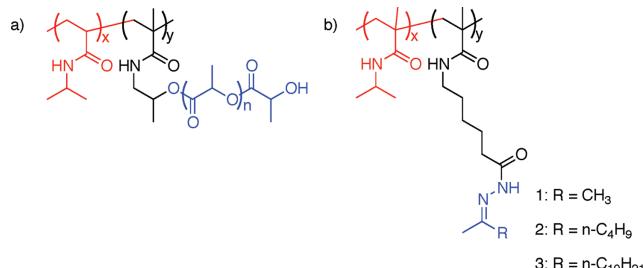
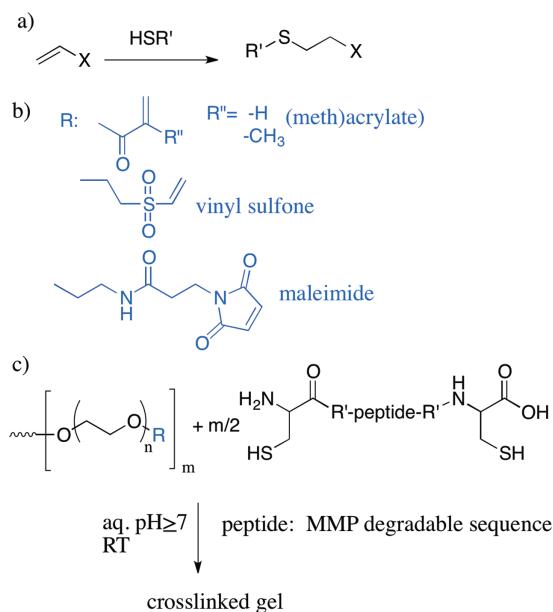


FIGURE 11 Thermo-responsive copolymers with hydrolysable groups: (a) poly(NIPAM-*co*-HPMAM-lactate) and (b) copolymers of *N*-isopropylmethacrylamide with comonomers containing hydrophobic alkyls of three different sizes bonded with hydrolytically labile hydrazone.



SCHEME 9 (a) General pseudo-Michael reaction, (b) commonly used Michael acceptors, (c) Michael addition to produce cross-linked enzymatically degradable gels.

a combination of PAA and poly(acrylamide) (PAAm) or poly(acrylamide-*co*-butyl methacrylate). They were mostly investigated as hydrogels, which shrink at lower temperatures and swell at higher temperatures.^{107,108} To the best of our knowledge, no UCST polymers showing any evidence of a temperature-induced degradation or disruption of the systems have been developed.

While thermo-responsive polymers have been extensively explored for drug delivery, their use *in vivo* faces some practical limitations. Although the body tightly regulates temperature, patient-to-patient differences exist, and the variation within different parts of the body is not large even in diseased states (except at skin/extremities). The physiologically relevant temperature window is quite narrow and would require polymeric materials with very narrow phase transitions. Furthermore, although it is possible to induce local temperature changes, such techniques can also damage surrounding healthy tissue, and even if no damage occurs, again only a very narrow temperature window is accessible. Therefore, temperature is perhaps not the best trigger for controlling biomaterial properties (other than for injectable gelling systems) because the accurate regulation of temperature remains a critical challenge for *in vivo* applications.

INTERNAL STIMULI FOR BIOMATERIAL MODULATION

As polymer chemists immerse themselves in the multi-disciplinary field of biomedical engineering, they have been able to view physiological systems not in terms of their biology, but rather in terms of potential for chemical reaction. In different parts of the body, different concentrations of reactive species are present. Furthermore, such differences also exist between healthy and diseased tissue. That is, cells are

genetically programmed to produce chemical reactants such as enzymes, ROS, and reducing species depending on their state of differentiation, overall health and in response to external cues. These chemical reactants can be exploited for biomaterials degradation. In this review, we term chemical reactants produced by cells as internal triggers.

Enzymolysis

In a biological system, many endogenous enzymes are present and may encounter polymeric materials. Reports of enzymatic degradation of polyesters began to appear in the 1950s and 60s,¹⁰⁹ and in 1984 Pitt et al. demonstrated *in vivo* enzyme enhanced degradation of polycaprolactone copolymers.¹¹⁰ Since then, many researchers have employed enzymatic degradation as a mechanism for controlling biomaterial properties.

Enzymatically Degradable Scaffolds

In nature, both specific and non-specific enzymes exist. For example, enzymes such as collagenase and hyaluronidase degrade specific substrates (collagen and hyaluronan, respectively), while other enzymes such as lysozyme are more promiscuous with respect to their substrates. In recent years, researchers have designed polymeric biomaterials incorporating enzyme-specific sequences for precise control over material degradation. Matrix metalloproteinases (MMPs) are zinc-dependent enzymes that typically degrade a cell's extracellular matrix, and are important in many biological processes, including wound healing, tissue remodeling, and cancer metastasis.¹¹¹ Many different MMPs exist, and exhibit differences in peptide substrate specificity. By incorporating a specific peptide sequence into a polymeric material, researchers are able to precisely control where and when the material is cleaved. Hubbell's group pioneered this approach by incorporating the MMP-sensitive linkages Ala-Pro-Gly-Leu (B block) into a BAB triblock copolymer with PEG (A block) with vinyl endgroups.¹¹² Hydrogels formed from these macromers degraded specifically in response to MMP I, but were immune to degradation by plasmin. In subsequent work, the Hubbell group developed MMP-sensitive sequences flanked by cysteines, and incorporated these peptides into hydrogels through Michael-addition to multifunctional PEG with acrylate, maleimide, and vinyl sulfone (Scheme 9) end groups.

Healy's group reported peptide-crosslinked poly(*N*-isopropylacrylamide-*co*-acrylic acid) networks that were both thermally responsive and susceptible to MMP-13.¹¹³ The authors incorporated thermoresponsiveness into their system to allow for injectable biomaterials, although this was not directly demonstrated. More recently, the Hubbell group has incorporated a SPARC (secreted protein acidic rich in cysteine) sequence into hydrogels.¹¹⁴ The SPARC sequence is sensitive to MMP-1 and MMP-2, in addition to other enzymes such as plasmin. Importantly, the kinetics of enzymatic degradation varies with the enzyme for identical substrates, allowing temporal control that can be tuned to specific tissues.

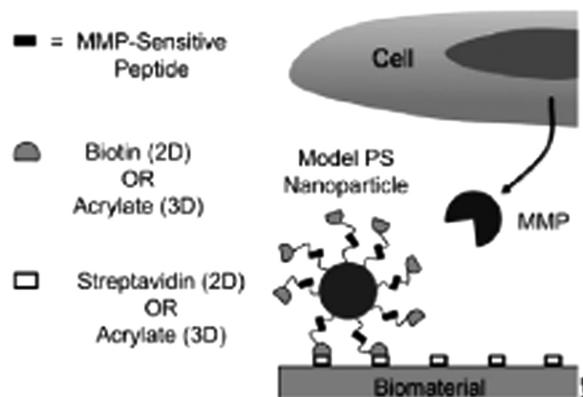
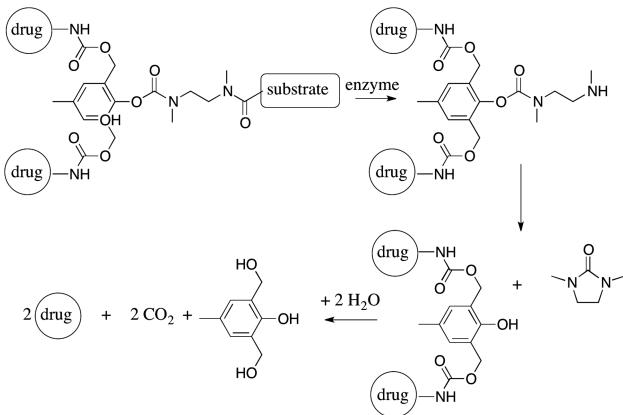


FIGURE 12 Enzymatic release of nanoparticles tethered to substrates through MMP-sensitive linkages. (Reproduced from ref. no. 115 with permission from Elsevier.)

The Segura group reported enzymatically degradable hydrogels entrapping polyplexes. Upon enzymatic degradation via MMPs, polyplexes were released, allowing gene transfer to infiltrating cells. In another report, her group immobilized nanoparticles to hydrogels through several different MMP-degradable linkers, and found that nanoparticle release depended on specific MMP expression of cells (Fig. 12).¹¹⁵ Although in this report the nanoparticles did not contain any payload, one can easily see the utility of cell-dictated release of nanoparticles that can be loaded with various therapeutics, such as DNA or siRNA.

Enzyme-Triggered Drug Release

MMP-degradable linkages have also been used to tether therapeutic agents into hydrogels. For example, growth factors such as vascular endothelial growth factor (VEG-F) can be released via enzymatic degradation of an MMP-sensitive tether to induce angiogenesis.¹¹⁶ Since these early reports, many other groups have fabricated enzymatically degradable polymers, and several reviews summarize activity in this area.^{9,117–119}



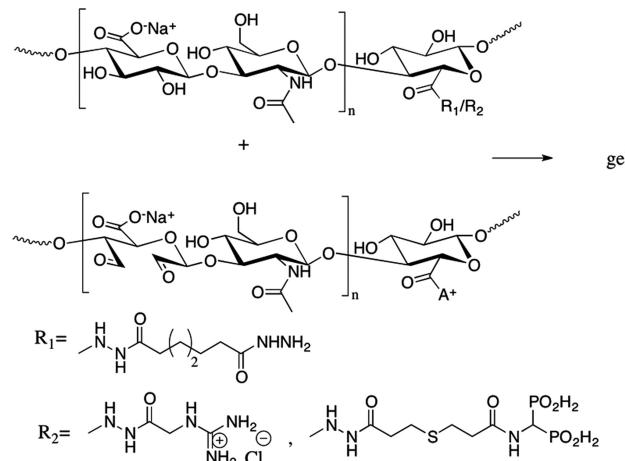
SCHEME 10 Enzymatically triggered self-immolation for drug release.

Several enzymes have been reported as the trigger for self-immolating polymers (including oligomers, linear polymers, and dendrimers), including plasmin, penicillin G amidase, bovine serum albumin, cathepsin, and β -glucuronidase (Scheme 10).¹²⁰ In one of the earliest reports, Shamas et al. conjugated the chemotherapeutic agents doxorubicin and camptothecin to self-immolating 2,6-bis-(hydroxymethyl)-*p*-cresol based AB₂ dendrimers in which the triggering event is a sequence of retro-aldo retro-Michael cleaving reactions catalyzed by the antibody 38C2.¹²¹ AB 38C2 is unique in that it reacts with substrates not recognized by human enzymes. This minimizes non-specific enzymatic degradation/pro-drug activation, however, it requires exogenous application of the enzyme.

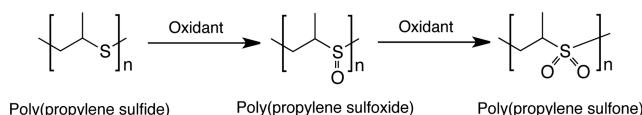
Varghese, Hilborn, and Ossipov have developed several HA pro-drugs susceptible to activation by hyaluronidase. HA is a non-sulfated linear glycosaminoglycan composed of repeating disaccharide units of glucuronic acid and *N*-acetylglucosamine. It is continually synthesized and degraded in the body. Specific cell-surface receptors exist for fragments of HA, including the CD44 receptor and the receptor for hyaluronic acid mediated motility (RHAMM). In the aforementioned report, the group first oxidizes HA with sodium periodate to produce aldehyde groups along the backbone. They then crosslink the gel with difunctional hydrazides. These gels can be used to either physically entrap a drug (i.e., BMP-2^{122,123}) or the drugs can be directly conjugated to the HA backbone through hydrazone linkages (bisphosphonate¹²⁴ and guanidinium,¹²⁵ Scheme 11). In the presence of hyaluronidase, the gel is cleaved into smaller fragments capable of interacting with the CD44 receptor, allowing internalization of drug-gel fragments by cells expressing the CD44 receptor through receptor-mediated endocytosis.

Oxidative Degradation

Oxidative stress is characterized by the overproduction of various oxidizing agents classified in two categories: (1) the reactive oxygen species (ROS) (superoxide radical anion,



SCHEME 11 Hyaluronan hydrogels containing hydrazone linkages to guanidinium groups and bis-phosphonate groups.



SCHEME 12 The different oxidation degrees of PPS.

hydrogen peroxide, and hypochlorite), and (2) the reactive nitrogen species (RNS) (nitric oxide or peroxynitrite).¹²⁶ Oxidative stress plays a pivotal role in the inflammatory process and it is associated with many diseases such as Parkinson's, Alzheimer's, atherosclerosis, rheumatoid arthritis, multiple sclerosis, ischemia, and tumors.¹²⁶ This redox imbalance can be used as a trigger to induce the degradation of oxidation-sensitive polymers in a specific inflammatory area.

The most commonly used oxidation-responsive polymers are polysulfides, specifically, poly(propylene sulfide) (PPS). Polysulfides are generally hydrophobic, making them useful as a core of a carrier for encapsulating hydrophobic drugs in micelle or nanoparticle structures. Interestingly, their oxidation leads to hydrophilic polysulfoxides or polysulfones (Scheme 12), which increases their solubility in water and facilitates their renal excretion from the body.

Hubbell and coworkers first demonstrated the utility of oxidation as a stimulus by fabricating polymeric vesicles from triblock poly(ethylene glycol)-poly(propylene sulfide)-poly(ethylene glycol) (PEG-PPS-PEG).¹²⁷ Oxidation converts the hydrophobic PPS block into hydrophilic poly(propylene sulfoxide) and poly(propylene sulfone) and induces a morphological change of the carrier without any loss of the block copolymer integrity. During the oxidation process, the polymeric carrier is disrupted from large vesicles to smaller particles (worm-like micelles and spherical micelles) to ultimately produce soluble unimolecular micelles with a size small enough (hydrodynamic radius approximately 3 nm) to ensure their clearance from the body. In this example, the core of the polymersomes is hydrophilic and is more suitable for encapsulating hydrophilic molecules. Since many drugs of interest are hydrophobic, the Hubbell group also pursued crosslinked polysulfide nanoparticles to allow delivery of hydrophobic drugs.¹²⁸ The nanoparticles were made from living emulsion polymerization of episulfides in the presence of Pluronic F-127 allowing the PEGylation and the stabilization of the nanoparticle surface. By varying the concentration of Pluronic, the nanoparticle size could be tuned from 25 to 225 nm. After the nanoparticles were exposed to H₂O₂, they exhibited increased swelling before their complete dissolution in water due to the oxidation of the disulfide bonds to sulfone groups. Owing to the amorphous character and the low glass transition temperature of PPS, the polar oxidants are able to readily diffuse through the nanoparticles.¹²⁶

For a specific application in the field of diabetes therapy, Hubbell and coworkers designed glucose-sensitive carriers based on their polysulfide vesicles¹²⁹ or nanoparticles.¹³⁰ Both systems carry glucose oxidase (GOx), an enzyme able to catalyze the oxidation of β -D-glucose into gluconolactone and H₂O₂. The H₂O₂ produced oxidizes the polysulfide,

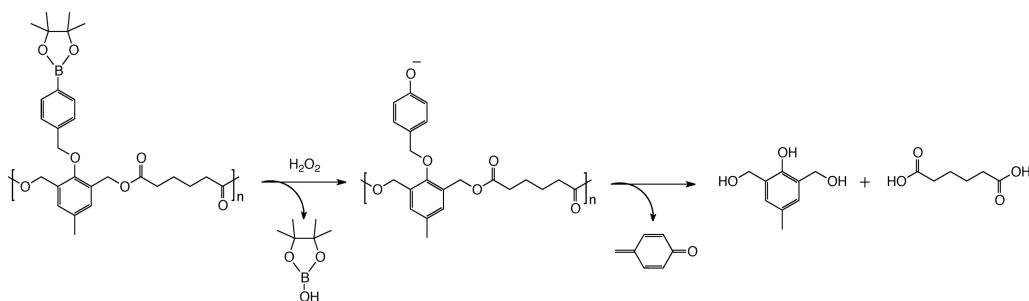
destabilizing the carrier. With the polymersomes, the GOx is encapsulated into the vesicles and the glucose diffuses through the permeable membrane yielding intravesicular H₂O₂. Accumulation of H₂O₂ and its reaction with the polymers changes the amphiphilic balance of the polymersome, leading to its dissolution and a complete and sudden (burst) substance release. In contrast, when GOx was covalently tethered to the crosslinked PPS nanoparticles through Pluronic, the nanoparticles absorb the glucose, inducing a gradual swelling of the nanoparticle more suitable for a sustained release of the encapsulated drug.

Since these early reports, many groups have studied polysulfides as oxidation-sensitive drug delivery systems in a variety of forms (vesicles, nanoparticles, micelles) able to deliver either hydrophilic or hydrophobic payloads. These nanocarriers can be decorated with imaging or targeting agents.^{131–135} The PPS nanoparticles have also been studied for antigen delivery to lymph node-resident dendritic cells (vaccine carriers) and they have been proven to be efficient in murine models.¹³⁶ The use of polysulfides is not limited to biodegradable nanocarriers and has been extended to bioresorbable implants such as neuroprostheses.¹³⁷

The residual fragments of the polysulfide material can be either water soluble polymers or depolymerized moieties depending on the oxidation conditions.¹³⁸ Indeed, a mild oxidative environment such as the presence of H₂O₂ oxidizes the polysulfides into non-toxic hydrosoluble polysulfoxides, while a stronger oxidant such as NaOCl further converts the polysulfoxides to polysulfones, which are prone to depolymerization. Although the oxidation products under NaOCl showed a higher cytotoxicity than those obtained under H₂O₂ oxidation, the toxicity remains relatively low and comparable to biocompatible polymers such as chitosan.¹³⁸

Although the oxidation of PPS crosslinked nanoparticles was determined to be twice as slow as that of the vesicles under identical conditions (10 vol% H₂O₂),^{127,128} the oxidative degradation is still rapid and the H₂O₂ concentration typically utilized to achieve oxidation in *in vitro* experiments may not represent pathological oxidative conditions. Recently, Gupta et al. have pointed out the difficulty in recapitulating the real oxidative environment.¹³⁵ Indeed, the oxidant concentration *in vivo* is difficult to evaluate since there might be continuous production, diffusional loss, and degradation of different reactive species. For some oxidants with short half-lives such as peroxynitrite, the concentration required to mimic the pathological environment has to be higher than the concentration observed *in vivo*. To better evaluate the practicality of polysulfide materials to respond to an oxidative stimulus, it is crucial to consider many factors: relevant concentrations, oxidation under multiple reactive species, enzyme-mediated oxidation, and ultimately *in vivo* experiments are required.

Other oxidation-sensitive polymeric materials have been designed and can be classified into two distinct forms: (1) nanoparticles and (2) scaffolds.

**SCHEME 13** Oxidative degradation of arylboronic-conjugated polymer.

Nanoparticles

Thioketal nanoparticles (TKNs) made of poly-(1,4-phenylene neacetone dimethylene thioketal) (PPADT) in which the thioketal groups are sensitive to ROS have been reported.¹³⁹ When TKNs are exposed to superoxide, it causes a drastic and rapid reduction of the polymer molecular weight. *In vitro* and *in vivo* studies reveal that TKNs are able to deliver genetic material in response to abnormally high levels of ROS into inflamed intestinal tissues. Finally, the delivered genetic information was able to silence the gene expression of the proinflammatory cytokine tumor necrosis factor-alpha (TNF- α) in the colon of mice. In another interesting report, an arylboronic ester has been used as an oxidation-responsive group pendent to a self-immolating polymer backbone.¹⁴⁰ Upon exposure to H_2O_2 , the arylboronic group is hydrolyzed to a phenol, which undergoes a quinone methide rearrangement and leads to the complete degradation of the polymeric nanoparticles (Scheme 13). A similar approach uses dextran modified with arylboronic ester in order to induce a solubility switch, and subsequently particle degradation under oxidative stress.¹⁴¹ These two last examples describe materials highly sensitive to relatively low and physiologically relevant H_2O_2 concentration (1 mM).

Scaffolds

While a relatively fast degradation rate is typically used for polymeric nanoparticles delivering therapeutic agents, implants used for tissue engineering require a slower degradation rate. For this reason, polymers used for the nanoparticles are typically not suitable for implant design. Oxidation-responsive polymers that may be suitable as scaffolds have been developed, but thus far, research in this area is limited to a handful of reports. Amino acids such as histidine, proline, arginine, and lysine are particularly susceptible to oxidative stress.¹⁴² Thus, polymeric scaffolds crosslinked with proline oligomers (Pn) have been synthesized, and exhibit an approximate 30% mass loss after 28 days of oxidative treatment. *In vitro*, these scaffolds were partially degraded after incubation with activated murine macrophages.¹⁴³ Poly(ester urethane) scaffolds have also been found to respond to oxidative stress, however their degradation is not only a consequence of oxidation but also occurs through hydrolysis of ester bonds.^{144,145}

Oxidation as a trigger has been widely investigated in the drug delivery field and has demonstrated utility in

controlling the degradation of various polymeric materials, and subsequently inducing drug release. Nevertheless, the relatively recent development of oxidation-responsive materials means that the *in vitro* and *in vivo* studies to confirm their real potential for a future clinical applications are still forthcoming.

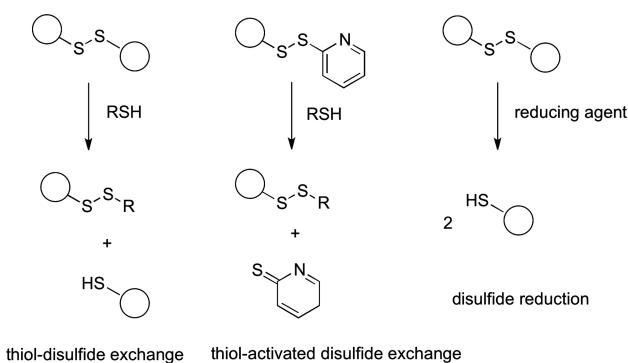
Disulfides Sensitive to Reducing Conditions

Just as materials may experience oxidative stress in a biological environment, reducing conditions also exist (often in response to oxidative stress). Many reducing molecules are produced by cells in a physiological system. For example, glutathione, a small tripeptide composed of L-glutamic acid, L-cysteine and glycine (with a gamma peptide linkage between cysteine and the side-chain carboxy group of L-glutamic acid) is endogenously produced by cells and may be present in concentrations up to 5 mM intracellularly.¹⁴⁶ It is an antioxidant and is able to reduce disulfide bonds in proteins found in the cytoplasm. Inspired by nature, several research groups have incorporated disulfide bonds into polymeric materials that undergo thiol-disulfide exchange with glutathione or other thiols. The general approach and different reactive structures are shown in Scheme 14. As with many other mechanisms, reviews on reduction sensitive polymeric biomaterials have been published,¹⁴⁷ and we will therefore only highlight the most recent advances.

Polymer Degradation

The Gillies group synthesized a self-immolating polymer with a backbone comprised of alternating *N,N'*-dimethylethylenediamine and 2-mercaptopropanol units linked through carbamate and thiocarbamate groups, with a disulfide end group (Scheme 15).¹⁴⁸ Upon reduction of the disulfide group, the entire polymer undergoes alternating cyclizations of 2-mercaptopropanol and *N,N'*-dimethylethylenediamine. In this report, degradation experiments were done in a 3:2 mixture of phosphate buffered $\text{D}_2\text{O}:\text{acetone}-d_6$ with periodic additions of dithiothreitol (DTT) at 37 °C. It is unclear how quickly the polymer will degrade under wholly aqueous conditions.

Galbis reported a copolyurethane composed of protected L-arabinitol, 2,2'-dithiodiethanol and 1,6-hexamethyldisocyanate (Scheme 16).¹⁴⁹ In the presence of glutathione at pH



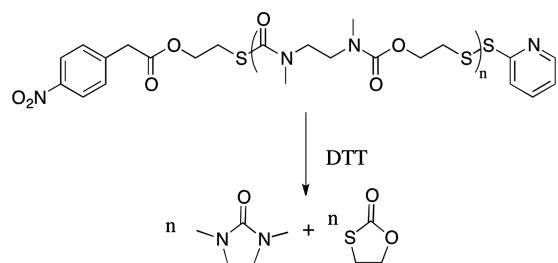
SCHEME 14 Disulfide reactions used in polymeric biomaterials include thiol-disulfide exchange, thiol-activated disulfide exchange, and disulfide reduction.

7.02 and 37 °C, the polymer fully degrades, and the rate of degradation depends on polymer composition (Fig. 13).

Therapeutic Release

Most research in reducible biomaterials focuses on delivery of therapeutic agents instead of simply material degradation. Reducible polymeric systems have been reported as carriers for proteins, DNA, siRNA, peptides, and chemotherapeutic agents.

In one example, the Hubbell and Swartz groups utilized PPS nanoparticles to deliver peptide antigens to dendritic cells. Peptide antigens conjugated to the nanoparticles through reducible disulfide linkages were able to be presented by the dendritic cells in the major histocompatibility complex I



SCHEME 15 Reducible, self-immolating polymer comprised of alternating *N,N*-dimethylethylenediamine and 2-mercaptopropanoate units.

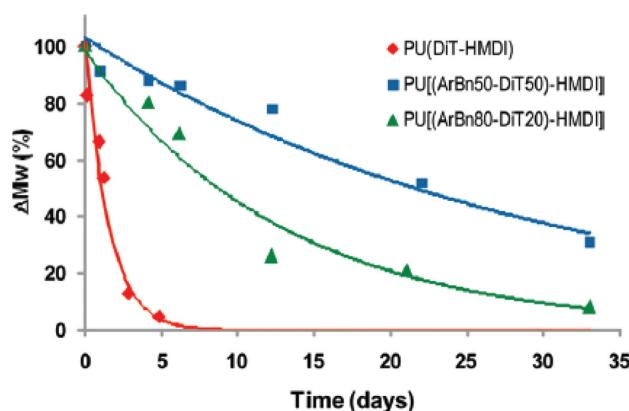
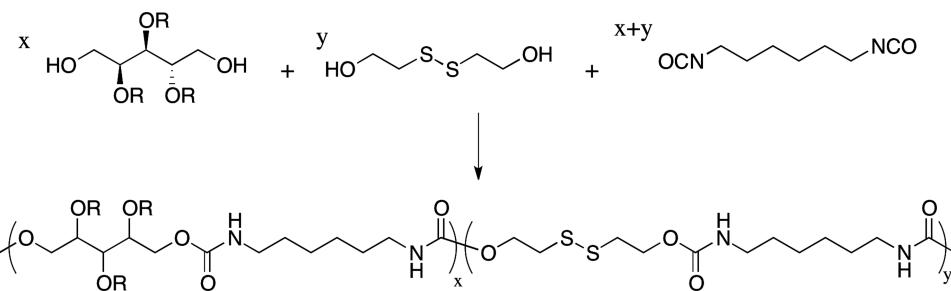


FIGURE 13 Mass loss of polyurethanes in the presence of GSH depends on composition (DiT: dithioethanol; HMDI: hexamethylene diisocyanate; ArBn: Arabinitol). (Reproduced from ref. no. 149, with permission from ACS Publications.)

(MHC I) and activate CD8+ T cells.¹⁵⁰ Notably, when the antigen was conjugated through a reducible disulfide linkage to the nanoparticle, less was required compared to nanoparticles in which the peptide antigen was tethered through a non-reducible linkage, indicating that the intracellular reducing environment allowed more efficient delivery of the peptide antigen to the dendritic cells.

Many groups have also explored reducible substrates for delivering genetic information to cells. Disulfide linkages linking together smaller poly(ethyleneimine) (PEI) chains have been reported by several groups.^{151–154} In most cases, transfection efficiency is compared to 25 kDa branched PEI, a common vector. In a typical example, Jiang et al. prepared hyperbranched disulfide-linked PEI, and used it to form polyplexes with plasmid pcDNA3-Luc and pEGFP (reporter genes for luciferase and green fluorescent protein, respectively).¹⁵¹ Compared to non-degradable branched PEI (25 kDa), the hyperbranched disulfide linked PEI was significantly less toxic to 293T cells. Furthermore, the reducible PEI showed higher transfection efficiency in HeLa cells when the N/P ratio was greater than 30. While these results are promising, the molecular weight of the reducible branched PEI was significantly lower (11.7 kDa) than the control (25 kDa).



SCHEME 16 Reduction-sensitive co-polyurethanes.

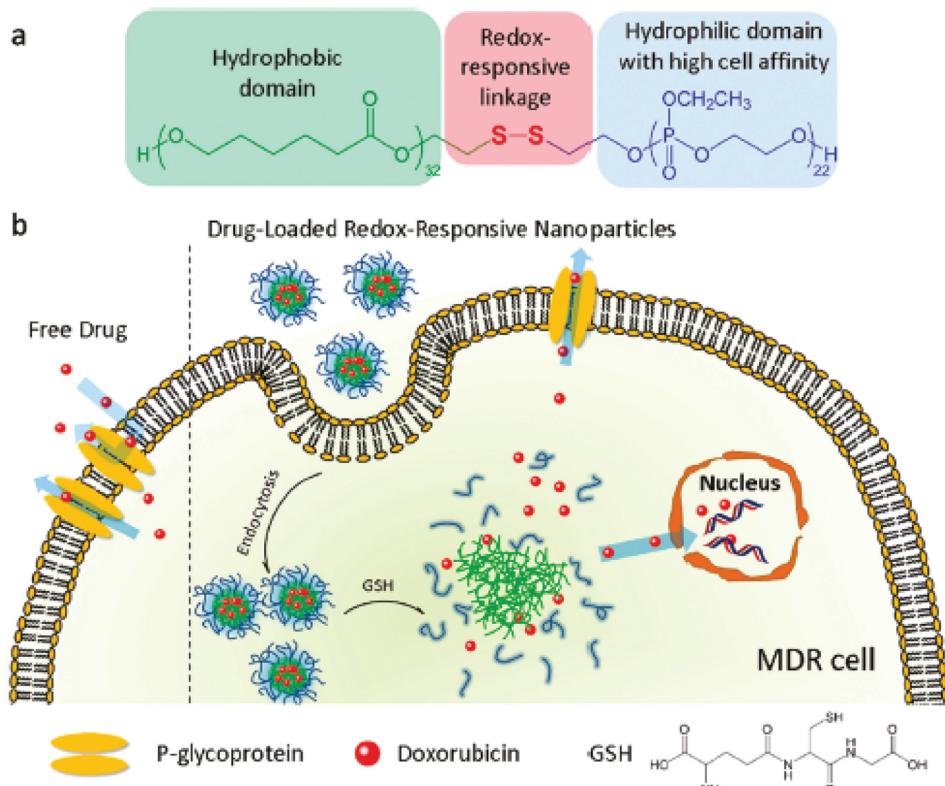


FIGURE 14 Receptor-mediated endocytosis of drug-loaded, reducible nanoparticles: (a) polymer structure and (b) cellular uptake. (Reproduced from ref. no. 158, with permission from ACS Publications.)

In another report, reducible and non-reducible crosslinked PEI were compared directly. Cell viability and transfection efficiency were found to be independent of reducibility, indicating that degradation of the disulfide bond may not play an important role in developing new PEI vectors for gene delivery.¹⁵⁴ Other reducible cationic vectors besides PEI have been reported.¹⁵⁵

In addition to gene delivery, reducible carriers have been used to deliver chemotherapeutic agents to cells. Many examples utilize micelles incorporating disulfide bonds between the hydrophilic and hydrophobic blocks.¹⁵⁶⁻¹⁶³ The hydrophobic chemotherapeutic agents (i.e., doxorubicin, paclitaxel) are sequestered in the hydrophobic core until the disulfide bond is reduced intracellularly and the micelle degrades. In tumor tissue, the intracellular concentration of glutathione may be two or more times higher than in healthy cells.¹⁶⁴ Both carrier size (EPR effect) and display of specific targeting ligands have been used to direct the carriers to cancerous tissue (Fig. 14).¹⁵⁸

EXTERNAL STIMULI FOR BIOMATERIAL MODULATION

In contrast to internal and environmental triggers, external triggers are particularly attractive to researchers as they inherently allow on-demand control over material properties in real time. Many triggers are possible, including light, magnetic field, electric field, and ultrasound. Other external

triggers are also possible; however, not all are physiologically relevant.

Photolysis

Photochemistry

Photochemistry has been used to form biomaterials for decades because light can be used in the presence of living cells and biomolecules without compromising viability or bioactivity. Photochemistry allows fabrication of complex biomaterial structures with well-defined chemical, mechanical, and physical properties. Light can be spatially focused in 2D and 3D, making it useful for creating complex patterned materials.

Photosensitive polymeric biomaterials can be classified in two ways—those in which covalent bonds are formed during exposure, and those in which covalent bonds are broken during exposure. In this work, we are interested in photodegradable materials, that is, materials in which covalent bonds are broken as a result of exposure to light. We can further classify reactions as photouncaging, photorelease, and photodegradation. In photorelease and photouncaging, exposure to light breaks a covalent bond that is not in the polymer backbone, but rather typically in a side chain. In photorelease, a therapeutic molecule is released from the polymer. In photouncaging, a functional group on the polymer chain is deprotected (uncaged) and the photolabile protecting group (cage) is released. In photodegradation, covalent bonds in the polymer backbone and/or crosslinks are broken; the polymer

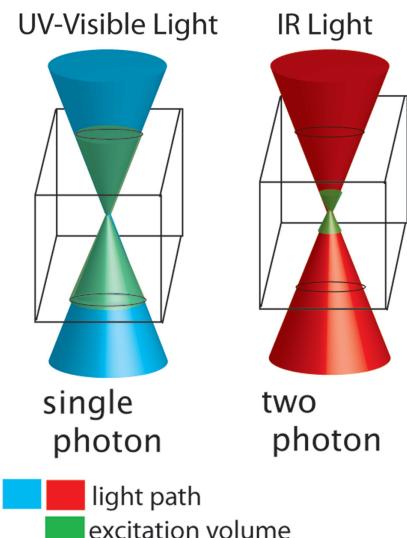


FIGURE 15 Single and two-photon excitation volumes.

behaves as a positive tone photoresist and degradation occurs only where the sample is exposed to light.

If a photodegradable system is to be used in the presence of live cells or tissues, the wavelengths, intensities, and exposure times are somewhat restricted so as not to cause damage to cells. Typically, only long-wave UV and longer light can be used, as shorter wavelengths can damage genetic information within the cell. Although this limitation can be somewhat overcome through short exposure times and/or low intensities, the possibility of damage still exists. The intensity of light and/or exposure time should also be minimized so as not to damage the cells. Biological structures may absorb light and become chemically altered, or the absorbed light may result in a local temperature change unhealthy for the cells. Finally, in complex systems such as living tissue or the human body, light is not always practical as it may be absorbed or scattered by other species in the physiological system.

Both single and multi-photon degradation can be used to chemically and/or physically pattern polymeric biomaterials over a broad range of size scales. In single-photon degradation, reactions occur in any part of the material exposed to light. In multi-photon degradation, multiple photons must be simultaneously absorbed, and therefore reactions only occur at the focal point of the light source (Fig. 15). The simultaneous absorption of multiple photons can result in an activity equivalent to that from the absorption of a single photon of the sum of their energies. Multi-photon photodegradation allows 3D control over material properties, and allows researchers to use lower energy, longer wavelength light. IR radiation holds several advantages over long-wave UV light. For example, at appropriate intensities it is not harmful to cells. Additionally, longer wavelength light can penetrate deeper into biological tissues and scatters less, which may allow for

better resolution. Photodamage of cells and other components of living systems in the out of focus regions is negligible in multi-photon experiments, due to their low absorptivity of IR light.

Light is an unique stimulus in that it can be both spatially and temporally controlled. Simply stated, degradation only occurs where you shine light, and when you shine line. Photomasks that physically limit the exposure of light are easily obtained and allow for rapid fabrication of patterned surfaces. More complex rastering or focusing techniques also allow 2D and 3D pattern generation.

Single Photon Uncaging

Photocaged surfaces have been utilized for patterning biomolecules for more than a decade. Initial efforts focused on caged biotins, as the affinity of avidins for uncaged biotin is one of the strongest known non-covalent interactions, allowing robust patterning post-exposure. One of the most commonly used photocaging moieties is an *ortho*-nitrobenzyl (*o*-NB) group, due to its availability and range of reactivity.^{165–167}

More recently, several research groups have reported photodeprotection of groups besides biotin that result in a chemically patterned surface. In an early example, Doh and Irvine generated patterned polyelectrolytes from a copolymer of methyl methacrylate, *o*-NB methacrylate, and PEG methacrylate.¹⁶⁸ Upon exposure to UV light, the *o*-NB group is removed to reveal a negatively charged carboxylate anion. Several review articles summarize efforts in photouncaging to generate patterned biomaterials.

Two-Photon Uncaging

Shoichet's group pioneered two-photon deprotection of polymeric biomaterials to control the spatial presentation of various ligands. Wylie and Shoichet used bromocoumarin-caged amines to modify agarose. After hydrogel formation, the coumarin cages were selectively removed via two-photon excitation to reveal free amines in spatially defined 3D regions in the agarose hydrogel.¹⁶⁹ Shoichet's group also reported agarose gels with 3D patterns of free thiols by using bromo-coumarin sulfide groups to modify the agarose gels.¹⁷⁰ After photodeprotection of the thiols, Wosnick et al. exploited their reactivity with maleimide groups to selectively immobilize a maleimide-functionalized fluorescent dye or biotin. Gels displaying 3D biotin patterns sequestered fluorescently labeled streptavidin through non-covalent interactions. Notably, the agarose-bound thiol groups were stable for prolonged periods of time.

Single Photon Drug Release

The Kasko lab reported a photosensitive polymerizable *o*-NB group conjugated to fluorescein as a model photoreleasable therapeutic agent.¹⁷¹ They copolymerized this macromer with PEG diacrylate to form hydrogels, and quantified the fluorescein release as a function of exposure conditions ($\lambda = 365\text{--}436\text{ nm}$, $I_0 = 5\text{--}20\text{ mW/cm}^2$, $t = 0\text{--}20\text{ min}$) and gel

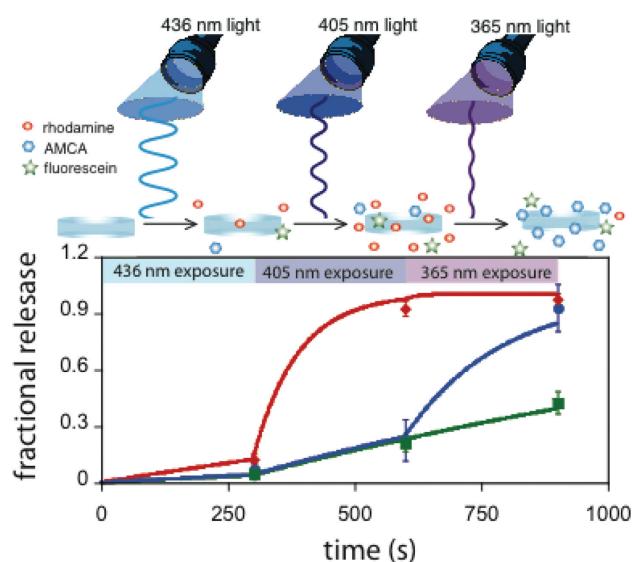


FIGURE 16 Fractional release of rhodamine, aminomethylcoumarin acetate (AMCA), and fluorescein from a hydrogel as a function of light exposure ($\lambda = 436$ nm, $I_0 = 44.6 \pm 1.0$ mW/cm 2 , $t = 5$ min, then $\lambda = 405$ nm, $I_0 = 21.4 \pm 1.1$ mW/cm 2 , $t = 5$ min, then $\lambda = 365$ nm, $I_0 = 5.53 \pm 0.14$ mW/cm 2 , $t = 5$ min); solid lines depict predicted release, actual release shown as data points. (Reproduced from ref. no. 173, with permission from ACS Publications.)

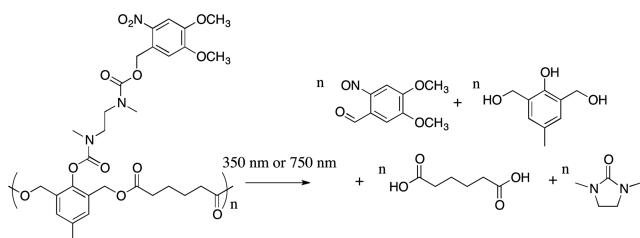
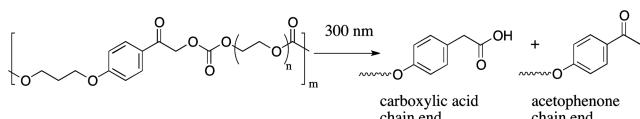
geometry. The predicted release matched the experimental release profiles for all exposure conditions and gel geometries. They next reported a series of *o*-NB linkers with different rates of photodegradation to allow the multistaged release of cells¹⁷² and model therapeutics (Fig. 16).¹⁷³ They also generated a library of *o*-NB linkers with different functionalities at the releasable site (Chart 1).¹⁷⁴ The *o*-NB macromers were used to sequester and release an amino acid (phenylalanine), peptides (glutathione, RGD), bovine serum albumin, and transforming growth factor beta, all without compromising bioactivity.

Johnson et al. reported brush copolymers with photoreleasable doxorubicin and camptothecin.¹⁷⁵ Simultaneous release of both drugs resulted in up to 30-fold higher toxicity than without light exposure, although the polymers exhibited some toxicity without triggering release of the chemotherapeutic agents.

Doxorubicin has also been conjugated to a PAMAM dendrimer through an *o*-NB linkage; in one example, the dendrimer also incorporated a ligand to target the folate receptor which is overexpressed by several types of cancer cells. Irradiation releases doxorubicin in a time-dependent manner, and *in vitro* studies indicate it is still therapeutically effective.¹⁷⁶

Macromer Structure	Reactive Group	Functional group reactivity	Functional		
			Macromer Structure	Reactive Group	Functional group reactivity
	Alcohol	Carboxylic acid, alkyl halides		NHS-ester	Amines
	Alkyl halide	Alcohols, nucleophiles		Pyridyl disulfide	Thiols
	Amine	Carboxylic acids		Biotin	Streptavidin
	Acrylate	Thiols, amines (pseudo-Michael addition)		Carboxylic acid	Amines, alcohols, alkyl halides

CHART 1 Reactivity of *o*-NB conjugates toward different functional groups found on biomolecules; R = -(CH₂)₃CO₂PEG526 methacrylate. (Adapted from ref. no. 175 and redrawn).

**SCHEME 17** Photodegradable polyester.**SCHEME 18** Photodegradation of alkoxyphenacyl esters.

Elisseeff's group conjugated isopropyl β -D-1-thiogalactopyranoside (IPTG) to PEG hydrogels through an *o*-NB group. Upon exposure to 302 nm light, IPTG is released and activates Chinese hamster ovary (CHO) cells transfected with LTR_i_EGFP (an inducible genetic switch), resulting in an increased EGFP expression by the CHO cells.¹⁷⁷

In addition to direct conjugation of therapeutics to photodegradable biomaterials, drugs may also be physically sequestered within materials via non-covalent interactions and released upon irradiation that causes breakdown of the entrapping polymer.

The Zhao group first reported photodegradable block copolymer micelles in 2004, and recently summarized the state-of-the art in this area.¹⁷⁸ For micelles, the photodegradable linker can connect two blocks, crosslink the core, be incorporated within the main chain of either block, or be used as a protecting group to allow triggered changes in hydrophobicity/hydrophilicity of one block. In a recent representative example of the last approach, Liu and Dong synthesized a block copolymer of PEG and poly(S-(*o*-NB)-L-cysteine). Upon UV irradiation, the *o*-NB linkage to the thiol in cysteine is cleaved, and the thiols in the poly(S-(*o*-NB)-L-cysteine) block form disulfide bonds, releasing doxorubicin loaded in the micelle.¹⁷⁹

Almutairi's group described the synthesis of an *o*-NB protected quinone-methide polymer that is stable in the absence of light. Upon exposure, nanoparticles fabricated from this polymer rapidly degrade and release a payload of Nile red, a model hydrophobic drug (Scheme 17).¹⁸⁰

Azagarsamy et al. physically entrapped proteins in cross-linked PEG-based nanoparticles incorporating *o*-NB groups. Upon irradiation, the mesh size of the crosslinked nanoparticle increased and released the protein.¹⁸¹ Although the authors only demonstrated single-photon degradation, two-photon degradation is also possible.

In a break from the typical *o*-NB group, Joy and coworkers developed an alkoxyphenacyl-based photodegradable polycarbonate. In a proof-of-concept experiment, Nile red was released (Scheme 18).¹⁸² Uniquely, these polymers have both a high modulus and high thermal stability, but are hydrolytically and photolytically degradable. However, the wavelength and exposure times required for photodegradation may be detrimental to cells ($\lambda = 300$ nm, 5.34 mW/cm², 5 min), and the products of degradation are not water soluble.

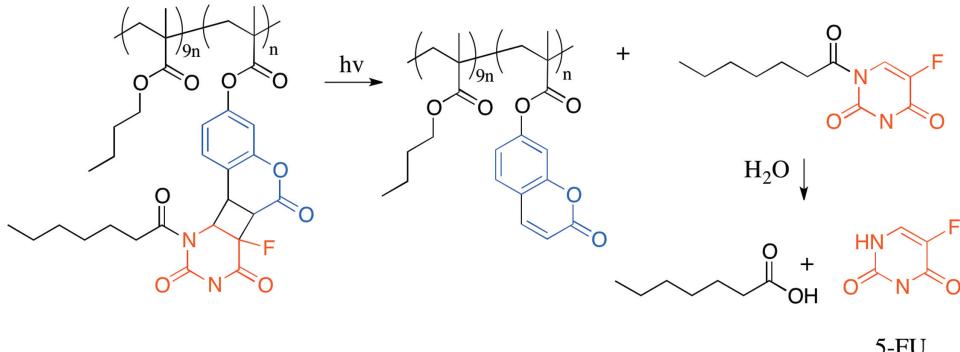
Two-Photon Drug Release

The *o*-NB capped polymer depicted in Scheme 17 is not only susceptible to single photon degradation (350 nm), but also susceptible to two-photon degradation (750 nm). However, this process is not efficient, as the two-photon absorption cross-section is very low (at $\lambda = 740$ nm, $\delta u = 0.01$ to 0.03 GM).¹⁶⁵ Therefore, other groups sensitive to two-photon activation/degradation (such as coumarins, where δu is on the order of 1 GM or higher¹⁸³) have been developed.

In a novel approach, a team at the University of Marburg covalently conjugated 5-fluorouracil (5-FU) to a polymethacrylate backbone through (reversible) photodimerization to a coumarin side group (Scheme 19). The polymer undergoes two-photon absorption at 532 nm, resulting in 5-FU release. This material has potential use as an intraocular lens, as 5-FU can be used to treat or prevent one of the most common post-implantation complications, posterior capsule calcification.¹⁸⁴

Single Photon Degradation

Photodegradable polymer networks have been reported by several groups, although not all systems are compatible with

**SCHEME 19** 5-FU releasing poly(methacrylate).

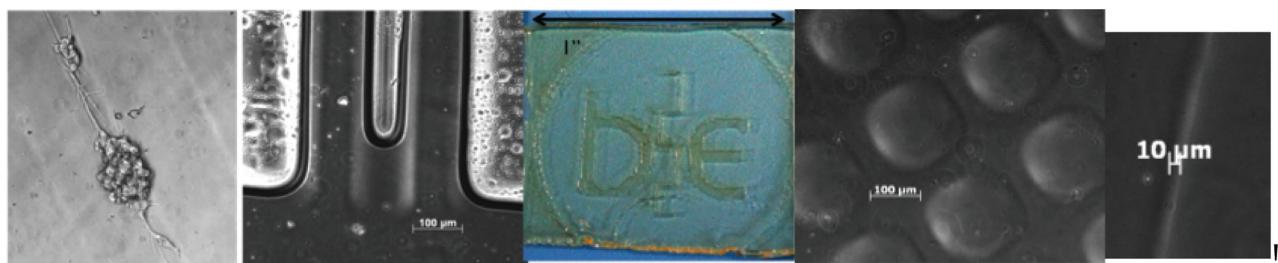


FIGURE 17 Multi-scale positive and negative features formed via single-photon etching (365 nm), L to R: Contact guidance of neurite outgrowth from PC-12 cells on 10 μm grooves; 100 μm channels formed in a device; macroscale logo; 100 μm square positive features protrude from hydrogel surface; 10 μm ridge. (Portions reproduced from ref. no. 195, with permission from ACS Publications.)

live cells. For example, in one report, macromers containing coumarin end groups are photodimerized at 365 nm.¹⁸⁵ This process is reversible at 254 nm. This can also be accomplished with anthracene end groups¹⁸⁶ and cinnamate end groups¹⁸⁷ (Scheme 20). These chemistries may be used to fabricate biomaterials and encapsulate cells, but are not suitable for photodegradation *in situ* because the wavelength used to reverse the photodimerization in these examples is not cell compatible (254 nm). Photodegradable hydrogels have been recently reviewed,¹⁸⁸ so we will highlight only the newest developments here.

Johnson et al. reported the synthesis of a photodegradable bifunctional initiator incorporating an *o*-NB group for atom transfer radical polymerization (ATRP) and used it to produce photocleavable poly(*t*-butyl acrylate).¹⁸⁹ Although they did not directly demonstrate its use as a biomaterial, PAA (easily obtained by hydrolyzing the *tert*-butyl esters) is a commonly used polymeric biomaterial.

Kloxin et al. incorporated an *o*-NB group into PEG macromers, and polymerized these macromers into hydrogel networks.^{190,191} They demonstrated surface erosion via single photon photolysis, channel formation via two-photon degradation, and control over cell phenotype through temporal control over the presentation of a cell adhesive ligand. The Anseth group further utilized this photodegradable system to investigate the effect of scaffold stiffness and topology on cell behavior.^{192–194}

The Kasko Lab explored the resolution limits of physical patterning of these systems via single and two-photon degradation, and uniquely utilized them as both positive and pseudo-negative photoresists (Fig. 17).¹⁹⁵

The Kasko group expanded the repertoire of PEG macromers containing *o*-NB linkers by synthesizing five different *o*-NB groups (chart 2) and comparing their degradation kinetics.¹⁷² Uniquely, incorporating multiple photodegradable groups into a single system allowed her group to achieve multi-staged degradation of the gel, resulting in the multi-staged release of cells (Fig. 18).¹⁷²

Moving away from the *o*-NB moiety, the Melman group synthesized alginate gels crosslinked with iron (III).¹⁹⁶ Both

chemical reduction (vitamin C) and photochemical reduction of iron (III) to iron (II) result in degelation. The photochemical reduction proceeds through co-oxidation of a coordinated ligand. In this report, the authors investigated several carboxylate ligands (butyric acid, methoxyacetic acid, formic acid, malic acid, and lactic acid) and observed the relative rate of oxidative decarboxylation of iron (III) and its concomitant reduction to iron (II). The rate of this reaction using lactic acid as a ligand was 2- to 86-fold higher than the other ligands. The light intensities and irradiation times used at 365 nm are comparable to the exposure used for *o*-NB photodegradation.

Two-Photon Degradation

In addition to *o*-NB groups, several other moieties are susceptible to two-photon excitation and degradation. The other most widely used moiety is coumarin, which has a high two-photon absorption cross-section.¹⁹⁷ However, researchers must carefully design systems containing coumarins, as they may themselves act as anticoagulants.

The Kasko group conjugated a coumarin fluorophore to the benzylic position of an *o*-NB group, and incorporated this conjugate into PEG macromers.¹⁹⁵ Conjugation of coumarins to *o*-NB groups enhances their two-photon action cross-section.¹⁹⁷ Hydrogels fabricated from the coumarin-*o*-NB PEG macromer exhibited increased degradation efficiency compared to hydrogels formed from an *o*-NB-PEG macromer without coumarin. Two-photon degradation of both samples (with and without coumarin) occurs at 730 nm and 810 nm but not at 872 nm, consistent with their single photon reactivities at 365 nm, 405 nm, and 436 nm. Submicron features are easily etched into the 3D volume of the hydrogels, including the fabrication of arbitrary features (Fig. 19).

Elisseeff and Montell combined the photodegradable *o*-NB/PEG hydrogel system with photoactivatable cells.¹⁹⁸ Channels were photoetched within the 3D volume of the gel, and MSCs transfected express a photoactivatable signaling pathway that enhanced migration.

Anseth's group has combined thiolene photoaddition reactions with *o*-NB photodegradation reaction in hydrogels to achieve 3D addition and subtraction of peptides in hydrogel

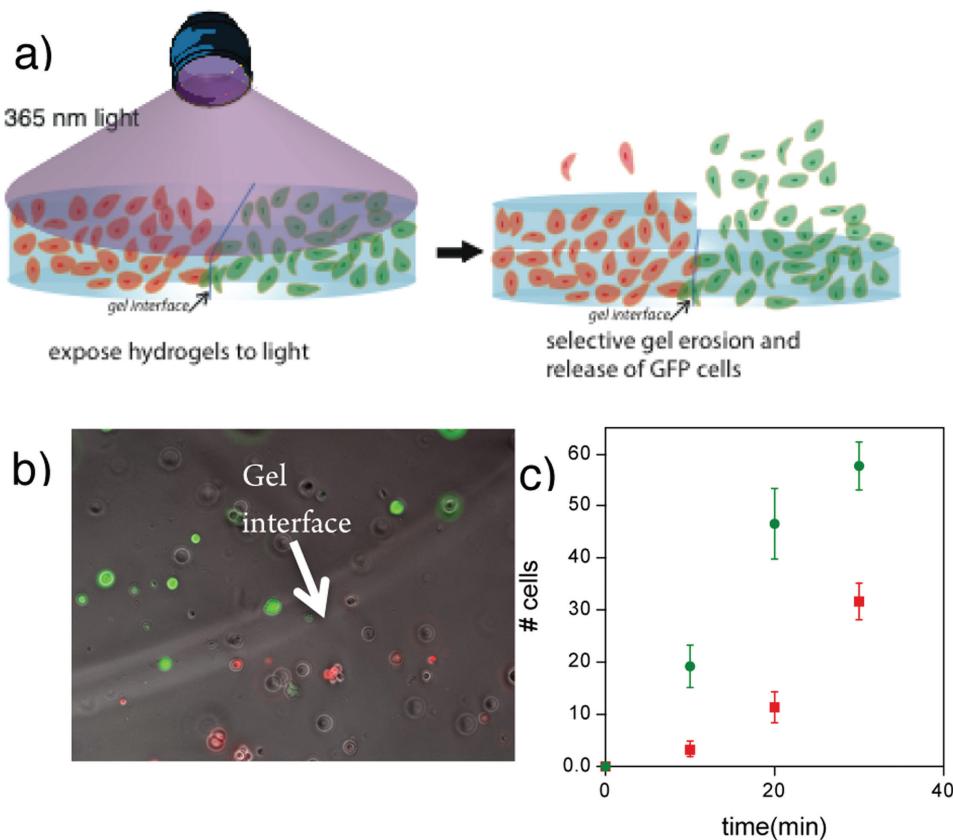


FIGURE 18 Wavelength-biased release of encapsulated cells. (a) Macromers containing either B (left of interface) or D (right of interface) were used to encapsulate RFG-expressing hMSCs or GFP-expressing hMSCs. (b) The interface between the two gels is directly observable in optical microscopy. (c) The rate of release of GFP cells from gel containing linker D is faster than the release of RFP-expressing cells from a gel containing linker B: this is consistent with ratio of the apparent rate constants of degradation ($k_{appD}/k_{appB} = 2.47$; $R_{GFP}/R_{RFP} = 2.36 \pm 0.68$). (Reproduced from ref. no. 172, with permission from ACS Publications.)

substrates¹⁹⁹ and to independently vary the mechanical and chemical properties of gels.²⁰⁰

The Seliktar group demonstrated non-specific two-photon photoablation of crosslinked PEGylated-fibrinogen gels, that is, no degradable linkages were incorporated and therefore photodegradation does not occur at a specific location or bond in the system.²⁰¹ Microchannels were photoablated in the gel at 840 nm close to dorsal root ganglia (DRG). Although the cell process from the DRG were able to invade the ablated regions, the light intensity utilized to fabricate the channels is too high for direct contact with cells, and may generate significant heat as the gel structure is ablated.

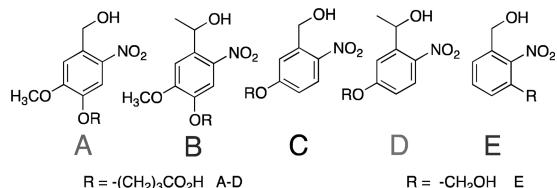


CHART 2 Series of *o*-NB linkers designed to have different degradation rates.

Electrical

Polymers that respond to an electrical stimulus have been utilized in numerous biomedical applications such as

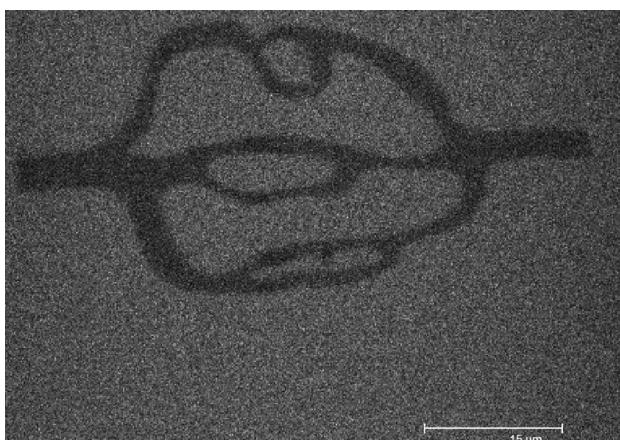
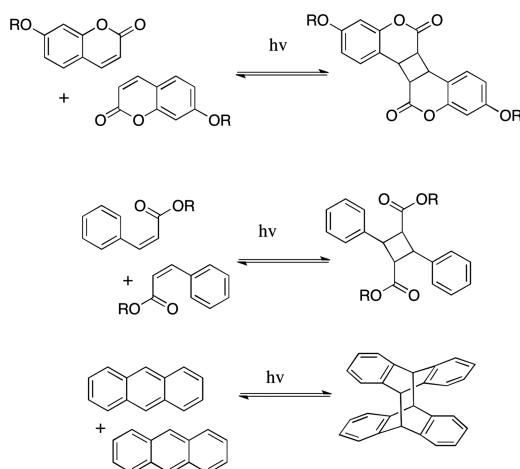


FIGURE 19 Two-photon lithography of a coumarin-conjugated macromer gel to obtain arbitrary features. Scale bar: 15 μm . (Reproduced from ref. no. 195, with permission from ACS Publications.)



SCHEME 20 Reversible photodimerization of coumarin, cinnamate groups and anthracene.

artificial muscle actuators,^{202–204} sensors,^{205,206} and drug delivery.^{207,208} Electroactive polymers can be classified in two major categories: electronic and ionic.^{202,209} Electronic electroactive polymers such as electrostrictive, electrostatic, piezoelectric, and ferroelectric polymers are able to hold strain under activation by a high DC voltage, which makes them useful for robotic applications. Ionic electroactive polymers are mainly polyelectrolytes, that is, polymers containing ionizable groups along their backbone that exhibit a change in the degree of ionization caused by the motion of ions upon low voltage. Synthetic and natural polyelectrolytes have been used including PAA, poly(vinyl alcohol), PAAm, poly(allylamine) (PAH), HA, chitosan, and alginate.

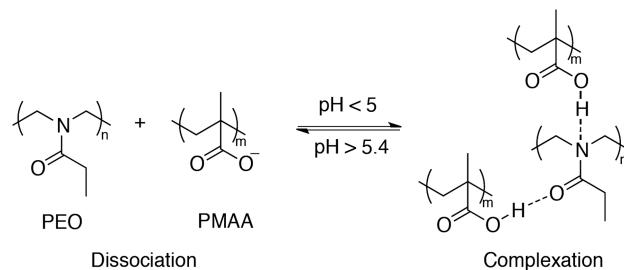
When stimulated by an electric field, electro-responsive polymers undergo structural changes such as crystalline/amorphous transition, deformation, swelling/deswelling, precipitation/dissolution, or erosion. Electrically induced degradation has been infrequently reported and usually involves the erosion of hydrogels.^{210,211} Typically, hydrogels are investigated for their ability to reversibly de-swell upon electrical stimulation.^{207,208} Because the response time of such hydrogels is long, their utility for rapid drug release is limited. To overcome that limitation, Kwon et al. prepared hydrogels able to quickly erode when exposed to an electric field.^{210,211} The erodible hydrogels were fabricated by complexation of water soluble polymers. Complexation in these systems was governed by either hydrogen bonds or ionic interactions in a pH-dependent manner. In one example, complexes of poly(ethyloxazoline) (PEOx) and poly(methacrylic acid) (PMAA) were formed by hydrogen bonds between the carboxylic and oxazoline groups at pH below 5, while above pH 5.4 the ionization of carboxylic groups led to the instantaneous dissolution of the complexes (Scheme 21).²¹⁰ After formation, the PEOx/PMAA hydrogels were immersed in a saline solution between two electrodes (attached only to the cathode). Application of an electric field

allowed the production of hydroxyls by electrolysis of water, resulting in an increase of the pH near the cathode and leading to the dissolution of the gel surface facing the cathode. The surface erosion could occur in a pulsed or continuous manner according to the employed electrical method. The authors demonstrated the utility of this system for pulsed release of insulin. While successful, it is difficult to imagine the utility of this approach in a physiological system, particularly because the system is restricted to low pH. The same group next developed a system operating at neutral pH. Complexes of PAH and heparin are formed by ionic interactions between the positive charges of amine groups and the negative charges of carboxylate and sulfate groups. These complexes are stable over a broad pH range (3–10), only undergoing dissociation below pH 2 and above pH 11, well out of normal physiological range (except for the stomach).²¹¹ Upon the application of an electric current, the production of hydroxyl ions at the cathode neutralized the cationic amine groups. As a result, the ionic bonds are disrupted and the gel surface disintegrates into hydrosoluble polymers.

To date, the progress concerning the electrically erodible gels remains limited and *in vivo* validation has not been established yet.

Magnetic Trigger

Polymeric materials that respond to an external magnetic field have recently drawn much interest for their potential use as artificial muscles,²¹² and in drug delivery.^{213–215} Magneto-responsive materials are particularly attractive as their response can be activated on demand by the on-off switch of a non-invasive magnetic field. Magnetically responsive polymers generally contain magnetic nanoparticles, which are either physically embedded into the polymeric matrix or chemically attached to the polymer chains. There are two main types of magnetic nanoparticles: iron oxide nanoparticles made of magnetite (Fe_3O_4), maghemite ($\gamma\text{-Fe}_2\text{O}_3$) or hematite (Fe_2O_3), and metallic nanoparticles made of iron, cobalt, or nickel. (One must carefully consider metal toxicity and/or allergy when designing such systems for *in vivo* use.) In the presence of an alternating current magnetic field, the magnetic moments of single-domain nanoparticles rotate. When they align with the field, the particles rotate as well, and ultimately generate heat governed by Néel and Brownian relaxation processes.^{216–218} Depending on the



SCHEME 21 Complexation between PEO and PMAA by hydrogen bonds at pH below 5 and dissociation at pH above 5.4.

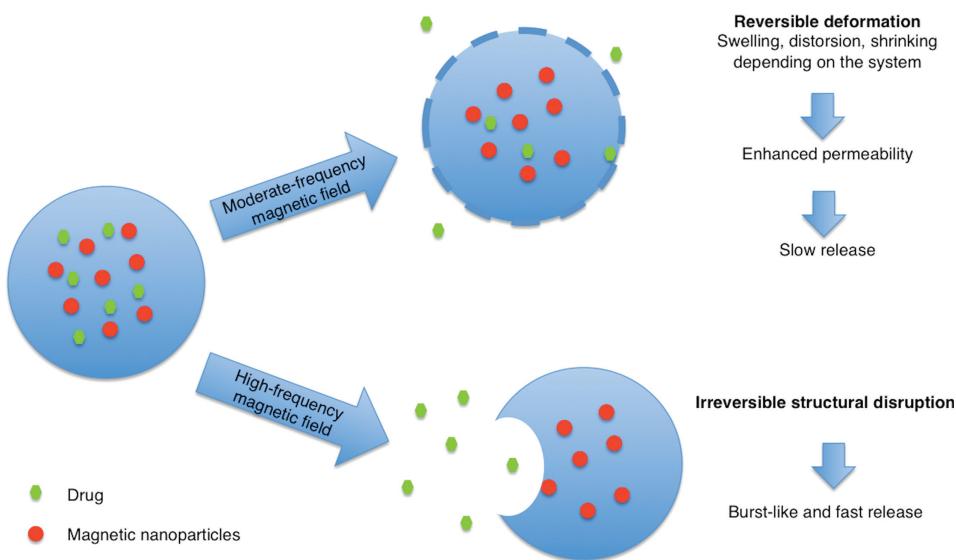


FIGURE 20 Schematic illustration of the structural changes of the magnetic-responsive polymeric carrier induced after exposure to a moderate- or high-frequency magnetic field and the resulting characteristics of drug release.

frequency of the magnetic field and the nature of the polymeric system, the responses induced upon magnetic field can be a deformation of the material,²¹⁹ possibly accompanied by a structural disruption. Despite the advantages of such a minimally invasive stimulus, to date, the demonstration of remote-controlled degradation using magnetic field stimulus has rarely been reported and the examples are mainly polymeric capsules.^{220–222}

Magnetic capsules exposed to a moderate magnetic field (100–300 Hz) show a layer structure distortion, which increases their permeability to entrapped molecules.^{223,224} The release rate of drugs from those capsules is relatively low and a faster response might be more desirable for some specific applications (immediate urgent physiological needs). Thus, a high-frequency magnetic field (50–300 kHz) can be used to accelerate the rotation of the embedded magnetic nanoparticles, and generate significant heat to burst the capsules and quickly release the drug (Fig. 20). For that purpose, magnetically degraded microcapsules made of Fe₃O₄ magnetic nanoparticles and PAH polyelectrolyte have been prepared by a LBL technique [Fig. 21(a)].²²⁰ Magnetic nanoparticles were physically entrapped within the thin PAH layer, which provided a rigid structure. Exposure of the microcapsules to a high-frequency magnetic field induced a three-stage shell structure evolution. First, the heat generation combined with magnetically induced stress in the shell causes a relaxation of polyelectrolytes, followed by the formation of nanocavities, which enhances the permeability of the microcapsules, and ultimately lead to a rupture of the shell. This structural evolution influences the drug release profile from a slow release to a burst-like behavior at different stages of stimulus [Fig. 21(b)]. The microcapsules show fast cellular uptake into human alveolar epithelial cells (A549 cell line) and exhibit low toxicity. In another report, Liu et al. combined both magnetic and thermal effects to

trigger controlled drug release caused by a disruption of nanocapsules.²²¹ They fabricated self-assembled nanocapsules made of a magnetic hydrophilic core inside a thermosensitive polymeric micelle.

Poly(ethyleneoxide)-poly(propyleneoxide)-poly(ethyleneoxide) (PEO-PPO-PEO) triblock copolymer was used as a thermoresponsive polymer to make the double-layer shell, which was stabilized by crosslinking, and iron oxide nanoparticles and vitamin B12 as a model drug were entrapped within the core. The nanocapsules exhibited a temperature-dependent contraction due to a hydrophilic/hydrophobic switch of the thermo-sensitive polymer above the critical micellization temperature. Upon high-frequency magnetic field, the magnetic nanocapsules underwent irreversible structural changes including iron oxide coarsening and core/shell disruption. These changes led to a fast burst-like release of the drug within a few minutes. In a similar approach, Hu et al. designed magnetically rupturable and thermosensitive yolk/shell capsules with an ultra-thin silica shell to prevent any drug release before application of the stimulus.²²² The main advantage of these thermo-responsive magnetic capsules is that they are more sensitive to smaller temperature fluctuations and can be degraded in a shorter time period, reducing the exposure to magnetic field. For a better overview of thermal and magnetic carriers, an excellent review published by Liu et al. should be consulted.²²⁵ Although magnetically responsive hydrogels have been extensively studied to trigger drug release,^{214,226–228} most rely on changes in swelling rather than magnetically dependent degradation. The first example has been reported by Hawkins et al.²²⁹ who developed nanocomposite hydrogels that degrade upon exposure to an external magnetic field. The hydrogels were synthesized by free-radical polymerization of a diacrylate macromer (Fig. 22) mixed with iron oxide (Fe₃O₄) nanoparticles. Application of a high-frequency field increases the degradation rate of the hydrogels due to a local heat produced by

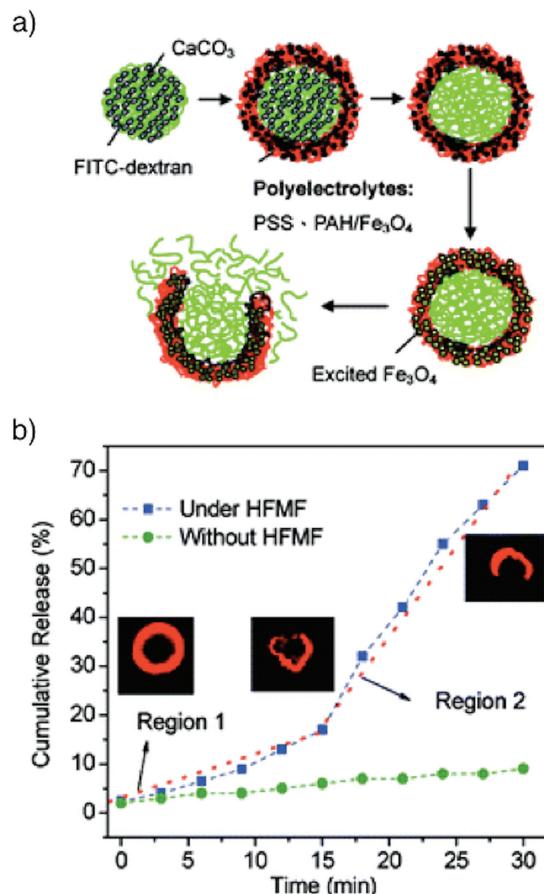


FIGURE 21 (a) Schematic representation of the encapsulation and release of substances in polyelectrolyte capsules. Calcium carbonate microspheres with fluorescein isothiocyanate (FITC)-dextran as the cores are coated with polyelectrolytes and nanoparticles using a LbL technique. After dissolution of the calcium carbonate, the hollow capsules are obtained. After a magnetic field treatment, the capsules should rupture and release the encapsulated substances. (b) The drug release behavior and morphologies of magnetic capsules under continuous high-frequency magnetic field (HFMF). (Reproduced from ref. no. 220, with permission from ACS Publications.)

the magnetic nanoparticles. Drugs physically entrapped within these nanocomposite gels undergo accelerated release in the magnetic field compared to the release from the control hydrogels. Although an *in vivo* application remains challenging at this stage, magnetically degradable hydrogels are promising for drug delivery as well as tissue engineering.

Magnetically induced degradation of magnetic-responsive polymers allows a sudden and rapid drug release on demand upon exposure to a high-frequency magnetic field. The magnetic properties of these systems can be multifunctional since they might also be used for hyperthermia in anti-tumor therapy and as contrast agents for magnetic resonance imaging (MRI). Nevertheless, the exposure of magnetic-responsive polymers to such high frequencies produces a significant elevation of the temperature, which could

harm the surrounding healthy tissues. Further *in vivo* studies should be considered to evaluate the effects of magnetically produced heat as well as the toxicity of the degradation products.

Ultrasound Trigger

The ability of ultrasound irradiation to induce polymer degradation via mechanical stress is well-established in the literature.^{230–233} When ultrasound is applied to a polymeric fluid, it causes acoustic cavitation (i.e., the creation, vibration, and implosive collapse of microbubbles), which produces intense shear on polymers. These shear forces can be strong enough to cause polymer chain scission, which occurs at the midpoint chain, in contrast to the random scission that occurs during thermal degradation.²³⁴ Ultrasound stimulus is relatively universal stimulus since it can be applied to a wide variety of polymers. However, it is difficult to define the specific structural characteristics of a polymer that result in ultrasound sensitivity. In comparison to magnetically responsive polymer composites, ultrasound does not require the addition of a specific agent. In comparison to photodegradable systems, ultrasound irradiation is a non-invasive technique that affords a much greater tissue penetration depth than light. Despite these advantages, the practical clinical use is restricted to high frequency ultrasound (>1 MHz), as the ultrasonic wave can be focused on the target site, minimizing the damage of surrounding tissues. However, at high frequency, cavitation is weaker, which may limit polymer degradation.

The effect of ultrasound has been broadly applied to increase drug release from both biodegradable and non-biodegradable polymeric carriers.^{235–240} Accelerated degradation of the polymers enhances drug release from biodegradable polymeric systems. In non-erodible polymeric matrices, enhancement of drug release is ascribed to the contribution of a convective term generated by cavitation without any destructive effect of the matrix.²⁴¹ In this section, we highlight advances in ultrasound-stimulated degradation of polymeric materials in the past decade. Ultrasound-induced degradation of polymeric devices is affected by numerous factors such as nature of the polymer, molecular weight, frequency, power, and irradiation time. It is important to note that it is difficult to directly compare different polymer chemistries, as the physical form of the polymer (solid, solution, micelle, capsule), along with its architecture and chemical structure affects its susceptibility to ultrasound-induced degradation (Fig. 23). Therefore, we have organized this section according to the physical form of

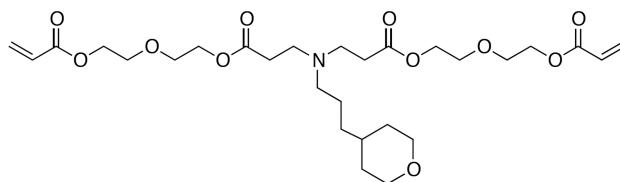


FIGURE 22 Diacrylate macromer used for the polymerization of magnetic hydrogels.²²⁹

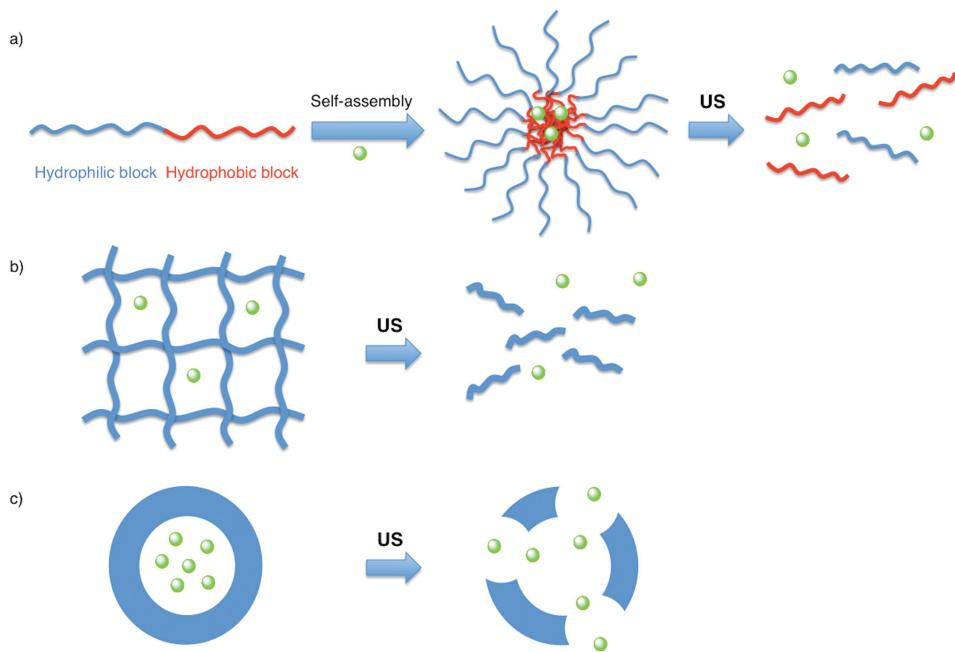


FIGURE 23 Degradation of polymeric architectures under ultrasound irradiation: (a) micelles, (b) hydrogels, and (c) capsules.

the polymeric system: micelles, erodible hydrogels, and microcapsules.

Micelles

Micelles and nanoparticles have been widely used for ultrasonic drug and gene delivery and have been already very well reviewed.^{242–244} Husseini et al. have greatly contributed to the understanding of the effects of ultrasound on polymeric micelles, and they have postulated that mechanical shear forces of shock waves caused by cavitation might result in the opening of the micelles leading to drug release.^{245,246} They also compared the kinetics of doxorubicin release from unstabilized and stabilized Pluronic P105 micelles.²⁴⁷ Stabilized micelles were synthesized using Pluronic P105 with a crosslinked network of *N,N*-diethylacrylamide. Doxorubicin release from unstabilized micelles is several times higher than the release from the stabilized micelles at the same frequencies and power intensities. Logically, the authors explained that the stronger integrity of the stabilized micelle cores renders them less susceptible to the shearing forces of cavitation. The group next investigated whether the release from stabilized micelles occurred by diffusion through the sheared network or by disruption of the micelles,²⁴⁸ and found that the covalent network of stabilized micelles was disrupted upon application of ultrasound. The degradation rates at two different frequencies but at the same mechanical index (measure of the probability and intensity of inertial cavitation) were found to be similar, suggesting a fundamental role of inertial cavitation. Although there is strong evidence that, upon application of an ultrasound field, mechanical shear forces disintegrate both unstabilized and stabilized micelles, the mechanism of degradation has not been clearly identified yet. Nevertheless, continued

efforts have been directed toward understanding the behavior of ultrasound-sensitive micelles.^{249,250} Zhang et al. investigated the irreversible release of Nile Red (NR) from poly(lactic acid)-*b*-poly(ethylene glycol) (PLA-*b*-PEG) micelles under high intensity focused ultrasound (HIFU).²⁴⁹ They hypothesized that if the ultrasound causes a simple physical disruption of the micelles, reassembly of the initial micellar structure should occur after switching off the signal. Since the molecular weight of the polymer chains decreased with increasing HIFU time, they concluded that the release of NR was due to a chemical chain breaking process of PLA-*b*-PEG induced by ultrasonic cavitation (note: they carefully excluded thermal effects and fluorescent dye degradation). Logically, the chemical structure of the polymer affects the rate and extent of degradation. The presence of labile or hydrolyzable bonds such as ester or acetal functions increases the sensitivity of polymer to ultrasound and favors a greater degree of degradation.²⁵⁰

Erodible Hydrogels

Precise spatial and temporal control over scaffold degradation remains a challenge for tissue engineering, and ultrasound is one of the most promising approaches to regulate the degradation of solid and hydrogel implants.²⁵¹ Agrawal et al. exposed biodegradable implants made of 50–50% poly(lactic-co-glycolic acid) (PLGA copolymer to ultrasound.²⁵² An increase in the ultrasound frequency enhanced the molecular weight loss, and less significantly mass loss, which contradicted previous studies showing a decrease in the degree of degradation with higher frequency for polymers in solution. The authors suggested that while the ultrasonic degradation of polymers in solution (or micelles as mentioned above) is due to a mechanochemical mechanism

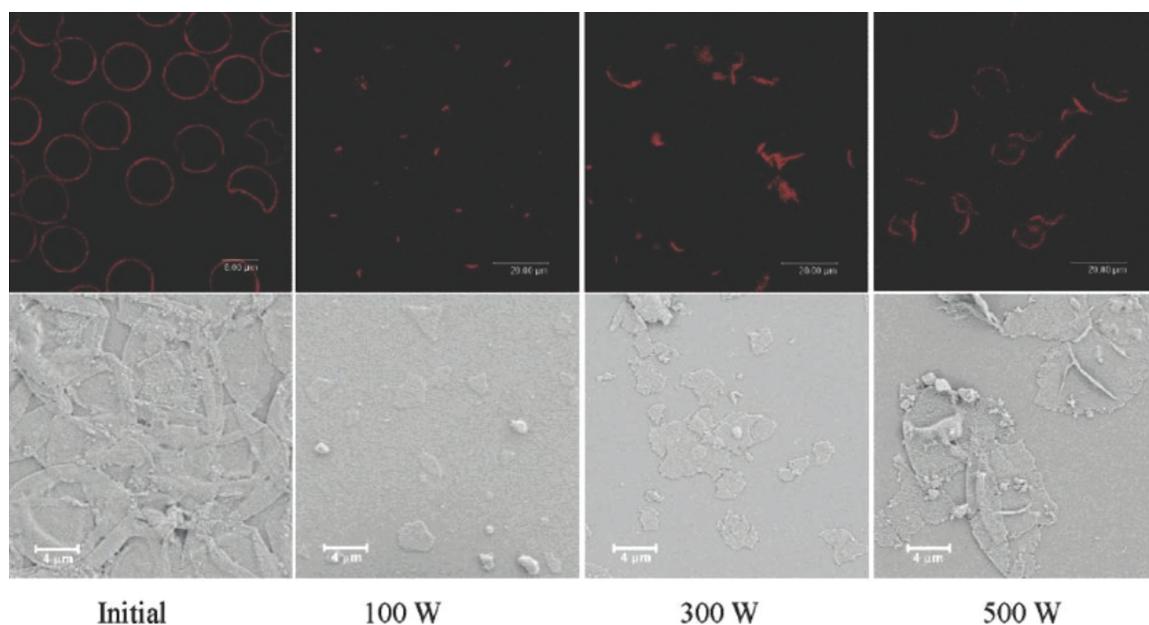


FIGURE 24 SEM (bottom panels) and fluorescence confocal (top panels) images of the initial Fe_3O_4 /polyelectrolyte capsules and Fe_3O_4 /polyelectrolyte capsules treated by ultrasound for 1 min at different powers. (Reproduced from ref. 253, with permission from ACS Publications.)

caused by the cavitation, the degradation of implants is primarily due to an increase in the rate of diffusion. Indeed, the energy supplied by the ultrasound could first increase the diffusion of water into the matrix promoting a faster hydrolysis of the polymer, and subsequently accelerate the transportation of degradation products out of the matrix. The authors observed that protein release from these matrices was three times greater when stimulated by ultrasound, further supporting the mechanism of ultrasound-enhanced diffusion in solid polymer matrices.

Microcapsules

The effect of ultrasound on microcapsules has also been studied, where it destroys the shell by mechanical shear forces.^{237,253} Multilayer hybrid and non-hybrid capsules were fabricated via LbL technique with different polyelectrolytes, PAH, and sodium poly(styrene sulfonate) (PSS), and gold nanoparticles (AuNPs).²³⁷ Ultrasound induces their destruction and the release of the encapsulated species. The level of degradation can be modulated by ultrasound power and irradiation time. Interestingly, the hybrid (AuNP/PAH) capsules were more stable toward ultrasound than capsules made solely of polyelectrolytes (PSS/PAH) owing to stronger interactions between the carboxyl groups on the surface of AuNPs and the cationic amino groups of PAH. A similar approach showed that the presence of magnetic (Fe_3O_4) nanoparticles within PSS/PAH polyelectrolyte capsules, besides imparting magnetic properties, increases the sensitivity of the capsules to ultrasound due to higher density contrast and lower elasticity of the shell.²⁵³ It is worth noting that the size of the shell fragments produced depends on the intensity of the acoustic waves; at low power (100 W), the capsules were disintegrated into smaller fragments than

at a power of 500 W (Fig. 24). Gas-filled microbubbles produced by sonication are commonly used as contrast agents for medical imaging. Furthermore, those microbubbles can also be employed as carriers for drug and genetic information but require protection with a lipid or polymeric shell.²⁵⁴ El-Sherif and Wheatley fabricated gas-filled microcapsules made of PLGA by a double emulsion method.²⁵⁵ Camphor was encapsulated in the oil phase and ammonium carbonate in the aqueous phase to help increase the porosity of the particles and achieve maximum acoustic enhancement. After fabrication, the camphor and ammonium carbonate are sublimed, leaving voids capable of being filled with a gas. El-Sherif et al. also investigated the effects of ultrasound on the degradation of PLGA microcapsule-based contrast agents.²⁵⁶ The degradation of the capsules was related to the acoustic efficiency, which is dependent on capsule morphology and frequency. In other words, the capsules degrade faster when they scatter more ultrasound. In addition, glycolic acid repeat units were shown to break down at a greater rate than lactic acid units due to higher hydrophilicity.

Ultrasound-responsive polymers have potential for use in imaging and drug delivery, and are particularly attractive because ultrasound is externally applied and minimally invasive. A polymer's susceptibility to ultrasound degradation is a function of its chemical structure as well as its physical form. While polymers in solution, micelles, and microcapsules are degraded by mechanical shearing forces due to cavitation, erodible solids are mostly degraded by enhanced diffusion of water within the matrix with a minimal effect of the cavitation. Although ultrasound-responsive systems have been extensively reported, structure–property relationships have not been well-established and thus many questions

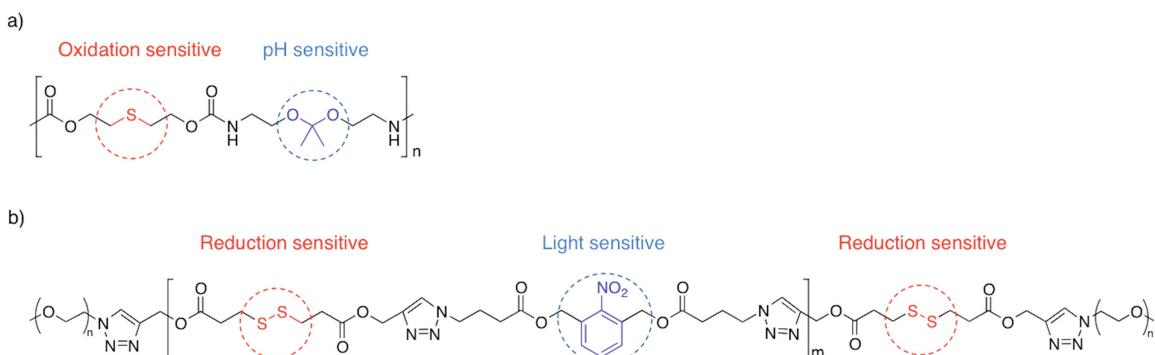


FIGURE 25 Dual-stimuli-responsive polymers: (a) oxidation- and pH-sensitive polymer, (b) reduction- and light-sensitive polymer.

remain. Further studies should focus on the elucidation of the degradation mechanism and the evaluation of the toxicity of degradation products.

DUAL-STIMULI-RESPONSIVE DEGRADATION

Many stimuli responsive polymers have been reported with demonstrated *in vitro* compatibility and efficiency. However, these systems may have limited relevance for *in vivo* applications, due to the complexity of the physiological system and the need for complex spatiotemporal control of the chemical and physical properties required to mimic natural processes. To better control polymer degradation, novel systems with a dual-stimuli-response to allow complex dynamic modulation of materials have emerged.

In dual stimuli systems, the degradation triggers may act in a cooperative manner or may act independent of one another. In an example of cooperativity, Mahmoud et al. designed polythioether ketal based nanoparticles that respond to both oxidative stress and the drop of pH that occurs in inflamed tissues [Fig. 25(a)].²⁵⁷ The degradation occurs in two stages; first, the hydrophobic thioether group is oxidized under exposure to ROS into more hydrophilic sulfones increasing the solubility of the polymer. Subsequently, that switch of solubility allows the rapid acid-catalyzed degradation of the ketal groups in mildly acidic environments. The nanoparticles remain intact when dispersed in acidic conditions without any oxidant for at least 24 h, and they swell in the presence of hydrogen peroxide at physiological pH due to an enhanced solubility of the polymer. Only the presence of both stimuli allows a complete degradation of the nanoparticles, leading to the release of encapsulated protein. Other systems responding to multiple stimuli include those responsive to pH/reduction,^{258,259} pH/temperature,⁷² or reduction/ultrasound²⁶⁰ to enhance a degradation that is too slow or does not occur with a single stimulus.

Both stimuli can also act independently of each other to induce two distinct responses, which might be useful to trigger the release of various substances at different time periods. Han et al. synthesized polymeric micelles made of PEO-*b*-poly(disulfide-alt-nitrobenzene)-*b*-PEO triblock copolymer

that could respond to two stimuli in different manners [Fig. 25(b)].²⁶¹ A disulfide bond and *o*-NB methyl ester group were incorporated by a two-step click chemistry approach in the hydrophobic middle block in order to trigger either a slow degradation of the micelles under the effect of a reducing agent, and as a result a slow release, or a fast degradation and a burst release by photolysis respectively.

CONCLUSIONS AND OUTLOOK

As our understanding of developmental biology, wound healing and disease has evolved, so has the sophistication of our approach in designing new polymeric biomaterials. In the past decade, polymer chemists have created a niche for biomaterials that degrade in response to physiologically relevant stimuli, including environmental triggers (water, pH, temperature), internal triggers (enzymes, ROS, reducing species), and external triggers (light, electric field, magnetic field). The incorporation of chemically degradable linkages in different polymer architectures and assemblies at the molecular level has advanced the biomaterials field, as degradation is important for both controlling release of therapeutic agents, and for eliminating biomaterials from the body once they are no longer needed.

Polymer chemists have exploited the chemical differences that have been observed between healthy and diseased cells and tissues (i.e., differences in intracellular concentration of ROS or reducing species) to deliver therapeutic agents in a controlled manner. Polymer chemists have also explored a wide variety of architectures (i.e., linear polymers, dendrimers, crosslinked networks) and structures (i.e., micelles, polymersomes, and solid nanoparticles) to aid in targeting carriers to diseased tissue (i.e., nanoparticles and the EPR effect) and releasing relevant doses of therapeutic agents. These new carriers for cells and therapeutic agents have been used in many applications, such the delivery of chemotherapeutic agents, other drugs, or genetic information, as polymeric pro-drugs, and as solid and hydrogel scaffolds for 2D and 3D cell culture. Although significant progress has been made, limitations still remain. It is important to note that majority of these degradable systems have not been tested *in vivo*. In a physiological system, many factors are

present that are not incorporated into *in vitro* testing, leading to discrepancies between *in vitro* and *in vivo* results. Specific limitations exist for each class of triggers. Internal stimuli such as enzymes, ROS, or reducing species are not always easy to predict or anticipate, in terms of both concentration and the timing of their appearance. Environmental stimuli such as water and temperature do not vary over a wide range, which makes them reliable, but typically the polymer's response to the stimulus is not instantaneous. Furthermore, once a polymeric biomaterial responding to these environmental stimuli is introduced into the body, its degradation rate cannot be altered or arrested. External stimuli are particularly attractive to researchers; however, these are the least explored stimuli *in vivo*, and practical challenges remain to deliver some stimuli into the body precisely. Despite these limitations, many opportunities exist for polymer chemists to design new dynamically responsive systems for biomedical applications. Future efforts will likely involve multiplexed systems to incorporate multiple material changes in response to multiple signals.

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