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Environment-Responsive Hydrogels for Drug Delivery

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87.1 Introduction

Diverse activities of biomolecules, cells, tissues, organs, and organisms are regulated through rapid, reversible, and repeated responses to a wide variety of environmental stimuli. For example, the mechanism of vision in human eyes is based on photoresponsive cis-trans isomerization of the retinal in rhodopsin. Influenza virus for infection and replication gains access to cytosol by pH-responsive conformational change of hemagglutinin protein (HA). A Venus flytrap closes its trap rapidly in response to the environmental stimulus of an insect landing on it. All living organisms, at their most basic levels, have macromolecules that vary physicochemical properties in response to environment stimuli.

Drug-delivery systems are intended to deliver drugs at proper times at proper sites. During the past two decades, a lot of work has been dedicated to the development of smart drug-delivery systems that can control drug release in response to environmental stimuli. Indeed, conventional drug formulations have moved forward to the smart drug-delivery systems that can sense disease signals, alter their physicochemical properties, and release the right amount of drug at the target sites at the right time. A variety of types of stimuli-responsive drug-delivery systems have been reported, which can respond to the external changes in environmental conditions, such as temperature, pH, light, glucose,

enzymes, antigens, inflammation, redox/thiol, ultrasound, magnetic, and electric field (Li and Keller 2009; Kojima 2010; Stuart et al. 2010). The term "environment-responsive" can be described in various ways. Typically, environment-responsive polymers in aqueous media vary their individual chain dimensions, secondary structures, ionization, solubility, intermolecular association, and supramolecular architecture. In most cases, the physical or chemical stimuli induce formation or destruction of secondary forces (hydrogen bonding, hydrophobic interaction, van der Waals forces, electrostatic interactions, etc.), chemical reactions of moieties pendant to the polymer backbone, or osmotic pressure differentials. Incorporating multiple environment-responsive groups along a polymer backbone can result in synergistic amplification for dramatic changes in macroscopic polymer properties. This approach is a form of biomimicry, since many biological macromolecules dramatically alter their conformation and three-dimensional (3D) structures in response to specific chemical species in their surroundings (Roy et al. 2010).

Water-soluble polymers can be physically or chemically cross-linked to form hydrogels, which are water-swollen polymeric materials with a 3D network structure (Kim et al. 1992). Owing to their high water content, the hydrogels exhibit excellent biocompatibility and have been widely used in the development of smart drug-delivery systems (Hamidi et al. 2008; Meng et al. 2009b; Oh et al. 2009). The 3D network can be formed by cross-linking polymer chains through covalent bonds, hydrogen bonding, hydrophobic interactions, or physical entanglements. Although individual polymer chains remain soluble in water, the cross-linking prevents individual molecules from dissolving in aqueous media. Instead, the polymer network swells as water diffuses into the interstices of the network while maintaining the physical integrity of the network itself. In this manner, the extent of cross-linking determines the extent of swelling and, also, the distance between chains within the cross-linked network. When entrapped molecules within the network are diffusing out, the rate of diffusion is dependent on the interchain separation and the size of the diffusing molecules.

Intelligent hydrogels have been extensively applied to the development of new drug-delivery matrices responding to several physiological stimuli arising from disease states or metabolic events in the human body (Onaca et al. 2009). One key strategy for drug-delivery systems was the spatiotemporal control of drug release responding to any changes in body physiology at specific sites (Kwon 2005). Stimuli-responsive hydrogels change their swelling degree or undergo phase transition in response to minimal changes in environmental conditions. Numerous monomers and cross-linking agents have been used for the synthesis of hydrogels with a wide range of chemical compositions. In this chapter, several types of stimuli-responsive hydrogels are introduced, and their applications to drug-delivery systems are discussed.

87.2 Thermoresponsive Hydrogels

87.2.1 Structure and Property

Temperature-responsive hydrogels are probably the most commonly studied class of environment-responsive systems in a drug-delivery field. Most natural polymers such as gelatin, agarose, and carrageenan show a thermoresponsive sol–gel phase transition with an upper critical solution temperature (UCST). That is, their aqueous solutions form a gel when the temperature is lowered. The sol phase is defined as a flowing fluid, whereas the gel phase is a nonflowing fluid, maintaining its integrity. These polymers undergo temperature-sensitive conformational change. Above UCST, they adopt a random coil conformation in the solution. Upon cooling, a continuous network is formed by partial helix conformation (Figure 87.1). However, the most extensively studied thermoresponsive hydrogels in smart drug-delivery systems are based on an inverse thermoresponsive sol–gel transition exhibiting a lower critical solution temperature (LCST). These polymers are readily soluble in water below LCST and form a gel above LCST. Figure 87.2 represents typical thermoresponsive polymers such as copolymers of *N*-isopropylacrylamide (NiPAAM), methylcellulose (MC), hydroxypropyl methylcellulose (HPMC), polyorganophosphazenes, and poly(*N*,*N*-diethylacrylamide) (PDEAAm). All these polymers share in

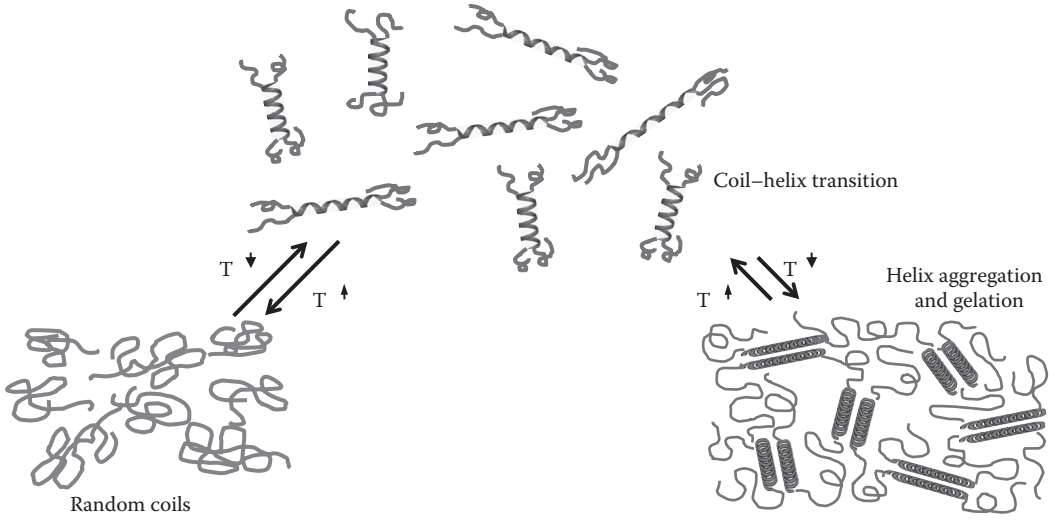

FIGURE 87.1 Schematic representation of thermoresponsive sol–gel phase transition of polymers with UCST.

common a unique hydration chemical structure in aqueous milieu that is metastable and can be altered by increasing thermal energy in the system. They are amphiphilic copolymers, having both hydrophilic and hydrophobic moieties, and their phase transitions are governed by the balance of hydrophilic and hydrophobic moieties on polymer chains. The main driving force for the thermoresponsive gelation is the temperature-triggered intermolecular association between hydrophobic groups. At lower temperatures, the polymers are fully hydrated in an aqueous medium, producing a clear solution. There are only simple entanglements of polymer chains that occur via weak polymer–polymer interactions. As the temperature rises, hydrogen bonds between water molecules and polymers gradually weaken, and hydrophobic association becomes more pronounced, thereby resulting in the formation of a hydrogel

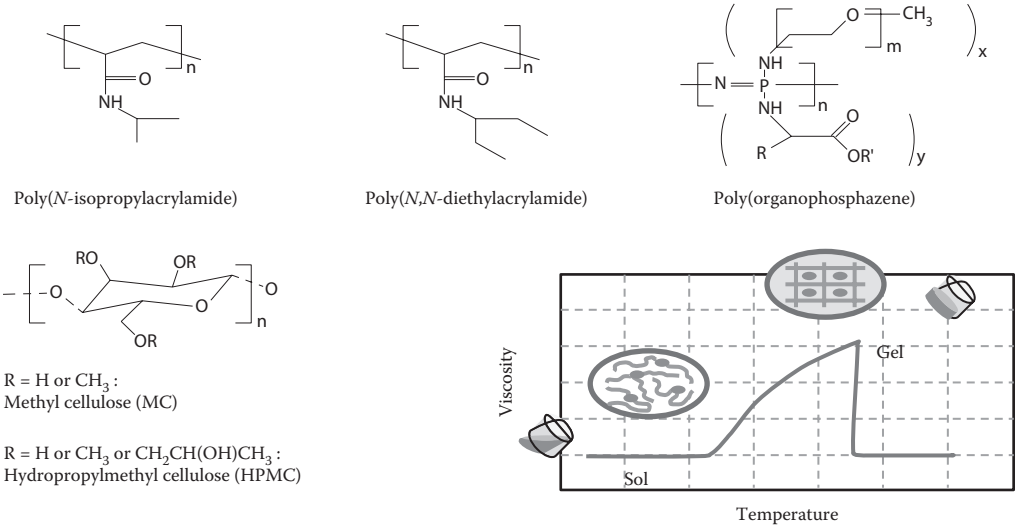

FIGURE 87.2 Structural formula of representative thermoresponsive polymers with LCST and schematic illustration of the sol–gel transition behavior.

structure. This phase transition is reversible, with some characteristic hysteresis, upon reversal of the temperature change.

As described above, the LCST of thermoresponsive polymers is governed by the balance between hydrophilicity and hydrophobicity. For example, the LCST of NiPAAM copolymers can be controlled by copolymerizing with other monomers with different hydrophobicity. The more hydrophobic comonomers, the lower the LCST. The presence of salts as a third component in aqueous solutions of thermosensitive polymers influences their thermogelling behaviors. The effect of salt is of importance, because salts are ubiquitous in biological systems. Salting-out solutes, such as NaCl, KF, $(NH_4)_2SO_4$, and Na_2CO_3 decrease the gelation temperature via their water-structure-making property, whereas salting-in solutes, such as KI, tend to result in increases in gelation temperature due to their water-structure-breaking properties.

Certain types of block copolymers including triblock copolymers of poly(ethylene oxide) and poly(propylene oxide) (PEO–PPO–PEO), and triblock copolymers of poly(ethylene glycol) and poly[(D,L-lactide)-co-(glycolide)] (PEG–PLGA–PEG) also exhibit an inverse temperature-responsive sol–gel phase transition (Zhao and Xu 2010) (Figure 87.3). A variety of PEO–PPO–PEO block copolymers are commercially available under the trade names of Pluronics® (or Poloxamer®) and Tetronics®, with varying compositions of PEO and PPO blocks (Wang et al. 2009b). The triblock copolymers form micelles above the critical micelle concentration (CMC). As the temperature increases, the equilibrium shifts from unimers to micelles, reducing the number of unassociated unimers in solution, leading to an increase in the micelle volume fraction (ϕ_m). The micelle volume fraction increases abruptly in a certain temperature range, which is dependent on the concentration. When the micelle volume fraction exceeds a critical value, the micellar solution becomes a gel by micelle packing.

Block copolymers composed of PEG and PLGA have received significant attention in a drug-delivery field, especially in an injectable *in situ* depot-forming system. PEG–PLGA–PEG triblock copolymers also show an inverse thermoresponsive gelation. Proper combinations of molecular weight and polymer architecture resulted in different LCSTs. Monomethoxy–PEG was used as a macroinitiator, to initialize the ring-opening polymerization of lactide and glycolide. Then two diblock copolymers (PEG–PLGA)

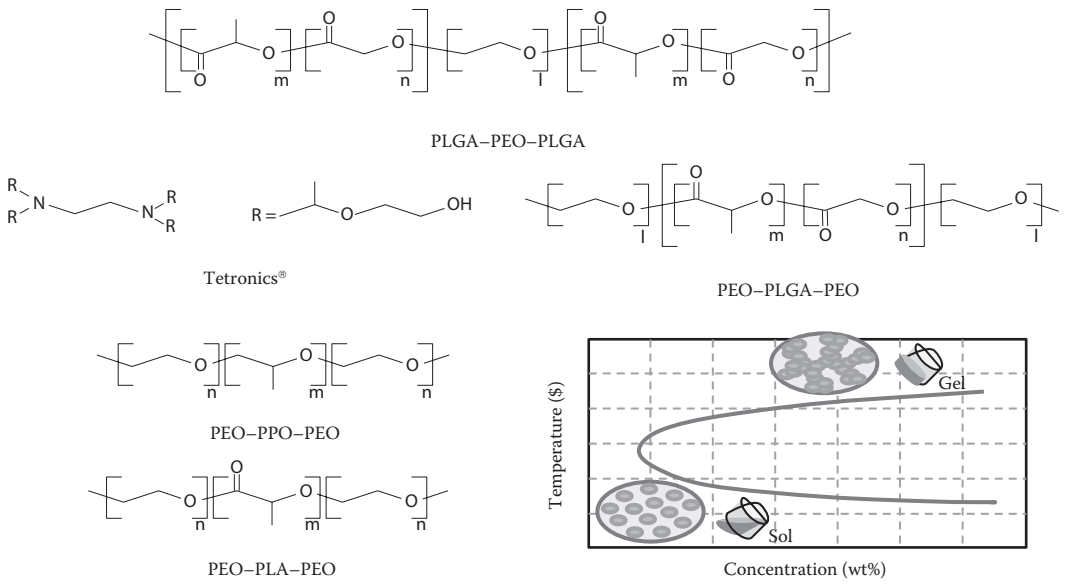

FIGURE 87.3 Structural formula of representative block copolymers with LCST and schematic illustration of the sol–gel transition behaviors.

were coupled using hexamethylene diisocyanate to form PEG–PLGA–PEG triblock copolymers that exhibited a relatively narrow molecular weight distribution (M_w/M_n ~ 1.2). The LCSTs were 30–36°C at polymer concentrations of 17–40 g/dL. A further increase in temperature affected gel appearance, from transparent to turbid, translucent, turbid, and then finally dissolving back to an opaque solution at a critical temperature ranging from 44°C to 70°C (Jeong et al. 1999b, 2002a).

PLGA–PEG–PLGA triblock copolymers can be synthesized via the bulk polymerization of PEG with lactide and glycolide in the presence of stannous 2-ethylhexanoate. These triblock copolymers had an analogous but inverted structure, compared to PEG–PLGA–PEG (Jeong et al. 1999a,b, 2000, 2002a). They showed three phases: solution, gel, and precipitate, depending on temperature. During the sol-to-gel transition, the aqueous solution of 23 g/dL showed large viscosity changes (approximately four orders of magnitude) from 0.4 P in the sol state to 5700 P at the onset of gelation at 13.6°C. Such a visco-elastic behavior was reproducible with repeated temperature changes. The *in vitro* degradation showed more rapid degradation at higher temperatures. Complete degradation occurred after 6–8 weeks at 37°C, whereas, at low temperatures, polymers were stable for 20–30 weeks at 5°C and more than 2 years at –10°C. The *in vivo* degradation after subcutaneous injection indicated that the hydrogel appearance dramatically changed between 2 and 4 weeks: initially, the polymer hydrogels decreased in size during the first 2 weeks; then, they became a mixture of gel and a viscous liquid, and then a completely viscous liquid with no gel; finally, the matrices were completely absorbed into the body, and followed a simple hydrolysis mechanism (Jeong et al. 1999a).

87.2.2 Application to Drug Delivery

Biodegradable, inverse thermoresponsive polymers with LCST are highly attractive as drug-delivery carriers because (1) their formulation requires no organic solvent; (2) the triblock copolymer matrices can be stored as dry, solid forms before administration; (3) highly hydrophobic, and thus practically insoluble drugs can be dissolved dramatically by simple mixing at ambient temperatures; and (4) drugs with a delivery vehicle can be injected directly by a syringe so that no surgical operation is necessary (Figure 87.4).

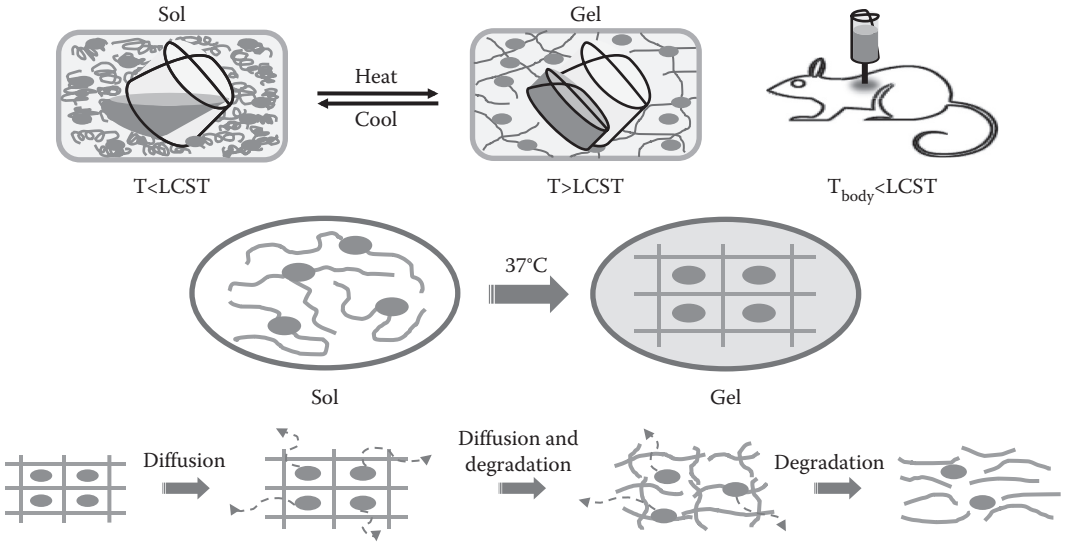

FIGURE 87.4 Schematic illustration of an injectable *in situ* depot-forming system using thermoresponsive sol–gel transition polymers.

Highly hydrophobic drugs such as paclitaxel (~1 μg/mL) and cyclosporine A (~4 μg/mL) can be dissolved by simply mixing these drugs with PLGA–PEG–PLGA triblock copolymer, a dramatic solubility increase over 2000-fold. These drugs were stable in the triblock copolymer formulations, with more than 85% stability. Paclitaxel release profiles showed a two-phase release pattern: a diffusion-governed mechanism for the initial 14 days, and the combined mechanism of diffusion and polymer degradation for the next 50 days. Direct injection of paclitaxel-containing triblock copolymer solutions to solid tumor in mice resulted in the gelation of polymers at injection sites, that is, within the tumor tissue. The drug remained at the tumor site for 42 days after injection, by which time it had gradually decreased to 20% (Zentner et al. 2001). PLGA–PEG–PLGA triblock copolymers were utilized for the delivery of peptide drugs including insulin (Kim et al. 2001), growth hormones (GHs), and granulocyte colony-stimulating factor (G-CSF). The *in vitro* protein drug release was monotonous and lasted for approximately 2 weeks after injection. The *in situ* forming hydrogels were applied to injectable cell delivery, so that cells stayed at desired sites. Such an approach has recently received a great deal of attention. Recent studies demonstrated that injected cells responded to external signals and produced bioactive compounds (Wang et al. 2009a).

Most polymers increase their water solubility as the temperature increases. Polymers with LCST, however, decrease their water solubility as the temperature increases. Hydrogels composed of LCST polymers shrink as the temperature increases above LCST. As described previously, the LCST can be changed by adjusting the ratio of the hydrophilic and hydrophobic segment. Such a strategy can be exploited so that a hydrogel's transition temperature can be controlled and then the drug release behavior can be regulated (Bromberg and Ron 1998). Below the LCST, drugs show diffusion-dependent release from a swollen gel (Figure 87.5a). The drug release profile can be altered when the temperature-triggered collapse of the gel occurs (Figure 87.5b). The entrapped drugs can be released by "squeezing-out effect" from the collapsed gel above the LCST. Figure 87.5c shows the "on–off" control of drug passage through

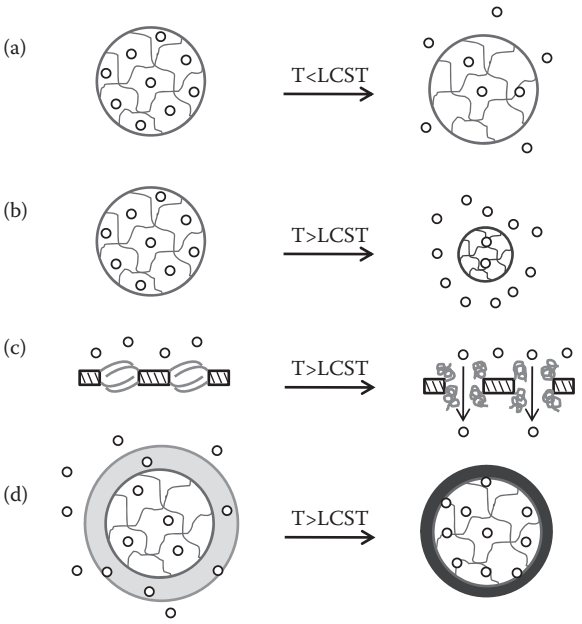

FIGURE 87.5 Smart drug-delivery systems using thermoresponsive polymers. (a) Diffusion-controlled drug release below LCST. (b) "Squeezing out" effect above LCST. (c) "On–off" control of drug release. (d) Heterogeneous microgels with a thermo-responsive shell. (Adapted from Bromberg, L. E., and Ron, E. S. 1998. Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. *Adv. Drug Deliv. Rev.*, 31: 197–221.)

the membranes that contain thermoresponsive hydrogel segments. Swollen gel blocks drug passage through the pores, and allows permeation when collapsed. Figure 87.5d describes another pulsatile drug release system. When a shell layer is made of thermoresponsive hydrogels with LCST, it may form a dense shell layer of the collapsed component while the core remains swollen.

The drug release behavior from PEG–PLGA–PEG *in situ* forming hydrogels has been extensively studied. For example, two low-molecular-weight compounds, ketoprofen and spironolactone, were used as model drug molecules having different hydrophobicities. The relatively hydrophilic ketoprofen was released monotonously through diffusion mechanisms, with approximately 90% of the drug released within 5 days. In contrast, the more hydrophobic spironolactone showed a sigmoid curve, with the release extending over 50 days. Given that the polymeric micellar structure was maintained within the triblock copolymer gels, the spironolactone molecules in PEG shell layers were released mainly by a diffusion process, while drugs preferentially existing in the hydrophobic micelle core were released via diffusion and bulk micelle matrix degradation. Thus, the longer-term sustained release of drugs was achieved using PEG–PLGA–PEG triblock copolymer hydrogels (Jeong et al. 2000, 2002b).

87.3 pH-Responsive Hydrogels

87.3.1 Structure and Property

The pH sensitivity has been one of the important parameters in designing a smart drug-delivery system because the pH change frequently occurs at pathological sites. For ionic hydrogels, the degree of swelling and drug release significantly depend on the environmental pH. The pH-responsive polymers can be classified as acidic weak polyelectrolytes containing pendant acidic groups (e.g., carboxylic and sulfonic acids) and basic weak polyelectrolytes with pendant basic (e.g., amine) groups (Figure 87.6). They accept or release protons in response to changes in environmental pH. Typical acidic pH-sensitive polymers containing carboxylic groups include poly(acrylic acid) (PAA), poly(methacrylic acid) (PMA), poly(L-glutamic acid), and alginate (Figure 87.7). Typical examples of the basic polyelectrolytes containing amine groups include poly(tertiary amine methacrylate), poly(2-vinylpyridine), poly(L-lysine), poly(L-histidine), poly(β-amino ester), and chitosan (Figure 87.7).

The presence of ionizable groups on polymer chains results in swelling of the hydrogels; far beyond that, it can be achievable by nonelectrolyte polymer hydrogels. Since the swelling of polyelectrolyte hydrogels is mainly due to the electrostatic repulsion among charges present on polymer chains, the extent of swelling is influenced by any changes that reduce or enhance electrostatic repulsion, such as pH, ionic strength, and type of counterions (Qiu and Park 2001). Swelling of ionic hydrogels sharply

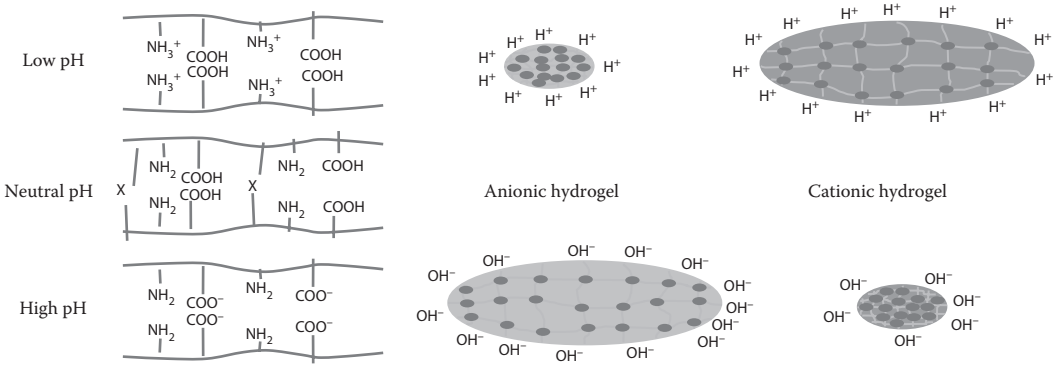

FIGURE 87.6 Schematic illustration of swelling/deswelling behaviors of different types of pH-responsive hydrogels.

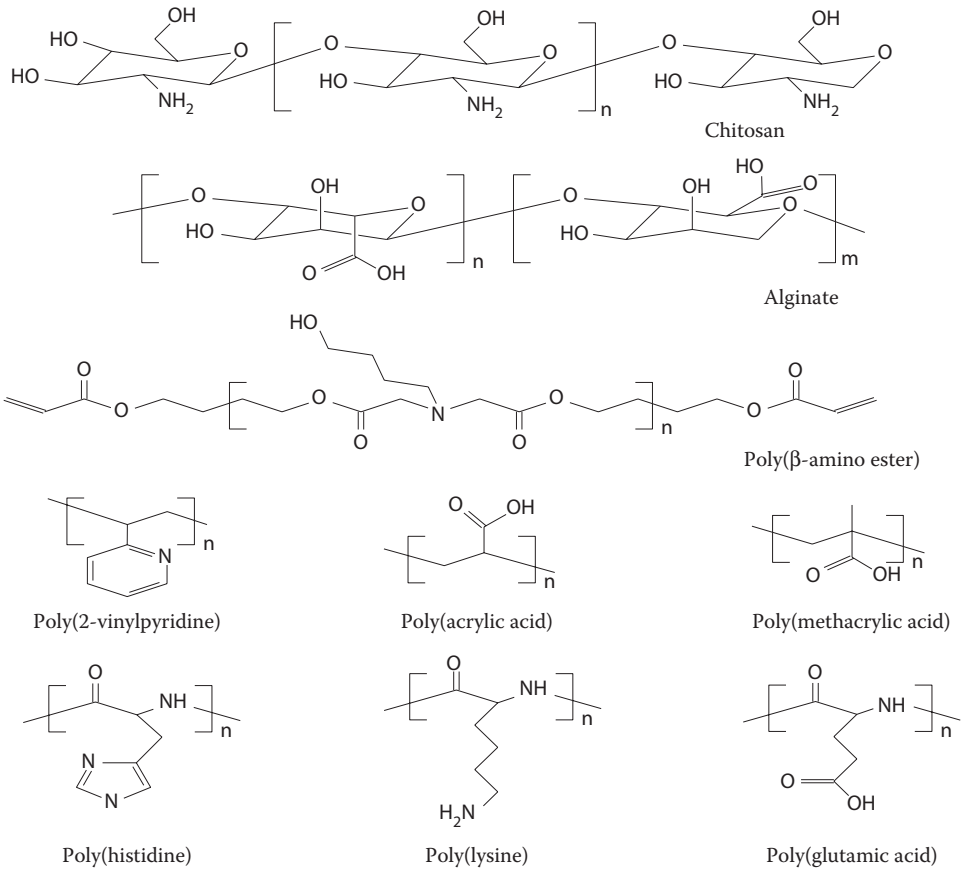

FIGURE 87.7 Structural formula of various pH-responsive polymers.

changes in the vicinity of their pK_a or pK_b values. Anionic hydrogels deprotonate and swell more when external pH is higher than pK_a of the ionizable groups bonded on polymer chains, while cationic hydrogels protonate and swell more when external pH is lower than the pK_b of the ionizable groups. By using two or more ionic monomers, the pH-dependent swelling curves can exhibit two or more inflection points near the pK_a/pK_b of the ionizable groups. As described above, the pendant acidic or basic groups on polymers undergo ionization such as acidic or basic groups of monomers. However, it should be noted that the ionization on the polymer is more difficult due to electrostatic effects exerted by other adjacent ionized groups. This tends to make the apparent dissociation constant (K_a) different from that of the corresponding monoacid or monobase.

The pH-responsive swelling/deswelling of polyelectrolyte hydrogels can be further manipulated by adding nonionic comonomers, such as 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate (MMA), and maleic anhydride (MA) (Zentner et al. 2001). Different comonomers provide different hydrophobicity to the polymer chain, leading to different pH-responsive behaviors (Kim et al. 2001). At low pH, the acidic protons of the carboxyl groups of PMA interact with the ether oxygen of PEG through hydrogen bonding, and such complexation results in shrinkage of the hydrogels. As the carboxyl groups of PMA become ionized at high pH, the resulting decomplexation leads to the swelling of the hydrogels. Cross-linked copolymer hydrogels of poly(L-glutamic acid) and PEO showed rapid swelling and deswelling behavior. The swelling of this hydrogel varied with pH and increased at higher ionization of the poly(L-glutamic acid), which resulted from not only the electrostatic effects but also the secondary

structural change associated with the polypeptide backbone. By modifying the hydrophobicity of polypeptide and the degree of ionization, the overall extent of pH-responsive swelling could be controlled.

Just like temperature-responsive sol–gel transition polymers, block copolymers composed of pH-responsive polymers and neutral polymers exhibit pH-sensitive sol–gel transition. Some triblock copolymers, such as poly(diphenylamine)–poly(2-methacryloyloxyethyl phosphorylcholine–poly(diphenylamine) (PDPA–PMPC–PDPA), form physical hydrogels at 37°C. At pH of 8 or less, the amino group is protonated and the block copolymers remain in solution, while under alkaline pH conditions, PDPA is sufficiently hydrophobic to form physical hydrogels. Triblock and three-arm star diblock copolymers can be synthesized with atom transfer radical polymerization (ATRP) initiated by bifunctional and trifunctional initiators, respectively, with the central block, poly(glycerol methacrylate) (PGMA), and the outer pH-responsive PDEA or PDPA blocks. The hydrogel from these block copolymers showed reversible, pH-responsive sol–gel transition; the free-standing gel formation was observed at neutral or higher pH, but dissolved in acidic solution. Armes and coworkers synthesized pH-responsive microgels with a diameter of approximately 250 nm by the emulsion polymerization of 2-(diethylamino)ethyl methacrylate (DEAEMA) with a bifunctional oligo(propylene oxide)-based diacrylate cross-linker and a PEO-based macromonomer. The microgels showed reversible swelling properties in response to pH. At low pH, microgels swelled due to the protonation of the tertiary amine units. On the contrary, compact latex particles due to the deswelling occurred when pH was >7.

87.3.2 Application to Drug Delivery

pH-responsive hydrogels have been frequently used to develop controlled-release formulations for oral administration. The gastrointestinal tract is known to possess a wide pH range, from a gastric pH of 1–2 to an intestinal tract pH of 7–8. Such significant changes can be utilized for the design of pH-responsive drug-delivery devices. Tumor sites and some sites of infection are known to have local acidic pH values amenable to pH-responsive release systems (Ghandehari et al. 1997). For polycationic hydrogels, the swelling is minimal at neutral pH, thus minimizing drug release from the hydrogels.

Hydrogels made of polyanions (e.g., PAA) cross-linked with azoaromatic cross-linkers were developed for colon-specific drug delivery. Swelling of such hydrogels in the stomach is minimal and thus, the drug release is also minimal. The extent of swelling increases as the hydrogel passes down the intestinal tract due to increase in pH leading to ionization of the carboxylic groups. But, only in the colon, can the azoaromatic cross-links of the hydrogels be degraded by azoreductase produced by the microbial flora of the colon (Ghandehari et al. 1997; Akala et al. 1998). The degradation kinetics and pattern can be controlled by the cross-linking density. The kinetics of hydrogel swelling can also be controlled by changing the polymer composition (Akala et al. 1998).

pH-responsive hydrogels can be placed inside capsules (Gutowska et al. 1997) or silicone matrices (Ghandehari et al. 1997; Akala et al. 1998) to modulate drug release. The release patterns of several model drugs having different aqueous solubilities and partitioning properties (including salicylamide, nicotinamide, clonidine HCl, and prednisolone) were correlated with the pH-dependent swelling pattern. At pH 1.2, the hydrogel swelling was low and the release was limited to an initial burst. At pH 6.8, the network became ionized and higher swelling resulted in increased release (Qu et al. 2006).

ABA-type triblock copolymers showing pH-responsive micelle formation and gelation were prepared through an ATRP. The A block consisted of either poly(2-(diisopropylamino) ethyl methacrylate) (PDPEA) or poly(2-(diethylamino) ethyl methacrylate) (PDEMA), and the B block contained poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC). At low pH regions, the amino groups in the A blocks were protonated and highly soluble in water, whereas they were deprotonated at neutral or higher pH ranges. At neutral pHs, the triblock copolymers became micelles in which the A blocks formed hydrophobic aggregated cores and the neutral hydrophilic B blocks formed the outer shell. At higher polymer concentrations in the basic pH solution, physical gels were formed. Thus, at physiological pH, drugs

could be incorporated into the micelle cores, and a slow release of the drugs was achieved. At pH 2, the polymer gels immediately dissolved and released drugs rapidly (Ma et al. 2003).

87.4 Light-Responsive Hydrogels

Photoresponsive polymers are macromolecules that change their physicochemical properties by irradiation with an appropriate wavelength (Kumar and Neckers 1989; Dai et al. 2009). Potential applications of the photoresponsive polymers include reversible optical storage, polymer viscosity control, photomechanical transduction and actuation, bioactivity switching of proteins, tissue engineering, and pulsatile drug- delivery devices (Shimoboji et al. 2002a,b). It is an important aspect of photoresponsive hydrogel systems that irradiation as a stimulus is a relatively straightforward and noninvasive method of inducing responsive behaviors. These types of polymers have been investigated for many years, but there has been a recent expansion in research to create complex macromolecular architectures.

Typically, the photoresponsive polymer is constructed by incorporating chromophores that can transfer light energy into a change in conformation. The chromophore should show a property change during isomerization large enough to cause a conformational change in the polymer. The chromophore is transformed under photoirradiation into isomers that return to the initial state either thermally or photochemically. This isomerization is called "photochromism." During the photochromism, some physicochemical properties of the chromophores are changed including geometrical structures, dipole moment, and charge generation. Figure 87.8 shows the typical chromophores that are often incorporated into the photoresponsive polymers (Irie 1990; He et al. 2009) into the backbone or side chains.

Azobenzene is the most frequently used chromophore. It undergoes isomerization from the trans- to the cis-form under ultraviolet (UV) irradiation (300–400 nm). The cis-form can return thermally or photochemically to the trans-form. The trans-to-cis isomerization causes three important physical property changes: (1) a change in the absorption spectrum—a decrease in the intense absorption at 320 nm and an increase in absorption at 440 nm, (2) a change in geometry—a shortening of the distance between the 4- and 4′-carbon from 0.9 nm (trans) to 0.55 nm (cis), and (3) a change in dipole moment from 0.5 D (trans) to 3.1 D (cis). The photomechanical effect of photoresponsive hydrogels that show a reversible contraction and expansion is based on the geometrical change of azobenzene embedded in the polymer hydrogel network. When the polymer hydrogel network containing azobenzene chromophores as a cross-linker is stretched, the azobenzene chromophores are preferentially oriented parallel to the stretching axis. With irradiation of such an oriented sample with UV light, the conformational change of azochromophores is expected to cause a macroscopic change in the overall hydrogel shape.

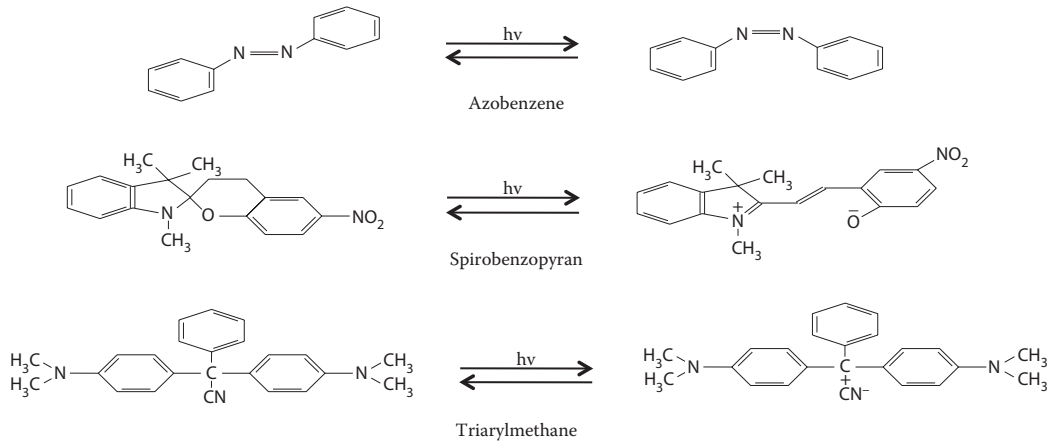

FIGURE 87.8 Structural formula of representative chromophores.

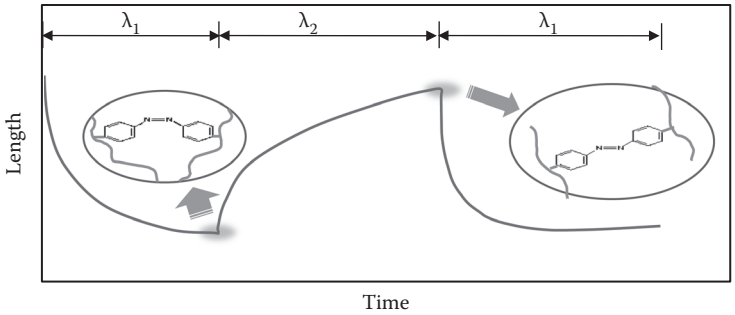

FIGURE 87.9 Schematic illustration of the photomechanical behavior of azobenzene-containing hydrogels on irradiation.

A typical photomechanical behavior of the polymer hydrogel network is described in Figure 87.9. By changing the irradiation wavelength to the visible region, the cis-form changes to the trans-form, and the dimension recovery is observed. This contraction recovery can be repeated many times.

Spirobenzopyran undergoes ring opening on UV irradiation, with the production of intensely colored merocyanine. The merocyanine can return thermally or photochemically to colorless spiropyran. Physical property changes associated with this isomerization are as follows: (1) a change in absorption spectrum, (2) a change in dipole moment, and (3) a geometric structural change. Spirobenzopyran can be incorporated into backbone or pendant groups to polymer hydrogel networks. A change in dipole moment caused by spiropyran–merocyanine isomerization would be expected to alter intramolecular interaction of polymer chains. The change of intramolecular interaction induces a conformational change in the polymer and thus causes swelling or shrinking polymer hydrogels. A change in dipole moment on irradiation could affect the adsorption–desorption behavior of drugs, particularly proteins on the polymer. This feature may be useful for the design of "on–off" control of drug release in a pulsatile delivery system. The photoresponsive hydrogels can also be synthesized by introducing triarylmethane into the polymer network (Mamada et al. 1990). The triarylmethane can be ionized upon UV irradiation. The hydrogels discontinuously swell in response to UV irradiation but shrink when the UV light is removed. The UV light-induced swelling is attributed to an increase in osmotic pressure within the gel due to the appearance of cyanide ions formed by UV irradiation.

A typical function of photoresponsive hydrogels is to change their volume reversibly on irradiation of UV or visible lights. Photoresponsive hydrogels respond to "on–off" stimulus of lights, which induces the gel swelling–shrinking that contributes to the release of drug molecules (Qiu and Park 2001). The molecular weight of the polymer may affect the photoresponsive property; in polymers with smaller molecular weight, the photoresponsive macroscopic change is induced more effectively. While the action of stimulus (light) is instantaneous, the reaction of hydrogels in response to such action is still relatively slow.

87.5 Electroresponsive Hydrogels

Electroresponsive hydrogels transform electrical energy directly into mechanical energy. Basically, electroresponsive hydrogels are made of polyelectrolytes, swellable polymer networks that carry cations or anions. The electroresponsive hydrogels change a macroscopic shape in response to an electric field (Figure 87.10) (Filipcsei et al. 2000). When a hydrogel is negatively charged, it swells near the anode and contracts near the cathode. Generally, the response rate is proportional to the external electric current. The commonly used electroresponsive polymers include conducting polymers, polyelectrolyte gels, and ionic polymer–metal composites. Electroresponsive polymers are an increasingly important class of smart materials (Kim et al. 1998; Ramanathan and Block 2001; Bajpai et al. 2008). They have promising

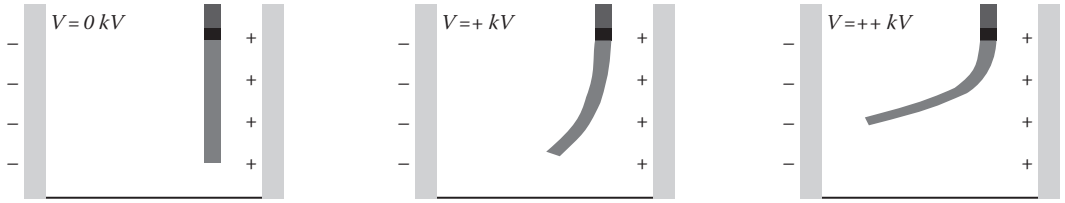

FIGURE 87.10 Schematic illustration of the bending phenomena of an electroresponsive polymer hydrogel.

applications in biomechanics, artificial muscle actuation, sensing, energy transduction, sound dampening, chemical separations, and controlled drug delivery. Gel deformation in an electric field is influenced by a number of factors, including variable osmotic pressure based on the voltage-induced motion of ions in the solution, pH or salt concentration of the surrounding medium, position of the gel relative to the electrodes, thickness or shape of the gel, and the applied voltage (Gao et al. 2008). Transforming the application of an electric field into a physical response by a polymer generally relies on collapse of a gel in an electric field, electrochemical reactions, electrically activated complex formation, ionic polymer–metal interactions, electrorheological effects, or changes in electrophoretic mobility (Filipcsei et al. 2000; Kim et al. 1998, 2004).

A typical function of electroresponsive hydrogels is to change their volume reversibly under the influence of an electric field. The volume change can be utilized for solute permeation control through hydrogels in controlled drug delivery. Electroresponsive hydrogels respond to "on–off" stimulus of electrical currents, which induces the gel swelling–shrinking that contributes to the release of drug molecules. The control of "on–off" drug release can be achieved by varying the intensity of electric stimulation. Hydrogels made of poly(2-acrylamido-2-methylpropane sulfonic acid-co-n-butylmethacrylate) were able to release edrophonium chloride and hydrocortisone in a pulsatile manner in response to electric current (Gong et al. 1994). Poly(sodium acrylate) microparticle gels containing pilocarpine showed a current-dependent pilocarpine release. However, a complete "on–off" drug release regulation is still challenging since it is difficult to completely stop drug release upon termination of the electrical stimulus (Kulkarni et al. 2010). Kwon et al. prepared cross-linked poly(2-acrylamide-2-methylpropanesulfonic acid-co-butyl methacrylate) [P(AMPS-co-BMA)] hydrogels and evaluated the feasibility of these hydrogels for electroresponsive drug-delivery devices (Kwon et al. 1991). They used a cationic drug molecule, edrophonium chloride, within the negatively charged hydrogel. Rapid drug release from the hydrogels resulted from an application of electric fields through the ion exchanges between positively charged drug molecules and protons at the cathode. The squeezing effects arising from the electric field application induced rapid drug release from the gels, which increased as the voltages increased in a dose-dependent manner. Using the P(AMPS-co-BMA) hydrogels, an "on–off" drug release regulation was achieved under an "on–off" application of electric current. The group further investigated the electric current-induced release of anionic heparin from a positively charged polyallylamine polyion complex. Rapid structural changes and an apparent dissociation of the polyion complex occurred upon application of an electric current. During the electric current application, the positively charged polyallylamine was neutralized at the cathode owing to the microenvironmental pH changes, and apparent dissociation of the polyion complex occurred. Although bioactive heparin was released by electric current application, polyallylamine was also released.

87.6 Glucose-Responsive Hydrogels

Insulin-dependent diabetes mellitus patients lack the pancreatic function that releases insulin in response to blood glucose levels. These patients require daily self-injections of an appropriate amount of insulin that helps them to avoid hyperglycemia. Diabetic patients suffer from a gradual decline in the efficiency of various organs, leading to vision loss and long-term diseases. Severe conditions may even

lead to patient death. Thus, injection of properly dosed insulin at proper times is required for insulin-dependent diabetes mellitus therapy. Self-injection of insulin, however, results in patient discomfort, varied bioavailability, and sometimes a hypoglycemic coma due to an overdose of insulin. Alternatively, insufficient insulin induces hyperglycemia and related complications. Therefore, the precise control of blood glucose levels with an effective, stimuli-responsive insulin release would be of great utility. A large number of formulations incorporating hydrogels for glucose concentration-dependent insulin release have been reported.

Glucose-responsive hydrogel systems are based on: (1) enzymatic oxidation of glucose by glucose oxidase (GOx), (2) binding of glucose to concanavalin A (Con A), and (3) reversible sol–gel phase transition hydrogels. In the glucose-responsive systems using GOx, the glucose sensitivity is not caused by direct interaction of glucose with the responsive polymer, but rather by the response of the polymer to the by-products that result from the enzymatic oxidation of glucose. The substrate glucose reacts with GOx, which produces gluconic acid and H_2O_2. Typically, a pH-responsive moiety is incorporated into glucose-responsive hydrogel networks, and the gluconic acid induces a pH-responsive swelling or collapse of the hydrogel matrix that contains insulin. Several insulin-release systems utilize the glucose-responsive hydrogels based on glucose–GOx. For example, Chu et al. reported the covalent modification of a cellulose film with GOx-conjugated PAA (Chu et al. 2004). At neutral and high pH levels, the carboxylate units of the PAA chains were negatively charged and extended due to electrostatic repulsion, which resulted in occlusion of the pores in the cellulose membrane. The gluconic acid that resulted from the addition of glucose led to a local pH reduction, protonation of the PAA carboxylate moieties, and concomitant collapse of the chains obscuring the membrane pores, with the latter event facilitating the release of entrapped insulin.

Con A has also been frequently used in modulated insulin delivery. In this type of the system, insulin molecules are attached to a support or carrier through specific interactions that can be interrupted by glucose itself. Kim and coworkers reported the synthesis of monosubstituted conjugates of glucosyl-terminal PEG (G-PEG) and insulin (Liu et al. 1997). The G-PEG–insulin conjugates were bound to Con A that was grafted along a PEG–poly(vinylpyrrolidone-co-acrylic acid) backbone. When the concentration of glucose in the surrounding aqueous media increased, competitive binding of glucose with Con A led to displacement and release of the G-PEG–insulin conjugates.

Obaidat and Park reported glucose-responsive systems that underwent sol–gel phase transition depending on the glucose concentration in the environment (Obaidat and Park 1996). The reversible sol–gel phase transition required glucose-responsive cross-linking. Since diffusion of insulin through the sol phase was an order of magnitude faster than that through the gel phase, the insulin release could be controlled by the glucose concentration in the environment. Another type of sol–gel transition polymers responsive to glucose was prepared using a water-soluble copolymer of acrylamide and allyl glucose. The resulting polymers were cross-linked in the presence of lectin and Con A. Since binding constants of native glucose molecules are higher than those of glucose moieties on the copolymer side chains, an exchange reaction occurred between added glucose and copolymer glucose moieties, inducing a gel-to-sol phase transition. Such changes can be utilized for the permeation control of insulin.

All the above-mentioned examples used proteins such as GOx and Con A. The exposure of these proteins and peptides to the body may cause an undesirable immune response upon contact. Therefore, these naturally derived proteins and peptides, and their whole systems, should be separated from the body using semipermeable membranes. Matsumoto et al. prepared synthetic polymers with glucose-responsive functions (Matsumoto et al. 2003). They focused on the unique characteristics of phenylboronic acid as a glucose-responsive moiety. Boronate is known to form reversible bonding with polyols such as *cis* diol sugar compounds such as glucose. They prepared water-soluble copolymers containing phenylboronic acid side chains using m-acrylamidophenylboronic acid (AAPBA) and various water- soluble monomers, including *N*-vinylpyrrolidone, acrylamide, and (*N*,*N*-dimethylacrylamide) DMAAm. The resulting copolymers formed reversible complexes with polyol compounds such as poly(vinyl alcohol) (PVA). These complexes dissociated with the addition of glucose in a concentration-dependent

manner. Such complex formation and dissociation could be attributed to the different dissociation constants of phenylboronate anions with PVA or glucose.

87.7 Enzyme-Responsive Hydrogels

Enzymes play a critical role in most biological pathways. Enzymes are highly selective and work under mild conditions present *in vivo* (aqueous, pH 5 – 8, 37°C). Generally, enzyme-responsive hydrogels consist of two components: (i) an enzyme-responsive substrate and (ii) a component that directs or controls interactions that cause macroscopic transitions (Figure 87.11) (Thornton et al. 2005; Ulijn 2006). The molecular interactions include hydrogen bonding, electrostatic interactions, van der Waals forces, hydrophobic interactions, π–π interactions, and their combinations. Catalytic action of the enzyme on the substrate can lead to changes in surface properties, self-assembly, supramolecular architectures, and swelling/collapse of gels (Ulijn 2006).

In situ depot-forming enzyme-responsive hydrogels were synthesized by using enzymatic dephosphorylation to induce a sol–gel transition (Yang et al. 2004; Yang and Xu 2004; Thornton et al. 2007). When fluorenylmethyloxycarbonyl (FMOC)–tyrosine phosphate was exposed to a phosphatase, the phosphate groups were removed, which resulted in reduction in electrostatic repulsions, supramolecular assembly by π-stacking of the fluorenyl groups, and eventually gelation. The incorporation of functional moieties that can react with enzymes is another typical approach to produce enzyme-responsive hydrogels. Exposure of the functional groups to a specific enzyme can lead to the creation of new covalent linkages that cause a change in macroscopic properties. For example, transglutaminase, a blood-clotting enzyme, had the ability to cross-link the side chains of lysine (Lys) residues with glutamine (Gln) residues.

Various approaches have been studied to prepare protease-responsive hydrogels. When the hydrogels are exposed to a protease, hydrolysis of protein or peptide leads to gel degradation and subsequent release of encapsulated drugs. Moore and coworkers prepared chymotrypsin-responsive hydrogels by incorporating a degradable (cysteine–tyrosine–lysine–cysteine) CYKC tetrapeptide sequence as a cross-linker within polyacrylamide hydrogels (Plunkett et al. 2005). The CYKC sequence contains a terminal cysteine conjugation site, a tyrosine residue that can be cleaved at the carboxyl side by chymotrypsin, and a Lys residue. When subjected to α-chymotrypsin, the micron-sized gels dissolved due to the degradation of CYKC by α-chymotrypsin. Ulijin reported protease-responsive hydrogels that were applicable to the removal of toxins or entrapment of drug molecules (Ulijn 2006). In this case, the response was caused by a change in osmotic pressure instead of cross-link degradation. Copolymer beads composed of acrylamide and PEG–macromonomers were modified via an enzyme-cleavable tripeptide comprising combinations of glycine, phenylalanine, and positively charged arginine residues that imparted swelling due to electrostatic repulsions. Upon the addition of proteases, the tripeptide was cleaved, and the resulting loss of arginine groups led to a reduction in electrostatic repulsions and subsequent collapse of the hydrogel.

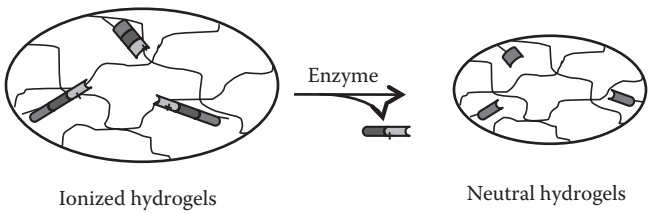

FIGURE 87.11 Enzymatic cleavage and drug release from enzyme-responsive hydrogels. (Adapted from Thornton, P. D., McConnell, G., and Ulijin, R. V. 2005. Enzyme responsive polymer hydrogel beads. *Chem. Commun.*, (47): 5913–5.)

87.8 Inflammation-Responsive Hydrogels

Inflammatory reactions are commonly observed at injury sites. Inflammation-responsive cells such as macrophages and polymorphonuclear leukocytes (PMNs) play a key role in normal healing processes after injury. The oxygen metabolites such as hydroxyl radicals (•OH) are produced from the inflammation-responsive cells at the injured tissues. Yui et al. designed a hydroxyl radical-responsive drug- delivery system (Yui et al. 1992,1993). They used hyaluronic acid (HA), a linear mucopolysaccharide consisting of repeating units of *N*-acetyl-D-glucosamine and D-glucuronic acid, for preparing inflammation-responsive hydrogels because the hydroxyl radicals produced at injured sites can effectively degrade HA. HA was cross-linked with ethylene glycol diglycidylether or polyglycerol polyglycidylether. HA degradation in response to hydroxyl radicals was observed only at the surface of the gel, indicating surface erosion degradation. Further utilization of these hydrogels involved the introduction of microspheres as model drug carriers in the hydrogels. The release of microsphere-encapsulating drugs followed the surface erosion of the gels. These HA gels could be useful *in vivo* for inflammation-induced drug-delivery systems, specifically for chronic inflammatory problems including rheumatoid arthritis.

87.9 Antigen-Responsive Hydrogels

Antigen–antibody interactions are highly specific and are associated with complex immune responses that help recognize and neutralize foreign infection-causing objects in the body. The high affinity and specificity of their interactions have been extensively used to yield a variety of antigen-responsive polymer systems. In most cases, antigen-responsive hydrogels have been prepared by physically entrapping antibodies or antigens in networks, chemical conjugation of the antibody or antigen to the network, or using antigen–antibody complexes as reversible cross-linkers within networks (Lu et al. 2003).

A typical antigen-responsive polymer can be synthesized by copolymerization of vinyl-functionalized antigen or antibody with acrylamide or *N*,*N*′-methylenebisacrylamide (MBA). The copolymerization results in a hydrogel cross-linked both covalently and by antigen–antibody interactions. When free antigen molecules are added to the solution with immersed antigen-immobilized hydrogels, the antibodies in the hydrogel network change partners with free antigen, owing to the difference in the binding constants. This antigen-competitive exchange results in a decreased number of cross-linking points in the hydrogels, and thus promotes the swelling of hydrogels. This antigen-responsive swelling behavior of an antigen-immobilized hydrogel is irreversible (Figure 87.12a), but, when both antigen and antibody are immobilized on polymer hydrogel networks, reversible swelling and deswelling occur (Figure 87.12b). Such changes are very antigen specific, so that the addition of other antigens does not alter hydrogel swelling.

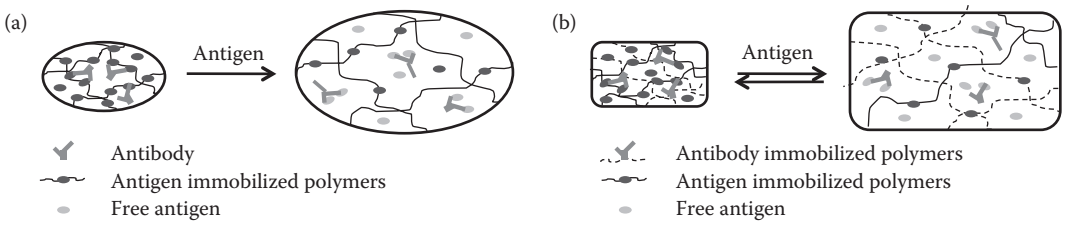

FIGURE 87.12 Irreversible (a) and reversible (b) antigen-responsive hydrogels. (Adapted from Miyata, T., Asami, N., and Uragami, T. 1999. *Nature*, 399: 766–9.)

87.10 Magnetoresponsive Hydrogels

For designing a magnetoresponsive hydrogel delivery system, several factors should be considered, including the magnetic properties of the delivery systems, field strength, field geometry, drug/gene-binding capacity, and physiological parameters such as the depth to target, the rate of blood flow, vascular supply, and body weight. Generally, inorganic magnetic particles are physically entrapped within or covalently immobilized to a 3D cross-linked network. In principle, in the presence of a magnetic field gradient, a translational force is exerted on the drug-delivery hydrogel complexes. This effectively traps the complex in the field at the target site and pulls it toward the magnet (Pankhurst et al. 2003).

A major challenge of chemotherapeutic approaches to cancer treatment is that they are nonspecific. Magnetoresponsive hydrogels have been explored extensively as possible drug carriers for site-specific drug delivery and controlled release. Although theoretically very effective, magneto drug-delivery systems still have numerous obstacles. Magnetoresponsive hydrogel systems generally require a relatively strong gradient in an external field. There is also the potential for embolization as the fraction of magnetoresponsive hydrogels may accumulate and block flow or they may also concentrate in the liver (Dobson 2006). There are other limitations such as the depth that the magnet may function, as is encountered when scaling up from small animals with near-surface targets to larger animals and humans.

Another type of magnetoresponsive hydrogels exhibit the shape and size distortions that occur reversibly and instantaneously in the presence of a nonuniform magnetic field (Zrinyi et al. 1997; Starodoubtsev et al. 2003; Wang et al. 2006). Such hydrogels have received significant attention for use as soft biomimetic actuators, sensors, cancer therapy agents, artificial muscles, switches, separation media, membranes, and drug-delivery systems (Zrinyi et al. 1997; Szabo et al. 1998, 2000; Zrinyi 2000; Babinocova et al. 2001; Starodoubtsev et al. 2003; Wang et al. 2006; Pyun 2007).

87.11 Ultrasound-Responsive Hydrogels

The application of stimuli-responsive polymers to drug delivery needs the target-specific delivery and the controlled release of therapeutic compounds at a specified rate. The drug-delivery systems are generally guided to the target site—which has an environment that stimulates release—by passive or active transport mechanisms. The change in environmental conditions spontaneously induces drug release at the target site. However, in most cases, to locally apply the stimulus at the targeted site is not simple. For instance, a change in temperature could lead to release in thermoresponsive hydrogels *in vitro*, but localized heating and cooling *in vivo* are not always trivial at sites deep within the body.

Ultrasound is a particularly effective stimulus that can be applied externally on demand and has proven to be effective at triggering drug release within the body. One of the pioneering approaches of exploiting ultrasound in drug delivery involves directing the ultrasound directly at the hydrogel matrix (Sershen and West 2002). This approach of ultrasound-responsive drug delivery achieved a 27 times increase in the release of 5-fluorouracil from a poly(ethylene-co-vinyl acetate) (PEVAc) matrix (Miyazaki et al. 1985). Ultrasound regulated drug delivery in which the release rates were repeatedly responsive (Kost and Langer 1988). Biodegradable polymers that have been used for ultrasound-responsive systems include poly(D,L-lactide) (PGA), poly(D,L-glycolide) (PLA), poly(bis(p-carboxyphenoxy))alkane–anhydrides (PCPX), and their copolymers with sebacic acid. When induced to ultrasound, these bioerodible polymer hydrogels responded rapidly and reversibly. It is believed that the ultrasound causes an increase in temperature in the delivery system, which facilitates diffusion (Mathiowitz and Cohen 1989). The concept of using ultrasound-responsive hydrogels for controlled drug delivery is attractive because the method is essentially noninvasive and has been successfully used in other areas of medical treatment and diagnostics (Noriis et al. 2005).

The success of ultrasonic mediation of drug delivery is generally ascribed to cavitation, which is the alternating growth and shrinkage of gas-filled microbubbles that results from high- and low-pressure

waves generated by ultrasound energy (Lentacker et al. 2006). Eventually, these cavitating microbubbles implode, generating local shock waves that can disrupt polymer assemblies in their vicinity.

87.12 Redox/Thiol-Responsive Hydrogels

Redox/thiol-responsive hydrogel materials are another class of responsive hydrogels that have recently received increased attention, especially in various fields of controlled drug delivery (Meng et al. 2009a). The interconversion of thiols and disulfides is a key step in many biological processes, plays an important role in the stability and rigidity of native proteins in living cells (Castellani et al. 1999), and has been commonly used for various bioconjugation protocols (Saito et al. 2003). Since disulfide bonds can be reversibly converted into thiols by exposure to various reducing agents (e.g., mercaptoethanol, dithiothreitol (DTT), and glutathione (GSH)) and/or undergo disulfide exchange in the presence of other thiols, polymers containing disulfide linkages can be considered both redox and thiol responsive (Oh et al. 2007). GSH, the most abundant reducing agent in most cells (Bulmus et al. 2003), has a typical intracellular concentration of about 10 mM, whereas its concentration is only about 0.002 mM in the cellular exterior (Jones et al. 1998). This significant variation in concentration has been utilized to design thiol/redox-responsive drug-delivery systems that specifically release therapeutics upon entry into cells (Ghosh et al. 2009).

87.13 Concluding Comments

Development of smart hydrogels as effective drug-delivery systems has been limited, mainly due to a few reasons. First, the chemical structures of most smart hydrogels contain functional groups that have not been used in clinical applications. This makes it rather difficult to use them in humans, as their safety has not been unequivocally demonstrated. Until the safety is proven, the pharmaceutical, as well as biomedical, industries are not willing to develop clinical products. Second, the smart hydrogels are obviously smarter than ordinary hydrogels by being able to sense the environmental changes and react to them, but they cannot overcome the inherent limitations of hydrogels in terms of drug loading and release. Unless a hydrogel can deliver a drug at a therapeutically significant level for a sufficiently long period of time, any control on drug release has no clinical meaning. Third, most of the smart hydrogels are not biodegradable, although they may be biocompatible. Thus, it is not practical using the smart hydrogels as implantable drug-delivery systems. Without biodegradation in the body, the utilization of smart hydrogels will be limited. Biodegradability can be achieved by employing synthetic polymers with biodegradable backbones or natural polymers.

Synthesizing new polymers to make environment-responsive hydrogels will continue. Synthetic polymers possess attractive potential because experts in the field can easily alter their design to achieve the desired characteristics of hydrogels that respond to different stimuli (Kwon 2005). The single environmental-responsive property of intelligent hydrogels would limit their practical applications. It would be favorable if the intelligent hydrogels could respond to more than one stimulus simultaneously. For example, more effective drug therapies for complicated diseases may require polymeric materials, the functions of which are variable or switchable in response to several kinds of stimuli. Indeed, the diagnosis of patients suffering from some diseases is generally achieved by monitoring several physiological changes. Therefore, multiresponsive hydrogels have attracted more and more attentions (Ji et al. 2007; Ju et al. 2009). The combination of two or more responses is particularly useful to optimize the control of drug release.

Recent advances in nanofabrication have allowed utilization of smart hydrogels in the nano-/microsystems. Application of smart hydrogels to microfluidic systems, bioseparation, and biosensors is a good example. Their application has been extended to high-throughput screening, such as peptide, protein, and deoxyribonucleic acid (DNA) array. Micropatterned hydrogels can be employed as a template for preparing nano-/microparticles of predefined size and shape. Nanofabricated smart hydrogels can be used in various aspects in tissue engineering, including cell sheet technique, artificial extracellular matrix materials, and 3D scaffolds with chemical patterning or microchannels.

Abbreviations

DEA 2-(diethylamine)
DPA 2-(diisopropylamine)
MPC 2-methacryloyloxyethyl phosphorylcholine

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