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Smart Polymeric Materials: Emerging Biochemical Applications

Review

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Smart polymeric materials respond with a considerable change in their properties to small changes in their environment. Environmental stimuli include temperature, pH, chemicals, and light. "Smart" stimuli-sensitive materials can be either synthetic or natural. This review discusses the application of smart materials as tools to solve biological problems such as bioseparation, drug delivery, biosensor design, tissue engineering, protein folding, and microfluidics. The goal for these endeavors is to mimic the "smartness" of biological systems and ultimately moderate complex systems such as immune responses at desired levels. The versatility and untapped potential of smart polymeric materials makes them one of the most exciting interfaces of chemistry and biology.

Introduction

Over the last decade, smart polymeric materials have been used in biochemical sciences in many ways. Since the term "smart polymeric materials" encompasses a wide spectrum of different compounds with unique potential for biological applications, and since interest in generating and manipulating these compounds is growing, we felt that it would be useful to assemble an overview of the field to aid in catalyzing cross-fertilization of ideas. The present review aims to bring together the exciting design of these materials and the ever-expanding range of their uses by focusing on hydrogels as a key example of this technology. Smart or stimuli-responsive polymers respond to small changes in their environment with dramatic changes in their physical properties (Table 1). Smart polymers are either reversibly soluble-insoluble (SIS) in aqueous media or crosslinked in the form of hydrogels. Specifically, hydrogels are networks of hydrophilic polymers that expand or swell by taking up "from 10%–20% up to thousands of times their dry weight in water" [1]. Physical hydrogels are held together with noncovalent forces, whereas chemical hydrogels are obtained by chemical crosslinking; both kinds of gels are structurally inhomogeneous. Physical hydrogels have hydrophilic and hydrophobic domains, whereas chemical hydrogels have "clusters" or regions of high crosslinking density (low swelling structure) present in an otherwise low crosslinking density (high swelling structure) polymeric network. During water uptake, hydration occurs first at hydrophilic moieties ("primary bound water"), then at hydrophobic sites ("secondary bound water") and at spaces between the chains and pores ("free or bulk water") [1]. SIS polymers can be synthetic [poly(N-isopropylacrylamide) and methylmethacrylates polymers] (Figure 1) or natural (alginate, chitosan, and κ-carrageenan [2, 3]), or a combination of both [collagen-acrylate and poly(polyethylene glycol-*co*-peptides)] [1]. Both SIS polymers and hydrogels have been shown to respond to a variety of stimuli such as changes in pH, temperature, ionic strength, light, electricity, and magnetic field (Table 1) [4]. The swelling (uptake of additional water) and solubility responses for hydrogels and SIS polymers, respectively, have a common mechanism. Application of stimulus changes the nanostructure and increases or decreases the overall hydrophilicity. For example, a pH-responsive polymer (or hydrogel) could be subjected to ionization of a free carboxyl group (like in methacrylates), and a thermosensitive polymer, like poly(N-isopropylacrylamide) [poly(NIPAAm)], would deswell as the temperature is raised beyond lower critical solution temperature (LCST). The change in network volume (the smartness readout) arises because there is a balance of hydrophilic (-CONH-) and hydrophobic [$-CH(CH_3)_2-$] moieties, therefore, below LCST; the gel is swollen, hydrated, and hydrophilic. However, above LCST it is collapsed, dehydrated, and hydrophobic.

Design and Synthesis of Smart Polymeric Materials

The thermosensitive gel poly(N-isopropylacrylamide) (NIPAAm) is one of the most commonly studied smart systems, and various strategies for synthesizing the hydrogel and its derivatives have been described. Efforts have been directed toward altering the swelling/shrinking behavior and preparing copolymers that also respond to other stimuli. Critical insight into the deswelling mechanism was gained by the Hoffman group's seminal work on PNIPAAm [5]. Researchers showed that gel collapse at LCST (38.5°C) is entropy driven since bound water molecules are freed. Collapse is accompanied by "skin" formation around the trapped pockets of water; therefore, deswelling kinetics are biphasic with faster expulsion of water through weakly densified gel portions followed by slower release of water through the more densified collapsed gel layer on the surface. The comonomers (along with NIPAAm) which have been assessed are acrylic acid, methacrylic acid, 2-methyl-2-acrylamidopropane sulfonic acid, trimethyl-acrylamidopropyl ammonium 3-methyl-1-vinylimidazolium iodide, sodium acrylate, sodium methacrylate, and 1-(3-sulphopropyl)-2-vinyl-pyridinium betaine [6–8]. The use of methacrylic acid, along with NIPAAm, not only changes LCST but also makes the hydrogels responsive to both temperature and pH [8].

Polymethacrylic acid chains do not expand readily before a critical charge density (due to ionized carboxyl groups) is reached. The copolymers of NIPAAm and methacrylic acid, in addition to hydrophobic and ionic binding, also involve hydrogen bonding between the amide and carboxyl groups of the two monomers [9]. Consequently, it was found that at any pH below pK_a of

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Table 1. Stimuli-Responsive Smart Polymeric Materials

Type of Stimulus	Responsive Polymer Material(s)	Reference(s)
pH	dendrimers	[71–74]
	poly(L-lysine) ester	[75]
	poly(hydroxyproline)	[76]
	lactose-PEG grafted poly (L-lysine) nanoparticle	[77]
	poly (L-lysine)-g-poly (histidine)	[77]
	poly (propyl acrylic acid)	[78]
	poly (ethacrylic acid)	[78]
	polysilamine	[79]
	Eudragit S-100	[80]
	Eudragit L-100	[81]
	chitosan	[82]
	PMAA-PEG copolymer	[83]
Ca^{2+}	alginate	[84]
Mg^{2+}	chitosan	[85]
Organic solvent	Eudragit S-100	[86]
Temperature*	PNIPAAm	[87]
Magnetic field	PNIPAAm hydrogels containing ferromagnetic material PNIPAAm-*co*-acrylamide	[88, 89]
$Ru^{2+} \rightarrow Ru^{3+}$ (redox reaction)	PNIPAAm hydrogels containing Tris (2,2′-bipyridyl) ruthenium (II)	[90]
Temperature[a] (sol-gel transition)	poloxamers	[32, 91, 92]
	chitosan-glycerol phosphate-water	[93]
	prolastin	[94]
	hybrid hydrogels of polymer and protein domains	[15, 95]
Electric potential	polythiophen gel	[96]
IR radiation	poly (N-vinyl carbazole) composite	[97]
UV radiation	polyacrylamide crosslinked with 4-(methacryloylamino)azobenzene Polyacrylamide-triphenylmethane leuco derivatives	[27, 98]
Ultrasound	dodecyl isocyanate-modified PEG-grafted poly(HEMA)	[99]
Dual-Stimuli-Sensitive Polymers		
Ca^{2+} and PEG	carboxymethyl cellulose	[100]
Ca^{2+} and temperature	Eudragit S-100	[87]
Ca^{2+} and acetonitrile	Eudragit S-100	[87]
32°C and 36°C	hydrogels of oligoNIPAAm and oligo(N-vinylcaprolactum)	[101]
pH and temperature	poly (N-acryloyl-N-propyl piperazine)	[102]
Light and temperature	poly(vinyl alcohol)-*graft*-poly-acrylamide-triphenylmethane leucocyanide derivatives	[103]

[a] Pressure sensitivity is a common characteristic of all temperature-sensitive gels, probably due to an increase in their LCST with pressure.

the methacrylate moiety, the copolymers reached lower swelling ratios as compared to either of the homopolymers, since hydrogen bonding presumably acts as additional crosslinks that keep water out.

Soluble copolymers of N-isopropylacrylamide and acrylic acid containing photodimerizable chromophores, like stilbene, styryl pyridinium, and acridizinium moieties, have been synthesized with a view to using light for inducing crosslinking [10]. Unfortunately, adequate reversibility of this photochemical switching could not be achieved in any of the cases.

The most frequently used method of polymerizing NIPAAm with N,N′-methylene bisacrylamide as a crosslinker via free radical crosslinking polymerization is exothermic [11]. The usual polymerization temperatures are close to LCST. Thus, any local or overall temperature increase above LCST results in pockets of phase separation and formation of spatially inhomogeneous gels. Real-time temperature and photon transmission measurements showed that polymerization even below LCST produced inhomogeneous network, whereas at temperatures higher than LCST, the gel system "undergoes a phase transition via a spinodal decomposition process" [11]. Kim et al. [12] have grafted PNIPAAm onto the surface of pH-responsive alginate to obtain a macroporous hydrogel with faster swelling/deswelling rates.

Much less work has been done with hydrogels, which respond to stimuli other than pH, temperature, and light. A hydrogel based upon ethylene-*co*-vinyl acetate, responsive to magnetic field, has been described [13]. Hydrogels based upon similar structures have also been described as responsive to ultrasonic radiation [13].

Of special interest are naturally occurring polysaccharides like chitosan, alginate, and κ-carrageenan that behave as reversibly soluble-insoluble polymers by responding to pH, Ca^{2+}, and K^{+}, respectively [2, 3]. Recently, a polymer sensitive to pH and temperature has been prepared by genetic engineering [14]. These are block copolymers containing repeating sequences from silk (GAGAGS) and elastin (GVGVP), where, in some cases, valine has been replaced by glutamic acid. By varying the extent of this change, the sensitivity to pH, temperature, and ionic strength could be controlled fairly precisely. Recombinant methods have also been used to design multidomain assemblies in which leucine zipper domains flank a central flexible polyelectrolyte [15]. pH/temperature stimuli trigger sol-gel transition. Extrapolation of this to create a seamless conjugate with specific biological activity is an exciting possibility.

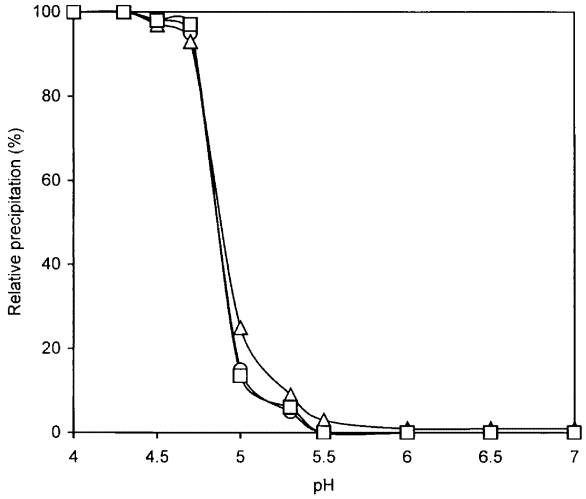

Figure 1. Reversible Precipitation of Eudragit S-100, a Methyl Methacrylate Copolymer, in Response to pH

Open circles, Eudragit S-100; open squares, after heating at 60°C for 30 min; and open triangles, after microwave treatment at 60°C for 30 min (our unpublished results).

Bioseparation of Proteins

The cost of the bioseparation step is a critical factor in determining the overall production cost of a protein. Use of smart polymers has led to some simple and economical strategies in bioseparation. In affinity precipitation, the smart macroaffinity ligand (soluble form) is mixed with the crude broth containing the target protein [2]. Usually, the smart macroaffinity ligand is prepared by chemical coupling of a suitable affinity ligand to a smart polymer. Occasionally, it is possible to exploit the inherent and fortuitous affinity of the polymer for the affinity capture of the desired protein. Applying specific stimuli for the smart polymer precipitates the complex of the macroaffinity ligand-target protein. The complex of the macroaffinity ligand-target protein is dissociated, and the smart macroaffinity ligand is usually recycled. The technique is scalable and does not require any costly equipment [2]. In many cases, selectivity of the affinity interactions ensures that the purity obtained is at least of the level of single band in SDS-PAGE [3, 4, 16].

The use of aqueous two-phase systems (such as PEG-dextran) for protein separation has been around for a long time. It has been shown that smart macroaffinity ligand-target proteins can be recovered from the PEG phase, and the two phases can be reused. This approach was successfully demonstrated by the purification of microbial xylanases, pullulanases, wheat germ α-amylase, and sweet potato β-amylase [17, 18]. Another attractive extension of this approach has been to separate animal cells by crafting the smart macroaffinity ligands by coupling an antibody (against a cell surface protein) to a smart polymer [19].

Macroaffinity ligand-facilitated three-phase partitioning (MLFTPP) converts three-phase partitioning (TPP) [4] into a more selective and predictable technique for bioseparation of proteins using smart affinity ligands [20–22].

Techniques like affinity precipitation, aqueous two-phase partitioning, and MLFTPP remain underexploited by biochemists, as their description is limited to journals devoted to biochemical engineering/biotechnology. By now, these techniques show sufficient promise to be adopted and routinely used by biochemists and molecular biologists.

Design of Reusable Biocatalysts

Over the years, immobilized enzymes have emerged as the preferred forms in which enzymes are used for various applications. Use of smart polymers for immobilization of enzymes allows reuse of the biocatalyst after homogeneous catalysis [23]. The separation (and reuse) of the bioconjugate can be simply done by precipitation. Thus, the benefits of homogeneous catalysis can be combined with the convenience of recovery/reuse of heterogeneous catalysts. The advantage of homogeneous biocatalysis (by using a soluble form of the smart polymer-enzyme bioconjugate) may be illustrated by the example of chitosan-α-L-rhamnopyranosidase [24]. The immobilized enzyme retained the kinetic parameters (Michaelis constant, K_m; and maximal velocity, V_{max}) of the free enzyme. The biocatalyst was used successfully for increasing the aroma of a model wine solution [24].

In nonaqueous media, immobilization has been widely used for stabilization of the enzyme against inactivation due to the media [25]. A photoresponsive copolymer linked to subtilisin was successfully used for a transesterification reaction in toluene [26]. Unfortunately, the rate of transesterification was no better than enzyme powders, showing that the bioconjugation failed to stabilize the enzyme.

Hydrogels have also been used for immobilizing enzymes. The pioneering work of Hoffman with β-galactosidase deserves special mention [27]. A more recent example is encapsulation of α-chymotrypsin in a photosensitive hydrogel [28]. When exposed to light of different wavelengths, the hydrogel changes its permeability to the amide substrate. This "on" and "off" cycle could be carried out repeatedly in a reversible fashion.

Drug Delivery

Most extensive efforts in this area have been made for developing insulin release systems in response to high glucose levels [29]. In an early approach, entrapped insulin was released from copolymers of allylglucose crosslinked with Concanavalin A. In many later designs, glucose oxidase has been used to generate H^+ (in response to the presence of glucose) and hence exploit pH-sensitive hydrogels. One common worry in all such cases is the slow response time. Thus, use of superporous hydrogels with fast swelling-deswelling kinetics ($<$1 min) is a step in the right direction [30].

The well-known interaction between boronic acid and sugars has been used to design glucose-responsive insulin-release systems [29]. To bring the pH of insulin release closer to the physiological conditions, it was necessary to coordinate the boron atom with an inbuilt amino group. It has also been possible to ensure that this system has a dosage capacity required for clinical use and is rechargeable once insulin is exhausted. Use of the presence of glucose to increase the LCST of the

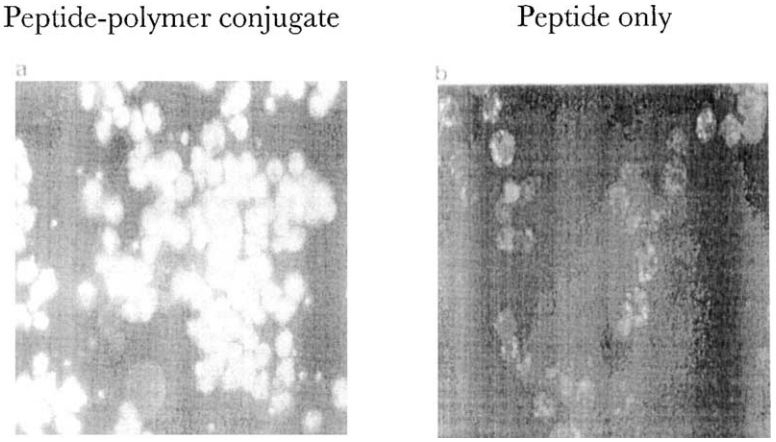

Figure 2. Manipulation of Intracellular Trafficking by a pH-Responsive Polymer for Drug Delivery

(A), Macrophages treated with an E 3 conjugated peptide; (B), lyososomal colocalization of the peptide. The peptide labeled with a fluorescent tag was incubated with macrophages overnight and viewed with a fluorescence microscope (40× magnification). Reproduced from [39] with kind permission of Prof. A.S. Hoffman.

thermoresponsive hydrogels of acrylamidophenylboronic acid and NIPAAm has also been evaluated. This system did exhibit the useful characteristic of requiring a threshold glucose concentration to release insulin [29].

In a slightly different approach, pH-sensitive poly(NIPAAm/MAA) nanoparticles were embedded along with glucose oxidase and catalase in an ethylcellulose-based membrane [31]. The rate of insulin release was modulated by glucose concentration due to volume changes in the embedded nanoparticles. The response lag time was about 5–15 min as reduction of polymer dimension to nanometer led to faster response. Unfortunately, the permeability cycles (to test reusability) were not reproducible since the design did not "allow sufficient time for removing the diffusants from the membrane" [31].

Polyoxamer-407, a nontoxic copolymer consisting of polyethylene and polyoxypropylene, blocks changes reversibly from a low viscosity solution (at 4°C) to a semisolid gel at body temperature [32]. In vivo results with intraperitonial administration of melanotar-I (an analog of α-MSH) along with polyoxamer showed that the polymer did prolong its half-life in plasma, presumably due to its slow release from the gel formed in the intraperitoneal cavity. A disadvantage of polyoxamers is that they are nonbiodegradable and are known to enhance plasma cholesterol and triacylglycerol after intraperitoneal injection in rats [33].

Jeong et al. [34] have described the synthesis of a thermoresponsive hydrogel consisting of blocks of poly(ethylene oxide) and poly(L-lactic acid). The polymer (at 45°C), upon subcutaneous injection and subsequent rapid cooling to body temperature, undergoes a sol-gel transition. The entrapped drug was released at first by diffusion and then at a faster rate as degradation mechanisms started operating. Unlike NIPAAm-based polymers or polyoxamers, these nontoxic polymers are biodegradable and form biocompatible and pharmacologically inactive products.

A pH-responsive hydrogel composed of polymethacrylic acid grafted with polyethylene glycol has been evaluated in vitro for calcitonin delivery [35]. This polypeptide is a therapeutic agent for bone diseases like Paget's disease, hypercalcemia, and osteoporesis. As the pH increased during the passage from the stomach to upper small intestine, the ionized pendant carboxyl groups caused electrostatic repulsion, the network swelled, and the hormone was released. The release behavior showed that movement of polymer chains was a key factor that controlled the solute transport.

Qiu and Park [36] have also reviewed various hydrogels responsive to various stimuli. An example worth quoting from their review uses the concept of release of antibiotics at the site and time of infection. The antibiotic, Gentamycin, was attached to the polyvinyl alcohol backbone through peptide linkers. Infected wounds produced a higher concentration of thrombin which snapped the peptide linkers and accelerated the release of the antibiotic. An example that mimics the chemical and biological design of a natural secretory granule is the anionic microgel, composed of a 1:4 mole ratio of methylene-bis-acrylamide and methacrylic acid and loaded with the cationic anticancer drug, doxorubicin [37]. These microgels showed $>$300% volume change when their pH was changed from 3.2 to 7.0 (diameter 6.5 μm) due to deprotonation of carboxyl groups. The coating of condensed microgel with a lipid membrane moved the design closer to a natural secretory granule but prevented pH-responsive volume expansion.

A major challenge in the delivery of biotherapeutics is in developing strategies for overcoming lysosomal degradation of internalized drug molecules. Poly(propylacrylic acid), a pH-responsive polymer, disrupts cell membranes at low pH values prevailing in endosomes [38]. It was shown that a ternary complex of biotinylated anti-CD3 antibody, streptavidin, and biotinylated polymer with a fluorescent label resulted in enhanced translocation to the cytoplasm of Jurkat cells.

Again, as better designs evolve, inspiration comes from biology [39]. A "bioinspired pH-responsive polymer" is modified polyethylene glycol (E3), which can deliver the linked peptide to lysosome (Figure 2). The graft on PEG is a membrane-disruptive copolymer of methacrylate and acrylate. The pH drop after endocytosis hydrolyzes the acetal link between PEG and the copolymer. Here, the workers have chosen macrophages, a rather challenging system to work with. Successful manipulation of intracellular trafficking overcame a significant problem in the delivery of protein therapeutics and vaccines.

The stimuli exploited in release systems include magnetic fields, ultrasonic radiation, electric fields, and pres-

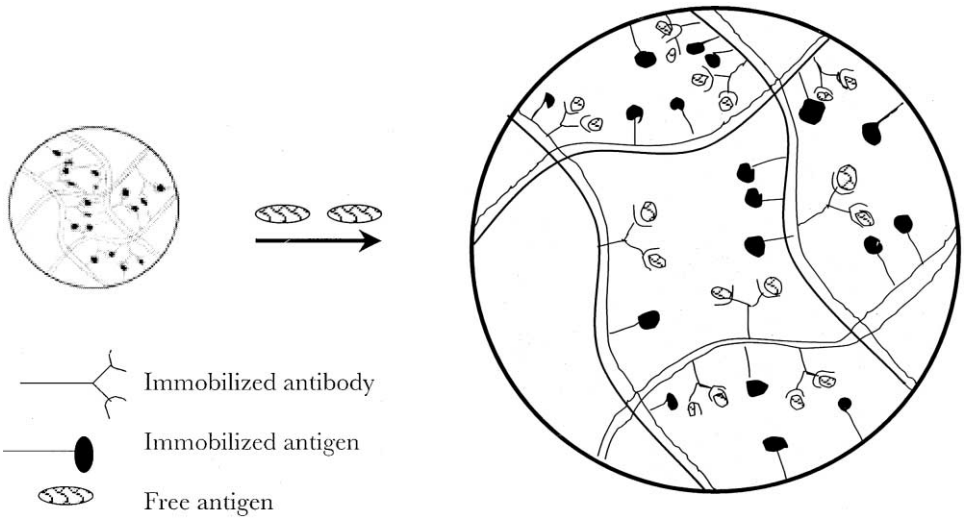

Figure 3. Schematic Diagram of the Swelling/Deswelling Properties of an Interpenetrating Antigen-Antibody Network, as Used in Immunoassays

ence of specific chemicals [13]. The release systems for naltrexone, amoxicillin, theophyllin, heparin, and calcitonin have been described [13]. The hydrogel-based products already available on the market target varied applications such as in hypertension, end-stage cancer pain, skin care, and wound and burn dressing [13].

Immunoassays and Antigen-Responsive Hydrogels

One of the earliest applications of poly(NIPAAm) in biology was in the area of immunoassays [40]. Miyata et al. [41] synthesized an antigen-antibody semi-interpenetrating network (semi-IPN) hydrogel by the copolymerization of vinyl(rabbit IgG) (obtained by chemical reaction between IgG and N-succinimidylacrylate) and acrylamide, along with N,N′-methylene bisacrylamide as a crosslinker in the presence of polymerized goat anti-rabbit IgG. The presence of an external antigen leads to the disruption of the intrachain antigen-antibody binding by displacing the bound antigen with the external one, causing the gel to swell (Figure 3). The response was reversible and showed "shape-memory effect" as a consequence of semi-IPN structure. Design of such gels requires a tradeoff between rigidity (possible by dense crosslinking) and efficient analyte binding kinetics (shown by loose networks) [42]. In yet another emerging approach, molecular imprinting, by polymerizing stimuli-responsive hydrogel in the presence of the template, imparts analyte sensitivity to the gel and allows memorization of their binding conformation and to be switched on and off by control of the external stimulus [42].

Designing Nonviral Vectors for Gene Therapy

The aims of gene therapy include curing genetic diseases and viral infections, slowing down tumor growth, and stopping neurodegenerative diseases [43]. As the basic principle is inserting the desired genetic material into the cell, finding an efficient method for the delivery of the gene and its sustained expression are crucial steps. While viral vectors are obvious choices and have been used most often so far, immune responses are a great obstacle. Two types of nonviral (synthetic) gene carriers, lipids and polymers, have been used. Both have to be cationic in nature in order to be able to form complexes with the anionic DNA, and the complex has to have net positive charge to interact with the anionic cell membrane and undergo endocytosis. The design has to conform to two contradictory requirements during endocytosis. While attaching to the cell and forming endosome, the binding between the carrier and the DNA has to be quite high. On the other hand, for DNA to move into the nucleus to initiate transcription, the complex should be easy to dissociate. It is here that stimuli-sensitive polymers are uniquely suited to fulfill the dual requirements, as the stimulus can control the binding to DNA. Furthermore, selective gene expression is possible in terms of site, timing, and duration by using temperature- or light-responsive polymers. The application of light-sensitive polymers is already illustrated by the use of L-lysine-modified polyazobenzene dendrimer for transfection of mammalian cells [44]. The temperature-responsive polymeric gene carrier utilizing PNIPAAm has also been described [45]. A copolymer with an LCST of 21°C was mixed with a plasmid DNA encoding the β-galactosidase gene at 37°C, added to the cells, and subjected to incubation for 3 hr at 20°C after preincubation for 20 hr at 37°C. The transfection efficiency was higher than the control, for which 20°C incubation was missing (Figure 4). This was the time period when the carrier formed extended chains and released DNA. In an alternative design, the carrier had an LCST above body temperature, and heat was applied for dissociation of DNA. It may be added that ultrasonic devices allow temperature change with 5 mm precision.

Another promising approach has been described by Stayton et al. [46], who found that pinocytosis of polylysine/poly(propylacrylic acid) nanoparticles could increase the transfection efficiency of polylysine/plasmid (expressing the green fluorescent protein) nanoparticles when both are taken up by NIH3T3 fibroblast cells. Poly (propylacrylic acid) becomes membrane destabilizing

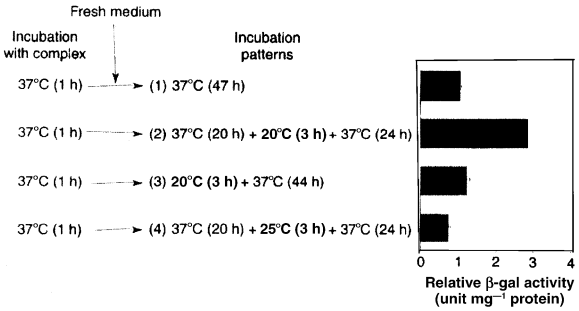

Figure 4. Plasmid Delivery by a Temperature-Sensitive Polymer

The copolymer-plasmid complex was added to cells. Incubation was followed by culturing under various conditions. The transfection efficiency was measured by assaying the enzyme activity. Reprinted from [45] with permission from Elsevier.

as the endosomal pH drops. The approach is aimed at enhancing transport across endosomal membrane to get the DNA to the right intracellular compartment. A more recent work from the same group shows that the approach works only with late endosomes and not with lysosomes [47]. Also, analogs of the polymer-like poly(2-methylacrylic acid) and poly(2-ethylacrylic acid) are ineffective. Thus, smart polymers provide potential for considerable innovation in designs for nonviral vectors for gene therapy. This assumes extra significance in view of the recent finding that treatment of severe combined immunodeficiency disease with gene therapy based upon retroviral vector is suspected to be associated with cancer risk [48].

Tissue Engineering

Tissue engineering is about delivery of appropriate cells for repair and/or development of new tissue by the use of scaffolds [1, 49]. Smart hydrogels constitute promising materials for such scaffolds for two reasons. First, their interior environment is aqueous. Second, they can release the cells at the appropriate place in response to a suitable stimulus. The difficulty in sterilizing the loaded hydrogels is an unsolved problem [1]. It has been shown that PNIPAAm above LCST could attach chondrocytes and release them below LCST [49]. A potential application is in repair of damaged cartilage sites as in rheumatoid arthritis. The coculturing of rat hepatocytes and human lung fibroblasts on polystyrene grafted with PNIPAAm has been carried out with a view to model studies on cell-cell communication [50]. Polystyrene surfaces and temperature-controlled hydrophobicity of grafted polymers could pick up the two different types of cells selectively.

Unlike in most of the other applications, the scaffolds for tissue engineering do not need to show the stimulus-dependent change in a reversible fashion. After the cells are delivered, one does not have to reverse the process. On the other hand, the biodegradability of the scaffold and lack of cytotoxicity and immunogenicity are the required traits [51]. It has also been realized that cells inside such scaffolds need effective cell-cell communications, and hence 3D constructs of such scaffolds should have appropriate geometries. Many of these considerations once again have led workers to look at biological structures for inspiration. The result has been some very thought-provoking work with self-assembling structures based on peptides, lipids, or hybrid peptide materials. These synthetic peptides also permit incorporation of features that promote cell attachment [51]. An example of a self-assembling peptide is RAD-16, a self-complementary amphiphilic peptide [51]. By varying the concentration of the peptide or salt, the former could be assembled to form a hydrogel with the shape of a tape, rope, or sheet (Figure 5). Such scaffolds have already been shown to support cell attachment of various mammalian and avian tissue culture cells. Future work will have to address the issue of adequate mechanical strength and controlling cell growth with defined 3D geometries. At present, the geometries of growing cell patterns can only be controlled in 2D [52]. The ultimate challenge will, of course, lie in designing "scaffolds that influence cell adhesion, differentiation, and migration of specific cell types to create artificial tissues" [51].

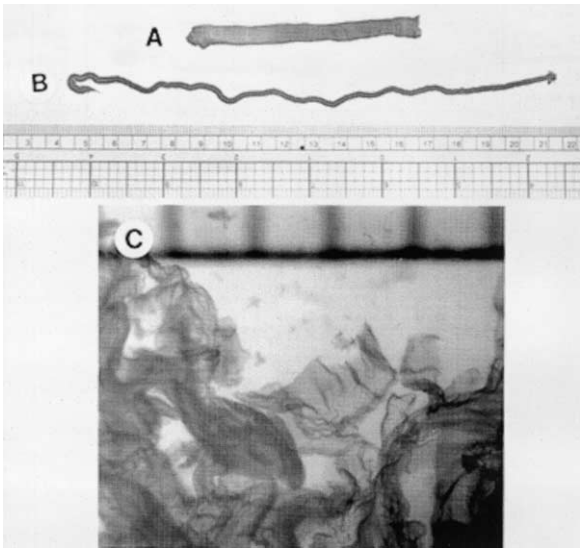

Figure 5. Amphiphilic Peptides Can Form Hydrogels of Different Shapes

A RAD16 self-assembling oligopeptide forming (A) tape- (B) rope-, and (C) sheet-shaped scaffolds after staining with Congo red. Reprinted from [51] with permission from the author. Original figure kindly supplied by Prof. T.C. Holmes.

Collier and Hessersmith [53] have designed a peptide that self-assembles into fibrillar structures and can further be crosslinked by transglutaminase. The attractive features of this design include the possibility of controlling transglutaminase action by Ca^{2+} concentration. While the N terminal of the peptide was susceptible to peptide action, the C terminal was tailored for cell attachment. The crosslinking imparted the mechanical strength just as disulphide crosslinks do in native proteins. The enzyme action can also be used in future for the decoration of these predominantly β-sheet fibrils with appropriate cell binding ligands or growth factors.

The peptides described above do show some limited β-sheet structure even in the absence of salt concentration as a stimulus. The extent of this self-assembled

structure becomes significant as the concentration of peptide increases. This may turn out to be a problem in real applications. It is not unlikely that a synergy of our understanding of synthetic hydrogels and such biomimetic structures would lead to the design of an ideal scaffold.

Molecular Gates and Switches

The Hoffman group has developed the concept of conjugating a stimulus-responsive polymer/hydrogel to a protein at a site near its ligand recognition site [54]. The carefully controlled placement of the polymer ensures that when a stimulus is applied, the collapse/swelling of the gel causes the active site of the protein to be blocked/"gated" or unblocked. This can also lead to the release of a small molecular weight ligand from the active site of the protein. In most of the cases, the polymer chosen is poly(NIPAAm), and frequently streptavidin (quite often suitably altered by protein engineering) has been the protein used. In one of the early examples, poly(NIPAAm) was linked to streptavidin at a site located just above its biotin binding site [54]. When the temperature is raised above the LCST of the hydrogel, it collapses, covering the active site. Biotin can no longer bind to streptavidin, thus the polymer effectively acts as a "molecular gate." Ding et al. [55] have conjugated poly(NIPAAm) to a site near a genetically engineered streptavidin that is involved in binding to biotin and is responsible for favoring the streptavidin-biotin interaction. Temperature-induced collapse of the polymer leads to the release of the bound molecule above its LCST. Thus, this streptavidin-polymer conjugate can act as a "trigger" for the release of biotin. Using pH as the stimulus can also bring about the release of biotin. In this case, a copolymer of acrylic acid and NIPAAm is conjugated to a genetically engineered streptavidin at a site near its biotin binding site. Changing the pH of the solution brought about the triggered release of biotin. In a novel manner of exploiting this approach, Ding et al. [56] have used the thermally sensitive polymer, poly (N,N-diethylacrylamide), and attached it to streptavidin at a site near the biotin binding site. Below its LCST (24°C), the polymer is in an extended coil conformation, thus acting as a "shield" and preventing the binding of the biotinylated protein to streptavidin. Using polymers of different sizes, one can control size selectivity of the shields. Hence, these shields can be used to discriminate among a mixture of biotinylated proteins on the basis of size. Collapse of the polymer upon increasing the temperature leads to exposure of the biotin binding site.

In a logical extension of this concept to thermal switching of enzyme activity, control of endoglucanase activity by site-directed conjugation of another acrylamide copolymer near the enzyme active site has been described recently [57]. This paper may also be consulted for some strategies for designing molecular switches, including some recent work on RNA switches and a redox switch for an endonuclease.

The concept of physical blocking of recognition sites by a collapsed form has also been utilized in design of photoswitches for ligand association. This has been shown to work with binding of biotin to streptavidin conjugate of a photoresponsive acrylamide-acrylate copolymer [58]. In a parallel work, photoregulation of endoglucanase activity by conjugation with the same copolymer has also been described [59]. Such molecular switches might be useful in bioprocessing, biosensors, prodrug therpeutic applications, and microfluidics.

With a goal to design composite systems by self-assembly for molecular switching, PNIPAAm and copolymers were also studied as adsorption layer on colloidal silica [60]. In the restricted geometry of the adsorbed layer, the phase transition was broader than in solution. In the case of the copolymer, NMR showed comparatively mobile arrangement even above the transition temperature, which was rightly interpreted in terms of electrostatic repulsion between the matrix and the polymer hindering globule formation.

Protein Folding

Another interesting recent discovery has been that smart polymers seem to simulate molecular chaperones to assist in correct protein folding [61]. Molecular chaperones act by binding to the protein folding intermediates with exposed hydrophobic residues, thus preventing aggregation and facilitating correct folded structure [62]. The smartness of the polymer is valuable, as the overall hydrophobicity of smart polymers can be varied by applying the appropriate stimulus. Also, it is possible to recycle the polymer by dissociating it from the folded protein molecule. Lin et al. [63] found that PNIPAAm increased the final yield of enzyme activity during renaturation of β-lactamase from its inclusion bodies. The presence of the polymer did not affect the initial renaturation rate, and the final yield increased with temperature. The latter is in agreement with the assumption that hydrophobicity of the polymer helps in protein folding. In a more recent work by the same group [64] but with guanidine hydrochloride-denatured carbonic anhydrase B, it was confirmed by fluorescence analysis and equilibrium studies that PNIPAAm enhances "protein refolding by the formation of complexes with aggregation-prone folding intermediates via hydrophobic interactions." An optimal molar ratio of polymer to enzyme was found, since higher polymer concentration led to protein precipitation. These workers also showed that addition of PNIPAAm after enzyme aggregation has taken place (within 1 min) did not lead to correct protein folding. Under optimum conditions, a refolding yield of 98.2% was reported. Kuboi et al. [61] showed that the presence of a thermoresponsive polymer, PPO-Ph-PEG [PEG containing poly(propylene oxide)phenyl group], increased the refolding yield of (guanidine-hydrochloride-denatured) carbonic anhydrase 1.7 times. It was also found that the polymer/dextran system increased the refolding yield of the enzyme as compared to the PEG/dextran system. The fact that 52°C was found to be optimal for this process was significant since (1) local hydrophobicity of the protein increased from 40°C onward, reached maximum around 60°C, and then declined, and (2) local hydrophobicity of the polymer PPO-Ph-PEG increased gradually with increasing temperature till 45°C, increased sharply at this phase

transition temperature, and ultimately reached a plateau beyond 45°C. Thus, 52°C, within the two values of 45°C and 60°C, presumably reflects the optimum interaction between the hydrophobic sites on the polymer and the enzyme. After the enzyme is refolded to native state, it is released from its complex with the polymer, since the folded protein has far less local hydrophobicity. Somewhat similar results have been obtained by Umakoshi et al. [65] while using a two-phase system consisting of a thermosensitive polymer, Breox (a random copolymer of ethylene oxide and propylene oxide), and dextran or a Breox/water system. It was found that unfolded chymotrypsin inhibitor 2 with a more hydrophobic surface partitioned to the relatively hydrophobic Breox phase in both systems. The inhibitor could be refolded in Breox phase and separated from it by precipitating out the polymer by increasing the temperature.

These investigations certainly help in gaining insight into the role of chaperones in protein folding and evolving efficient protein recovery protocols, especially in the case of many overexpressed proteins (inclusion bodies) in *Escherischia coli*.

Autonomous Flow Control in Microfluidics

The concept of lab-in-a-chip has evolved out of efforts to miniaturize analytical instruments. By using photolithography on a chip, one can create microchannels and work with very small volumes. As Mitchell [66] wrote, "Functional complexity brings with it a need to actively switch and control fluids, to change their flow rate and direction at different points on the chip at different stages of the process." Smart materials show considerable promise in designing microactuators for autonomous flow control inside these microfluidic channels. Saitoh et al. [67] have explored the use of glass capillaries coated with PNIPAAm for creating an on/off valve for the liquid flow. Below LCST, the PNIPAAm-coated capillary (PNIPAAm enhances the wettability at these temperatures) allowed the flow of water; above LCST, the flow was blocked as the coating was now hydrophobic. Beebe et al. [68], on the other hand, used a pH-sensitive methacrylate to control the flow inside the microchannels. The hydrogel-based microfluidic valve opened or closed depending upon the pH of the flowing solution. The design has the potential of being self-regulating/autonomous since the valve can be controlled by feedback by H^+ produced or consumed in the reaction. A good response time of less than 10 s was reported. Undoubtedly, we will see many other innovative designs for such applications in the coming years.

Conclusions

In this review, we have provided only a glimpse into the complexities and utility of smart polymeric biomaterials; however, with this snapshot we have strived to illustrate the versatility and potential of these materials. Indeed, one could postulate that the versatility of smart materials is limited only by the imaginations of their designers and by the scientists who use them. Interestingly, a recently published article discussing the "intelligence" of smart materials hypothesized that if current smart materials were rated using a standard IQ test, they would have an IQ of 2 [69]—impressive for inanimate compounds. And researchers are continuing to develop smarter, more useful compounds—we like to think that we are getting closer to designing T-1000 (the robot from Terminator 2: Judgment Day that changes from solid to liquid) [70]. One may question the reasoning of our goal of developing novel smart polymers to perform chemical and biological functions, since existing natural compounds can already meet these needs. We argue that purposefully designed biomaterials offer additional attractions such as predictability (a designed artificial polymer is a known entity) as well as an improved ability to manipulate desirable (and undesirable) traits present in assays. Drug design and medicine will profit both financially and in terms of providing high quality health care, with the ability to precisely craft artificial organs and drug delivery vehicles that "intelligently" interface with cells and organs. An area of key interest to the smart polymeric biomaterial field is the immune system. For example, using smart materials one could imagine ways to regulate the immune response to control hypersensitivity without impairing the overall immune system. Smart materials are poised for take off and will certainly promise an exciting future at the interface of chemistry and biology.

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