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Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology**

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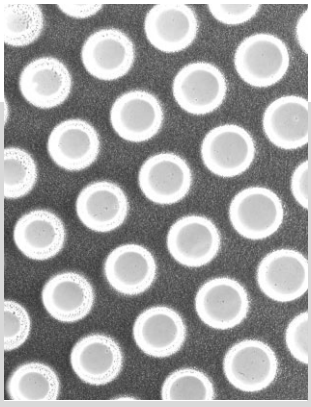

Hydrophilic polymers are the center of research emphasis in nanotechnology because of their perceived "intelligence". They can be used as thin films, scaffolds, or nanoparticles in a wide range of biomedical and biological applications. Here we highlight recent developments in engineering uncrosslinked and crosslinked hydrophilic polymers for these applications. Natural, biohybrid, and synthetic hydrophilic polymers and hydrogels are analyzed and their thermodynamic responses are discussed. In addition, examples of the use of hydrogels for various therapeutic applications are given. We show how such systems' intelligent behavior can be used in sensors, microarrays, and imaging. Finally, we outline challenges for the future in integrating hydrogels into biomedical applications.

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1. Introduction

In the last ten years there has been an explosion of advances in the fields of structured and intelligent materials science and nanomaterials properties. The variety of chemical structures, together with the precise control of the molecular architecture and morphology, rationalize the numerous uses of polymers in high-technology and biological applications. For example, photodegradable polymers and photopolymerizable monomers are utilized as photoresists in microlithography, and polymer liquid crystals find applications in nonlinear optics. Thin polymeric films are part of electronic devices and separation membranes, while biocompatible polymers are the basis of artificial organs.

Interfacial phenomena are very important in biopolymer science and engineering. The properties of these interfaces determine the applicability of various polymers. Chemical and physical interactions are essential for the development of desirable interfacial stability. Significant interest has been shown in the use of natural, synthetic, and biohybrid hydrophilic polymers as biomaterials and as carriers for drug delivery. The study and understanding of the fundamental phenomena and molecular mechanisms associated with the formation of new surfaces and interfaces is therefore neces-

sary to respond to emerging technology problems for high-performance materials.

In medical diagnostics and therapeutics, the need to improve patient care is always present; thus, there is a continuous effort to enhance methods, materials, and devices. In recent years, the development of novel biomaterials and their application to medical problems have dramatically improved the treatment of many diseases.[1,2] Biomaterials such as poly-

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mers, ceramics, and metals have been used for many years in medical applications. In addition to the over 40 000 pharmaceutical preparations in use, it is estimated that currently there are over 8000 medical devices and 2500 diagnostic products that employ biomaterials being used in various medical applications.[1] Despite the widespread use of materials in medicine, many biomaterials lack the desired functional properties to interface with biological systems and have not been engineered for optimized performance. Therefore, there is an increasing need to develop new materials to address such problems in medicine and biology. Hydrophilic polymers, and especially their crosslinked forms, known as hydrogels, are a class of biomaterials that have demonstrated great potential for biological and medical applications.

The ability to engineer traditional hydrophilic polymers with specific material properties is hampered by lack of control of molecular weight, chain configuration, and polymerization kinetics. Hybrid materials have been developed to preserve the bulk properties of traditional polymers while making their molecular chains look more like proteins. The elusive goal of molecular recognition in synthetic polymer systems has been reached in certain cases. For example, acrylic gels have been designed with recognition capabilities by incorporating non-covalently crosslinked antibodies. These proteins couple the reversible-swelling character of the networks with molecular recognition by only swelling in the presence of a specific antigen. The advantage of using synthetic polymeric materials based solely on proteins or peptides is that it offers a high degree of control over properties. Peptides and proteins can be coded for specific properties using a basic knowledge of inter- and intrachain interactions. While these interactions are understood in other polymer systems, there is much less of an ability to control them. The present and future of biomedical materials development requires this degree of control prediction in the design, synthesis, and function of next-generation materials. Recent work with this principle in mind has resulted in protein-based materials with properties analogous to more widely used polymers, as well as new properties. These new materials have been generated with a variable degree of efficiency and complexity.

Many of these hydrophilic polymer networks have a high affinity for water but are prevented from dissolving due to their chemically or physically crosslinked network. Water can penetrate in between the polymer chains of the polymer network, subsequently causes swelling and the formation of a hydrogel.[1] Hydrogels are appealing for biological applications because of their high water content and biocompatibility. In the last couple of decades, hydrogels have attracted a great deal of attention, and significant progress has been made in designing, synthesizing, and using these materials for many biological and biomedical applications. Recent developments include the design and synthesis of novel hydrogels and their use in tissue engineering, drug delivery, and bionanotechnology.

2. Hydrogel Design, Structure, and Characterization

The suitability of hydrogels as biomedical materials and their performance in a particular application depend to a large extent on their bulk structure. The most important parameters used to characterize the network structure of hydrogels are the polymer volume fraction in the swollen state ($v_{2,s}$), the molecular weight of the polymer chain between two neighboring crosslinking points ($\overline{M}_c$), and the corresponding mesh size (ξ).

The polymer volume fraction in the swollen state is a measure of the amount of fluid imbibed and retained by the hydrogel. The molecular weight between two consecutive crosslinks, which can be either chemical or physical in nature, is a measure of the degree of crosslinking of the polymer. It is important to note that due to the random nature of the polymerization process itself only average values of $\overline{M}_c$ can be calculated. The correlation length or distance between two adjacent crosslinks, ξ, provides a measure of the space available between the macromolecular chains (e.g., for drug diffusion); again, it can be reported only as an average value. These parameters, which are related to one another, can be determined theoretically or through the use of a variety of experimental techniques. Two methods that are prominent among the growing number of techniques utilized to elucidate the structure of hydrogels are equilibrium-swelling theory and rubber-elasticity theory.

The structure of hydrogels that do not contain ionic moieties can be analyzed by the Flory–Rehner theory.[1] This combination of thermodynamic and elasticity theories states that a crosslinked polymer gel that is immersed in a fluid and allowed to reach equilibrium with its surroundings is subject only to two opposing forces: the thermodynamic force of mixing and the retractive force of the polymer chains. At equilibrium these two forces are equal. This physical situation is defined in terms of the Gibbs free energy:

$$\Delta G_{\text{total}} = \Delta G_{\text{elastic}} + \Delta G_{\text{mixing}} \quad (1)$$

Here, $\Delta G_{\text{elastic}}$ is the contribution due to the elastic retractive forces developed inside the gel, and ΔG_{mixing} is the result of the spontaneous mixing of the fluid molecules with the polymer chains. The term ΔG_{mixing} is a measure of the compatibility of the polymer with the molecules of the surrounding fluid. This compatibility is usually expressed by the polymer–solvent interaction parameter, χ_1.

Differentiation of Equation 1 with respect to the number of solvent molecules while keeping temperature and pressure constant results in

$$\mu_1 - \mu_{1,o} = \Delta\mu_{\text{elastic}} + \Delta\mu_{\text{mixing}} \quad (2)$$

In Equation 2, μ_1 is the chemical potential of the solvent in the polymer gel and $\mu_{1,o}$ is the chemical potential of the pure

solvent. At equilibrium, the difference between the chemical potentials of the solvent outside and inside the gel must be zero. Therefore, changes in the chemical potential due to mixing and elastic forces must balance each other. The change of chemical potential due to mixing can be expressed using heat and the entropy of mixing.

The change in chemical potential due to the elastic retractive forces of the polymer chains can be determined from the theory of rubber elasticity. Upon equaling these two contributions, an expression for determining the molecular weight between two adjacent crosslinks of a neutral hydrogel prepared in the absence of a solvent can be written as

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\left(\frac{\bar{v}}{V_1}\right)\left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1\upsilon_{2,s}^2\right]}{\left(\upsilon_{2,s}^{1/3} - \frac{\upsilon_{2,s}}{2}\right)} \tag{3}$$

Here, $\overline{M}_n$ is the molecular weight of the polymer chains prepared under identical conditions but in the absence of the crosslinking agent, $\bar{v}$ is the specific volume of the polymer, and V_1 is the molar volume of water.

We have modified the original Flory–Rehner theory for hydrogels prepared in the presence of water.[1] The presence of water effectively modifies the change of chemical potential due to the elastic forces. This term must now account for the volume fraction density of the chains during crosslinking. The molecular weight between crosslinks in a neutral hydrogel prepared in the presence of water is determined by

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\left(\frac{\bar{v}}{V_1}\right)\left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1\upsilon_{2,s}^2\right]}{\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} - \left(\frac{\upsilon_{2,s}}{2\upsilon_{2,r}}\right)\right]} \tag{4}$$

Here, $\upsilon_{2,r}$ is the polymer volume fraction in the relaxed state, which is defined as the state of the polymer immediately after crosslinking but before swelling.

The presence of ionic moieties in hydrogels makes the theoretical treatment of swelling much more complex. In addition to the contributions of ΔG_{mixing} and $\Delta G_{elastic}$ in Equation 1, there is an additional contribution to the total change in Gibbs free energy due to the ionic nature of the polymer network, ΔG_{ionic}.

$$\Delta G_{total} = \Delta G_{elastic} + \Delta G_{mixing} + \Delta G_{ionic} \tag{5}$$

Upon differentiating Equation 5 with respect to the number of moles of solvent, keeping T and P constant, an expression similar to Equation 2 for the chemical potential can be derived as

$$\mu_1 - \mu_{1,o} = \Delta\mu_{elastic} + \Delta\mu_{mixing} + \Delta\mu_{ionic} \tag{6}$$

Here, the $\Delta\mu_{ionic}$ is the change in chemical potential due to the ionic character of the hydrogel. We have developed expressions for the ionic contributions to the chemical potential, which exhibit strong dependencies on the ionic strength of the surrounding media and on the nature of the ions present in the solvent. Equations 7 and 8 are expressions that have been derived for swelling of anionic and cationic hydrogels, respectively, prepared in the presence of a solvent:

$$\frac{V_1}{4IM_r}\left(\frac{\upsilon_{2,s}}{\bar{v}}\right)^2\left(\frac{K_a}{10^{-pH}+K_a}\right)^2 = \left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1\upsilon_{2,s}^2\right] + \left(\frac{V_1}{\bar{v}\overline{M}_c}\right)\left(1-\frac{2\overline{M}_c}{\overline{M}_n}\right)\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} - \left(\frac{\upsilon_{2,s}}{2\upsilon_{2,r}}\right)\right] \tag{7}$$

$$\frac{V_1}{4IM_r}\left(\frac{\upsilon_{2,s}}{\bar{v}}\right)^2\left(\frac{K_b}{10^{pH-14}-K_a}\right)^2 = \left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1\upsilon_{2,s}^2\right] + \left(\frac{V_1}{\upsilon\overline{M}_c}\right)\left(1-\frac{2\overline{M}_c}{\overline{M}_n}\right)\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} - \left(\frac{\upsilon_{2,s}}{2\upsilon_{2,r}}\right)\right] \tag{8}$$

In these expressions, I is the ionic strength, K_a and K_b are the dissociation constants for the acid and base, respectively, and M_r is the molecular weight of the repeating unit.

Hydrogels resemble natural rubbers in their remarkable property to elastically respond to applied stresses. A hydrogel subjected to a relatively small deformation (less than 20 %) will fully recover to its original dimension in a rapid fashion. This elastic behavior of hydrogels can be used to elucidate their structure by utilizing the rubber-elasticity theory. Here, only the form of rubber-elasticity theory used to analyze the structure of hydrogels prepared in the presence of a solvent is presented, and it is left to the reader to consult a standard reference for detailed derivations.[3]

$$\tau = \frac{\rho RT}{\overline{M}_c}\left(1-\frac{2\overline{M}_c}{\overline{M}_n}\right)\left(a-\frac{1}{a^2}\right)\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} \tag{9}$$

Here, τ is the stress applied to the polymer sample, ρ is the density of the polymer, R is the universal gas constant, T is the absolute experimental temperature, and $\overline{M}_c$ is the desired molecular weight between crosslinks. In order to perform analysis of the structure of hydrogels using rubber-elasticity theory, experiments need to be performed using a tensile testing system. Interestingly, rubber-elasticity theory has been used not only to analyze chemically and physically crosslinked

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hydrogels, but also hydrogels exhibiting temporary crosslinks due to hydrogen bonding.

An important structural parameter for analyzing hydrogels is the space available between macromolecular chains. This space is often regarded as the molecular mesh or pores. Depending upon the size of these pores, hydrogels can be conveniently classified as i) macroporous, ii) microporous, and iii) nonporous. A structural parameter that is often used in describing the size of the pores is the correlation length (ξ), which is defined as the linear distance between two adjacent crosslinks and can be calculated using

$$\xi = a(\bar{r}_o^2)^{1/2} \tag{10}$$

Here, a is the elongation ratio of the polymer chains in any direction and $(\bar{r}_o^2)^{1/2}$ is the root-mean-square, unperturbed, end-to-end distance of the polymer chains between two neighboring crosslinks. For an isotropically swollen hydrogel, the elongation ratio (a) can be related to the swollen polymer volume fraction, ($\upsilon_{2,s}$) by

$$a = \upsilon_{2,s}^{-1/3} \tag{11}$$

The unperturbed end-to-end distance of the polymer chain between two adjacent crosslinks can be calculated using Equation 12, where C_n is the Flory characteristic ratio, L is the length of the bond along the polymer backbone (for vinyl polymers, 1.54 Å), and N is the number of links per chain that can be calculated by Equation 13.

$$(\bar{r}_o^2)^{1/2} = l(C_n N)^{1/2} \tag{12}$$

$$N = \frac{2\overline{M}_c}{M_r} \tag{13}$$

In Equation 13, M_r is the molecular weight of the repeating units of which the polymer chain is composed. Finally, when one combines Equations 10 through 13, the correlation distance between two adjacent crosslinks in a swollen hydrogel can be obtained as

$$\xi = \upsilon_{2,s}^{-1/3}\left(\frac{2C_n\overline{M}_c}{M_r}\right)^{1/2} l \tag{14}$$

The ability to tailor the molecular structure of hydrogels (e.g., $\upsilon_{2,s}$, $\overline{M}_c$, and ξ) enables the tailoring of their mechanical, responsive, and diffusive properties. As a result, hydrogels have been, and continue to be, an attractive material choice for a wide variety of biological and medical applications. In addition, the type of crosslinking can greatly modify the properties of the hydrogel. The crosslinking structure of the hydrogel can be due to a number of factors such as covalent bonding, entanglements, hydrogen bonding, ionic bonding, and formation of crystallites. Covalent crosslinks can lead to stable hydrogels, while other types of crosslinks could be used to reverse the gelling properties of the hydrophilic polymers under the desired conditions. Hydrogels can be characterized based on their derivation and composition as synthetic, biological, or a hybrid. In the following section, the various types of hydrogels are introduced.

2.1. Synthetic Hydrogels

Polymer networks can be synthesized using various chemical methods (e.g., photo- and thermal-initiated polymerization). The polymer engineer can design and synthesize polymer networks with molecular-scale control over structure such as crosslinking density and with tailored properties, such as biodegradation, mechanical strength, and chemical and biological response to stimuli.

Neutral synthetic polymers can be generated from derivatives of poly(hydroxyethyl methacrylate) (PHEMA), poly(ethylene glycol) (PEG), and poly(vinyl alcohol) (PVA) (Scheme 1). PEG hydrogels are one of the most widely studied and used materials for biomedical applications. PEG hydrogels are nontoxic, non-immunogenic, and approved by the US Food and Drug Administration for various clinical uses. In many cases, PEG has been applied as a "stealth material" since it is inert to most biological molecules such as proteins. Some of the earliest work on the use of PEG and poly(ethylene oxide) (PEO) as hydrophilic biomaterials was performed by Merrill et al.,[4] who showed PEO adsorption onto glass surfaces prevented protein adsorption. Since then, many forms of PEG surface modification have been used in order to render a surface protein resistant and to enhance surface biocompatibility.[5] Commonly used methods of PEG surface modification include covalent bonding through silane, acrylate, and thiol linkages, adsorption, and ionic and hydrogen bonding, all of which have been reviewed elsewhere.[5,6]

PEG polymers can be covalently crosslinked using a variety of methods to form hydrogels. A particularly appealing method of crosslinking PEG chains is through photopolymerization using acrylate-terminated PEG monomers.[7] In the presence of cells, PEG hydrogels are passive constituents of the cell environment since they prevent adsorption of proteins. However, numerous methods of modifying PEG gels have made PEG gels a versatile template for many subsequent conjugations. For example, peptide sequences have been incorporated into PEG gels to induce degradation[8] or modify cell adhesion (Fig. 1).[9] In addition to chemical modification, block copolymers of PEG, such as triblock copolymers of PEO and poly(propylene oxide) (henceforth designated as PEO-*b*-PPO-*b*-PEO), degradable PEO, poly(lactic acid) (PLA), and other similar materials, can be used to add specific properties to the PEG hydrogels.[10]

PHEMA is another hydrogel that has been extensively studied and used in biomedical applications such as contact

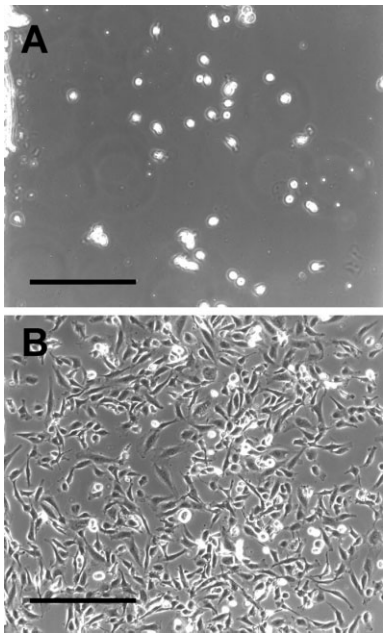

Figure 1. Light microscopy images of endothelial cells attached (3 h after seeding) to the surface of PEG hydrogels fabricated A) without RGDS and B) with 5.0 mM Acr-PEG-RGDS. Scale bars correspond to 200 μm. Reproduced from [9].

lenses[11] and drug delivery.[12] The attractive features of PHEMA include its mechanical properties, its optical transparency, and its stability in water. Like PEG, various modifications can be made to PHEMA derivatives to modify its properties. For example, dextran-modified PHEMA gels have been synthesized to modulate the degradation properties of the gel.[13] Also, copolymerization of HEMA monomers with other monomers, such as methyl methacrylate, can be used to modify properties such as swelling and mechanical properties. Using these approaches, PHEMA and its derivatives have been used in drug-delivery and tissue-engineering applications.[14]

Another major synthetic polymer is PVA.[15] PVA hydrogels are stable, and elastic gels that can be formed by the repeated freezing and thawing process or chemically crosslinked.[16] They can be formed by both physical and chemical crosslinking methods.[17] The physically crosslinked versions of PVA hydrogels are biodegradable, and thus can be used for various biomedical applications.[17–22] PVA must be crosslinked in order to be useful for a wide variety of applications, specifically in the areas of medicine and pharmaceutical sciences. Crosslinking may be achieved by chemical, irradiative, or physical mechanisms.

PVA can be crosslinked through the use of difunctional crosslinking agents. Some of the common crosslinking agents that have been used for PVA hydrogel preparation include glutaraldehyde, acetaldehyde, formaldehyde, and other monoaldehydes. When these crosslinking agents are used in the presence of sulfuric acid, acetic acid, or methanol, acetal bridges form between the pendent hydroxyl groups of the PVA chains. As with any crosslinking agent, however, residual amounts are present in the ensuing PVA gel. It becomes extremely undesirable to perform the time-consuming extraction procedures in order to remove this residue. If the residue is not removed, the gel is unacceptable for biomedical or pharmaceutical applications because, if it were placed directly in the body, the release of this toxic residue would have obvious undesirable effects. Other methods of chemical crosslinking include the use of electron-beam or gamma irradiation. These methods have advantages over the use of chemical crosslinking agents as they do not leave behind toxic, elutable agents. In addition, photocrosslinkable PVA hydrogels have been synthesized that facilitate cell adhesion in tissue-engineering applications.[23,24]

2.1.1. Responsive Hydrogel Systems

By tailoring their molecular structure, polymer networks can be created that interact with their environment in a preprogrammed and intelligent manner. Environmentally responsive hydrogels have been synthesized that are capable of sensing and responding to changes to external stimuli, such as changes to pH, p*I*, and temperature.[25] Recent reviews have highlighted the extensive research focused on the development and application of new environmentally sensitive hydrogels, especially those sensitive to temperature, pH, and specific analytes.[3,26–28]

The response mechanism is based on the chemical structure of the polymer network (e.g., the functionality of chain side groups, branches, and crosslinks). For example, in networks that contain weakly acidic or basic pendent groups, water sorption can result in ionization of these pendent groups depending on the solution pH and ionic composition. The gels then act as semipermeable membranes for the counterions, thereby influencing the osmotic balance between the hydrogel and the external solution through ion exchange, depending on the ion–ion interactions. For ionic gels containing weakly acidic pendent groups, the equilibrium degree of swelling increases as the pH of the external solution increases, while the degree of swelling increases as the pH decreases for gels containing weakly basic pendent groups. Numerous properties (e.g., ionic content, ionization equilibrium considerations, nature of counterions, and nature of the polymer) contribute to the swelling of ionic hydrogels, and these have been extensively studied.[29–31] Examples of some commonly studied ionic polymers include poly(acrylic acid), poly(methacrylic acid), polyacrylamide (PAAm), poly(diethylaminoethyl methacrylate), and poly(dimethylaminoethyl methacrylate) (Scheme 1).

Temperature-responsive hydrogels are one of the most widely studied responsive hydrogel systems. These systems, which are mostly based on poly(*N*-isopropylacrylamide) (PNIPAAm) and its derivatives, undergo a reversible volume-phase transition with a change in the temperature of the envi-

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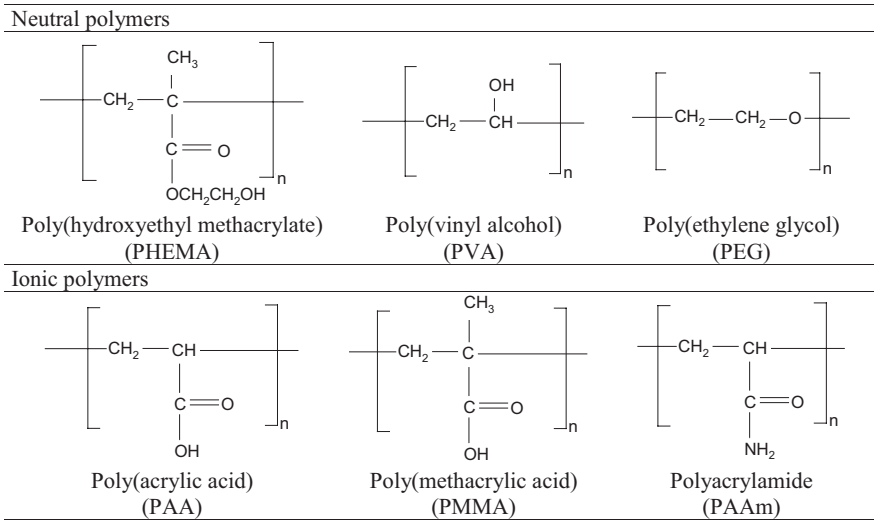

Scheme 1. Representative chemical structures of synthetic neutral and charged polymers.

ronmental conditions. This type of behavior is related to polymer phase separation as the temperature is raised to a critical value known as the lower critical solution temperature (LCST). Networks showing a lower critical miscibility temperature tend to shrink or collapse as the temperature is increased above the LCST, and the gels swell upon lowering the temperature below the LCST. For example, PNIPAAm exhibits a LCST around 33 °C. PNIPAAm and other thermosensitive hydrogels have been studied for variety of applications, including drug delivery and tissue engineering.[27,32]

2.1.2. Imprinted Hydrogels

In many applications, it is desirable to control the molecular-recognition properties of hydrogels for various biological analytes and physiological processes. The design and synthesis of such molecular-recognition schemes requires techniques in which the chemical functionality and structure can be organized in a precise 3D configuration. Polymer networks exhibiting these desired characteristics can be prepared using template-mediated polymerization techniques (e.g., molecular imprinting), resulting in recognition domains that can specifically bind template molecules with high affinity.[33] Although the field of molecular imprinting is more than three decades old, only recently have researchers applied these techniques to hydrogel systems, to biologically significant target molecules, and to the creation of controlled drug-delivery systems.[34–37] This field of research shows great promise and could be used to synthesize synthetic gels that recognize particular analytes.

2.2. Biological Hydrogels

In general, hydrogels from natural sources can be derived from polymers such as collagen, hyaluronic acid (HA), fibrin, alginate, agarose, and chitosan.[38] Depending on their origin and composition, various natural polymers have specific utilities and properties. Many natural polymers, such as collagen, hyaluronic acid, and fibrin, are derived from various components of the mammalian extracellular matrix. Collagen is the main protein of the mammalian extracellular matrix, while HA is a polysaccharide that is found in nearly all animal tissues. Alternatively, alginate and agarose are polysaccharides that are derived from marine algae sources. The advantages of natural polymers include low toxicity and biocompatibility.

Collagen and other mammalian-derived protein-based polymers are effective matrices for cellular growth because they contain many cell-signaling domains present in the in vivo extracellular matrix. Collagen gels can be created through natural means without chemical modifications. However, in many cases these gels are mechanically weak. To synthesize gels with enhanced mechanical properties, various methods have been developed such as chemical crosslinking,[39,40] crosslinking with UV or temperature,[39,41] or mixing with other polymeric agents.[39,42] Collagen degradation is mediated through natural means by proteins such as collagenase.

HA is a glycosaminoglycan (GAG) that is composed of repeating disaccharide units and is particular prevalent during wound healing and in joints. Covalently crosslinked HA hydrogels can be formed by means of multiple chemical modifications.[43–46] HA is degraded by cells through the release of enzymes such as hyaluronidase.

Alginate is a linear polysaccharide that is derived from brown seaweed and bacteria. It gels under benign conditions, which makes it attractive for cell encapsulation. Alginate gels are formed upon formation of ionic bridges between divalent cations (i.e., Ca^{2+}) and various polymer chains of the alginate. The crosslinking density of alginate gels is a function of the monomer units and molecular weight of the polymer. Alginate gels degrade slowly in a process in which the mechanical properties of the gels are altered with time.

Chitosan is another naturally occurring linear polysaccharide derived from chitin. Dissolved chitosan can be crosslinked by increasing pH, by dissolving in a nonsolvent[47] or by photocrosslinking.[48] Chitosan can be degraded by the lysosome and is therefore degraded in humans.[49] Chitosan gels can be used for many applications, including drug delivery.[50,51]

2.3. Biohybrid Hydrogels

By integrating biological entities with synthetic hydrogels, novel systems can be created that synergistically combine well-evolved biological mechanisms, such as high affinity and specificity of binding, with tailorable hydrogel properties (e.g., mechanical stability and environmental-responsive properties). For example, biologically active molecules can be incorporated into polymer networks (e.g., by physical or chemical entrapment) to produce conjugated biomaterials.[52]

Research groups have immobilized enzymes within the network structure of hydrogels. For instance, activated glucose oxidase has been incorporated into pH-sensitive cationic hydrogels.[53] The glucose oxidase converts glucose into gluconic acid, thereby lowering the pH of the local environment, which then causes the hydrogel network to swell in the case of a cationic gel.

In other work, stimuli-responsive hybrid materials consisting of hydrogels and genetically engineered proteins have been demonstrated (Fig. 2).[54] The stimuli-responsive hydrogel exhibited gating and controlled transport of biomolecules across the network, demonstrating its potential for microfluidics and drug delivery.

Hydrogels have been synthesized so that they contain functional groups for enhancing cellular adhesion.[9,55] In this scheme, the addition of such modalities can dramatically change the properties of the hydrogels. The most common peptides used to modify hydrogels are amino acid sequences derived from natural proteins, such as RGD (derived from proteins such as fibronectin, laminin, or collagen), IKVAV, and YIGSR from laminin. Using these approaches, PEG[9,55,56] and other hydrogels, such as alginate,[57] have been modified with RGD to enhance cellular adhesion (Fig. 1). Also, PVA gels have been modified to enhance cellular adhesion by incorporation of GHK[58] or RGDS[59] sequences for adhesion of hepatocytes and epithelial cells, respectively.

In addition, the degradation property of hydrogels may be modified through incorporation of degradable linkers. Many synthetic gels have been modified in various ways to change their properties. For example, Hubbell, Anseth, West, and others have synthesized degradable PEG hydrogels based on a number of schemes, such as synthesis of block copolymers with degradable blocks[10,60,61] and the incorporation of proteases.[8] Furthermore, other hydrogels have also been linked with degradable units to render them degradable. For example, dextran has been incorporated into PHEMA gels to form enzymatically degradable gels.[13]

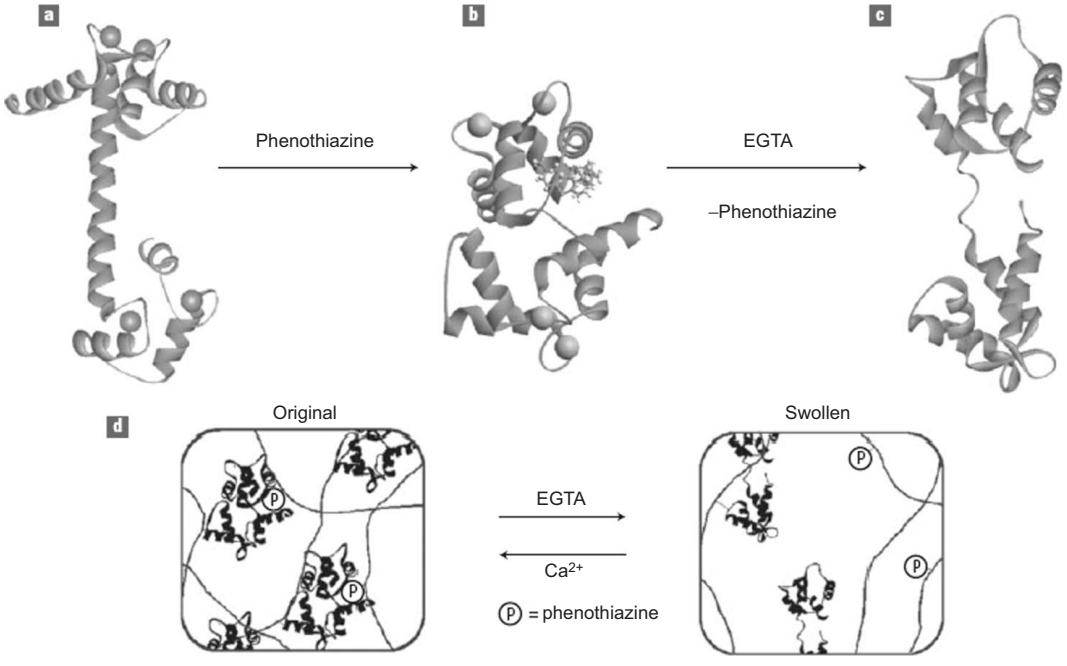

Figure 2. Genetically engineered biohybrid hydrogel and swelling of stimuli-responsive hydrogel. Calmodulin (CaM) can have three different conformations a) dumbbell (spheres represent four bound Ca^{2+} ions and are larger for emphasis); b) CaM with bound phenothiazinebound (Ca^{2+} and ball-and-stick structure for phenothiazine shown); c) native conformation in the absence of Ca^{2+}. d) Hydrogel swelling mechanism in response to ethylene glycol tetraacetic acid (EGTA). CaM is originally bound to phenothiazine. In the presence of EGTA, Ca^{2+} is removed from its binding sites in CaM and the noncovalent crosslinking is broken, resulting in expansion of the hydrogel network. Reproduced with permission from [54]. Copyright 2005 Nature Publishing Group.

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Another form of modification of hydrogels is the incorporation of growth factors into the gel. Growth factors can be covalently attached to the hydrogels. For example, transforming growth factor beta (TGF-β) has been tethered to PEG to regulate smooth muscle cell function;[62] in addition, other TGF-β related proteins such as bone morphogenic protein 2 (BMP-2) have been covalently attached to alginate to regulate osteoblast migration and calcification into the gels.[63]

Natural monomers such as peptides have also been used to synthesize hydrogels. For example, genetic-engineering approaches to synthesize peptides have also been used to fabricate hydrogels made from artificial peptides and artificial proteins.[64–67] Polypeptides designed using genetic engineering have many advantages over synthetic peptides, including its ease of synthesis using established protocols. Polypeptides have been used to synthesize silklike structures with pH-sensitive variations that incorporate GA modalities.[65] Other polypeptide variations include elastin-based materials.[68] In addition, Zhang has developed a series of hydrogels made from self-assembling peptides.[64] The self-assembly can be controlled by microenvironmental features such as pH that allow the gels to be formed in situ as required. Although current approaches to synthesize these gels are expensive, it is anticipated that new processes to enhance such techniques will be valuable in making these gels more economically feasible.

3. Hydrogels in Therapeutics

Hydrogels have been applied as fundamental components in a variety of therapeutic applications. In the following, we briefly highlight examples in tissue engineering and controlled drug delivery. For example, attention has recently been given to the use of micro- and nanofabrication approaches to deliver drugs and to fabricate vascularized tissue-engineering scaffolds. Naturally, because of their biocompatibility, compatibility with microscale fabrication approaches, and their responsiveness to their environment, hydrogels and hydrophilic polymers are becoming important for materials for constructing these devices.

3.1. Tissue Engineering

Tissue engineering aims to replace, repair, or regenerate tissue or organ function and to create artificial tissues and organs for transplantation.[69] Scaffolds used in tissue engineering mimic the natural extracellular matrix (ECM) and provide support for cell adhesion, migration, and proliferation. They also allow for differentiated function, new tissue generation, and its 3D organization. Of course, scaffolds need to be completely biodegradable so that after tissue is grown, the resulting structures are made entirely from biological components.

Cell-laden hydrogels are interesting scaffolding materials. Their high water content, biocompatibility, and mechanical properties that resemble natural tissues make hydrogels particularly attractive for tissue-engineering applications. By adding cells to a hydrogel before the gelling process, cells can be distributed homogeneously throughout the resulting scaffold. Cells have been encapsulated in both natural hydrogels, such as collagen and fibrin materials, as well as in synthetic hydrogels made from PEG. Also, combinations of natural and artificial polymers can be used to provide proper scaffold degradation behavior after implantation. Fibroblasts, osteoblasts, vascular smooth muscle cells, and chondrocytes successfully immobilize and attach to these hydrogel scaffolds. With a combination of microfluidic channel technology and photopatterning of hydrogels, these scaffolds can facilitate increased growth-factor delivery and shape sculpting that is only limited by its molded housing.[70]

Hydrogels have been used as scaffolds for tissue engineering[38,57] and as immunoisolation barriers for microencapsulation technology (Fig. 3).[71–73] In microencapsulation, allogeneic or xenogeneic cells are protected from the host's

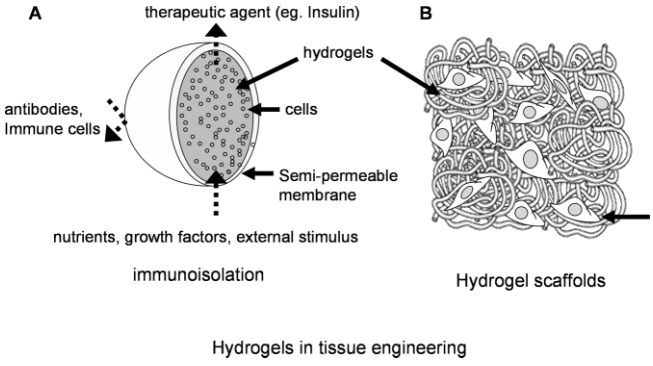

Figure 3. Hydrogels and tissue engineering. Schematic diagram of the use of hydrogels in microencapsulation (A) and as a tissue-engineering scaffold (B).

immune system through separation from the immune components using a semipermeable membrane (Fig. 3A). Lim and Sun demonstrated the use of alginate-based polymers that were crosslinked using calcium ions for the treatment of diabetic animals.[73] In addition to calcium alginate based microcapsules, the use of PEG coatings as a method of coating cells has also been shown.[74] Additionally, polymeric microcapsules containing cells can be immobilized in hydrogels such as agarose gels to enhance the functionality of transplanted constructs.[71] In tissue-engineering scaffolds, hydrogels can be used to deliver signals to the cells, act as support structures for cell growth and function, and provide space filling (Fig. 3B).[38,57,75,76] Desired characteristics of hydrogel scaffolds include physical parameters such as mechanical strength and degradability, while biological properties include biocompatibility and the ability to provide a biologically relevant microenvironment.

Natural hydrogels, such as collagen, were some of the first materials used in tissue engineering. Collagen has been used for tissue engineering of various organs such as liver,[77] skin,[78] and blood vessels.[79] Natural materials such as hyaluronic acid hydrogels can be crosslinked to form degradable hydrogels.[45,80] Natural materials have also been used in conjunction with poly(L-glutamic acid) (lactic-*co*-glycolic acid) tissue-engineering scaffolds to provide more support for cell growth. For example, fibrin-filled PLGA scaffolds have been used to modulate tissue invasion in vivo for bone regeneration.[81] Also, natural materials such as alginate have been modified with RGD peptides to modify osteoblast adhesion in bone-tissue engineering.[57] Hubbell and co-workers have modified specific groups in fibrin such as factor XIIIa so that the properties of this natural hydrogel, such as degradability, can be controlled.[82]

In addition to natural hydrogels, synthetic hydrogels have also been widely used in various tissue-engineering applications. PEG is the most commonly used synthetic polymer for tissue engineering. PEG gels are inherently cell repellent; however, with chemical modification it is possible to incorporate various peptides or other signaling molecules into the gels that reduce their cell-repulsion behavior.[83]

Recently, photopolymerizable hydrogels[9,84] have been used for many tissue-engineering applications, including growth of bone,[56,85,86] cartilage,[87–89] vascular tissues,[90] and other tissues.[9] Photocrosslinking allows the polymers to be gelled in situ, enabling the polymer to conform to the shape of the implantation site. In addition, cells within photocrosslinked hydrogels are uniformly distributed.[91] Various parameters of these polymers can be controlled to modify cell behavior. For example, UV exposure, photoinitiator concentration, monomer chain length, and conjugation of various biological molecules can be used to modify gel properties.

3.2. Controlled Drug Delivery

Hydrogels have been widely applied as intelligent carriers in controlled drug-delivery systems.[3,26,92,93] Researchers have engineered their physical and chemical properties at the molecular level to optimize their properties, such as permeability (e.g., sustained-release applications), enviro-responsive nature (e.g., pulsatile-release applications), surface functionality (e.g., PEG coatings for stealth release), biodegradability (e.g., bioresorbable applications), and surface biorecognition sites (e.g., targeted release and bioadhesion applications), for controlled drug-delivery applications (Fig. 4).

Control of hydrogel swelling properties can be used as a method to trigger drug release.[94] One example of how the change in the swelling properties of hydrogels can be used in drug delivery is a PVA and PEG system.[95] By controlling the polymer chain length, polymer composition, and initiation concentration and other factors, it is possible to control the density and degree of network crosslinking.

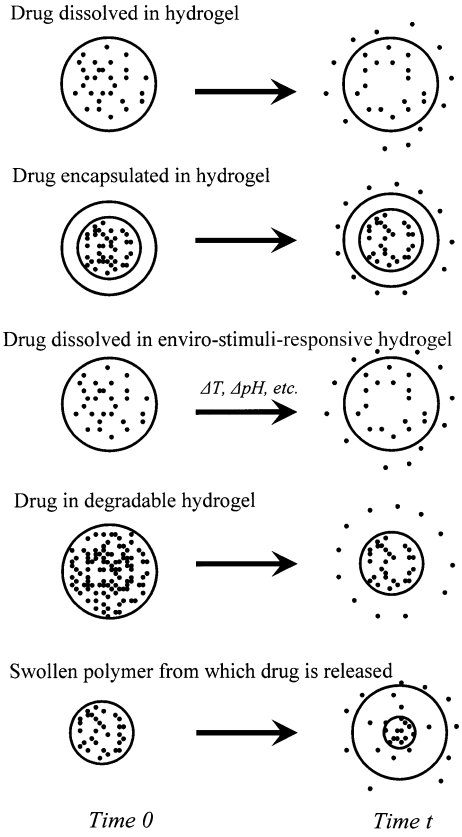

Figure 4. Various delivery and release mechanisms of hydrogels.

Environmentally responsive hydrogels have been applied in a wide variety of controlled drug-delivery applications. The intelligent response of these systems allows for release that is controlled by the conditions of the environment. Temperature-responsive hydrogels (e.g., PNIPAAm, which is the most widely studied) have been widely used to create drug-delivery systems that exhibit a pulsatile release in response to temperature changes.[27,32] In addition, pH-responsive hydrogels have been applied in numerous controlled-release applications. For example, pH-responsive hydrogels composed of PEG-containing ionic networks have been applied for the oral delivery of proteins such as insulin[96,97] and calcitonin.[98,99]

By incorporating enzymes within environmentally responsive hydrogels, researchers have created drug-delivery systems that are responsive to biological analytes. For example, an important class of polymers for drug delivery are glucose-responsive hydrogels that are based on polymers incorporating glucose oxidase within their network.[53,100,101] These polymers exhibit release kinetics that could be useful as "smart" materials for diabetes applications in which the materials can sense changes in glucose concentration and release insulin in response.

Another area of drug delivery where hydrogels have proven beneficial is in systems where molecular recognition is utilized for enhanced residence times, sustained delivery, and/or targeted drug delivery. Over the last twenty years,[102] bioadhe-

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sion has been the focus of extensive research. In particular, bioadhesive and mucoadhesive systems have been the focus of research where enhanced residence times will lead to improved drug delivery and activity.[103] In recent years, biomimetic systems that mimic biological recognition processes have proven valuable in drug-delivery applications, and these systems have shown great potential for enhanced drug-delivery systems.[36,37]

4. Hydrogels in Diagnostic Devices

Hydrogels can also be used as integral components in microdevices. This is because hydrogels can be incorporated within microdevices using photolithographic, molding, or other approaches. The ability to easily integrate hydrogels is particularly appealing given the breadth of smart hydrogels that have been developed. Environmentally responsive hydrogels have been used as functional components of microdevices, including as biosensors and valves. pH-sensitive photocrosslinkable PEG-based hydrogels have been shown to function as functional valves within microfluidic channels by sensing the pH of the solution and in response changing their swelling, which results in actuation.[104] Other methods of actuating valves using smart materials are now being investigated, such as the use of photoactive (Fig. 5), temperature-dependent, or electrically and chemically sensitive polymers that change their properties using controlled external stimuli.

PEG hydrogels have also been used within microchannels to fabricate structures capable of adding functional capability. The integration of PEG hydrogels within microfluidic channels has been shown to control the location of proteins and cells within a microfluidic channel for controlled microreactors.[105,106] In addition, PEG hydrogels can be used to form microstructures within channels capable of capturing and localizing cells in regions of low shear stress (Fig. 6).[107] The ability to capture cells from flowing solutions can be used for many applications such as sensing, cell separation, and cell-based microreactors.

Microchannels can also be used to synthesize hydrogels with unique properties. One recently illustrated example is in controlling the spatial properties of materials. Controlling the spatial properties of materials could be potentially useful for a variety of applications such as tissue engineering and drug delivery.[108] Previously, synthesis of gels with spatially distinct properties required cumbersome methods such as generating gradients of ligands within hydrogels and then using photoreactive domains to covalently anchor the ligands to the gel. For example, by using lasers, specific regions within an agarose gel could be tethered with RGD peptide, which allowed for neurite extension within peptide-modified regions.[109] Recently, microfluidic systems have been used to control the spatial properties of materials. By generating a concentration gradient of the photocrosslinkable monomers within a micro-

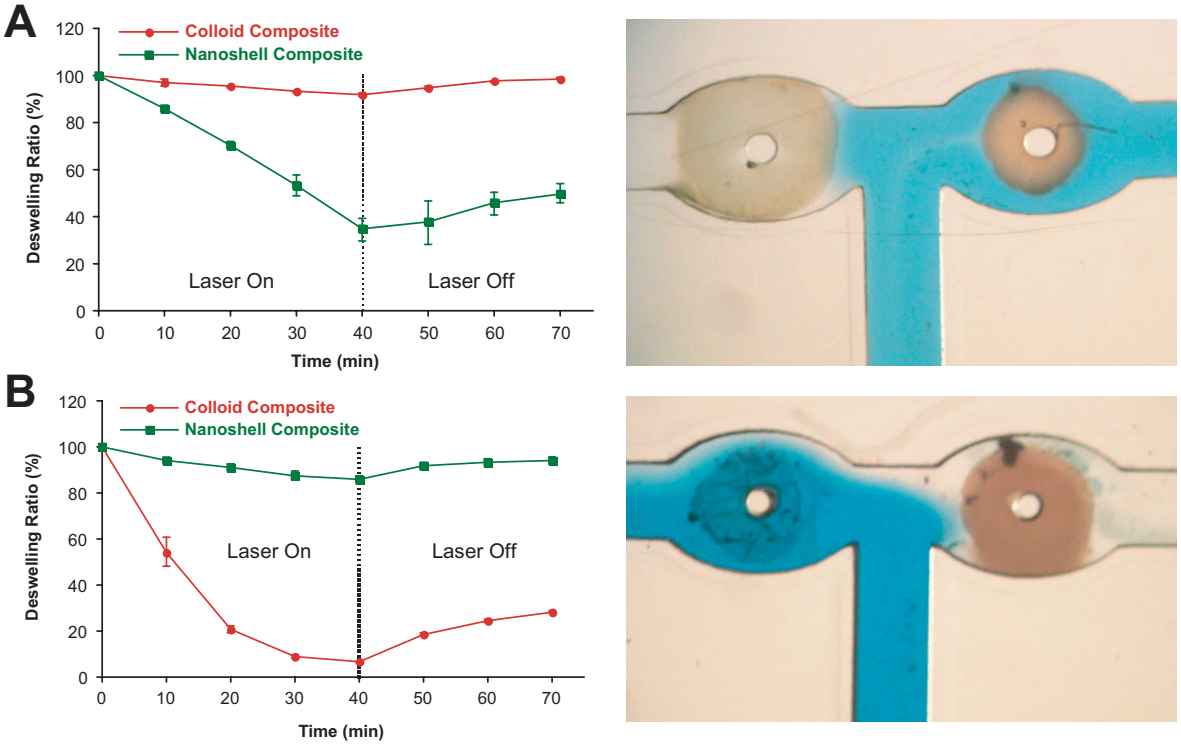

Figure 5. The collapse and reswelling of gold–colloid composite hydrogels (red circles) and gold-nanoshell composite hydrogels (green squares) during and after irradiation at A) 832 nm and B) 532 nm. Images in the right panel indicate how the flow within the channels can be altered by swelling of the hydrogels. Reproduced from [70].

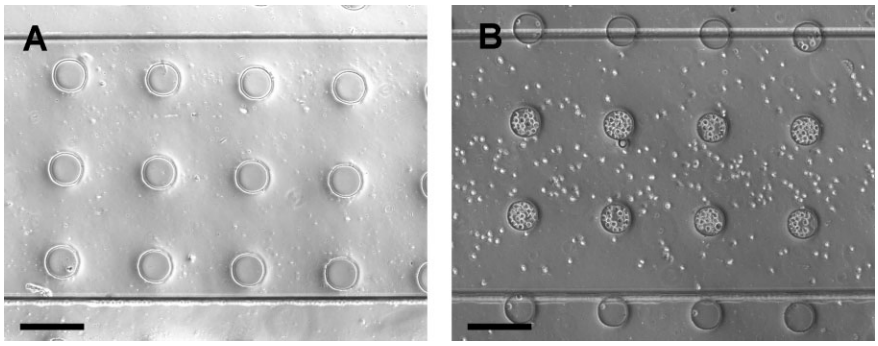

Figure 6. PEG hydrogel microstructures within microfluidic channels. A) PEG microwells were fabricated within microfluidic channels. B) As cells flowed in the channels, they docked within the low-shear-stress regions within the microwells. Reproduced with permission from [107]. Copyright 2004 Royal Society of Chemistry.

fluidic channel, it is possible to fabricate gels with control over their spatial properties (Fig. 7).[55] Gels can be synthesized with gradients of signaling or adhesive molecules or with varying crosslinking density across the material. Such gels can be used to release drugs in a spatially dependent manner, to induce directed cell migration or adhesion within the gel, or to study biological systems.[55,110]

It is anticipated that the ability to enclose biological detection molecules, such as antibodies within hydrogels can be used to enhance biological signals from these analysis since a higher number of targeting molecules can be immobilized within a particular region of a channel.[105] The increase in the density of the desired targeting receptor in the device increases its sensitivity in comparison to direct immobilization of the antibody on a surface.

4.1. Medical and Biological Sensors

In addition to pumps and valves, hydrogels can be used as integrated sensors within microdevices. Recently, microelectromechanical systems (MEMS) sensor platforms, specifically those based on microcantilevers, have been applied in a wide variety of applications due their miniature size and ultrahigh sensitivity. For example, environmentally responsive hydrogels have been micropatterned onto silicon microcantilevers to develop an ultrasensitive bioMEMS sensor platform (Fig. 8).[111,112] This was the first demonstration of a microscale MEMS sensor device where actuation is controlled by an intelligent polymer network. In similar work, Thundat and co-workers[113] have recently demonstrated a variation on this novel sensor platform by integrating hydrogels responsive to CrO_4^{2-} with commercial silicon microcantilevers to create CrO_4^{2-} sensors. More recently, another variation has been demonstrated where hydrogels containing benzo-18-crown-6 coated on microcantilevers were used to create Pb^{2+} sensors.[114]

In other work utilizing the actuation response of hydrogels, Grimes and co-workers[115,116] demonstrated wireless pH sensors based on integrating pH-responsive hydrogels with magnetoelastic thick films. The sensor device functioned by remotely monitoring the change in resonance frequency resulting from an applied mass load of the magnetoelastic sensor device. Recently, Han et al.[117] demonstrated a constant-volume hydrogel osmometer as a novel sensor platform. The concept was illustrated with a device where a pH-responsive hydrogel was confined between a rigid semipermeable membrane and the diaphragm of a miniature pressure sensor. Changes in the osmotic swelling pressure of the hydrogel resulting from changes in pH were accurately measured via the pressure sensor. Although the device had macroscale dimensions, the design could be easily miniaturized for microscale sensor development. Other groups have demonstrated macroscale sensor platforms for pH[118] and CO_2[119] using pressure sensors to transduce the swelling response of hydrogel systems. These systems also have the ability to be miniaturized, which would greatly enhance their applicability.

Several research groups have patterned hydrogels containing immobilized oxidoreductase enzymes, such as glucose oxidase, lactate oxidase, and alcohol oxidase, onto electrodes using photolithography to create biosensors for monitoring various analyte levels.[120–125] In other work, Sheppard and co-

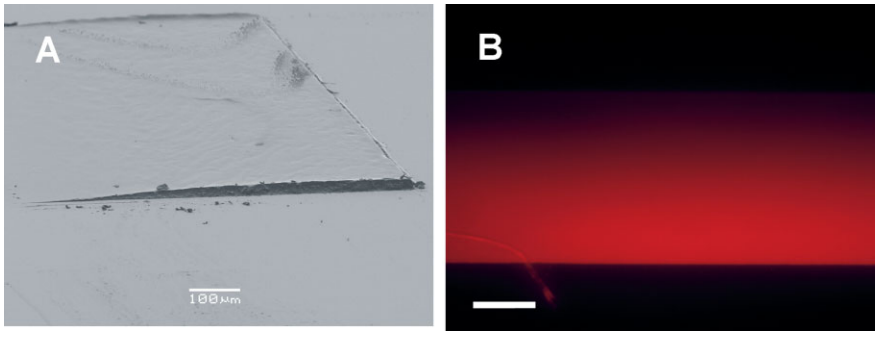

Figure 7. Gradient hydrogels using photopolymerization within microfluidic channels. A) Scanning electron microscopy image of a hydrogel with a concentration gradient of crosslinking density across the width of the gel. B) Fluorescence microscopy image of rhodamine concentration gradient within a photopolymerized hydrogel (scale bar: 200 µm).

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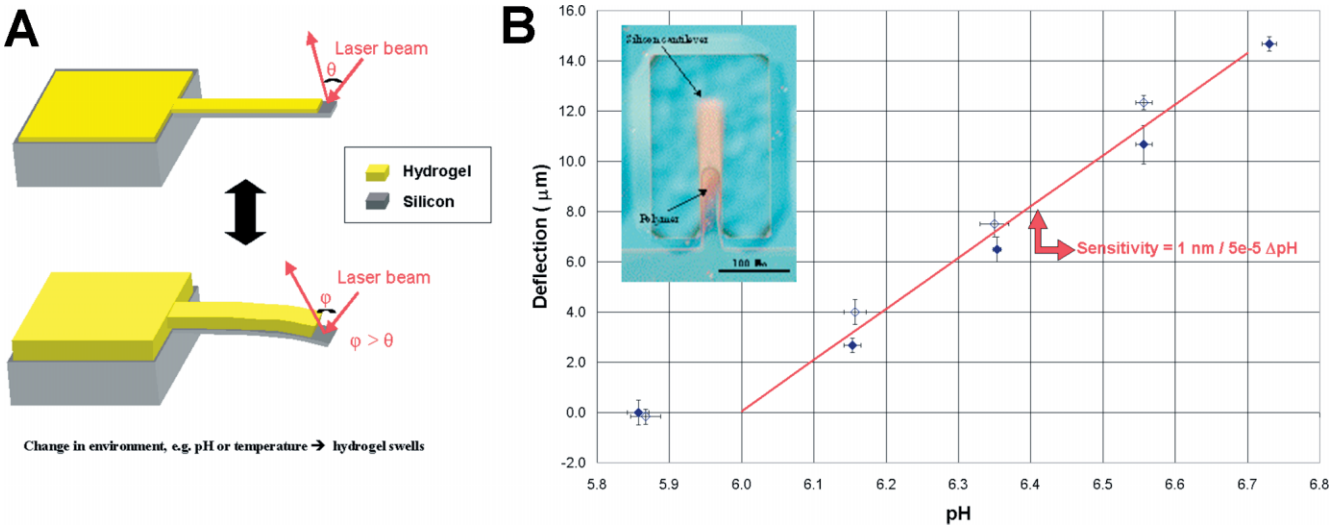

Figure 8. A) Schematic of the bioMEMS sensor platform based on a microcantilever patterned with an environmentally responsive hydrogel. B) Detailed examination of the equilibrium bending data versus pH (constant ionic strength of 0.5 M). Reproduced with permission from [112]. Copyright 2003 Springer.

workers developed miniature conductimetric pH sensors based on the measurement of the conductivity of pH-sensitive hydrogels that were photolithographically patterned onto planar interdigitated electrode arrays.[126,127] The sensor detection was based on the measurement of changes in the electrical conductivity of the hydrogel membrane that resulted in its swelling/collapsing. In related work, Guiseppi-Elie and co-workers[128] demonstrated chemical and biological sensors that applied conducting electroactive hydrogel composites as recognition elements and utilized electrochemical detection.

4.2. Microarrays

Control of surface properties and spatial location of hydrophilic polymers is of great importance for a variety of applications ranging from high-throughput systems to cell biology studies. Hydrogels and hydrophilic polymers have been used for patterning surfaces, immobilizing cells and protein within hydrogel microstructures, and controlling cell–cell interactions in hydrogels. Various methods of grafting PEG to the substrate have been used based on the surface properties of the substrates. Self-assembled monolayers (SAMs) of PEG-terminated alkanethiol molecules have been used extensively to form dense layers of PEG on surfaces.[129,130] More recently, various modification schemes have been used to form stable biofouling-resistant surfaces. For example, immobilizing poly(L-lysine) to PEG has been used to form PEGylated layers on metal surfaces.[131,132]

With the advent of surface-patterning approaches such as microcontact printing and photolithography, PEG has been routinely used to pattern surfaces. In most schemes, PEG-terminated SAMs are used to resist surface adhesion.[133–136] Because of the thiol linkage, these monolayers have typically been limited to gold substrates. This has resulted in research into other types of PEG-based polymers for surface modifications. For example, PEG has been grafted to poly(L-lysine) to facilitate immobilization of PEG molecules on metal oxide surfaces.[131,132] Other modification approaches, such as silane and acrylate chemistries, have been used to anchor PEG on surfaces for patterning cells and proteins.

More recently, we have synthesized PEG-based molecules that can form multivalent anchorage sites on various substrates such as glass and oxides.[137] These molecules were shown to form stable layers on surfaces of various substrates based on silane chemistry. In addition, we demonstrated the use of these polymers for patterning surfaces and fabricating nanostructures.[138] Photocrosslinkable PEG hydrogels have been used to immobilize cells in particular regions of a substrate. This has been done by either patterning PEG on a substrate[138–141] or by directly capturing cells within the PEG.[142,143] Also, collagen gels have been molded to pattern cells.[144]

Controlling the degree of cell–cell contact is an important area of microscale cell control that could have applications in cell-based screening and drug-delivery applications. Some of the pioneering work using micropatterns to control cell–cell contact was performed by Toner and co-workers.[145–147] Natural hydrophilic polymers such as polysaccharides have also been used as patterning materials. We have shown that hydrophilic polysaccharides such as HA can form adsorbed monolayers on surfaces of hydrophilic substrates.[148–150] In addition, the use of layer-by-layer deposition of hydrophilic polymers (i.e., HA and poly(L-lysine)) can also be used to generate patterned co-cultures.[149]

Temperature-responsive polymers such as PNIPAAm are also useful for patterning cells.[151–154] Yamato et al. have reported the use of PNIPAAm-patterned polystyrene sub-

strates.[151] The PNIPAAm-grafted surface exhibits dehydrated properties above this polymer's LCST(32 °C), as well as hydrated properties below the LCST. Cells are allowed to adhere only to the PNIPAAm-lacking polystyrene areas below the LCST. After the culture temperature is increased over the LCST, the second cell type is allowed to attach to the PNIPAAm-grafted areas.

4.3. Diagnostic Imaging

In medical imaging, delivering the correct dose of imaging agent and targeting the delivery to the correct location are critical for successful diagnosis. Therefore, the same properties that make hydrogels attractive carriers for therapeutics in controlled drug-delivery applications can be applied for delivery and targeting in controlled imaging applications. In particular, hydrogels can be applied as carriers with tailored release properties, as coatings with targeting capabilities, as coatings with stealth properties, or as combinations of these.

Only recently have researchers begun to apply hydrogel systems in imaging applications. For example, a novel carrier for quantum dots based on nanogels was demonstrated for intracellular imaging (Fig. 9).[155] In other work, hydrogel microspheres were applied to confine water-soluble semiconductor nanocrystals.[156] These hybrid hydrogels systems could be

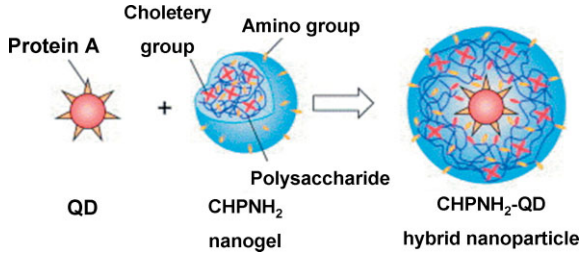

Figure 9. Schematic of nanoscale imaging device based on a amino-group-modified cholesterol-bearing pullulan ($CHPNH_2$)–quantum dot (QD) hybrid nanoparticle. Reproduced with permission from [154]. Copyright 2000 Elsevier.

applied as fluorescent probes in biological-imaging applications. In other research, hydrogels have been applied as carriers for the delivery of radiochemotherapy agents.[157] Additionally, hydrogel coatings have been applied to encapsulate radioisotopes for radiation-delivery devices.[158]

5. The Future: Hydrogels and Bionanotechnology

The design and synthesis of "smart" hydrophilic polymers and hydrogels has significant potential in future biomedical and nanotechnology applications. The future success of these materials relies on the development of novel materials that can address specific biological and medical challenges. This development will occur through synthesis of new polymers or by modifying natural polymers. For tissue engineering, the desired tissue should be used as the model to engineer the desired chemical, mechanical, and biological properties into the hydrogel. Hydrogels being used for cartilage or tissue engineering should be capable of providing mechanical properties and loading as well as the molecular signals that are present in the native or regenerating organ. In addition, other properties of gels, such as pore sizes and degradation properties, must also be optimized. Novel tissue-engineering approaches should incorporate temporal and spatial signals that are present during the normal healing process.

With respect to drug delivery, the continued development of "smart" biocompatible materials that can respond to their environments will provide new and improved methods of delivering molecules for therapeutic applications. Finally, advancing the knowledge and the use of hydrogels and smart polymers for nanotechnology is an important area with significant potential that remains to be fully investigated. The incorporation of functional hydrogels into microdevices and the use of microdevices to engineer hydrogels will continue to provide new methods for fabricating improved hydrogel-based systems.

The above examples represent some of the approaches that can be used to synthesize and use hydrophilic polymers and hydrogels for biological and medical problems. With the development of new materials and novel methods of engineering chemical, mechanical, and biological functionality into hydrophilic molecules, we anticipate that in the future hydrophilic polymers will play an even greater role in biomedical applications and nanotechnology.

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