Annu. Rev. Biomed. Eng. 2000. 02:9–29

PHYSICOCHEMICAL FOUNDATIONS AND STRUCTURAL DESIGN OF HYDROGELS IN MEDICINE AND BIOLOGY

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Key Words controlled release, diffusion, polymer network, responsive hydrogels, swelling

■ **Abstract** Hydrogels are cross-linked hydrophilic polymers that can imbibe water or biological fluids. Their biomedical and pharmaceutical applications include a very wide range of systems and processes that utilize several molecular design characteristics. This review discusses the molecular structure, dynamic behavior, and structural modifications of hydrogels as well as the various applications of these biohydrogels.

Recent advances in the preparation of three-dimensional structures with exact chain conformations, as well as tethering of functional groups, allow for the preparation of promising new hydrogels. Meanwhile, intelligent biohydrogels with pH- or temperature-sensitivity continue to be important materials in medical applications.

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1523-9829/00/0825-0009$14.00

INTRODUCTION

Hydrogels are water-swollen polymeric networks containing chemical or physical cross-links. Physical cross-links may be entanglements, crystallites, or weak associations such as hydrogen bonds or van der Waals interactions (1).

The importance of hydrogels in biomedical applications was first realized in the late 1950s with the development of poly(2-hydroxyethyl methacrylate) (PHEMA) gels as a soft contact lens material (2). These PHEMA gels had a high water content at equilibrium and exhibited rubbery behavior and good biocompatibility, thus resembling natural tissues more closely than other synthetic materials. Today, hydrogels are used in numerous biomedical applications including ophthalmological devices, biosensors, biomembranes, and carriers for controlled delivery of drugs or proteins (1, 3, 4).

Hydrogels are either neutral or ionic, depending on the ionization of their pendant groups. The network morphology may be amorphous or semicrystalline. Additional network structures include hydrogen-bonded or supermolecular structures. In addition, the network structure may be cast in the form of macroporous, microporous, or nonporous gels (5). Table 1 lists some of the common polymeric hydrogels and their medical applications.

In addition to their promising biocompatibility characteristics, certain hydrogels are particularly desirable in the biomedical field due to their sensitivity in the physiological or biological environment where they are used. In recent years, much research has focused on the development and analysis of environmentally responsive hydrogels (1), i.e. hydrogels that can exhibit swelling changes due to the external pH, temperature, ionic strength, nature of the swelling agent, or electromagnetic radiation. It is therefore important to summarize the structural characteristics of biomedical gels.

STRUCTURE OF BIOMEDICAL HYDROGELS

Structural Parameters

Three important parameters can define the structure of biomedical hydrogels, the polymer volume fraction in the swollen state, $v_{2,s}$, the number average molecular weight between cross-links, $\bar{M}_c$, and the correlation length, ξ, also known as the network mesh (or pore) size (6).

The equilibrium polymer volume fraction in the gel, $v_{2,s}$, is the ratio of the volume of polymer, V_p, to the volume of the swollen gel, V_{gel}, and the reciprocal of the volume swelling ratio, Q:

$$v_{2,s} = V_p / V_{\text{gel}} = Q^{-1} \tag{1}$$

It can be determined by equilibrium swelling experiments (7). The number-average molecular weight between cross-links, $\bar{M}_c$, can be related theoretically to the

degree of cross-linking, X:

$$\bar{M}_c = M_0/2X \tag{2}$$

where M_0 is the molecular weight of the repeating unit of the polymer. However, $\bar{M}_c$ represents the true level of cross-linking and is best determined by swelling experiments and theories that are summarized later.

Finally, the correlation length or the network mesh size, ξ, is indicative of the distance between consecutive junctions, cross-links, or tie points. All of these network parameters can be measured through a range of experimental techniques or calculated by application of network deformation theories.

Models for Network Prediction

The hydrogel network structure is important in determining mechanical properties of the material and in evaluating the material for particular applications. Numerous researchers have developed models to predict the network structure of various biomaterials and to aid in understanding the structure at the molecular level. There are presently three distinct models used for examining network structure formation: kinetic models, statistical models, and Monte Carlo simulations.

Kinetic models consist of a set of differential equations representing the mass balances on the species. These equations are then solved to obtain the final conversion, the average molecular weight, and other properties of the gel. Zhu et al (8) have developed detailed models to examine the cross-linking and cyclization in network formation. Okay (9) developed a kinetic-thermodynamic model to examine the formation of macroporous styrene-divinylbenzene copolymer networks. This theoretical model can be applied to biomedical hydrogels.

The kinetic models are all mean-field models in that they determine average properties for the system. Therefore, statistical (10) and Monte Carlo models (11) have been developed to provide more insight into the network formation process and the heterogeneities that occur. These models also provide information about both the sol and the gel regions. Both regions are important to consider, especially when developing hydrogels for biomedical applications.

Swelling Behavior

The swelling behavior of biomedical hydrogels in biological fluids can be described by a variety of nonideal thermodynamic frameworks. The ultimate goal of all these theoretical models is the prediction of the swelling behavior, the mesh size for solute diffusion, and related parameters.

Due to the highly nonideal thermodynamic behavior of polymer networks in electrolyte solutions, no theory can predict exact behavior. However, the Flory-Rehner analysis, and its various modifications, continues to be used with reasonable success (12). This theoretical framework describes gels as neutral, tetrafunctionally–cross-linked networks with polymer chains exhibiting a Gaussian distribution.

TABLE 1 Important hydrogel polymers in medicine

Hydrogel polymer	Medical applications
Poly(vinyl alcohol) [PVA]	
Polyacrylamide [PAAm]	
Poly(N-vinyl pyrrolidone) [PNVP]	
Poly(hydroxyethyl methacrylate) [PHEMA]	**Blood-Compatible Hydrogels**
Poly(ethylene oxide) [PEO]	
Poly(ethylene glycol) [PEG]	
Poly(ethylene glycol) monomethyl ether [PEGME]	
Cellulose	
Poly(hydroxyethyl methacrylate) [PHEMA] copolymerized with:	
NVP	
Methacrylic acid [MAA]	**Contact Lenses**
Butyl methacrylate [BMA]	
Methyl methacrylate [MMA]	
3-methoxy-2-hydroxypropylmethacrylate [MHPM]	
PHEMA/poly(ethylene terephthalate) [PTFE]	**Artificial Tendons**
	Other Medical Applications
Cellulose acetate	Artificial kidney
PVA and cellulose acetate	Membranes for plasmapheresis
PNVP, PHEMA, cellulose acetate	Artificial liver
PVA and PHEMA	Artificial skin
Terpolymers of HEMA, MMA and NVP	Mammaplasty
PHEMA, P(HEMA-co-MMA)	Maxillofacial reconstruction
PVA	Vocal cord reconstruction
P(HEMA-b-siloxane)	Sexual organ reconstruction
PVA, poly(acrylic acid) [PAA], poly (glyceriyl methacrylate)	Ophthalmic applications
PVA, HEMA, MMA	Articular Cartilage
	Controlled Drug Delivery*
Poly(glycolic acid) [PGA], Poly(lactic acid) [PLA], PLA-PGA, PLA-PEG, Chitosan, Dextran, Dextran-PEG, polycyanoacrylates, fumaric acid-PEG, sebacic acid/1,3-bis(p-carboxyphenoxy) propane [P (CPP-SA)]	*Biodegradable hydrogels*
	Non-Biodegradable Hydrogels
PHEMA, PVA, PNVP, poly(ethylene-co-vinyl acetate) [PEVAc]	Neutral
Poly(acrylamide) [PAAm], Poly (acrylic acid) [PAA], PMAA, poly (diethylaminoethyl methacrylate) [PDEAEMA], poly (dimethylaminoethyl methacrylate) [PDMAEMA]	pH-Sensitive

(*continued*)

TABLE 1 (*Continued*)

Hydrogel polymer	Medical applications
Poly(methacrylic acid-grafted-poly(ethylene glycol)) [P(MAA-g-EG)], poly(acrylic acid-grafted-poly(ethylene glycol) [P(PAA-g-EG)]	Complexing hydrogels
Poly(N-isopropyl acrylamide) [PNIPAAm]	Temperature-sensitive
PNIPAAm/PAA, PNIPAAm/PMAA	pH/Temperature-sensitive

*These drug delivery applications have been used for the controlled release of several therapeutic agents such as contraceptives, antiarrhythmics, peptides, proteins, anticancer agents, anticoagulants, antibodies, among others. This table does not include all the copolymers of such hydrogels.

When a biopolymer network is in contact with an aqueous solution or a biological fluid, the network starts to swell due to the thermodynamic compatibility of the polymer chains and water. The swelling force is counterbalanced by the retractive force induced by the cross-links of the network. Swelling equilibrium is reached when these two forces are equal.

Clearly, the water chemical potential change can be calculated at constant temperature and pressure as follows:

$$\mu_1 - \mu_{1,0} = \Delta\mu_{\text{mix}} + \Delta\mu_{\text{elastic}} \tag{3}$$

Here, μ_1 is the chemical potential of water in the system, $\mu_{1,0}$ is the chemical potential of pure swelling water, and $\Delta\mu_{\text{mix}}$ and $\Delta\mu_{\text{elastic}}$ are the mixing and elastic contributions to the total chemical potential change. The chemical potential difference, $\Delta\mu_{\text{mix}}$, can be determined from the thermodynamics of the biomedical polymer-water mixing process. The other parameter, $\Delta\mu_{\text{elastic}}$, can be determined from application of the rubber elasticity theory (13) as follows:

$$\Delta\mu_{\text{mix}} = RT\left[\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2\right] \tag{4}$$

and

$$\Delta\mu_{\text{elastic}} = \frac{RTV_1}{\bar{v}\bar{M}_c}\left(1 - \frac{2\bar{M}_c}{M_n}\right)\left(v_{2,s}^{1/3} - \frac{v_{2,s}}{2}\right) \tag{5}$$

Here, χ_1 is the biomedical polymer-water interaction parameter, V_1 is the molar volume of water, $\bar{v}$ is the specific volume of the biomedical polymer, $v_{2,s}$ is the volume fraction of the swollen gel, $\bar{M}_c$ is the number-average molecular weight between the cross-links, and $\bar{M}_n$ is the molecular weight of linear polymer chains prepared at the same conditions without cross-linking. Equation 5 is written for the hydrogels that were cross-linked in the absence of a solvent.

Equations 3 through 5 lead to the expression for the true $\bar{M}_c$ of a nonionized biomedical hydrogel:

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{(\bar{v}/V_1)\left[\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2\right]}{\left(v_{2,s}^{1/3} - (2/\varphi)v_{2,s}\right)} \tag{6}$$

For the case of biomedical hydrogels cross-linked in the presence of water, Equation 6 is modified to account for the water-induced elastic contributions to swelling:

$$\frac{1}{M_c} = \frac{2}{M_n} - \frac{(\bar{v}/V_1)\left[\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2\right]}{v_{2,r}((v_{2,s}/v_{2,r})^{1/3} - ((2/\varphi)v_{2,s}/v_{2,r}))} \tag{7}$$

Here, $v_{2,r}$ is the volume fraction of the polymer in the relaxed state, i.e. immediately after cross-linking but prior to swelling/deswelling (14), and φ is the functionality of the cross-linking agent.

Yet most biomedical hydrogels contain ionizable pendant groups. In these gels, the force influencing swelling may be greatly increased due to localization of charges within the hydrogel. These ionizable groups may be partially or completely dissociated in solution. Due to the existence of ionic interactions, an ionic contribution term, $\Delta\mu_{\text{ion}}$, is added to the right-hand side of the chemical potential term of Equation 3. These ionic interactions are strongly dependent on the degree of ionization, ionization equilibrium, and the nature of the counterions.

The equilibrium between the swollen biogel and water can be described according to the Donnan membrane equilibrium (15), which is associated with the description of the osmotic pressure effect (16).

By considering the ionic interactions in the ionic gels, the governing equation of swelling was modified for anionic biogels (17) as follows:

$$\begin{aligned}
&\frac{V_1}{4IM_r}\left(\frac{v_{2,s}}{\bar{v}}\right)\left(\frac{K_a}{10^{-\text{pH}} + K_a}\right)^2 \\
&\quad = \left[\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2\right] \\
&\qquad + \left(\frac{V_1}{\bar{v}M_c}\right)\left(1 - \frac{2\bar{M}_c}{\bar{v}M_n}\right)v_{2,r}\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{v_{2,s}}{2v_{2,r}}
\end{aligned} \tag{8}$$

Diffusive Characteristics

Our ability to control solute diffusion through hydrogels is the basis for various applications of such gels in bioengineering (5, 18). Drug or protein diffusion is best described by Fick's equation or by the Stefan-Maxwell equations (5), which correlate the solute's flux with its chemical potential gradient in the system. Thus, the structure and pore size of the gel, the polymer composition, the water content, and the nature and size of the solutes are all taken into account by the diffusion coefficient of solute.

Solute diffusion coefficients can be described as depending on the states of hydrogels, i.e. dried or swollen states, and glassy or rubbery states. For porous gels with pore sizes much larger than the molecular dimensions of the solute, the diffusion coefficient is related to the porosity and the tortuosity of the porous structure (5). For porous hydrogels with pore sizes comparable to the solute molecular

size and for nonporous hydrogels, various expressions have been proposed for the diffusion coefficients (5, 18, 19). The general form of these expressions is

$$\frac{D_{123}}{D_{13}} = f(r_1, \phi, \xi) \tag{9}$$

Here, D_{13} is the corresponding diffusion coefficient of solute in the pure solvent phase, r_1 is the size of the solute molecules, ϕ is the polymer volume fraction of the gel, and ξ is the mesh size of the gel.

The interaction among solutes, gel polymers, and solvents has an important effect on the diffusion process. For example, the attraction between the solute and gel polymers will slow down the diffusion (20). The diffusion of polymers in hydrogels is largely different from that of small molecules with rigid shapes (21). When the polymer size is much larger than the mesh size of the hydrogel, the diffusion may be due to reptation, as in gel electrophoresis of DNA and proteins (22, 23). The molecular-level understanding of this mechanism is yet to be clarified, especially when compared to the important role of diffusion in biological studies (24).

Surface Properties

Hydrogels have a complex surface structure with numerous dangling chains and only one end attached to the network. At the hydrogel/water interface, the structure changes gradually (25). The dangling chains have more mobility than the backbone chains in the bulk gel phase, and more importantly, they behave similarly to "polymer brushes" (26).

The properties of hydrogel surfaces can be understood based on these structural features. For example, it has been shown that hydrogels may exhibit different surface properties when in contact with different solvents (27), since the surface chains have enough mobility to adjust their conformations in order to minimize the surface energy in different environments.

Interactions between protein/cell and hydrogel surfaces have been studied (28, 29) and suggest that the amount of protein adsorption on hydrogel surfaces is much lower than on hydrophobic surfaces (30), and that the local structure determines the adsorption of different proteins. The cell studies also suggest that highly hydrophilic hydrogel surfaces are poor substrates for cell adhesion (31, 32).

The surface friction of hydrogels has also been investigated (33). When a gel slides against a solid surface, the friction force is only slightly dependent on the normal load but is strongly dependent on the sliding velocity. Frictional coefficients of gels are generally much lower than those of solids. The unique friction behavior of hydrogels is related to the strong water solvation of the network (33).

Hydrogel surface structures can be molecularly designed for specific applications (34). Hern & Hubbell (35) incorporated adhesion-promoting oligopeptides (RGD peptide sequence) into hydrogels and gave the hydrogels the ability to mediate cell adhesion properties. In their study, the functional sequences

were incorporated into the network structure by a poly(ethylene glycol) (PEG) chain spacer. Figure 1 shows the generalized approach of tethered-chain surface modifications. Hydrogel surfaces can also be modified to mimic cell membranes by using modified lipid bilayers (36). Hydrogels containing immobilized bilayer membranes were recently synthesized (37).

MEDICAL AND PHARMACEUTICAL APPLICATIONS OF HYDROGELS

Molecularly designed hydrogels can be used in a wide range of pharmaceutical and biomedical applications, as indicated in Table 1. Most of these applications utilize one or more of the characteristics described above, such as specific diffusive properties, presence of tethered functional groups, or specific three-dimensional structures. In this section, we summarize some major recent developments in the field, with emphasis on novel developments that have been the result of molecular design of such biogels.

Molecularly designed hydrogels are used in delivery systems that should be able to maintain the blood concentration of a drug inside the therapeutic region for an extended period of time. There are situations where it is necessary to release the therapeutic agent only when needed or only in the affected area. This requires the use of an "intelligent" biomaterial. Hydrogels have a structure that can be tethered, allowing for control of drug diffusion, the sensitivity to its environment, or the recognition of a specific target by incorporation of functional groups in the matrix. All of these properties make hydrogels excellent candidates for controlled release applications (38).

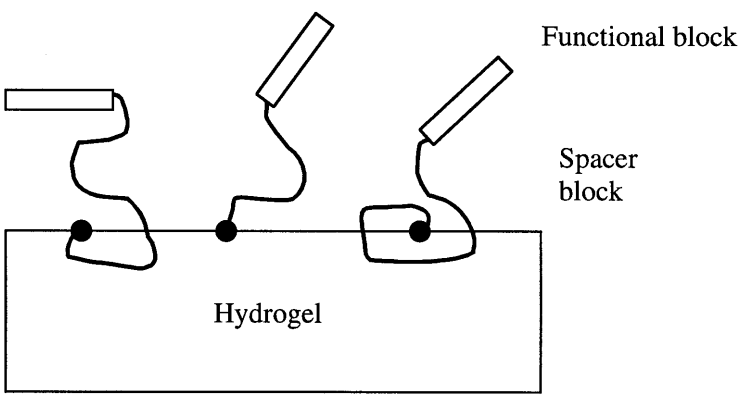

Figure 1 Tethered structure surface modification of hydrogels. The tethered chains may be tailored for various surface interactions. Examples for spacer blocks are Poly(ethylene glycol), and for functional blocks are oligopeptide sequences (to modify cell adhesion) and heparins (to introduce nonthrombogenic properties).

Diffusion-controlled hydrogel-based systems (1, 39) can be matrix or reservoir systems. The active agent diffuses through a hydrogel membrane and then passes to the biological fluid. In the reservoir system, the active agent is located in a core, and a polymer membrane surrounds it. In matrix systems, the drug or protein is homogeneously distributed throughout the membrane and is slowly released from it. The polymer membrane can be biodegradable.

In erodible systems the polymer matrix is hydrolyzed either by bulk or surface erosion mechanisms (39a). Bioerodible systems are attractive delivery systems, especially for implants, because they don't need to be surgically removed, and, depending on the erosion kinetics, the drug can be released for an extended period of time. The most studied bioerodible polymers are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers (39a). In both cases the final degradation byproducts are water and carbon dioxide. Other examples of bioerodible hydrogels include dextran and chitosan.

Swelling-controlled release systems (1, 39) are particularly popular for controlled release applications. The governing transport mechanism is not Fickian diffusion (1, 39). As the water penetrates to the dried glassy polymer, the glass transition temperature of the local region is lowered and the polymer becomes rubbery due to the rearrangement of the macromolecular chains. Case II, anomalous, or relaxation controlled transport prevails. The most common hydrogels that exhibit swelling controlled behavior are poly(2-hydroxyethyl methacrylate) (PHEMA), poly(vinyl alcohol) (PVA), poly(N-vinyl-2-pyrollidone) (PNVP), poly(ethylene glycol) (PEG), and their copolymers (1, 39).

Environmentally responsive systems (38) are characterized by their ability to respond to changes in pH, temperature, ionic strength, solvent composition, and magnetic fields, among others. Hydrogels that respond to any of these changes in their surroundings show a dramatic change in their swelling behavior. These changes in swelling are usually characterized by a transition point where the behavior occurs. Also, these changes in the network are completely reversible. The release behavior of therapeutic agents is more complex and usually is a combination of diffusion and anomalous transport.

The most commonly used environmentally sensitive hydrogels in controlled release are pH-sensitive and temperature-sensitive ones. Hydrogels that contain ionizable moieties such as carboxylic and amine are pH- and ionic strength–sensitive (38). These hydrogels swell when the ionic moieties ionize, and the electrostatic repulsion causes the network to expand and water is allowed to enter. The most common pH-sensitive hydrogels are poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), poly(diethylaminoethyl methacrylate) (PDEAEMA), and poly-(dimethylaminoethyl methacrylate) (PDMAEMA), and their copolymers (38).

The most studied temperature-sensitive hydrogel is poly (N-isopropylacrylamide) (PNIPAAm) (38). This hydrogel has a lower critical solution temperature (LCST) of 34.3°C (40). Therefore, it swells at temperatures below the LCST. Comb-type grafted PNIPAAm hydrogels were developed by Okano and associates (41). Such systems show major promise for rapid or oscillatory release of

drugs, peptides, and proteins. Peppas and associates (42) synthesized a terpolymer hydrogel composed of N-isopropylacrylamide (NIPAAm), acrylic acid (AA), and 2-hydroxyethyl methacrylate (HEMA) with combined pH- and temperature-sensitivity. This hydrogel exhibited pulsatile release under oscillatory pH and temperature conditions in the streptokinase controlled release studies, as shown in Figure 2. Hoffman and associates have also developed novel pH- and temperature-sensitive hydrogels by grafting temperature-sensitive side chains onto a pH-sensitive backbone and by copolymerizing temperature- and pH-sensitive units (43).

Novel applications of hydrogels come from the development of stimuli-responsive systems using pH- and temperature-sensitive hydrogels, specifically in the development of polymeric carriers that could release peptides and proteins though different delivery routes. These systems are very promising not only in the area of controlled delivery of proteins and other agents, but also in the development of biosensors. The challenges in the delivery of peptides and proteins are their poor chemical stability in aqueous solutions and their low bioavailability as a consequence of degradation in the different biological barriers (44). Therefore, they have to be administered mainly by frequent intramuscular injections, limiting

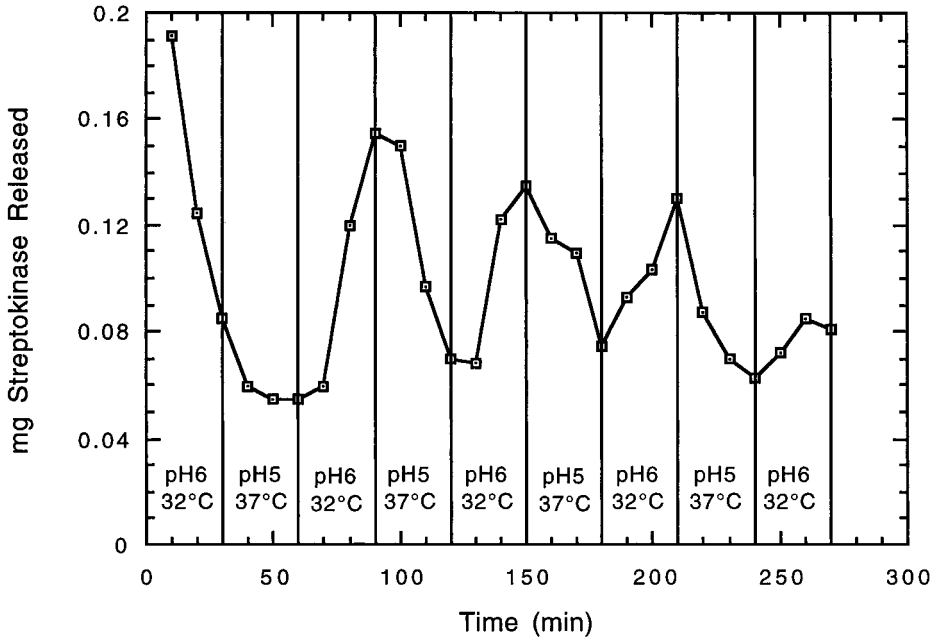

Figure 2 Streptokinase release under conditions of pulsatile pH and temperature from hydrogel with a 10:10:80 NIPAAm:AA:HEMA molar ratio, which was loaded with drug during polymerization into a random terpolymer hydrogel sample. The gels, starting from the dry state, were kept alternatively in pH and temperature environments at 32°C and pH 6, or at 37°C and pH 5, for 30-min time intervals. (Adapted from Ref. 42).

their use as therapeutic agents. Of particular interest is the release of these agents through the gastrointestinal tract.

Temperature- and pH-sensitive hydrogels have been the focus of extensive research in the past years, especially in the area of controlled release of peptides and proteins. Insulin has been used extensively as the model drug for developing these new systems. In the case of insulin, Schwarte et al (45, 46) developed a system able to respond to high glucose levels in blood. This system consists of cationic hydrogels containing a poly(diethylaminoethyl methacrylate) grafted with poly(ethylene glycol). The hydrogel contains glucose oxidase, catalase, and insulin. The enzymes give the system specific glucose sensitivity due to the reaction of the glucose oxidase with glucose to produce gluconic acid. The local decrease in pH causes the network to swell and insulin to be released. Ramkissoon-Ganorkar et al (47) investigated pH- and temperature-sensitive hydrogels composed of poly (N-isopropyl acrylamide), butyl methacrylate (BMA), and acrylic acid (AA) (NIPAAm/BMA/AA). The in vitro release of insulin showed that it is dependent on the molecular weight of the terpolymer owing to the polymer's higher dissolution rate at lower molecular weights.

Another pH- and temperature-sensitive system composed of poly (N-isopropyl acrylamide) and N,N′-dimethylaminopropylmethacrylamide (DMAPMAAm) (NIPAAm/DMAPMAAm) was investigated by Park (48). In this system, the release behavior in vitro was strongly affected by the changes in temperature and to a lesser extent by changes in pH. Lowman & Peppas (49, 50) have investigated complexation hydrogels as possible delivery carriers for insulin. Poly(methacrylic acid) grafted poly(ethylene glycol) [P(MAA-g-EG)] hydrogels were synthesized (49). The feasibility of these hydrogels as a possible oral carrier for insulin was investigated both in vitro (50) and in vivo (51); these hydrogels showed an enhancement in the transport of insulin through the epithelial cell lining of the small intestine in vivo. Figures 3 and 4 show the release behavior of insulin in such systems in vitro and in vivo, respectively. Torres-Lugo and Peppas (52) have also tested these systems in vitro as possible delivery carriers for salmon calcitonin. Salmon calcitonin was successfully released in vitro from the system at a constant rate for approximately 7 h, as shown in Figure 5.

Biodegradable systems have also been extensively used for the delivery of peptides and proteins (39a). These systems contain, but not exclusively, dextran, PLA, PGA, and PLGA, and their copolymers with PEG have been investigated for the delivery of peptide and proteins. Hydrogels composed of glycidyl methacrylate derivatized dextran (dex-GMA) were studied for the controlled release of proteins such as lysozyme and bovine serum albumin (53, 54). For this system, the release behavior was affected by the amount of water in the system, the degree of GMA substitution, and the protein size. Moriyama and Yui (55) used dextran and dextran hydrogels containing PEG to release insulin in vitro. The release of insulin from hydrogels containing PEG was governed by surface degradation, whereas for hydrogels containing only dextran, the release was controlled by diffusion.

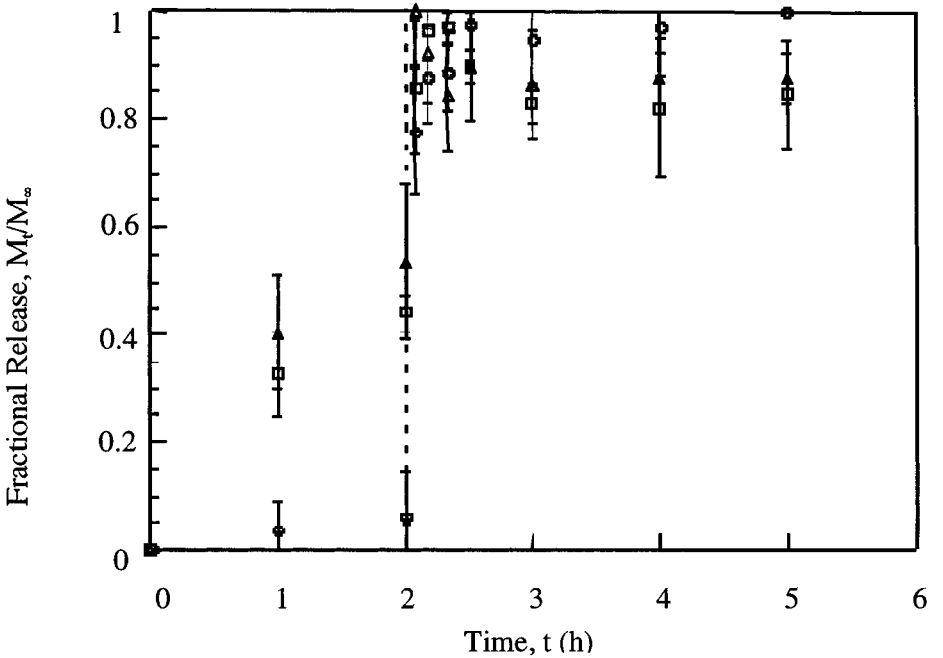

Figure 3 Pulsatile release of insulin in vitro from P(MAA-g-EG) microparticles containing graft PEG chains of molecular weight 1000 and MAA/EG ratios of (✪) 1 and (◘) 4 in simulated gastric fluid (pH = 1.2) for the first two hours and phosphate-buffered saline solutions (pH = 6.8) for the remaining three hours at 37°C. The release from P(MAA) microparticles is represented by ▲.

As shown in the previous paragraphs, PEG containing hydrogels have gathered much attention in this particular of area of protein delivery due to PEG's characteristics (56). PEG is water soluble, nontoxic, not recognizable by the immune system, and an adhesion promoter when used with bioadhesive polymers. Also, PEG is suspected to protect peptides and proteins from proteolytic attack because it prevents protein adhesion on surfaces. PEG is a special molecule because, when it is covalently bonded to another molecule, it retains its own characteristics and transfers them to the new material (56).

MOLECULAR ENGINEERING OF FUNCTIONAL BIOGELS

Hydrogels are found in numerous applications in the fields of biology and medicine (57, 58). For example, hydrogels have been used as electrophoresis materials for biomolecular separation (24), drug delivery devices (59), and packing materials for liquid chromatography (60). More information about these applications can be found in these recent reviews. A new direction of medical applications is toward functional hydrogels, which are desirable materials for numerous biorelated applications.

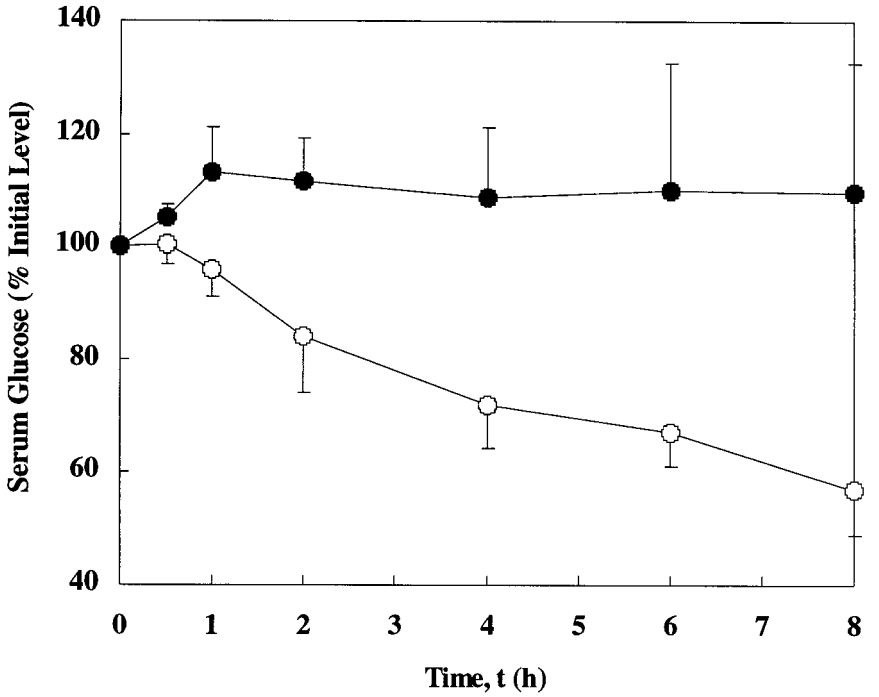

Figure 4 Blood glucose response in diabetic male Wistar rats following oral administration of 25 IU/kg body weight doses contained in (○) P(MAA-g-EG) microspheres and (●) insulin solutions (n = 5).

By "functional," we mean that hydrogels may recognize the existence of specific molecules in their environment and undergo notable corresponding changes. By the definition, the simple pH- and temperature-sensitive hydrogels do not belong to this category, because neither pH changes nor temperature changes are related to the existence of specific molecules (for example, the addition of any kind of acid or base molecules will introduce pH changes to the environment). A typical example of a functional hydrogel is a hydrogel prepared by grafting an antigen and the corresponding antibody to the network structure (61). The binding between the antigen and the antibody introduces extra cross-links in the network. When the hydrogel is in contact with free antigen solutions, competitive binding of the diffused free antigen triggers a change in gel volume owing to the breaking of the noncovalent cross-links. By exploiting the antigen-antibody type interaction, this hydrogel responds to only specific kinds of antigens in the solution, and the response is reversible.

The development of functional hydrogels represents an effort to design biological recognition materials (62). The ultimate goal is to design artificial materials that are able to serve as functional proteins, i.e. they can recognize and respond to slight changes in their environment. Hydrogels are suitable for these applications

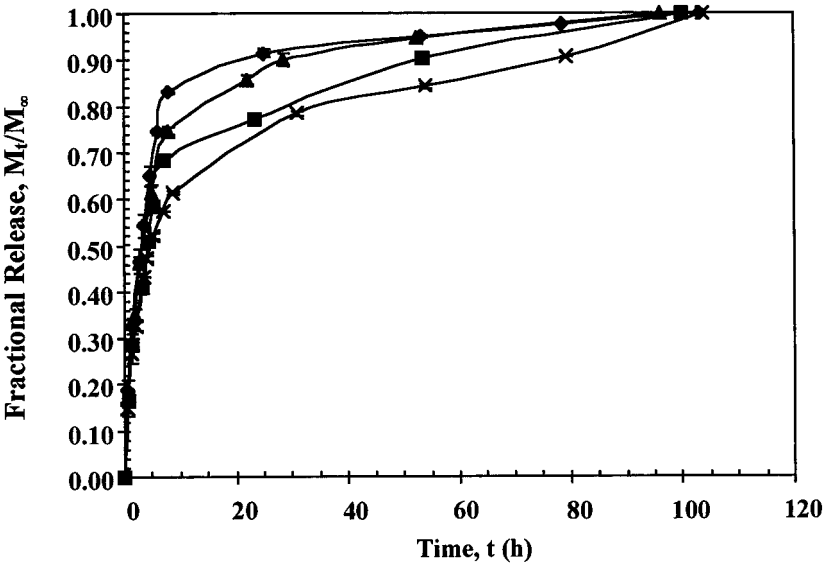

Figure 5 Release behavior of sCT in P(MAA-g-EG) for different solvent volume fractions, 0.17 (◆), 0.23 (■), 0.34 (▲), 0.57 (×) in phosphate buffer at pH = 7, I = 0.1 M, T = 37°C.

because they undergo dramatic and reversible changes as a result of weak external perturbations. Moreover, hydrogels are generally biocompatible (63). However, to act as successful functional biomaterials, hydrogels have to overcome two problems: the introduction of molecule-recognition ability and the slow dynamics.

One method to introduce molecule-recognition ability is to graft bioselective molecules on the backbone polymer chains of hydrogels (64). Such selective molecules include enzymes, antigens (61), crown ethers (65), and lectins (66), etc. When these side-chain molecules combine with their corresponding molecules, their property change causes a change in the swelling properties of the gel. A successful example of this method is the glucose-sensitive hydrogel (61): The enzyme glucose oxidase (GOx) is attached to the hydrogel, and the reaction of the enzymes with glucose will ionize the oxidase. Therefore, the hydrogel changes from a neutral to a charged one, and the hydrogel volume increases as a result.

Another possible way to introduce molecule-recognition ability is to directly use such biomolecules as proteins in the hydrogel backbone chains (67, 68). The current synthesized protein hydrogels can respond only to pH- or temperature-changes. However, with further understanding of the structure-property relationships of functional proteins (69) and hydrogels (70), this method may allow us to precisely control the structure of hydrogels at the molecular level.

An alternative method is the use of molecular imprinting techniques (71–74). This method allows for the formation of specific recognition and catalytic sites

in polymers by the use of templates, and it is a promising technique to use for functional materials. The template, which is the molecule to be recognized, is first allowed to form bonds with monomers. These are subsequently cross-linked in situ (73). Subsequent removal of the templates leaves cavities with a complementary size, shape, and spatial distribution of functional groups. Compared with the other two methods discussed previously, the molecular imprinting method has such advantages as robustness, low cost, and no requirement of recognizable molecules (72).

So far, most molecularly imprinted materials, some of which themselves are water-swellable hydrogels, do not function in aqueous solutions (74). This is possibly because the swelling process damages the structure of complementary cavities and because water molecules themselves readily form hydrogen bonds, which in turn greatly decreases the imprinting efficiency. The design of molecularly imprinted gels that function in water is one of the major challenges in this field (74).

Since both hydrogels as materials and molecular imprinting as a method have promising properties for functional applications, it is tempting to design molecularly imprinted hydrogels to function in aqueous solutions. Several groups are working in this direction (73, 75–77).

Another area under investigation is the development of nano- or micro-patterned materials for selective protein adsorption, cell adhesion, and bioactive substance immobilization. It has been proposed by our group and others to form hydrogels on surfaces in specific micropatterns. Figure 6 displays one possible method for developing the patterns. In a similar method, Nakayama and coworkers (78) were able to immobilize bioactive substances, and their materials show promise for obtaining the desired biocompatibility on a given part of a fabricated medical device.

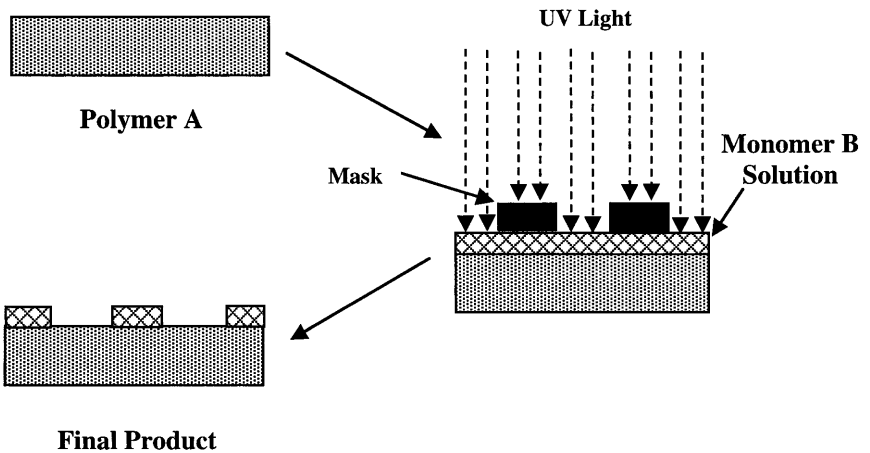

Figure 6 Method for fabricating micro-patterns by UV polymerization.

Another creative method for incorporating a large number of functional groups into a small volume is the development of dendrimers and star polymers (79). Biogels of poly(ethylene oxide) (PEO) star polymers have been prepared (80) with large biological functionality. The star polymers have also been used as materials for molecular imprinting (81).

It is well known that preparation conditions have nontrivial effects on the properties of hydrogels (13). We want to exploit this fact to design functional hydrogels that memorize the existence of specific molecules in the preparation process. To achieve this, we need a more detailed understanding of hydrogels. New theoretical methods beyond Flory's treatment (13) are needed to treat the more complex gel structures.

The problem of slow dynamics is essential to any applications of functional hydrogels. For example, the specific molecules have to diffuse into the hydrogel in order for it to respond. The required time may be estimated by a simple diffusion equation, $t \sim l^2/D$. Here, l is the characteristic length of the hydrogel sample, and D is the diffusion coefficient of the molecules in the hydrogel. A typical time example for gel collapse is on the order of milliseconds for l of μm magnitude and weeks for l of cm magnitude. Therefore, the first thing we can do to improve the response dynamics of hydrogels is to decrease their sizes. Moreover, carefully designed hydrogel architectures (82, 83) have proven helpful.

In summary, though we are only at the beginning, hydrogels can be designed to act as successful functional biomaterials. However, further understanding of their structure-property relationships is needed to bring a breakthrough in this field.

FUTURE DIRECTIONS

The scientific contributions of the biomedical and pharmaceutical academic and industrial world are leading to major new solutions of significant medical problems. Thus, the treatment of diabetes, osteoporosis, asthma, cardiac problems, cancer, and other diseases is based on seminal developments of molecular sciences that have opened new opportunities for curing diseases. Similarly, the design of new medical devices calls for advanced methods of molecular design and optimization. In this changing world, the field of hydrogel preparation and utilization is expected to move toward a more molecular and rational design of new systems.

For example, a significant opportunity has appeared in the therapy of diseases over the past five years with the examination of advanced hydrogel-based systems. These formulations do not simply deliver a protein at some characteristic rate, but they do so in a way that the molecular designer wants. For example, insulin can be delivered only when needed, calcitonin can be directed to bypass the stomach and be delivered only in the upper small intestine, and large-molecular-weight, genetically engineered molecules can be delivered across tissues at acceptable rates. The network structure and the thermodynamic nature of the components of these networks play a key role in their biological interactions, diffusional behavior,

molecular mesh size changes (especially in environmentally responsive hydrogels), and the associated molecular stability of the neighboring bioactive agents.

The development of biomedical hydrogels has been an evolving process. Many biomaterials in clinical use were not originally designed as such. For example, dialysis tubing was originally made of cellulose acetate. These materials allowed serious medical problems to be addressed, yet they also introduced complications. Such dialysis tubing can activate platelets and the complement system. In the next few years, combinatorial chemistry screening and imaginative synthetic techniques will lead to new gels that will impart desirable chemical, physical, and biological properties to biomaterials. Materials will be synthesized directly, so that desirable chain segments or functional groups will be built into the material, or indirectly, by chemical modification of existing structures to add desirable segments or functional groups. In addition, synthetic approaches will involve genetic engineering for the preparation of artificial proteins of uniform structure.

We expect an increase of efforts toward chemical modification of polymer surface or bulk properties by various treatments such as grafting PEO segments onto or within existing hydrogels to enhance biocompatibility or reduce protein adsorption. In addition, hydrogels may be synthesized that promote a desirable interaction between themselves and surrounding cells.

Advances in the development of neutral and ionic hydrogels are expected to concentrate on several aspects of their synthesis, characterization, and behavior such as:

1. synthetic methods of preparation of hydrophilic polymers with desirable functional groups;
2. synthetic methods of preparation of multifunctional or multiarm structures including branched or grafted copolymers and star polymers;
3. understanding of the criticality and the swelling/syneresis behavior of novel anionic or cationic polymers;
4. development of ultrapure polymers, such as cross-linking-free gels produced by freezing/thawing processes;
5. synthesis and characterization of biomimetic hydrogels;
6. understanding of the relaxational behavior during dynamic biological processes; and
7. modeling of any associated dissolution or biodegradation.

Novel and challenging properties of various star hydrogels will attract additional attention of researchers in the biological, medical, and pharmaceutical fields. This is because of their unusual “architecture” and their three-dimensional character. Star polymers are characterized by a central core, which may be a slightly cross-linked polymer “sphere” from which a large number of branches of the same or different molecular structure propagate. These polymeric systems are particularly promising because they can serve as micro- or nanoparticulate carriers. In

addition, because of the very large number of free arms, they can be used for immobilization of drugs, cells, enzymes, or antibodies, whereby a very high density of biological agent is attained in a very small volume. Thus, heparinase can be immobilized to give nanoparticulate star polymers that can be used for postoperative blood purification and removal of heparin. Fibrinolytic enzymes such as streptokinase and urokinase can be immobilized and used for lysis of thrombi in the blood. Urease can be immobilized to produce microparticles that will be useful in blood purification by hemoperfusion.

In the last few years there have been developed new creative methods of preparation of hydrophilic polymers and hydrogels that may be used in the future in biomedical and drug delivery applications. Such efforts include synthesis of self-organized nanostructures based on triblock copolymers that may have applications in controlled drug delivery. Such gels may be promising as carriers for drug delivery if combined with techniques of molecular imprinting. Indeed, there have been several reports of the use of cross-linked polymers as templates for drug imprinting and subsequent release. Still, this field is relatively new and its applications may not be immediately available.

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