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MICROSCALE HYDROGELS FOR MEDICINE AND BIOLOGY: SYNTHESIS, CHARACTERISTICS AND APPLICATIONS

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Microscale hydrogels with dimensions of 200 μm or less are powerful tools for various biomedical applications such as tissue engineering, drug delivery, and biosensors, due to their size, biocompatibility, and their controllable biological, chemical, and mechanical properties. In this review, we provide a broad overview of the approaches used to synthesize and characterize microgels, as well as their applications. We discuss the various methods used to fabricate microgels, such as emulsification, micromolding, microfluidics, and photolithography. Furthermore, we discuss the effects of porosity and crosslinking density on the mechanical and biological properties of hydrogels. In addition, we give specific examples of the use of hydrogels, such as scaffolds and cell encapsulation for tissue engineering, controlled release materials for drug delivery, and environmentally sensitive sensors for microdevices. Finally, we will discuss the future applications of this technology.

1. Introduction

Hydrogels are crosslinked hydrophilic polymers that swell greatly in water. Hydrogels can be synthesized from a wide range of natural or synthetic polymers [Peppas et al. 2006]. Examples of common natural hydrogels include fibrin, hyaluronic acid (HA), agarose, and alginate. Similarly, common synthetic polymers that can be crosslinked to form hydrogels include poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), and polystyrene. Since the chemical and mechanical properties of hydrogels can be engineered, they are well suited to address problems in medicine and biology. For example, each year thousands of people die from organ failure due to shortages of transplantable organs; hydrogels can potentially be used to engineer tissue constructs. To minimize the toxic effects of high drug doses, it is often desirable to deliver drugs in a controllable manner: this too can be achieved with hydrogels synthesized with controlled porosity and crosslinking density.

Despite their widespread potential, macroscale hydrogels have a number of limitations for medical and biological applications. In tissue engineering, for example, hydrogel scaffolds may cause cell necrosis due to diffusion limitations, even though these artificial scaffolds closely mimic the chemical and mechanical properties of natural extracellular matrix (ECM). In large hydrogels it is difficult to control the three-dimensional (3D) architecture and cell-cell interactions, which makes it difficult to replicate the complexity of real tissues. Microscale hydrogels, in contrast, have no such limitations. For example, by using hydrogels of controlled sizes and shapes [Yeh et al. 2006], it is possible to minimize diffusion limitations while fabricating tissues with complex microvasculature and microarchitecture [Sefton and

Keywords: BioMEMS, tissue engienering, biomaterials, drug delivery, hydrophilic polymer, stem cells, regenerative medicine, biosensor.

	(+)	(−)
Micromolding	Controlled shape and size	Batch process
Photolithography	Controlled shape and size	Batch process Cell toxic photoinitiator
Microfluidics	Homogeneous, continuous	Nonscalable
Emulsification	Easily scalable	Limited to spherical shapes

Table 1. Advantages and disadvantages of microgel fabrication methods.

McGuigan 2006]. The use of microscale hydrogels is also useful for drug delivery applications, since the size and shape of the delivery vehicle can be used to control the rate of release and to target delivery to specific locations in the body.

This review presents a broad summary of the science and applications of microscale hydrogels. It discusses various methods for fabricating microgels, and introduces techniques for characterizing and engineering the mechanical and chemical properties of microgels. We also demonstrate the application of engineered microgels to tissue engineering, diagnostics, microdevices, and drug delivery.

2. Synthesis and fabrication of microgels

Microgels can be manufactured by a variety of techniques, including micromolding, emulsification, microfluidic drop formation, and photolithography (see Figure 1 and Table 1). A combination of manufacturing method, hydrogel precursor, and crosslinking agent determine the eventual mechanical, physical, and chemical characteristics of a hydrogel. All of the manufacturing methods outlined in Figure 1 can be used with a wide range of hydrogel precursors as well as crosslinking agents.

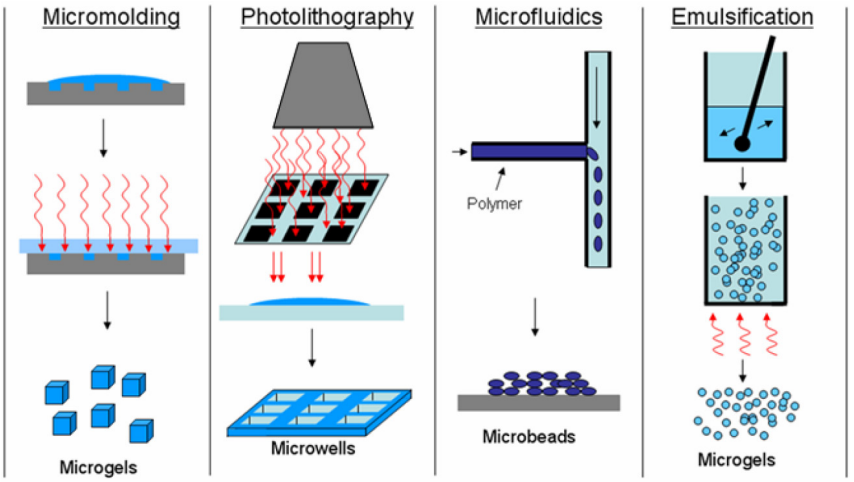

Figure 1. Methods of fabricating microgels.

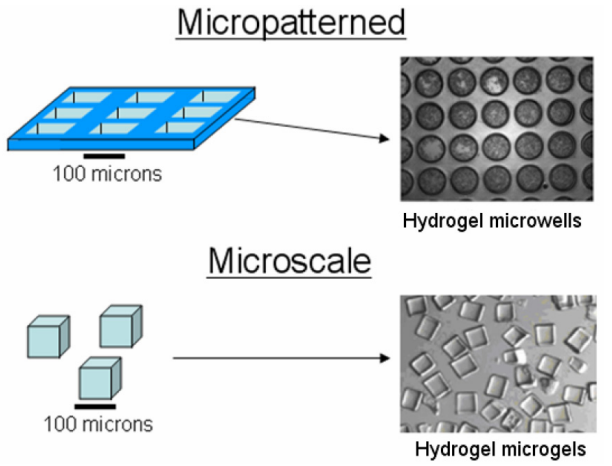

Figure 2. Microscale versus micropatterned hydrogels.

In this review we distinguish between microscale and microfeatured hydrogels. We define microscale hydrogels (microgels) as hydrogels with dimensions of $\approx 200\,\mu$m or less, and define microfeatured hydrogels as hydrogels that have been patterned with microscale features (see Figure 2). In this section we will discuss a few methods of manufacturing both microscale and microfeatured hydrogels.

Emulsification. Emulsification is the most common method used to manufacture microgels. The emulsification process typically uses a two-phase system by mixing two dissimilar substances such as a hydrophilic hydrogel precursor solution and a hydrophobic phase. Mechanical shearing forces the aqueous hydrogel prepolymer to emulsify in the hydrophobic phase (such as oil) and form a suspension of hydrogel microbeads. Varying the fluid viscosity and shear rate controls the size of the resulting microbeads. The prepolymer microbeads can then be crosslinked by a variety of methods such as heat or light.

Emulsification is used in drug delivery for fabrication of alginate microgels for insulin delivery [Reis et al. 2006], as well as for gene therapy [Alexakis et al. 1995]. Emulsification can also be used to encapsulate cells within microgels for bioreactor and immunoisolation applications [Dang and Zandstra 2005].

Photolithography. A common method to fabricate microgels of controlled sizes and shapes is photolithography. In photolithography, a hydrogel precursor is mixed with a photoinitiator which, when exposed to ultraviolet (UV) light, catalyzes the crosslinking reaction.

In one method, a thin film of hydrogel precursor and photoinitiator is placed underneath a photomask containing opaque patterns. The photomask is then exposed to UV light. The light reaches the underlying hydrogel precursor solution through the transparent regions of the photomask, causing the microgels to crosslink in those regions. This technique can be used to create microgels of various sizes and shapes based on the features on the mask. Photolithographic patterning of photocrosslinkable hydrogels can also be used to localize cells and generate cell-laden microstructures. For example, HepG2 cell-laden microstructures have been fabricated by encapsulating cells within crosslinkable PEG hydrogel [Liu and Bhatia 2002]. It is also possible to use this approach to generate more complex structures by combining multiple cell types into 3D structures [Koh et al. 2003]. Additionally, photolithography can create

functional components within microfluidic channels. For example, Beebe et al. [2000] manufactured microvalves by photopolymerizing hydrogels directly inside microchannels. These valves can be engineered from environmentally sensitive hydrogels, so that only specific stimuli will actuate them.

Despite the merits of photolithography for various applications, it has a number of limitations. For example, currently established photolithographic processes only produce output in batches. Recently, however, Dendukuri et al. [2006] adapted microgel photolithography into a continuous flow process. In this method, a photopolymerizable polymer is flowed through a microfluidic channel and subsequently exposed to UV light through a photomask to form microgels of specific sizes and shapes.

Micromolding. Micromolding is a useful technique for forming microgels and micropatterned hydrogels. Most micromolding techniques utilize a micropatterned master to mold replicas for repeated fabrication. The shape of the mold determines the shape of the resulting structures. It is now possible to fabricate microstructures as smaller than 1 μm. To fabricate microscale or microfeatured hydrogels, a hydrogel precursor is molded on the master, which is made from materials such as glass, PDMS, or silicon.

Micromolding of cell-laden hydrogels have been used to fabricate shape- and size-controlled tissue pieces that may be useful for tissue engineering [Khademhosseini et al. 2006a; 2006b; 2006c]. In these experiments, micromolded HA microwells were formed and used as docking regions for cell patterning and microarrays. The cells trapped inside the wells not only remained viable but could also be retrieved. The authors demonstrated that cell-laden microscale HA structures could be molded for incorporation into microdevices and biosensors. Fukuda et al. [2006] adopted micromolding techniques to create hydrogel microarrays and cocultures. By micromolding chitosan, they showed that various low shear-stress surface patterns could be created for the entrapment and organization of cells. Yeh et al. [2006] demonstrated the possibility of using photocrosslinkable polymers and micromolding to form stem cell seeded microgel tissue building blocks.

Microfluidics and droplet formation. Microfluidic techniques have been widely explored for their ability to form a variety of microgel constructs. Droplet formation within microfluidic channels can be used to fabricate highly homogeneous microgels. In one droplet-formation technology, a solution of prepolymer precursor is diverted into a larger microfluidic channel filled with a flowing solution containing the crosslinking agent [Nisisako et al. 2002]. The shear forces of the flow in the larger channel detach individual droplets of prepolymer solution, causing them to solidify into microgel constructs. The constructs are geometrically dependent on the flow rate and respective concentrations of the two microfluidic streams being mixed (see Figure 1). Other techniques utilize a microfluidic flow focusing device (MFFD) to generate reproducibly sized hydrogel spheres as small as 20 μm [Xu et al. 2005]. In addition to microfluidic channels, a number of authors have explored methods of forming droplets at the air-solution interface. For example, microneedles that are filled with a prepolymer solution can be induced to form picoliter droplets at a rate of hundreds of hertz, due to piezoelectrically induced pressure waves [Demerci et al. 2003].

Microfluidic approaches can also create macroscale hydrogels with unique spatial properties, such as concentration of density gradients. For example, hydrogels can be synthesized with spatially regulated patterning of adhesive or signaling molecules, or with an elasticity that varies from region to region. Such gels could be used for studying biological systems, directing stem cell differentiation, spatially or time-regulated drug delivery, or cell migration direction for tissue engineering [Burdick et al. 2004].

3. Mechanical properties of microscale hydrogels

Tissue engineering and biomedical needs call for fine control of the mechanical properties of hydrogels. For example, it might be necessary to develop a hydrogel tissue scaffold of varying mechanical rigidity or porosity. Alternatively, it may be desirable to create a hydrogel with nonuniform crosslinking for more effective drug delivery. The mechanical property of hydrogels is a function of many parameters such as the type of hydrogel, concentration, and crosslinking density. In this section we analyze the theoretical and experimental aspects of some of these parameters and examine their effect on the mechanical properties of hydrogels.

Crosslinking and porosity. A synthesized hydrogel has three main properties: the swollen-state polymer fraction $\nu_{2,s}$, the size ξ of the polymer mesh, and the average molecular weight $\overline{M}_c$ of an intercrosslinked section of polymer chain [Peppas and Khare 1993]. The latter is the most important factor in hydrogel formation and governs how much a particular hydrogel solution is crosslinked. This in turn can drastically affect mechanical and chemical performance characteristics [Anseth et al. 1995]. To determine $\overline{M}_c$, the Flory–Rehner theory can be used for hydrogels prepared in a nonionic aqueous solvent. The theory states that

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{(\bar{\nu}/V_1)\big(\ln(1-\nu_{2,s}) + \nu_{2,s} + \chi_1 \nu_{2,s}^2\big)}{\nu_{2,r}\left(\left(\dfrac{\nu_{2,s}}{\nu_{2,r}}\right)^{1/3} - \dfrac{\nu_{2,s}}{2\nu_{2,r}}\right)},$$

where $\bar{\nu}$ is the specific polymer volume, $\nu_{2,r}$ is the relaxed state polymer volume fraction, and V_1 is the molar water volume [Langer and Peppas 2003]. The Flory–Rehner theory for hydrogels dissolved in ionic aqueous solvents is more complex; it predicts the equations

$$\frac{V_1}{4IM_r}\left(\frac{\nu_{2,s}^2}{\nu}\right)\left(\frac{K_a}{10^{-\mathrm{pH}} - K_a}\right)^2 = \big(\ln(1-\nu_{2,s}) + \nu_{2,s} + \chi_1\nu_{2,s}^2\big) + \frac{V_1}{\nu\overline{M}_c}\left(1 - \frac{2\overline{M}_c}{\overline{M}_n}\right)\nu_{2,r}\left(\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)^{1/3} - \frac{\nu_{2,s}}{2\nu_{2,r}}\right),$$

$$\frac{V_1}{4IM_r}\left(\frac{\nu_{2,s}^2}{\nu}\right)\left(\frac{K_b}{10^{\mathrm{pH}-14} - K_a}\right)^2 = \big(\ln(1-\nu_{2,s}) + \nu_{2,s} + \chi_1\nu_{2,s}^2\big) + \frac{V_1}{\nu\overline{M}_c}\left(1 - \frac{2\overline{M}_c}{\overline{M}_n}\right)\nu_{2,r}\left(\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)^{1/3} - \frac{\nu_{2,s}}{2\nu_{2,r}}\right),$$

where K_a and K_b are the acid and base dissociation constants, I is the ionic strength, and M_r is the repeating unit molecular weight [Peppas et al. 2006]. This theory enables custom design of hydrogels and microhydrogels to satisfy particular mechanical and chemical characteristics.

Spacing between macromolecular chains is another important characteristic of hydrogels. These spaces are called *pores.* Hydrogels are generally categorized into three porosity categories: (i) nonporous, (ii) microporous, and (iii) macroporous. The polymeric mesh, or pore size of a particular hydrogel, can be calculated using the following equation:

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

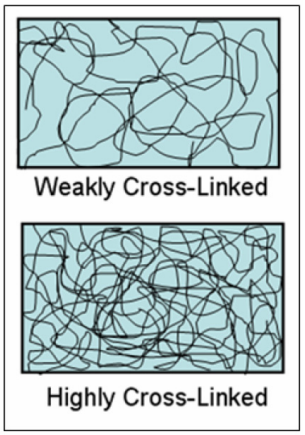

Figure 3. Highly crosslinked hydrogels and weakly crosslinked hydrogels.

where C_n is the Flory characteristic ratio and l is the length of the polymer backbone bond [Peppas et al. 2006].

By altering such factors as the pore size or the molecular weight between crosslinks (see Figure 3), it is possible to tailor individual hydrogel constructs to exhibit particular thermal, diffusive, or mechanical properties.

Mechanical performance. Hydrogels' adaptable mechanical properties makes them especially useful for drug delivery and tissue engineering. For example, mechanical forces are known to affect cell viability, gene expression, and stem cell differentiation pathways [Engler et al. 2006]. Because human tissues are highly organized structures, any engineered tissue will need to have the same degree of mechanical and physical complexity. Thus, by utilizing hydrogels of varying mechanical properties, it may be possible to mimic natural tissue.

Mechanically, hydrogels are remarkably similar to both human tissue as well as natural rubbers. Hydrogels generally exhibit excellent elastic characteristics: when loaded to deformations of 20% or less, they typically rebound instantaneously [Peppas et al. 2006]. Thus, rubber elasticity theory effectively characterizes the deformation of hydrogels. The rubber elasticity theory for solvent-based hydrogels is given as

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

where τ is the stress applied to the polymer sample, T is the absolute experimental temperature, ρ is the density of the polymer, R is the universal gas constant, and $\overline{M}_c$ is the molecular weight between crosslinks [Peppas 1997].

Much work has been done to characterize mechanical characteristics of hydrogels [Anseth et al. 1995] and to determine how to synthesize a gel with desired mechanical properties [Cohen et al. 1992; Davis 1989; Greenberg 1980; Moussaid et al. 1994]. The innumerable possible combinations of composite hydrogels have inspired new techniques for high-throughput mechanical testing of polymers. In one example, over 1700 photopolymerizable materials were tested in only a few days by using an automated

nanomechanical screening system [Tweedie et al. 2005]. Using nanoindentation, an entire library of nanoliter samples placed on a glass slide was quickly tested for mechanical characteristics such as elastic modulus E, hardness H, and P-h load-displacement hysteretic curves.

4. Tissue engineering

Tissue engineering is "an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function or entire organs" [Langer and Vacanti 1993]. Since hydrogels are made mostly of water and natural (or synthetic biocompatible) polymers, they are often biologically compatible [Bruck 1973]. Accordingly, most hydrogels exhibit good compatibility when seeded with cells or when implanted *in vivo*.

The use of microscale hydrogels (such as microrods or microbeads), as well as microfeatured hydrogels (such as microchannels or microvasculature), has been useful in various tissue engineering applications. In the following sections we will highlight a few examples of hydrogels in tissue engineering research.

Microscale scaffolds. Cells require a suitable growth environment and a biomimetic 3D architecture in order to form tissues [Nguyen 2002; Peppas et al. 2006]. One method of constructing tissues from cells is by spatially orienting the cells in a desired 3D geometry with a hydrogel scaffold. Hydrogel scaffolds provide cells with environmental conditions favorable for growth by allowing nutrient transport and oxygen diffusion. Additionally, they offer temporary mechanical support for cells, until the cells can deposit their own ECM molecules. Many hydrogel materials are also biologically degradable. Consequently, as cells proliferate within the scaffold and form tissues, the scaffold itself breaks down, leaving transplantable tissues containing only natural cellular components.

Scaffold-based tissues may be built either from the top down, or from the bottom up. In the former, macroscale hydrogels are micropatterned to enable nutrient perfusion and cell adhesion. The bottom-up approach, in contrast, uses cell-laden microgels or tissue aggregates that can be combined to form larger, tissue-like constructs.

In one top-down example, a vessel was loaded with PMMA microspheres and then filled with the hydrogel precursor [Stachowiak et al. 2005]. Upon crosslinking the hydrogel, the PMMA microspheres were dissolved to create a microporous hydrogel scaffold. The resulting scaffold was then seeded with cells (see Figure 4).

Sefton and McGuigan [2006], in contrast, used a bottom-up approach: a packed bed of microgels facilitated the development of a perfusable tissue-like construct. Submillimeter hydrogel rods were seeded with endothelial cells (ECs) and then packed within a larger tube that was perfused with blood. The microscale gel rods merged to form a tissue-like construct with vascularization, due to the interstitial spaces between the microrods (see Figure 5).

Another bottom-up method due to Yeh et al. [2006] showed the potential use of HA or PEG microgel blocks as building blocks for tissues. Using photopolymerized micromolding, cell-laden microgels were fabricated with various shapes and sizes. These gels could be subsequently assembled to form complex 3D structures.

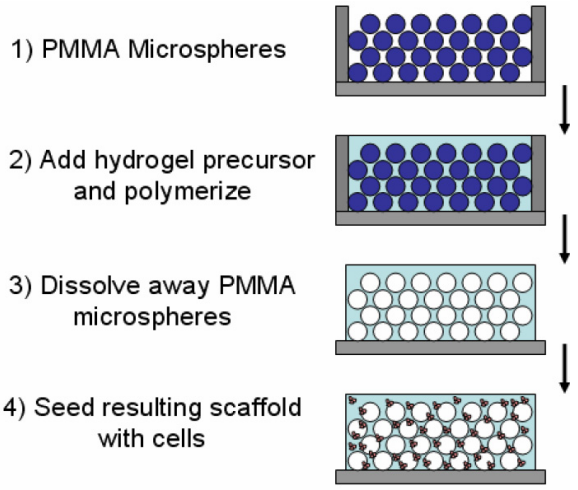

Figure 4. Tissue scaffold formation via microsphere dissolution.

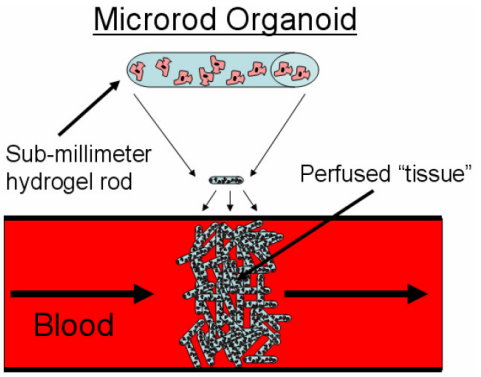

Figure 5. Packed bed of hydrogel microrods form a tissue-like organoid.

Microvasculature. One of the most difficult problems in tissue engineering is vascularization. Many attempts have been made to engineer microvasculature within tissue engineering constructs [Borenstein et al. 2002; Kaihara et al. 2000]. Although initial work was performed using nonbiodegradable PDMS and silicon, recent research has demonstrated that it is possible to create biomimetic capillary channels using micromolded biodegradable poly(glycerol-sebacate) (PGS). In this technique, hydrophilic PGS was molded onto microfabricated silicon. Multiple layers of micropatterned PGS were then stacked on one another and subsequently bonded. The resulting 3D tissue-like construct contains biomimetically sized vasculature. Eventually, this may enable the construction of perfusable engineered tissues.

Recently, Cabodi et al. [2005] demonstrated the feasibility of using sealable calcium alginate hydrogel microfluidic channels. The authors fabricated sealed microfluidic pathways (as small as 25 μm by 25 μm) within micromolded calcium alginate hydrogels. These hydrogel-based microchannels also have significant potential for generating microvasculature-like tissue structures.

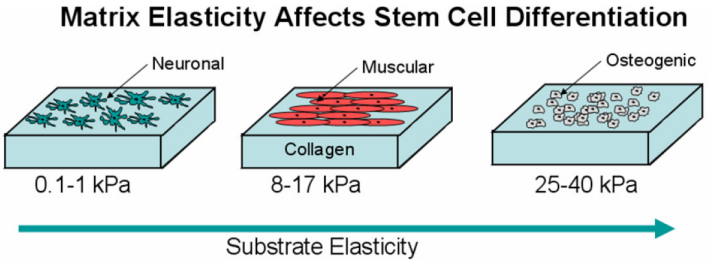

Figure 6. The mechanical properties of hydrogels induce directed stem cell differentiation.

Regulation of stem cell fate. Embryonic stem (ES) cells are a potential source of transplantable tissues because they can both renew themselves, and differentiate into a number of desirable cell types, such as hepatocytes, pancreatic cells, cardiomyocytes, otsteoblasts, endothelial cells, and neural cells. Currently, however, it is difficult to induce ES cell differentiation uniformly in a scalable process. The challenge is that ES cells proliferate and differentiate in response to a large number of microenvironmental factors, such as soluble growth factors, matrix components, forces, and cell-cell interactions. Interactions among these various factors play different roles in affecting the resulting cell lineage at different stages of development in a highly regulated manner.

Microscale hydrogels offer a possible means of finely controlling the microenvironment of differentiating ES cells. Nonadhesive microwells fabricated from photocrosslinkable PEG have been used as part of a template-based approach for reproducible generation of uniform microtissues [Khademhosseini et al. 2006a; 2006b; 2006c]. Formation of cell aggregates called embryoid bodies (EBs) was initiated by seeding cells within photopolymerized PEG microwells. EBs could then be harvested for further differentiation of the cells. In addition, aggregates of various cell types, shapes and sizes have been manipulated by simply altering the geometry of the PEG microwells.

In addition, Dang et al. [2004] demonstrated that the microencapsulation of stem cells within hydrogels can help produce tissue progenitor cells at scalable rates. In these applications, cells encapsulated within microscale hydrogels can be separated from each other to prevent aggregation, thus enabling large-scale production of EB-derived cells.

Another recent study showed that the mechanical characteristics of the growth substrate can drastically affect stem cell differentiation [Engler et al. 2006]. Depending on the concentration of the polymer and the nature of the crosslinks, gels were manufactured with elasticities between 0.1 kPa and 40 kPa thus simulating the range of elasticities found in human tissues. In general, brain tissues exhibit an elasticity between 0.1 kPa and 1 kPa, muscle tissues are around 10 kPa, and collagenous bone is around 100 kPa. It was demonstrated that the stem cells seeded onto various substrates differentiated with great regularity into the tissue precursor mechanically similar to the underlying substrate. In these experiments, mesenchymal stem cells (MSCs) that were seeded onto soft hydrogels (0.1–1 kPa) tended to develop into neuronal precursors, while MSCs that were seeded onto stiffer gels (8–17 kPa) differentiated into muscular precursors. At the high end of the range of substrate stiffness (25–50 kPa), the MSCs tended to differentiate into collagenous bone-like tissues precursors (see Figure 6) [Engler et al. 2006].

A similar study gave insight into oncogenic stem cells differentiation by demonstrating how the mechanical properties of a 3D hydrogel matrix affects tumor cell migration speed [Zaman et al. 2006]. In

this study, it was demonstrated that tumor cells in a 3D fibronectin hydrogel matrix migrate quickly in highly elastic substrates. In contrast, tumor cells on 2D surfaces migrate quickly when the underlying substrate is nonelastic.

Cell encapsulation and immunoisolation. Hydrogels can also be used to encapsulate cells in microcapsules. This can prevent cell aggregation, which can be useful for stirred bioreactor experiments. Furthermore, cell-laden hydrogels can be coated with various polymers to immunoisolate the encapsulated cells from the surrounding environment [Peppas et al. 2006].

Hydrogel materials express physical characteristics that are similar to the ECM and exhibit high permeability to oxygen, nutrients, and other metabolites, thus providing a favorable environment for cell survival. Typically, the procedures used to encapsulate cells within a hydrogel result in high cell viability, often only requiring that a cell suspension be mixed with the hydrogel precursor prior to crosslinking of the network.

When encapsulating cells within hydrogels, it is important to consider the photoinitiator concentration, UV exposure length, macromer concentration, and thermal exposure, since they all affect cell viability. When forming a gel, a balance is needed between the desired mechanical characteristics and long-term cell viability. Several studies have demonstrated long-term viability for hydrogel-encapsulated cells, especially in microgel structures which encourage effective nutrient and oxygen perfusion; see [Khademhosseini et al. 2006a; 2006b; 2006c]. These studies also show that hydrogel cell immunoisolation is useful because it can protect allogenic or xenogeneic cells from the host's immune system within a semipermeable membrane. For example, functional pancreatic cells may be immunoisolated in hydrogel and implanted into an allogenic host [Lim and Sun 1980].

5. Diagnostics and microdevices

Hydrogels can be used as functional components in microdevices and diagnostic tools. Due to the ease of photolithographic and micromolding techniques, it is possible to incorporate hydrogels cheaply into devices and sensors. The wide range of mechanically and chemically responsive *smart* hydrogels makes this integration particularly appealing.

"Smart" hydrogels. Engineering the chemical or physical makeup of a hydrogel can predetermine their response to environmental stimuli. These so-called *smart* or *environmentally responsive* hydrogels can be designed to respond to a wide range of stimuli, such as changes in pH, *p*I, and temperature [Jeong et al. 2002; Miyata et al. 2002; Peppas 1997; Peppas and Khare 1993; Peppas et al. 2000].

Thermally responsive hydrogels, such as poly(N-isopropyl acrylamide) (PNIAAm) and its derivatives, have a highly reproducible response to temperatures. Generally, as the temperature of a hydrogel is increased, its volume will increase until it reaches a critical point, called the lower critical solution temperature (LCST). As the temperature of the gel exceeds the LCST, the gel undergoes a volumetric phase change and begins to shrink. This process is reversible; when the temperature is lowered below the LCST, the hydrogel will return to its original volume. For example, PNIPAAm exhibits a LCST around 33° C. PNIPAAm and other thermoresponsive hydrogels are being studied for a wide variety of tissue engineering and drug delivery applications [Jeong et al. 2002; Sershen and West 2003].

Another response mechanism is ionic activation. Examples of ionically responsive hydrogels are poly(acrylic acid), poly(methacrylic acid), polyacrylamide (PAam), poly(diethylaminoethyl methacrylate), and poly(dimethylaminoethyl methacrylate). In general, hydrogels with weakly acidic pendent groups will exhibit swelling as the pH of the surrounding medium increases, whereas hydrogels with weakly basic pendent groups will swell as the pH of the surrounding medium decreases. The determining factors for ionic swelling of hydrogels have been widely studied and include ionization equilibrium, ionic content and polymer structure [Khare and Peppas 1993; Podual and Peppas 2005; Scott and Peppas 1999].

Hydrogels as components of microdevices. Environmentally responsive hydrogels, whether chemically, thermally, or mechanically activated, have been used in microdevices for a variety of purposes such as controlled microreactors, valves, and pumps [Beebe et al. 2000; Miyata et al. 2002; Yu et al. 2001]. For example, pH-sensitive photocrosslinkable PEG-based hydrogels have served as functional microvalves. As the pH of the microfluidic solution changes, so does the geometry of the valve, therefore allowing for effective sealing and opening of the microfluidic pathway [Beebe et al. 2000]. Another method used differential swelling between basic and acidic ionic gels to enable controllable valves [Yu et al. 2001]. By utilizing a bimetallic strip-like construct, it was possible to force a hydrogel construct to open in a particular direction, depending on the pH of the surrounding medium. Other signaling methods, such as photoactivity and thermal, chemical and electrical stimulation, have also been demonstrated [Beebe et al. 2000]. While valves are only one example of environmentally responsive hydrogel structures, the potential implication of microactuated hydrogel constructs could have far reaching applications. For example, chemically actuated hydrogel pumps may one day enable tissue engineering constructs that self perfuse.

Micropatterned PEG hydrogels also have applications in microdevices. For example, micropatterned PEG hydrogels embedded within microfluidic channels have been shown to enable control over the location of cells and proteins within the microfluidic channel. The ability to precisely control both cell and protein location can be used to perform cell- or protein-based assays or to create controlled microreactors [Heo et al. 2003; Zhan et al. 2002]. It has also been shown that PEG microstructures within microfluidic channels are capable of capturing and localizing cells in regions of low shear stress [Khademhosseini et al. 2004]. Capturing cells from flowing solutions is useful for many applications, such as sensing, cell separation, and cell-based microreactors.

Hydrogels as integral components of microsensors. The incorporation of hydrogels into biological sensors could also result in a new class of sensing technologies. Hydrogels' perfusability enables the embedding of a large number of biological detection factors, such as antibodies, within a gel's 3D structure. When compared to antibody immobilization on a 2D surface, microgels should provide a significant sensing advantage by increasing the density of the receptor molecule [Zhan et al. 2002]. One example is a protein-sensitive, environmentally responsive hydrogel MEMS sensor (see Figure 7). In this sensing mechanism, an antibody-laden hydrogel is micropatterned onto a MEMS microcantilever [Bashir et al. 2002; Hilt et al. 2003]. Then, as the hydrogel absorbs the target protein, the hydrogel swells or contracts, causing the MEMS cantilever to deflect. The degree of deflection is measured using refractive optics.

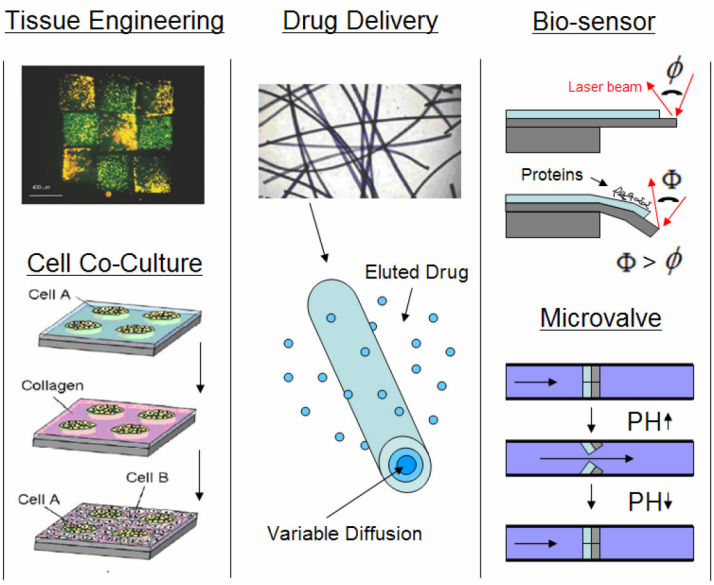

Figure 7. Applications of microscale and microfeatured hydrogels for tissue engineering, cell coculture, drug delivery, biosensors, and microfluidics.

Micropatterned hydrogel MEMS cantilever sensors have been used for a variety of sensing applications. For example, similar techniques have been demonstrated using pH- or thermally sensitive hydrogels, in which pH or temperature changes (respectively) cause swelling that deflects a microcantilever. Similar work has also demonstrated microcantilever hydrogel sensors capable of accurately sensing CrO_4^{2-} [Zhang et al. 2003] and Pb^{2+} [Liu and Ji 2004].

To develop analyte sensing technologies, several groups have micropatterned hydrogels onto MEMS electrodes using photolithography. By localizing oxidoreductase enzymes onto the microelectrodes, analyte levels can be accurately detected by measuring changes in the conductivity of the micropatterned hydrogel [Jimenez et al. 1997; Jobst et al. 1996; Sirkar et al. 2000].

Cell-based diagnostics and screening. In addition to providing a source of viable cells for tissue replacement therapies, the use of microscale hydrogels for close regulation of the cellular microenvironment may also be utilized in high-throughput experimentation and diagnostic tools. Cells *in vivo* are exposed to various 3D microenvironmental conditions closely monitored by the body. *In vitro* culturing conditions often differ vastly from those experienced by cells in native organ systems. In traditional cell culture systems, cell-cell, cell-ECM and cell-soluble factor interactions are often too complicated to control, making it difficult to mimic the native spatial and temporal distribution of cell signaling. In addition, culture dishes offer only a 2D environment, as opposed to the 3D environments encountered by cells in the body.

Cells cultured in microscale hydrogels come into contact with a microenvironment much more comparable to that experienced by cells *in vivo*. As a result, this technique may provide a better tool for

in vivo studies on cell-environment interactions. The microscale nature of this technique permits combination with high-throughput technologies when studying many microenvironmental factors at once [Khademhosseini 2005].

One particularly promising application for microgels and micropatterned hydrogels is for cellular coculture experiments. By using natural hydrogel polymers such as HA and collagen, it has been demonstrated that effective cell cocultures can be performed using micropatterned hydrogels. In one example [Khademhosseini et al. 2006a; 2006b; 2006c], a microwell patterned layer of HA was used as a template to control cell-cell interactions (see Figure 7).

Drug delivery. Many current drug delivery mechanisms are invasive, painful, or ineffective. Microscale hydrogels may provide an intelligent means of controlled drug delivery that solves these problems.

Drug-infused microscale hydrogels can deliver drug therapies in a sustainable and controllable manner [Langer 2000]. Furthermore, the drug release kinetics may be tailored by manipulating the shape, size, and density distribution of the microgels during the fabrication process. Microgels may also be fabricated from many different hydrogel polymers; this results in a dramatic variability of drug release mechanisms, many of which are environmentally responsive.

Hydrogels exhibiting pH sensitivity, temperature sensitivity, and swelling properties have all been exploited for drug release purposes. For example, pH-responsive microgels comprised of ionic networks containing PEG can be used for the oral delivery of medically relevant proteins such as insulin and calcitonin [Peppas et al. 2006]. Additionally, microgels with specific degradation characteristics can be induced to demonstrate pulsatile release responses upon breakdown. For instance, drugs encapsulated within alginate microgels can be released upon depolymerization of the alginate network, which is triggered through removal of divalent cations in the network.

Control over drug release systems can be used in the formation of intelligent materials, which may be utilized in targeted drug delivery methods [McCarthy et al. 2005; Peppas 1997; Peppas and Khare 1993]. By engineering the material composition, size, and shape of hydrogel drug delivery vehicles, not only can rates of drug diffusion be methodically managed, but release mechanisms can be made responsive to the surrounding environment. For example, *smart* microgels infused with cancer drugs could delay elution of their payload until they reach cancer cells. Such systems have great potential to increase the safety and effectiveness of future drugs, while decreasing the invasiveness of delivery mechanisms.

6. Future applications

It is widely expected that a majority of future tissue engineering techniques will be based on hydrogel technology. No other class of materials has the flexibility or biological compatibility to enable significant advances in tissue engineering. For example, microscale hydrogels let engineers precisely control the cellular microenvironment, which may lead to significantly more effective stem cell differentiation techniques. Additionally, the ability to create micropatterned, vascularized, cell-laden hydrogel scaffolds will enable more effective tissue engineering therapies. Finally, the versatile chemical and mechanical properties of microscale hydrogels provide a unique platform for the future development of more accurate and effective biological sensors and microdevices.

Many scientists believe that it may be possible one day to print an entire three-dimensional, functional organ. Novel 3D printing techniques, currently under development, may make this a reality. Due to the

exceptional mechanical and biological properties of hydrogels, many of these new technologies utilize hydrogel materials as their *ink*. By printing sequential layers of cell-laden hyrdogels, it may be possible to start to print entire ogranoids or tissues.

7. Conclusion

Microgels, as well as microfeatured hydrogels, have demonstrated great promise in biomedical engineering applications. Their unique, easily regulated mechanical and chemical characteristics solve many problems in tissue engineering, drug delivery and microdevice applications. In tissue engineering, microgels have shown promise for the construction of both scaffolds (for top-down methods) and the building blocks needed for bottom-up approaches. For drug delivery, the ability to fine-tune microgel drug release mechanisms has been shown widely effective for controlled-release applications. Finally, since hydrogels are easily customized, they make ideal components of microdevices and biosensors. Hydrogels, especially those with microscale features or sizes, have rapidly become an indispensable tool for solving some of the most difficult problems in medicine and biology. As research utilizing microgels progresses, it is expected that this platform will enable numerous advances in medicine and biology.

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