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# Manipulations in hydrogel chemistry control photoencapsulated chondrocyte behavior and their extracellular matrix production

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Stephanie J. Bryant,<sup>1</sup> Kevin L. Durand,<sup>1,2</sup> Kristi S. Anseth<sup>1,2</sup>

<sup>1</sup>Department of Chemical Engineering, University of Colorado, ECCH 111, Boulder, Colorado 80309-0424

<sup>2</sup>Howard Hughes Medical Institute, University of Colorado, Boulder, Colorado 80309-0424

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**Abstract:** In engineering a cell-carrier to support cartilage growth, hydrogels provide a unique, largely aqueous environment for 3-dimensional chondrocyte culture that facilitates nutrient transport yet provides an elastic framework dictating tissue shape and supporting external loads. Although the gel environment is often >90% water, we demonstrate that slight variations in hydrogel chemistry control gel degradation, evolving macroscopic properties, and ultimately the secretion and distribution of extracellular matrix molecules. Specifically, biodegradable poly(ethylene glycol)-*co*-poly(lactic acid) hydrogels were fabricated via photopolymerization. When chondrocytes were photoencapsulated in these gels, changes in the poly(ethylene glycol)-*co*-poly-

(lactic acid) repeat unit ratio from 19 to 7 increased total collagen synthesis 2.5-fold after 6 weeks *in vitro*. Furthermore, the ratio of collagen to glycosaminoglycans varied from glycosaminoglycan-rich,  $0.33 \pm 0.13$ , to collagen-rich,  $4.58 \pm 1.21$ , depending on gel chemistry and *in vitro* versus *in vivo* culture environment. By tuning scaffold chemistry, and subsequently, gel structure and degradation behavior, we can better guide tissue evolution and development. © 2003 Wiley Periodicals, Inc. *J Biomed Mater Res* 67A: 1430–1436, 2003

**Key words:** photopolymerization; poly(ethylene glycol); cartilage; tissue engineering; degradation

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## INTRODUCTION

Cartilage is one of the few tissues found in the body that has a limited capability to regenerate as a result of injury, congenital abnormalities, or arthritis all of which can lead to further degradation of the tissue and excessive joint pain.<sup>1</sup> Many approaches have been explored over the years to develop surgical techniques to heal damaged cartilage. However, current therapies available to patients are laden with shortcomings and to date no method provides a perfect solution. Traditional therapies include autogenic and allogenic implants in which the former is limited by donor tissue availability and donor site morbidity, whereas the latter poses a risk for disease transmission. More recently, subchondral penetration has been used to stimulate a healing process, but the resulting tissue (fibrocartilage) that is formed is often mechanically inferior.

The latest advance in cartilage repair is autologous chondrocyte implantation, a promising therapy, but its success is limited by the defect size and location.<sup>2,3</sup>

A new potential therapy for cartilage repair, which has led to a plethora of research activity, is *tissue engineering*. Through this therapy, chondrocytes are seeded on or encapsulated in degradable cell-scaffolds. However, the design of the cell-scaffold is challenging with many important considerations. In this regard, we are interested in *in situ* forming photocrosslinkable and degradable hydrogels. Hydrogels, in general, are highly hydrophilic, insoluble polymer networks that swell in the presence of water and mimic the highly aqueous environment of biological tissues whereas photopolymerization enables *in situ* scaffold formation providing a minimally invasive vehicle for cell transplantation. Specifically, a liquid macromer (or pre-hydrogel) solution can be combined with cells and polymerized under mild cytocompatible conditions to form a solid three-dimensional (3D) network.

One particular hydrogel chemistry that has been investigated as a scaffold for 3D culture of chondrocytes is photocrosslinkable poly(ethylene glycol) (PEG).<sup>4,5</sup> When chondrocytes were photoencapsulated

Correspondence to: K. S. Anseth; e-mail: kristi.anseth@colorado.edu

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in these PEG gels, the chondrocytes' phenotype and function were maintained both *in vitro* and *in vivo*.<sup>6,7</sup> Furthermore, the degradation behavior of these gels can be controlled by incorporating degradable linkages, such as poly(lactic acid) (PLA) or enzymatically susceptible peptides, into the gels.<sup>8-10</sup>

Although these PEG-based gels are predominately water, slight variations in the gel chemistry greatly affect the gel structure, properties, and degradation behavior,<sup>8,11</sup> but the question remains as to the effects of these changes on cell behavior and tissue evolution. To investigate this issue, degradable PEG hydrogels were fabricated from a triblock copolymer of PLA-*b*-PEG-*b*-PLA endcapped with methacrylates.<sup>8</sup> Chondrocytes were photoencapsulated in these gels, and the effect of the scaffold chemistry, particularly changes in the PEG/PLA ratio, on the cell behavior and extracellular matrix (ECM) type and distribution was surprisingly dramatic. Even though these gels are largely water, this work underscores the importance of tailoring the gel chemistry with high fidelity to engineer tissues with desirable and equivalent properties to their native tissue counterpart.

## METHODS

### Macromers

Triblock copolymers, PLA-*b*-PEG-*b*-PLA with methacrylate end groups (PEG-LA-DM) were synthesized using PEG (Aldrich) with molecular weights (MWs) of 4600 or 8000 g/mol.<sup>8</sup> The number of lactic acid units incorporated into the PLA-*b*-PEG-*b*-PLA macromers was determined by <sup>1</sup>H NMR in which the area under the integral for the lactide resonances ( $\delta = 5.1$  ppm,  $\delta = 1.3$  ppm) was compared with the resonance for the PEG backbone (methylene protons,  $\delta = 4.2$ ). On average, 7 and 3 lactic acid repeat units were added to each side of the 4600 and 8000 MW PEGs, respectively. The macromers were purified by repeated precipitations in ethyl ether.

### Hydrogel preparation and characterization

The *homopolymer* hydrogels were formulated by dissolving the 4600-7 macromer in phosphate-buffered saline (Gibco) to a final concentration of 10% (w/w). The *copolymer* hydrogels were formulated using a 10% (w/w) macromer solution consisting of 50 mol % 4600-7 and 50 mol % 8000-3 in phosphate-buffered saline. Previously determined cyto-compatible photoinitiating conditions were used.<sup>12</sup> A small amount of concentrated photoinitiator, 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone (Ciba-Geigy), was added to the macromer solution to a final concentration of 0.05% (w/w). The solutions were filter-sterilized with a 0.2- $\mu$ m syringe filter. The macromer solution was photopo-

lymerized using a longwave ultraviolet lamp (UVP, model XX-20) at an intensity of  $\sim 4$  mW/cm<sup>2</sup> for 10 min. Homopolymer and copolymer hydrogel discs (11 mm in diameter and 2 mm in thickness) were degraded in chondrocyte medium (see below) at 37°C on an orbital shaker. During degradation, 2-3 discs were removed from the medium every 12-36 h and weighed to determine the equilibrium swollen mass. The discs were lyophilized to determine the dry, polymer mass. The equilibrium volumetric swelling ratio, *Q*, can readily be determined from the mass swelling ratio (equilibrium swollen mass/dry polymer mass).<sup>13</sup>

### Chondrocyte encapsulation and *in vitro* studies

Chondrocytes were isolated from the femoral-patellar groove of a young calf (Research 87, Marlboro, MA).<sup>14</sup> Isolated chondrocytes were combined with the sterile macromer/initiator solutions at a concentration of  $75 \times 10^6$  cells/mL and photoencapsulated under the cyto-compatible conditions described above. The resulting cell-hydrogel constructs (5 mm in diameter and 2 mm in thickness) were incubated at 37°C on an orbital shaker in a humid environment with 5% CO<sub>2</sub> in 12-well plates. The constructs were cultured for 6 weeks in chondrocytes medium [Dulbecco's modified Eagle medium (Gibco), without phenol red, supplemented with 10 mM HEPES, 0.04 mM L-proline, 50 mg/L L-ascorbic acid, 0.1M Eagle minimum essential medium non-essential amino acids (Gibco), 1% penicillin-streptomycin (Gibco), 0.5  $\mu$ g/mL fungizone (Gibco), and 10% fetal bovine serum (Gibco)], which was replaced biweekly. Two constructs were removed for histological analysis at 2 and 4 weeks. At 4 and 6 weeks, three gels were removed for biochemical analysis.

### *In vivo* implantation and harvest

Fifteen athymic female mice (nu/nu, 6 weeks old; Charles River laboratories) were anesthetized with isoflurane, and three subcutaneous pockets were created on the upper left and right and lower left or right sides of each mouse using aseptic techniques. Cell-hydrogel constructs were prepared as described above and immediately placed into a subcutaneous pocket. For the *in vivo* studies, three experimental conditions were used: 1. homopolymer hydrogels without chondrocytes (control); 2. homopolymer hydrogels with chondrocytes; and 3. copolymer hydrogels with chondrocytes. One cell polymer-construct at each experimental condition was placed into each mouse. Incisions were closed with staples, which were removed 1 week postoperation. Five mice were euthanized with CO<sub>2</sub> inhalation at 2, 4, and 6 weeks. Control gels were completely degraded by 4 weeks. Histology samples were taken from one mouse, and the remaining four mice (12 constructs) were used for biochemical analysis. All procedures performed were approved by the University of Colorado at Boulder Institutional Animal Care and Use Committee.

## Neotissue analysis

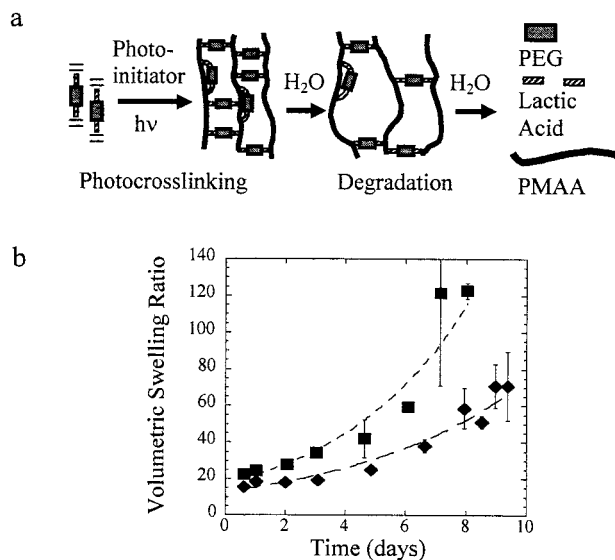
The constructs were weighed to determine their wet weight, lyophilized for 24 h to determine their dry weight, and then digested in a papain solution [125  $\mu\text{g}/\text{mL}$  papain type III (Worthington), 10 mM L-cysteine (Aldrich), 100 mM phosphate, and 10 mM ethylenediaminetetraacetic acid at pH 6.3] at 60°C for 15 h. Total glycosaminoglycan (GAG) content was determined using the dimethylmethylene blue dye method.<sup>15</sup> Total collagen content was determined using the hydroxyproline assay<sup>16</sup> in which hydroxyproline is assumed to comprise ~10% of collagen.<sup>17</sup> The GAG and total collagen contents were normalized by the wet weight. For the biochemical results, statistical analysis was performed using a Student's *t* test with a confidence level of 0.05. All values are reported as the mean  $\pm$  standard deviation.

Cell-hydrogel constructs were fixated overnight in 10% formalin, embedded in paraffin, and microtomed in 8- $\mu\text{m}$  sections following standard histological technique. The sections were stained with Safranin-O and fast green for negatively charged proteoglycans (GAGs), which stain red, and with the Masson's trichrome stain for collagen, which stains blue. Immunohistochemical analysis was performed on sections from the implants using a labeled streptavidin-biotin immunoenzymatic antigen detection system (UltraVision detection system; NeoMarkers, Inc.). The paraffin sections were digested with pepsin (1 mg/mL in 0.01N HCl) for 30 min at 37°C before applying the primary antibody, collagen type II mouse monoclonal antibody (NeoMarkers, Inc.).

## RESULTS AND DISCUSSION

In this study, two PEG MWs of 4600 and 8000 g/mol were investigated. Biodegradable PLA was grafted to the PEG molecules to obtain ~7 lactic acids per side on the 4600 MW PEG (4600-7) and ~3 lactic acids per side on the 8000 MW PEG (8000-3). The triblock copolymers, PLA-*b*-PEG-*b*-PLA, were end-capped with methacrylate groups to form crosslinkable macromers. Two degrading hydrogels were formulated via photopolymerization 1. from 100% 4600-7 macromer to form *homopolymer* gels with a PEG/PLA repeat unit ratio of 7, and 2. from 50 mol % 4600-7 and 50 mol % 8000-3 to form *copolymer* gels with an average PEG/PLA repeat unit ratio of 19. By copolymerizing macromers with different structures and chemistries in the presence of cells, the eroding gel properties can be manipulated to guide and enhance tissue formation. For example, fast-degrading crosslinks create "open" space for macroscopic tissue growth whereas slow-degrading crosslinks maintain scaffold integrity and allow for tissue development.

A typical schematic for the formation and degradation of these photocrosslinked PEG hydrogels is shown in Figure 1(a). These hydrogels are formed through a chain polymerization reaction in which photoinitiated radicals propagate through the carbon-



**Figure 1.** (a) The ideal network structure of hydrogels formed from PLA-*b*-PEG-*b*-PLA macromers, their degradation behavior, and degradation products after hydrolysis. During degradation, the network structure evolves as the crosslinks are broken. (b) The equilibrium water content (volumetric swelling ratio) as a function of degradation time where  $\blacklozenge$  and  $\blacksquare$  represent the homopolymer and copolymer gels, respectively. PMAA, poly(methacrylic acid).

carbon double bonds within the methacrylate endgroups on the macromer to form polymethacrylate kinetic chains. These kinetic chains are connected via PLA-*b*-PEG-*b*-PLA crosslinks to form a 3D network. The length of the crosslink can be varied by changing the MW of the macromer, and this change will influence the overall gel structure. During degradation in aqueous medium at physiological pH, the ester bonds within the PLA blocks of the crosslinker are hydrolytically cleaved. As the crosslinks are broken, the gel swells imbibing more water and the equilibrium water content (or equilibrium volumetric swelling ratio,  $Q$ ) increases.<sup>18</sup> When an ester bond is cleaved on each end of the crosslink, the crosslink is eroded from the hydrogel network and the gel loses polymer mass.

The PEG hydrogels were characterized in the absence of cells, and during degradation, the equilibrium volumetric swelling ratio for both gels increased exponentially with degradation time as shown in Figure 1(b). In the homopolymer gels,  $Q$  increased from 15 to 70 just before gel dissolution, whereas  $Q$  was initially higher at 22 in the copolymer gels and increased to 120. A  $Q$  of 15 corresponds to a gel that is >93% water, so these systems are highly swollen at all stages of degradation. Furthermore, the exponential increase in  $Q$  with time is evidence of pseudo-first-order hydrolysis kinetics of the PLA blocks in the network crosslinks,<sup>9</sup> which is attributed to the fact that the gels are predominately water. Overall, the copolymer gels had higher  $Q$ s, and therefore, exhibited a

**TABLE I**  
**Biochemical Composition of the Neotissue Formed in the Homopolymer (H) and Copolymer (C) Gels**

	<i>In Vitro</i>				<i>In Vivo</i>			
	4 Weeks		6 Weeks		4 Weeks		6 Weeks	
	H	C	H	C	H	C	H	C
Collagen	1.20 ± 0.24	1.26 ± 0.11	2.47 ± 0.22	1.46 ± 0.80	3.25 ± 0.44	3.39 ± 0.75	5.81 ± 0.66	6.06 ± 0.53
GAG	2.92 ± 0.45	4.04 ± 1.2	0.96 ± 0.04	1.57 ± 0.20	1.63 ± 0.05	1.95 ± 0.29	1.47 ± 0.32	1.37 ± 0.25
BCR	0.42 ± 0.07	0.33 ± 0.1	2.58 ± 0.13	0.92 ± 0.40	2.00 ± 0.31	1.78 ± 0.52	4.14 ± 1.16	4.58 ± 1.21

Collagen, percent total collagen content/wet weight; GAG, glycosaminoglycans content/wet weight; BCR, biochemical composition ratio (total collagen/GAG contents).

lower crosslinking density.<sup>19</sup> As a result of this decreased crosslinking density, the copolymer gels degraded slightly faster (8 days) than the homopolymer gels (10 days). It was not possible to fully characterize gel degradation *in vivo* because of the infiltration of host cells.

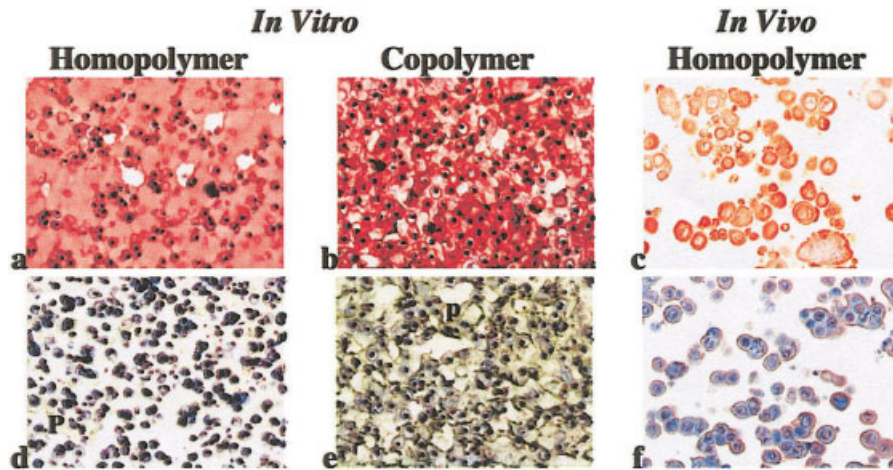
After characterizing the gels, isolated chondrocytes were photoencapsulated in these gels and cultured either *in vitro* or *in vivo* for up to 6 weeks. The resulting neocartilaginous tissue produced by the chondrocytes was measured by quantifying the main components of native cartilage, that is, GAGs (the main component of proteoglycans) and collagen molecules. The results are summarized in Table I. Overall, the total collagen content, normalized by construct wet weight, increased, whereas the GAG content decreased, with culture time and implantation time. The biochemical data are also represented as a ratio, termed the biochemical compositional ratio (BCR), of collagen to GAG. This ratio was used as a unique measure to demonstrate how changes in the local cell environment influence the compositional make up of the resulting neotissue. With different polymer gel compositions under both *in vitro* and *in vivo* conditions, the BCR increased significantly from 4 to 6 weeks, that is, there was a greater increase in collagen synthesis compared with GAG synthesis. *In vitro*, the differences in polymer gel composition did not affect the BCR at 4 weeks, in which the cartilaginous tissue was rich in GAGs, but by 6 weeks, the gels with a lower PEG/PLA ratio had a BCR that was 2.8-fold higher (a collagen-rich tissue). The gels *in vivo* guided the formation of a collagen-rich tissue with BCRs significantly greater than their *in vitro* counterparts.

In addition to bulk biochemical characterization of the neotissue, the spatial distribution of specific ECM molecules was assessed by staining tissue sections for GAGs and collagen. Representative micrographs from 2 and 4 weeks are shown in Figures 2 and 3, respectively. First, the chondrocytes appear healthy and located in a lacuna, characteristic of native cartilage. GAGs were distributed throughout the gel constructs *in vitro* with heavy staining within the lacunae in the homopolymer gels, whereas in the copolymer gels, the

degree of staining in the lacunae varied from heavy to light. Contrary to the *in vitro* results, the distribution of GAGs was localized in the pericellular regions *in vivo*. In both culture environments, collagen was localized pericellularly; however, the pericellular matrix was more elaborate *in vivo*. By 4 weeks *in vitro*, the distribution of ECM appeared similar in both hydrogels with GAGs and collagen located throughout the scaffold resembling that of native cartilage. *In vivo*, nodules of cartilaginous tissue rich in GAGs and collagen have formed which were surrounded by void spaces (i.e., acellular regions with no tissue formation). The presence of type II collagen shown in Figure 4 confirms the differentiation of encapsulated chondrocytes and the formation of cartilage-like tissue *in vivo*.

Interestingly, the degree of gel degradation can be qualitatively assessed by the Masson's trichrome stain, which happens to stain the PEG polymer brown. This artifact enables us to understand better the degradation behavior of the PEG hydrogels in the presence of cells. A significant amount of polymer was present in the copolymer gels compared with the homopolymer gels at 2 weeks [Fig. 2(d,e)] suggesting that the scaffold degradation is slower in the presence of cells, and furthermore, is slower in the copolymer gels. This observation is contrary to the gel characterization studies in the absence of cells. *In vivo*, no polymer staining was observed suggesting that the *in vivo* environment enhanced degradation.

Thus, this study demonstrates that the degradation behavior of these PEG hydrogels depends on the presence of cells and culture environment. Simply characterizing the gels in cell absentia may not be sufficient and accurate for understanding gel degradation in more complex environments. In general, a decrease in macromer MW results in a more highly crosslinked gel (increasing degradation time), whereas an increase in the PLA block increases the probability that an ester bond will be cleaved (decreasing degradation time). Overall, when cells and growing ECM were present, both gels degraded more slowly. However, the dominating gel property with respect to degradation appears to be the crosslinking density in the absence of



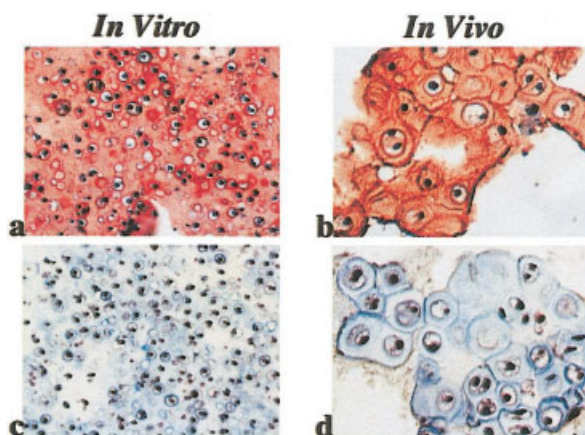
**Figure 2.** Histological analysis of chondrocytes photoencapsulated in homopolymer and copolymer hydrogels that were cultured either *in vitro* or *in vivo* for 2 weeks. Safranin-O was used to stain proteoglycans red (a–c) and Masson's trichrome was used to stain collagen blue (d–f). Hematoxylin was used to stain nuclei black. Histological results for copolymer gels cultured *in vivo* (data not shown) were similar to that observed for the homopolymer gels *in vivo*. The PEG polymer stains brown using the Masson's trichrome stain and P represents the polymer present in the cell-scaffolds. Original magnification, 200 $\times$ .

cells (i.e., the copolymer gels with a lower crosslinking density degraded first) and the PLA length in the presence of cells (i.e., the homopolymer gels with a longer PLA block degraded first).

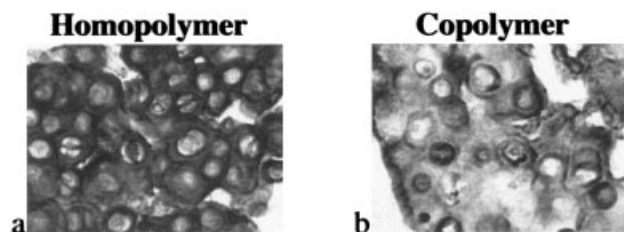
To better understand this phenomenon, the water content was examined in the cell-laden gels by ratioing the wet weight to the dry weight ( $q$ ). Quickly, it becomes obvious that the water content in the cell constructs ( $q < 20$ ) was considerably lower over the duration of degradation compared with the gels alone ( $14 \leq q \leq 115$ , where  $q$  is the mass swelling ratio). This difference in the local environment (i.e., lower water

content, cells, and ECM) surrounding the hydrolytically labile PLA blocks appears to significantly change the hydrolysis kinetics. Furthermore, the *in vivo* environment, which is known to accelerate cleavage of the ester bonds,<sup>20,21</sup> decreased the overall degradation time significantly. As a result of the fast *in vivo* gel degradation (i.e., <2 weeks), no differences in tissue composition were observed as a function of gel composition.

Although these hydrogels contain <7% polymer, small changes in the gel chemistry greatly affected the gel degradation time, the biochemical composition of the regenerated tissue, and the spatial distribution of ECM components. For example, when the PEG/PLA ratio was increased from 7 to 19, the concentration of PEG and PLA in the hydrogels changed only slightly by +0.9% and -0.9%, respectively. Gels, which exhibited a lower PEG and higher PLA concentration (i.e., degrading more quickly), guided the development of a collagen-rich neotissue whereas the neotissue developed in gels that degraded more slowly (i.e., a lower



**Figure 3.** Representative histological micrographs of chondrocytes encapsulated in the homopolymer gels and cultured *in vitro* and *in vivo* for 4 weeks. Safranin-O was used to stain proteoglycans red (a,b) and Masson's trichrome was used to stain collagen blue (c,d). Hematoxylin was used to stain nuclei black. Similar histological results were found *in vivo* and *in vitro* for the copolymer gels (data not shown). Original magnifications, 200 $\times$  (a,c) and 400 $\times$  (b,d).



**Figure 4.** Immunohistochemical staining for type II collagen (shown in grayscale) after 4 weeks *in vivo* for the homopolymer (a) and copolymer (b) gels. Original magnification, 400 $\times$ .

concentration of PLA) was comprised of similar amounts of collagen and GAG. The *in vivo* environment containing many hormones, growth factors, and other signaling factors significantly enhanced collagen synthesis, which may be attributed, in part, to the increased gel degradation, and in part, to its complex environment.

During tissue development, proteoglycans and collagen molecules are synthesized intracellularly and excreted into the pericellular regions where they are further developed before diffusing into the extracellular regions.<sup>22,23</sup> Diffusivity within these crosslinked gels is closely related to the network structure and, with increased crosslinking density, the diffusivity decreases.<sup>24</sup> It has been shown that the release of high MW solutes can be controlled through the eroding network structure.<sup>25</sup> Thus, the gel crosslinking density may influence the release of the ECM molecules into the extracellular regions. For instance, collagen fibers are significantly larger than proteoglycans, and therefore, we would expect that the proteoglycans would be able to diffuse more readily than the collagen molecules. In fact, we observe this at early culture time (2 weeks) *in vitro* [Fig. 2(a–d)]. If the distribution of GAGs *in vitro* as a function of gel composition is examined more closely, differences in GAG distribution are evident, particularly in the lacunae. Proteoglycans of many different sizes are synthesized by the cells and secreted into the pericellular regions.<sup>22</sup> It is likely that larger proteoglycans are unable to diffuse into the extracellular regions of the gel because of the gel's crosslinked nature. This localization of proteoglycans and collagen pericellularly may well influence cell expression and explain the differences in the overall tissue composition. The lower crosslinking density in the copolymer gels may allow for increased diffusion of larger proteoglycans. However, with increased degradation, a critical network structure was reached that promoted both proteoglycan and collagen diffusion throughout the gels [Fig. 3(a,c)].

The evolution of tissue development from a spatial perspective was also considerably different *in vivo* compared with *in vitro*. First, both proteoglycans and collagen molecules were localized in the pericellular regions *in vivo* even though no polymer was present at 2 weeks. The *in vivo* environment may promote the production of larger proteoglycans and/or the aggregation of proteoglycans into aggrecan, and furthermore, promote increased interactions between collagen and aggrecan molecules,<sup>23</sup> which may delay the release of these ECM molecules. It is possible that a delayed response may occur from the time when the network reaches a critical mesh size for ECM diffusion to the time when the chondrocytes sense and respond to the change in the network. By 4 weeks, an ECM had formed.

By altering the hydrogel chemistry only slightly, significant changes in the type of cartilaginous tissue

produced were found to vary from GAG-rich to collagen-rich tissues. This feature can potentially be a very powerful tool for developing an engineered tissue in which the composition and organization are key to restoring function. With further understanding of how the gel chemistry influences tissue formation both *in vitro* and *in vivo*, we can begin to develop more sophisticated cell-scaffolds that guide the formation of an organized and functional tissue.

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## References

1. Langer R, Vacanti JP. Tissue engineering. *Science* 1993;260:920–926.
2. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994;331:879–895.
3. Grande DA, Pitman MI, Peterson L, Menche D, Klein M. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. *J Orthop Res* 1989;7:208–218.
4. Elisseeff J, Anseth K, Sims D, McIntosh W, Randolph M, Langer R. Transdermal photopolymerization for minimally invasive implantation. *Proc Natl Acad Sci USA* 1999;96:3104–3107.
5. Bryant SJ, Anseth KS. Hydrogel properties influence ECM production by chondrocyte photoencapsulated in poly(ethylene glycol) hydrogels. *J Biomed Mater Res* 2001;59:63–72.
6. Elisseeff J, Anseth K, Sims D, McIntosh W, Randolph M, Yaremchuk M, Langer R. Transdermal photopolymerization of poly(ethylene oxide)-based injectable hydrogels for tissue-engineered cartilage. *Plast Reconstr Surg* 1999;104:1014–1022.
7. Bryant SJ, Anseth KS. The effects of scaffold thickness on tissue engineered cartilage in photocrosslinked poly(ethylene oxide) hydrogels. *Biomaterials* 2001;22:619–626.
8. Sawhney AS, Pathak CP, Hubbell JA. Bioerodible hydrogels based on photopolymerized poly(ethylene glycol)-*co*-poly(alpha-hydroxy acid) diacrylate macromers. *Macromolecules* 1993;26:581–587.
9. Metters AT, Anseth KS, Bowman CN. Fundamental studies of a novel, biodegradable PEG-*b*-PLA hydrogel. *Polymer* 2000;41:3993–4004.
10. West JL, Hubbell JA. Polymeric biomaterials with degradation sites for proteases involved in cell migration. *Macromolecules* 1999;32:241–244.
11. Metters AT, Anseth KS, Bowman CN. A statistical kinetic model for the bulk degradation of PLA-*b*-PEG-*b*-PLA hydrogel networks: Incorporating network non-idealities. *J Phys Chem B* 2001;105:8069–8076.
12. Bryant SJ, Nuttelmen CR, Anseth KS. Cytocompatibility of ultraviolet and visible light photoinitiating systems on cultured NIH/3T3 fibroblasts *in vitro*. *J Biomater Sci Polym Ed* 2000;11:439–457.
13. Peppas NA. *Hydrogels in medicine and pharmacy*. I. Boca Raton, FL: CRC Press; 1986.
14. Shortkroff S, Spector M. Isolation and *in vitro* proliferation of chondrocytes, tenocytes, and ligament cells. Morgan JR, Yarmush ML, editors. *Tissue engineering methods and protocols*. Totowa, NJ: Humana Press Inc.; 1999. p 195–204.
15. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulfated glycosaminoglycans by use of dimethylmethylene blue. *Biochim Biophys Acta* 1986;883:173–177.

16. Woessner JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys* 1961;93:440–447.
17. Hollander AP, Heathfield TF, Webber C, Iwata Y, Bourne R, Rorabeck C, Poole AP. Increased damage to type-II collagen in osteoarthritic articular-cartilage detected by a new immunoassay. *J Clin Invest* 1994;93:1722–1732.
18. Metters AT, Bowman CN, Anseth KS. A statistical kinetic model for the bulk degradation of PLA-*b*-PEG-*b*-PLA hydrogel networks. *J Phys Chem B* 2000;104:7043–7049.
19. Flory PJ, Rehner R Jr. Statistical mechanics of crosslinked polymer networks. I. Rubberlike elasticity. *J Chem Phys* 1943;11:521.
20. Catiker E, Gumusderelioglu M, Guner A. Degradation of PLA, PLGA homo- and copolymers in the presence of serum albumin: a spectroscopic investigation. *Polym Int* 2000;49:728–734.
21. Kurono Y, Maki T, Yotsuyanagi T, Ikeda K. Esterase-like activity of human-serum albumin: structure-activity relationships for the reactions with phenyl acetates and *p*-nitrophenyl esters. *Chem Pharm Bull* 1979;27:2781–2786.
22. Buckwalter JA. Cartilage. In: Dulbecco R, editor. *Encyclopedia of human biology*. San Diego, CA: Academic Press; 1997. p 431–445.
23. Poole CA. Articular cartilage chondrons: Form, function, and failure. *J Anat* 1997;191:1–13.
24. Canal T, Peppas NA. Correlation between mesh size and equilibrium degree of swelling of polymeric networks. *J Biomed Mater Res* 1989;23:1183–1193.
25. Lu SX, Anseth KS. Release behavior of high molecular weight solutes from poly(ethylene glycol)-based degradable networks. *Macromolecules* 2000;33:2509–2515.