

Encapsulating Chondrocytes in Degrading PEG Hydrogels With High Modulus: Engineering Gel Structural Changes to Facilitate Cartilaginous Tissue Production

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Abstract: A major challenge when designing cell scaffolds for chondrocyte delivery in vivo is creating scaffolds with sufficient mechanical properties to restore initial function while simultaneously controlling temporal changes in the gel structure to facilitate tissue formation. To address this design challenge, degradable photocrosslinked hydrogels based on poly(ethylene glycol) were investigated. To alter the gel's initial mechanical properties, hydrogels were fabricated by varying the initial macromer concentration from 10% to 15% to 20%. A twofold increase in macromer concentration resulted in an eightfold increase in the initial compressive modulus from 60 to 500 kPa. Gel degradation was tailored by incorporating fast-degrading crosslinks that enable maximal extracellular matrix (ECM) diffusion with time and a minimal number of nondegrading (or slowly degrading) crosslinks to maintain scaffold integrity and prevent complete gel erosion during tissue formation. Chondrocytes encapsulated in these gels produced cartilaginous tissue rich in glycosaminoglycans and collagen as seen biochemically and histologically. Interestingly, mass loss appeared to more closely match tissue secretion in gels fabricated from a 15% macromer concentration. However, the spatial ECM distribution was grossly similar in all three gels. By tailoring gel degradation and controlling network evolution during degradation, gels with optimal properties can be fabricated to support initially physiologic compressive loads while simultaneously supporting the formation of a neotissue. © 2004 Wiley Periodicals, Inc.

Keywords: chondrocytes; PEG-hydrogels; cartilage production

INTRODUCTION

Over the last decade, several chemistries and scaffold concepts have been explored to tissue engineer cartilage. A significant amount of research has investigated porous scaffolds prepared from poly(glycolic acid), poly(L-lactic acid),

and poly(lactic-co-glycolic acid) (Athanasίου et al., 1998; Freed et al., 1993; Gugala and Gogolewski, 2000). Although promising for in vitro tissue development, their application to in vivo treatments that deliver scaffolds and cells to repair cartilage defects is problematic with substandard mechanical properties. Conversely, natural hydrogels, such as alginate and fibrin glue, provide an easy means by which to transplant cells in situ and form three-dimensional (3D) cell-laden matrices. However, natural materials, in general, are inherently variable in composition, and their mechanical properties and degradation rates are limited and difficult to control.

As an alternative to these more common cell carriers, synthetic hydrogels can be used as 3D scaffolds where scaffold structure and degradation rates and profiles can be intimately controlled. The network structure of crosslinked hydrogels formed from multifunctional macromers has been studied extensively, and the initial network structure is routinely controlled by variations in the processing conditions and/or macromer structure and functionality (Merrill et al., 1993; West and Hubbell, 1995). Hydrogel erosion (or mass loss) further influences the gel structure during degradation (Lu and Anseth, 1999; West and Hubbell, 1995) and, correspondingly, influences many of the gel properties, including the gel's mechanics and solute diffusion. More recently, the degradation behavior and mass loss profiles of crosslinked gels prepared from multivinyl macromers were studied in detail, and a statistical kinetic model was developed to describe these systems (Martens et al., 2001; Metters et al., 2000b).

Multivinyl macromers are useful for many biorelated applications, because, when exposed to light in the presence of a photoinitiator, crosslinked networks can be formed in a matter of minutes (or even seconds) with high degrees of conversion (Martens and Anseth, 2000). Many investigators have focused on using these systems for drug delivery, and this work routinely relates gel structure and degradation profiles to the release of various solutes. Furthermore, the

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crosslinking density of photocrosslinked poly(ethylene glycol) (PEG) hydrogels was found to immunoprotect encapsulated islet cells (Cruise et al., 1998). However, little attention has focused on tailoring the broad array of gel properties, especially as a function of degradation, for tissue evolution.

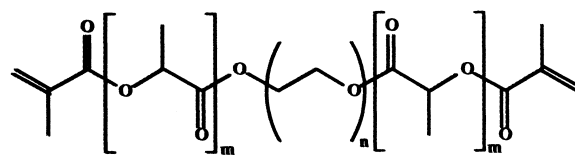
For example, when designing an in situ-forming gel carrier for chondrocytes and cartilage tissue engineering, many criteria must be satisfied. From a materials perspective, the scaffold properties should closely match those of the surrounding cartilage so that the tissue's function may be restored immediately. However, to generate new tissue, the microstructure of the scaffold must promote diffusion of both nutrients and extracellular matrix (ECM) molecules, while the degradation of the gel must be tailored to match macroscopic tissue formation. For tissues where their function requires good mechanical properties, as in the case of articular cartilage, these scaffold design requirements are often contradictory.

Recent studies by the present investigators have shown the first set of studies demonstrating the influence of PEG gel properties (Bryant and Anseth, 2001) and the importance of degradation (Bryant and Anseth, 2003) on cartilage tissue development. In this study, our aim was to utilize our understanding of the gel structure and the evolution of the gel structure during degradation to develop sophisticated cell carriers, which can simultaneously support function in vivo and guide tissue development. To accomplish this goal, PEG gels were fabricated where the initial crosslinking density was varied by reacting the macromers at different concentrations to optimize the initial gel properties. The degradation profiles of the resulting gels were fine-tuned by copolymerizing macromers that degrade on different time-scales to capture essential features of the gel erosion profile that are important for tissue regeneration.

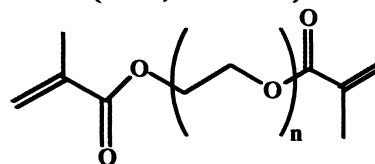
MATERIALS AND METHODS

Macromer

Poly(ethylene glycol) (PEG; MW 3000) was purchased from Fluka and used without further purification. Poly(ethylene glycol) dimethacrylate and poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) (PLA-*b*-PEG-*b*-PLA), endcapped with methacrylate groups, were synthesized as described by Sawhney et al. (1993) to form the nondegrading and degrading macromers, respectively. Briefly, PLA-*b*-PEG-*b*-PLA was synthesized by the ring opening polymerization of D,L-lactide by stannous octoate. An excess of methacryloylchloride was then reacted with the PLA-*b*-PEG-*b*-PLA and PEG molecules in the presence of triethylamine. The solution was precipitated in ethyl ether three times to recover and purify the macromers. The resulting PLA-*b*-PEG-*b*-PLA macromers contained, on average, seven lactic acid repeat units flanking each side of the PEG with ~100% methacrylation (for both macromers) as determined by ¹H NMR. The macromer chemical structures are shown in Figure 1.



PLA-*b*-PEG-*b*-PLA Dimethacrylate
(*n*=68, MW 3000)
(*m*=7, MW 490)



PEG Dimethacrylate
(*n*=68, MW 3000)

Figure 1. Chemical structures of macromers.

Hydrogel Preparation

The PEG macromers were dissolved in phosphate-buffered saline (PBS; Gibco, pH 7.4) to a final concentration of 10% (w/w) or distilled water to final concentrations of 15% and 20% (w/w) for all experiments. The ultraviolet (UV) photoinitiator, 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone (I2959, Ciba Geigy), shown to be cytocompatible under the given conditions (Bryant et al., 2000), was added to the macromer solution to produce final initiator concentrations of 0.05%, 0.022%, and 0.0125% (w/w) for the 10%, 15%, and 20% gels, respectively. The macromer/initiator solution was irradiated with 365-nm light at ~4 mW/cm² for 10 min. In the cell encapsulation experiments, the macromer/initiator solution was filter-sterilized using a 0.2- μ m filter, and isolated chondrocytes were suspended in the solution before irradiation.

Gel Degradation

Hydrogels of varying crosslinking densities (i.e., polymerized from macromer solutions at different concentrations) and erosion profiles (i.e., polymers with different PEGDM: PEG-LA-DM ratios) were photopolymerized and placed in a 2.5 M NaOH solution on a shaker for 24 h. The basic solution accelerates the rate of hydrolysis of the ester linkage within the PLA block, but no degradation of the ester in the PEGDM macromer was observed on this time scale.

Hydrogel Mechanics

The compressive modulus of elasticity for the PEG hydrogels was measured using a dynamic mechanical analyzer (DMA-7, Perkin Elmer) in the absence of cells. The gels were measured in unconfined compression at a constant rate of 40 to 100 mN/min. The change in compression rate did

not affect the compressive modulus values indicating the moduli were in the elastic region of deformation. Hydrogels with a diameter of 5 mm and height of 1 mm were used to obtain the initial compressive modulus before degradation. The mechanics were measured after polymerization and after 1-h immersion in PBS buffer. A sample size of five was used.

Determining Mesh Size

Gels were fabricated from a 10% (w/w) solution of the degradable macromer (PLA-*b*-PEG-*b*-PLA dimethacrylate) and degraded in PBS solution at 37°C. Gels were removed every 1 to 2 days and their equilibrium swollen mass was measured (m_s), then lyophilized to determine their dry polymer mass (m_d). From the equilibrium swelling ratio (m_s/m_d), the mesh size was calculated using a modified Flory–Rehner equation neglecting chain ends (Bryant and Anseth, 2001; Flory, 1953; Peppas, 1986).

Chondrocyte Encapsulation

Chondrocytes were harvested from the patellar–femoral groove of calf legs, as described by Freed and Vunjak-Novakovic (1995). The cells were combined with aforementioned sterile macromer/initiator solutions at a concentration of 75 million cells/mL and polymerized under the cytocompatible photoinitiating conditions described earlier. Gels were polymerized as disks, 5 mm in diameter and ~2 mm in thickness. The constructs were cultured in nontreated (24-well) tissue culture plates on an orbital shaker using Dulbecco’s modified Eagle medium (Gibco) with 1% penicillin–streptomycin (Gibco), 0.5 mg/mL Fungizone (Gibco), 0.01 M MEM nonessential amino acids (Gibco), 10 mM HEPES, 0.04 mM 1-proline and 10% fetal bovine serum. The gels were incubated at 37°C and 5% CO₂, and the medium was replaced every 2 or 3 days.

Histology

Two gel constructs were removed at 2 and 8 weeks, fixed in formalin, embedded in paraffin, and analyzed via standard histological techniques. Cross-sections of 8- μ m thickness were stained with Safranin-O, which stains proteoglycans red, or with Masson trichrome stain, which stains collagen blue. In both stains, hematoxylin was used to stain the nuclei black.

Biochemical Assay

Three gel constructs were removed at 2, 4, and 6 weeks, lyophilized for 24 h, and weighed to determine the construct dry weight. The dried constructs were crushed and digested in a papain solution (125 mg/mL papain type III [Worthington], and 10 mM L-cysteine [Aldrich] in PBE buffer [10 mM phosphate, 10 mM ethylene-diamine tetraacetic acid, pH 6.5]) for 16 h at 60°C on an orbital shaker. Total

glycosaminoglycan (GAG) content was assayed with dimethylmethylene blue dye (Farndale et al., 1986). Total collagen content was assayed by measuring the hydroxyproline content (Woessner, 1961) and assumed to be 10% of the total collagen content (Hollander et al., 1994).

Statistical Analysis

Statistical analysis was performed on the biochemical data where time and gel composition were the two factors studied, using two-factor effects and analysis of variance.

RESULTS AND DISCUSSION

To guide cartilage tissue regeneration *in vivo*, cell scaffolds must be designed to function in lieu of the tissue initially, but must simultaneously afford space that increases with time for tissue deposition. We aimed to accomplish both design requirements by carefully engineering the properties and chemistry of crosslinked PEG hydrogels. In what follows the structural aspects of these PEG networks, especially as a function of degradation, are described with an emphasis on developing gels for tissue engineering scaffolds. This discussion is followed by a detailed description of ways to manipulate the gels to support tissue evolution. Finally, chondrocytes are encapsulated in gels with a range of initial properties and degradation profiles, and the resulting cartilaginous tissue is examined.

Fundamentals of Crosslinked Hydrogels

Formation and Structure

PEG hydrogels were formed via a photoinitiated radical-chain polymerization of divinyl macromers. Specifically, radicals propagate through the carbon–carbon double bonds that reside in the methacrylate endgroups on the macromolecular monomers (Fig. 1) to form polymethacrylate kinetic chains that are covalently linked by PEG crosslinks (Fig. 2). The network crosslinking density controls many of the hydrogel properties, including diffusion coefficients, compressive modulus, and degradation rates (Martens et al.,

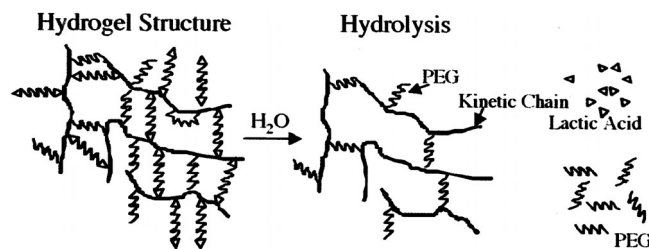


Figure 2. Network structure of degrading PEG hydrogels comprised of PEG and PLA-*b*-PEG-*b*-PLA crosslinks. As lactides are hydrolyzed, many of the crosslinks are released, increasing the average mesh size of the gel. However, the crosslinks without lactides do not degrade on the time scale of the experiment.

2001; Mason et al., 2001; Metters et al., 2000a). For example, the average distance between the crosslinks or the mesh size, ξ , controls diffusion of molecules through the gel (Lu and Anseth, 2000). In general, an increase in gel crosslinking density results in gels with a higher compressive modulus, a smaller mesh size, and longer degradation times (Metters et al., 2000a). Thus, to fabricate hydrogels that enable functional restoration of damaged tissues, the initial gel crosslinking density must be optimized. However, to facilitate new tissue growth, degradation and the resulting temporal changes in the gel structure must also be optimized.

Degradation

Degradable hydrogels were formed from triblock copolymer macromers of PLA-*b*-PEG-*b*-PLA (Fig. 1). The degradation mechanism occurs via hydrolysis of the ester bonds that reside in the PLA blocks. A typical plot of mass loss as a function of degradation time for these hydrogels is shown in Figure 3A (Martens et al., 2003; Metters et al., 2000a). When PLA ester groups on both ends of a PEG linker are cleaved, the PEG, lactides attached to the PEG, and unattached monomeric lactic acids are released from the network and diffuse out of the gel. This slow and relatively steady rate of erosion is observed for ~ 10 days in this example, but the gel erosion can be fine-tuned to last from days to months, depending on the gel chemistry and structure, to match the rate of tissue secretion (Sawhney et al., 1993). When fewer than two crosslinks per kinetic chain remain on average, the system transitions from an insoluble gel to a highly branched, soluble polymer. At this point, a sharp increase in mass loss is observed as the gel solubilizes. This process is referred to as *reverse gelation* and, from a tissue engineering perspective, the time to reach reverse gelation should couple strongly with the time required for the encapsulated cells to produce a “functional” tissue.

By controlling the gel’s structure, the point at which reverse gelation occurs can be controlled. For example, the critical fraction of hydrolyzed PLA blocks that leads to gel dissolution is dependent only on the number of crosslinks per kinetic chain, N , and can be calculated by (Metters et al., 2000b):

$$P_c = 1 - \left[\frac{2}{N} \right]^{1/2} \quad (1)$$

where P_c is the critical degradation conversion of the PLA blocks at which reverse gelation occurs. N is related to the length of the kinetic chain, and this parameter can be controlled easily through the photoinitiation conditions (e.g., light intensity and/or photoinitiator concentration). As the kinetic chain length increases, the critical degradation conversion increases, as shown in Figure 3B, meaning a larger fraction of the network crosslinks must be cleaved before the gel reaches its reverse gelation point. As N becomes extremely large, the critical degradation conversion asymptotically approaches that of 1. For networks formed from the polymerization of divinyl macromers, the length of the kinetic chains is a major structural component of the crosslinked network (Fig. 2).

These gel structural components—that is, the PEG crosslinks and the kinetic chains—create a mesh of “open” space that controls ECM diffusion through the gels. Figure 2 illustrates schematically that when crosslinks are cleaved from the network, the gel “opens up” and the mesh size increases (thus increasing the diffusion of ECM molecules through the gel). For highly swollen gels, the mesh size (ξ) is directly related to the degradation time by (Mason et al., 2001):

$$\xi \sim \rho_{xl}^{-7/10} \sim e^{7/5} \quad (2)$$

where ρ_{xl} is the network crosslinking density. In a bulk degrading hydrogel, ρ_{xl} temporally changes as a function

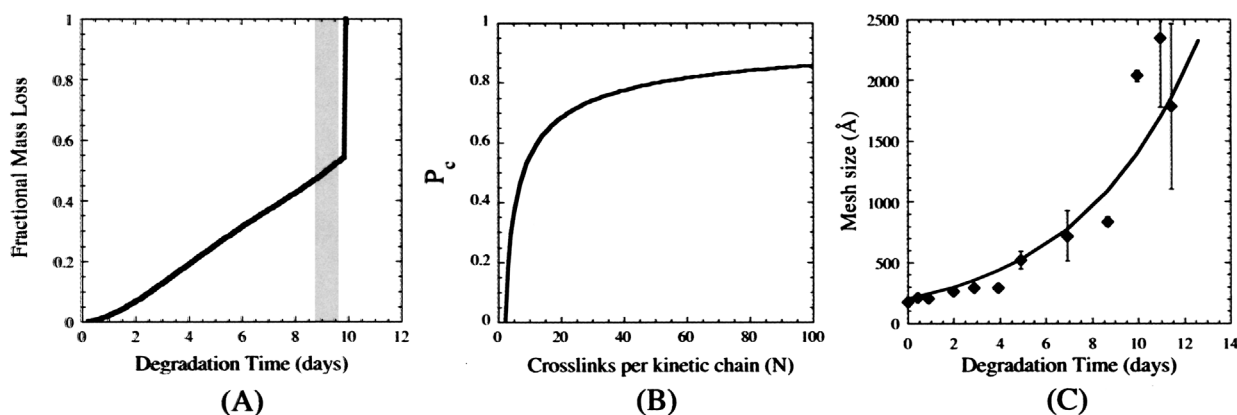


Figure 3. Typical degradation behavior for crosslinked PEG hydrogels. (A) Erosion (or mass loss) profiles for crosslinked networks. The fractional mass loss versus degradation time is modeled from a gel formulated from the 10% PLA-*b*-PEG-*b*-PLA macromer, where $N = 70$. The shaded area represents the region just before the gel solubilizes and where the mesh size is maximized. (B) Critical degradation conversion at which reverse gelation occurs (P_c) versus the number of crosslinks per kinetic chain (N). (C) Evolution of mesh size with degradation time for a gel prepared from the 10% PLA-*b*-PEG-*b*-PLA macromer (solid line represents an exponential fit for the data).

of the hydrolysis kinetic constant (k') and degradation time (t). Figure 3C plots the calculated change in mesh size with degradation time for a gel fabricated from a 10% solution of PLA-*b*-PEG-*b*-PLA. During degradation, the mesh size increases by an order of magnitude. By tailoring the initial network structure and chemistry of the degradable linkers in the crosslinks, the evolution of the gel's structure can be controlled to match tissue secretion.

Tailoring Macroscopic Gel Properties

Degradation

To design a cell carrier for tissue engineering, it is important that the cell carrier degrade at a rate to support facile ECM diffusion, but not completely erode until “enough” tissue has formed and organized into a functional tissue. We hypothesize that if a 3D support structure is maintained during tissue development, the composite tissue–polymer mechanics will better support the physical loads *in vivo*. Therefore, one goal of this study was to capture and prolong the gel properties just before reverse gelation occurred (shaded region in Fig. 3A). At this point, the gel retains a 3D structure with a *minimum* crosslinking density and a *maximum* mesh size. In this way, a 3D environment is maintained to preserve scaffold integrity during tissue development, whereas a large mesh size is sustained to allow ECM diffusion.

To determine the shaded region in Figure 3A, hydrogels were formulated by copolymerizing nondegrading (i.e., do not degrade on the time scale of the experiment) PEG macromers with degrading PLA-*b*-PEG-*b*-PLA macromers (i.e., degrade in 10 to 11 days for a gel prepared from a 10% [w/w] macromer solution [Martens et al., 2003]). By varying the ratio of PEG:PLA-*b*-PEG-*b*-PLA, the goal was to determine the minimum number of nondegrading crosslinks necessary to prevent gel dissolution as a function of a single processing parameter, the macromer concentration. A range of PEG:PLA-*b*-PEG-*b*-PLA ratios for each macromer concentration was investigated and the final results are summarized in Table I. Overall, the concentration of nondegradable macromer necessary to maintain a 3D

Table I. Macromer concentrations and ratios of non-degrading (PEG) to degrading (PLA-*b*-PEG-*b*-PLA) macromers used to form gels that maintained a 3D network after complete hydrolysis of the PLA-*b*-PEG-*b*-PLA crosslinks.

Macromer	PEG	PLA- <i>b</i> -PEG- <i>b</i> -PLA	Theoretical PEG ^a
10%	20%	80%	2.2%
15%	10%	90%	2.2%
20%	2%	98%	2.2%

^aBased on the theoretical model fit for a gel formulated from a 10% PLA-*b*-PEG-*b*-PLA macromer solution with 70 crosslinks per kinetic chain. The theoretical percent of nondegrading macromer required is based on the fact that 2 crosslinks are needed per kinetic chain to maintain a 3D insoluble network [see Eq. (1)].

network decreased from 20% to 2% (w/w), with an increase in macromer concentration from 10% to 20% (w/w). To better understand these differences, a previously developed model was used to determine that $N = 70$ was required to fit the mass loss data (Martens et al., 2001) for a 10% gel. Based on this N , the theoretical minimum amount of PEG required to maintain a gel with 2 crosslinks per kinetic chain was determined to be 2.2%. This theoretical value was significantly lower than the experimentally determined value for the gels with low crosslinking density (i.e., low macromer concentration). This deviation is likely due to the formation of cycles as a result of the high solvent concentration and the decrease in the overall crosslinking efficiency (Elliott et al., 2001). It is also possible that the basic degrading medium may hydrolyze a small number of the nondegrading crosslinks, which would be more pronounced in the 10% gels. Gels with a higher crosslinking density, which typically have fewer cycles (due to the decrease in solvent concentration) and longer kinetic chains (Metters et al., 2000b), will require a smaller amount of nondegrading crosslinks to maintain a 3D network.

Mechanics

In addition to optimizing the degradation profiles, the initial gel properties must also be optimized, particularly when designing an *in situ* forming scaffold that aims to restore function to the patient. To accomplish this goal, the scaffold properties should be similar to the surrounding tissue. We are particularly interested in articular cartilage, which has an equilibrium modulus of elasticity of 500 to 1000 kPa (Armstrong and Mow, 1982). One way to match this high compressive modulus of cartilage in our PEG hydrogels is to increase the macromer concentration before polymerization. An increase in macromer concentration generates gels with a higher crosslinking density. To assess the ability of these gels (i.e., described in Table I) to serve as a functional scaffold in cartilage tissue engineering, the compressive modulus was measured initially to degradation

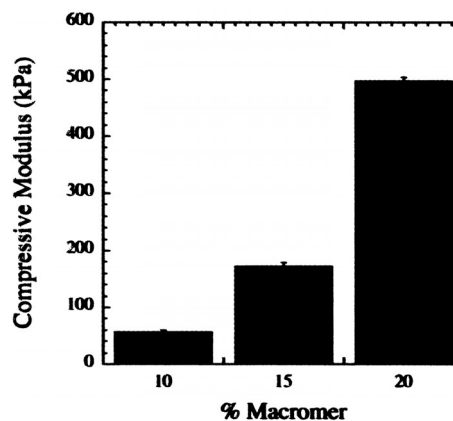


Figure 4. Initial compressive modulus of PEG hydrogels as a function of macromer concentration. The PEG:PLA-*b*-PEG-*b*-PLA ratios are given in Table I.

and in the absence of chondrocytes. The results are shown in Figure 4. A dramatic and statistically significant increase in compressive modulus can be seen as the macromer concentration was increased. A twofold increase in macromer concentration resulted in an eightfold increase in the gel compressive modulus. Furthermore, these results suggest that gels formulated with a macromer concentration of at least 20% (w/w) are sufficient to match the compressive modulus of native articular cartilage.

Chondrocyte Encapsulation and Cartilaginous Tissue Growth

To understand the influence of gel stiffness and, subsequently, a slower degradation (with increased number of

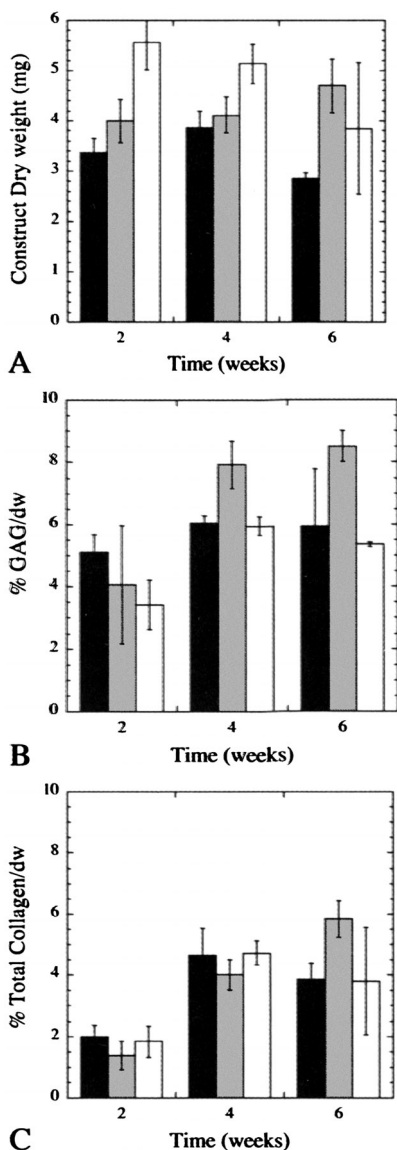


Figure 5. Biochemical content of the neocartilaginous tissue formed in gels with initial macromer concentrations of 10% (■), 15% (▣), and 20% (□). (A) Construct dry weight. (B) Glycosaminoglycan (GAG) content. (C) Total collagen content as a function of time.

Table II. Statistical analysis of biochemical data.

Factor	Measure	SS	MS	VR	P-value
Gel composition	Construct weight	10.09	5.04	15.43	<0.005
	GAG	16.87	8.43	8.47	<0.005
	Total collagen	0.43	0.22	0.35	>0.05
Time	Construct weight	1.74	0.87	2.66	>0.05
	GAG	35.05	17.52	17.60	<0.005
	Total collagen	45.17	22.58	36.95	<0.005
Interaction	Construct weight	5.37	1.34	4.11	<0.05
	GAG	12.03	3.01	3.02	<0.05
	Total collagen	9.16	2.29	3.74	<0.05

SS, sum of squares; MS, mean of squares; VR variance ratio.

crosslinks) on ECM formation, chondrocytes were photo-encapsulated in these PEG hydrogels with the compositions listed in Table I. The biochemical composition of the cartilaginous tissue is summarized in the graphs in Figure 5, and the statistical analysis given in Table II. The dry weights of the PEG constructs (Fig. 5A) were comprised of both the degrading gel and growing ECM. During initial culture times, the mass of the polymer dominated, but with increased culture time the growing ECM began to dominate. At 2 weeks, the dry mass increased accordingly with higher polymer content (Fig. 5A). Interestingly, over the culture time, the dry weights of the 15% gels increased, whereas the

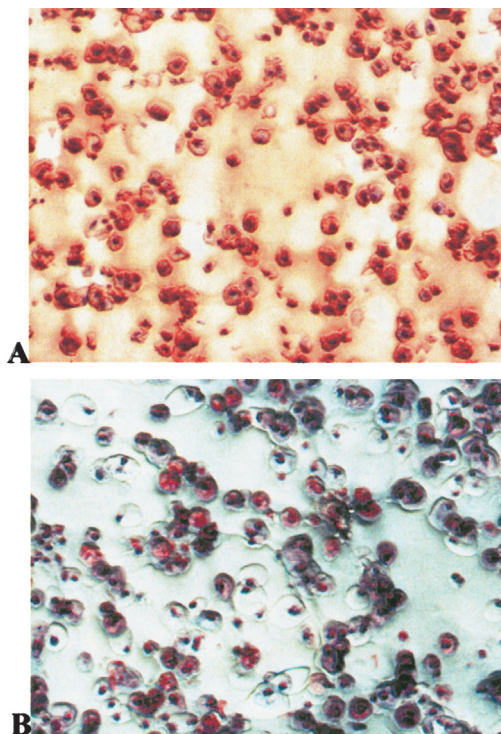


Figure 6. Representative histological micrographs of ECM distribution after 2 weeks in vitro. These sections are from the 15% gel and (A) shows Safranin-O staining proteoglycans red and (B) shows Masson trichrome staining collagen blue. Original magnification $\times 200$. Note that the light blue staining in (B) is PEG, not collagen; heavy blue staining for collagen is observed only in lacunae.

dry weights of the 10% and 20% gels decreased. This observation suggests that the degradation rate of the 15% gels may be more closely linked to ECM secretion, yielding a statistically significant interaction between time and gel composition for the construct dry weights as indicated by $P < 0.05$ in the interaction column of Table II.

The GAG and total collagen contents are shown in Figure 5B and C, respectively, as a function of time in culture and gel composition. The GAG and total collagen content increased from 2 to 4 weeks for all gels. From 4 to 6 weeks, the results varied and depended on composition, resulting in a significant interaction. In the 15% gels, there was a statistically significant increase in total collagen content from 4 to 6 weeks. In addition, the GAG content was highest in the 15% gels at 4 and 6 weeks compared with the other gel compositions. This result further suggests that scaffold environment and degradation behavior of the 15% gels provides an environment that promotes the continued secretion of ECM molecules.

In addition to quantifying the biochemical content of ECM in the constructs, it is also important to understand the spatial distribution of ECM molecules. Representative histological micrographs illustrating the distribution of

proteoglycans and collagen after 2 weeks of culture are shown in Figure 6. Each gel composition shows a similar ECM distribution. The embedded cells had the rounded chondrocyte phenotype and were seen in isolated pockets (or lacunae). Figure 6A shows that, even at this early stage, GAGs diffused into the extracellular regions of the hydrogel. Interestingly, heavy staining for GAGs was present within the lacunae, suggesting that larger proteoglycans were unable to diffuse into the gel. However, collagen molecules were restricted to the pericellular regions, and no diffusion was observed. These data suggest that, by 2 weeks, the networks have not degraded sufficiently to allow diffusion of large proteoglycans and collagen molecules.

Of greater interest is the ECM diffusion after the gels have had time to degrade and “open up” with increased mesh size. Figure 7 illustrates the GAG and collagen distribution after 8 weeks in vitro. In all gels, the GAGs were more uniformly distributed by 8 weeks compared with 2 weeks. Furthermore, little or no staining for GAGs was observed within the lacunae, appearing more like native cartilage. However, the larger ECM components, such as collagen, had not diffused to the same extent as the proteoglycans. In the 10% gels, collagen had begun to

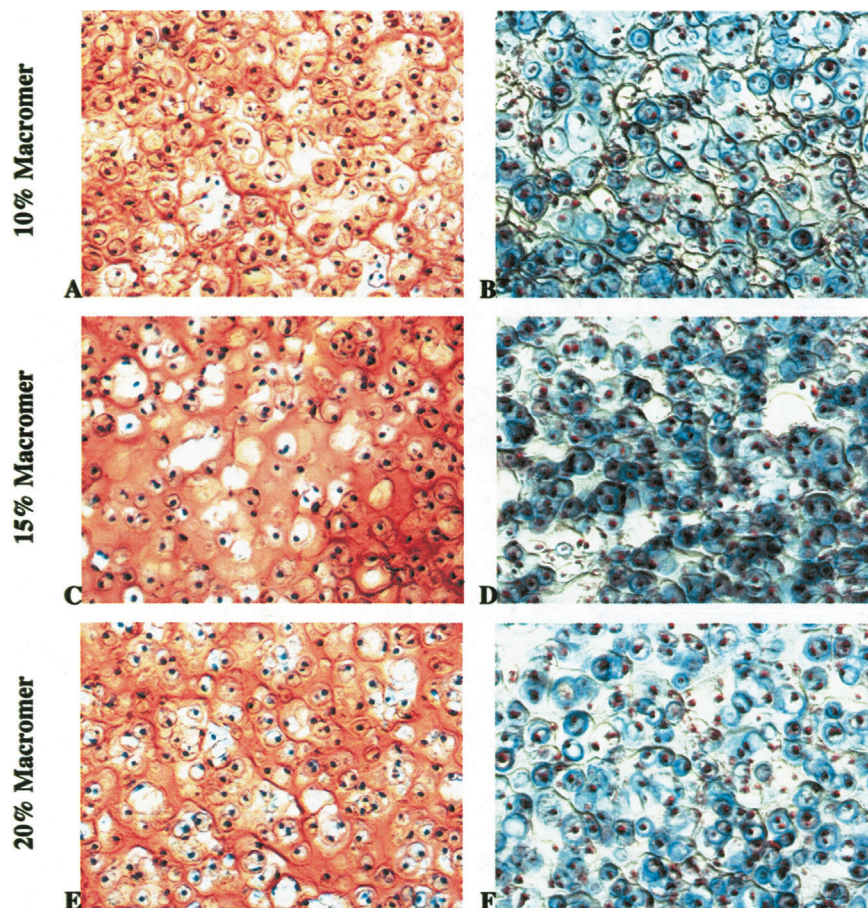


Figure 7. Histological analysis of chondrocytes photoencapsulated in PEG hydrogels with (A, B) 10%, (C, D) 15%, and (E, F) 20% macromer after 8 weeks in vitro. (A), (C), and (E) were stained with Safranin-O, which stains proteoglycans red; (B), (D), and (F) were stained with Masson trichrome, which stains collagen blue. Original magnification $\times 200$.

diffuse out past the pericellular regions and into the hydrogel. However, in the gels with initially higher crosslinking densities and slower degradation, collagen molecules accumulated at the edge of the pericellular regions, suggesting slower diffusion in these gels. It is possible that, by 8 weeks, not all of the degradable crosslinks degraded in the gels with increased crosslinking density. Polymer was present in all three systems as seen by the Masson trichrome stain, which stained the PEG polymer brown. Interestingly, an increase in polymer staining was observed with increasing macromer concentration, which further suggests that degradation and mass loss was incomplete in gels with an initially higher number of crosslinks. With longer culture times, the collagen molecules may be able to diffuse further into the extracellular regions in the 15% and 20% gels, resembling the 10% gels.

Future studies will aim to fine-tune the degradation rate of these hydrogels to better match tissue secretion, particularly in gels with a higher crosslinking density. Fine-tuning can be done in a variety of ways, such as changing the length of the PLA block (i.e., an increase in PLA length decreases degradation time) or changing the core PEG length (an increase in PEG molecular weight will increase the length of the crosslinker, and thus, the mesh size). Furthermore, to generate gels that are more clinically acceptable, the nondegrading crosslinks can easily be exchanged for slow-degrading crosslinks, such as triblock copolymers of ϵ -caprolactone-*b*-PEG-*b*- ϵ -caprolactone. Although further optimization is necessary, we have clearly demonstrated the ability to fabricate scaffolds with appropriate properties (i.e., compressive modulus) that match those of the native tissue while maintaining cell viability and cell function.

Although this study has focused on tissue engineering cartilage, the knowledge of gel degradation and network evolution during degradation and its impact on tissue formation could be expanded to engineering of other tissues. By understanding the nuances of such systems and the key parameters for engineering temporal structural changes in gels, these cell scaffolds may be fine-tuned to match many cell- and tissue-specific types of growth.

CONCLUSIONS

The macroscopic properties and degradation behavior of PEG hydrogels can be fine-tuned to optimize scaffold properties for enhanced cartilage tissue regeneration. Specifically, by increasing the overall macromer concentration, gels with initial compressive moduli of 60 to 500 kPa were obtained. A unique advantage of photopolymerization is the ease with which macromers of varying degradation kinetics can be incorporated into the network. Scaffold integrity was maintained by copolymerizing a small amount of slowly degrading crosslinks into the network (and delaying reverse gelation). The incorporation of degradable crosslinks into the network facilitated diffusion of proteoglycans into the extracellular regions of the hydrogel

scaffold. Collagens were localized primarily around the pericellular regions, but were more diffuse in the loosely crosslinked and faster degrading network. By tailoring the composition of the gels and controlling degradation and temporal changes in the network structure, gels can be processed with initially high compressive moduli yet be conducive to tissue formation with time.

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