
Encapsulating chondrocytes in copolymer gels: Bimodal degradation kinetics influence cell phenotype and extracellular matrix development

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Abstract: Hydrogels provide an ideal environment for encapsulating chondrocytes and facilitating the production of cartilaginous tissue. However, the deposition of extracellular matrix (ECM) and ultimate tissue function are significantly affected by degradation of gel scaffolds. It was hypothesized that a bimodal degradation process would capture the critical features necessary for neotissue development. Specifically, most of the initial crosslinks would degrade quickly and enable ECM deposition, whereas a critical amount would remain or degrade much more slowly to provide structural integrity over a longer time period. In this study, chondrocytes were encapsulated in copolymer gels of nondegradable [poly(ethylene glycol) dimethacrylate] and degradable [poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) dimethacrylate] macromers to investigate the effects of gel degradation on ECM evolution. All gels were synthesized from 10 wt % total macromer solutions consisting of 0, 19, 21, 23, 25, or 100 mol % nondegradable units. The copolymer constructs were found to have lower DNA content than completely degradable constructs after 8

weeks. However, total biochemical content was very similar among the various copolymer constructs. Histological analysis gave more interesting insight, showing a more uniform spatial distribution of ECM components in copolymer samples than in constructs with 100 mol % nondegradable units. In addition, a number of major structural defects were present in constructs with 0 mol % nondegradable units that became less apparent as the amount of nondegradable units was increased. Overall, the copolymer gels had a higher compressive modulus during neotissue development and also showed no evidence of chondrocyte dedifferentiation. With their bimodal degradation profile, copolymer gels with carefully selected ratios of degrading to slow or nondegrading crosslinks provide distinct advantages for ECM development in tissue-engineered cartilage. © 2004 Wiley Periodicals, Inc. *J Biomed Mater Res* 70A: 560–568, 2004

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

INTRODUCTION

In native articular cartilage, chondrocytes maintain a rounded morphology and exist in small pockets in the tissue called lacunae.¹ The lacunae are surrounded by an extracellular matrix (ECM) that has a network-like structure of insoluble collagen surrounded and permeated by an aqueous phase containing proteoglycans.² Hydrogels are a natural scaffold choice for tissue-engineered cartilage for a few reasons. First, the physical properties of the gel capture many features of

native cartilage. Hydrogels have very high water content and a network structure that can be adjusted to control the initial diffusivity and compressive properties of the gel. Next, the encapsulation of chondrocytes in hydrogels allows them to maintain their rounded morphology and desired cell behavior. Finally, hydrogels have the potential to be formed *in situ*, exactly replicating and adhering to a defect, by injecting an aqueous macromer solution and then initiating polymerization or gelation.

Although there are many types of hydrogels,³ of interest here is the formation of hydrogels with degradable crosslinks created by photopolymerizing multifunctional macromers with a hydrophilic polymer core attached to degradable blocks capped with vinyl end groups. Macromolecular monomers composed of poly(ethylene glycol) (PEG) or poly(vinyl alcohol) (PVA) with or without degradable poly(lactic acid) (PLA) blocks and acrylate or methacrylate end

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groups have been explored previously in cartilage tissue engineering applications.⁴⁻⁷ Increasing the initial crosslinking density of these types of gels by increasing the initial macromer concentration or functionality has been shown to decrease the equilibrium swelling ratio and diffusion coefficient of ECM molecules while increasing the compressive modulus.⁷⁻⁹ The diffusion coefficient and compressive modulus are especially important in the development of a chondrocyte delivery system because cartilage is an avascular tissue with load-bearing responsibilities. To synthesize gels that have initial compressive moduli on the order of native cartilage (~ 500 – 1000 kPa),¹⁰ the crosslinking density must be relatively high, which limits the facile distribution of ECM components secreted by encapsulated chondrocytes. As a result, a number of recent studies have explored the development and distribution of cartilage ECM in degrading PEG-based hydrogels.^{4,11,12} In PEG-based systems, histological results have shown that gel networks degrading too slowly cause collagen to localize only in the pericellular region, although gel networks that degrade too quickly often result in major defects in the developing tissue.^{7,12} Thus, the degradation profile of the gels in which chondrocytes are encapsulated has a major effect on the quality of neocartilage that will be produced.

Hydrogel networks formed from the multifunctional macromers described above follow a bulk degradation pathway in which mass loss initially advances slowly and somewhat linearly as individual crosslinks are cleaved from the network and diffuse out of the gel. When the weight-average number of crosslinks per kinetic chain becomes less than two, the network itself can become solubilized, and the remaining mass is lost in one final burst. The point at which this final burst occurs has been previously described as the reverse gel point.¹³ Recently, statistical kinetic models have been developed to describe the mass loss in this type of hydrogel network.^{8,13,14} A plot of mass loss with time, using a simplified version of this model for an ideal network structure, is shown with solid and dotted lines in Figure 1. The solid line in Figure 1 represents a homopolymer gel with completely degradable crosslinks, and it exhibits a reverse gel point. It is important to note that the region on this curve just before the reverse gel point represents the time when the gel has maximum diffusion properties but also retains some semblance of structural integrity that could help developing neocartilage maintain proper shape and a degree of load-bearing function. Addition of a critical amount of very slowly degrading poly(ethylene glycol) dimethacrylate (PEG-DM) has been shown to alter the degradation behavior of copolymer gels. Specifically, a bimodal mass-loss profile results in which crosslinks are degraded quickly, but enough very slowly degrading crosslinks remain to prevent

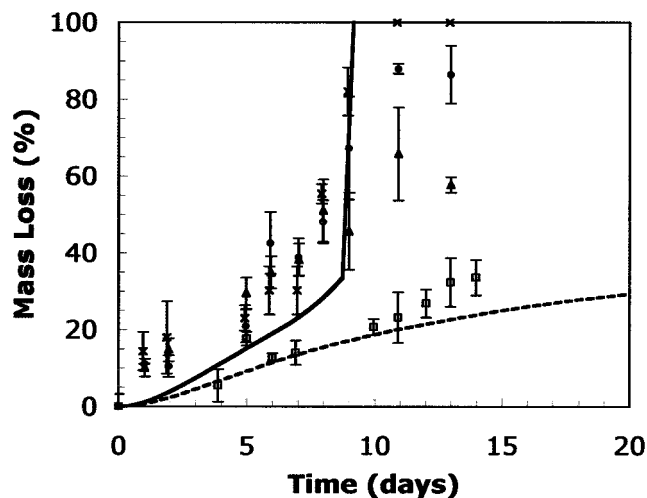


Figure 1. Predicted and experimental mass-loss profiles as a function of degradation time. Predicted profiles were calculated for a bulk degrading homopolymer gel (solid line) and copolymer gel (dashed line) with a critical amount of nondegradable crosslinks that prevent complete degradation. Experimental mass-loss profiles include the following copolymer molar ratios (moles PEG-DM:moles PEG-LA-DM): crosses, 19:81; circles, 21:79; triangles 23:77; and squares, 25:75 gels.

reverse gelation and allow diffusion of ECM components.¹⁵ Over a much longer period of time, these slowly degrading crosslinks are eroded and the gel is completely resorbed. An example of the fast-degrading part of this bimodal degradation profile is shown as the dotted line in Figure 1. At longer times, this profile levels out, as the only mass loss comes from the very slow degradation of the remaining crosslinks.

This study aims to give new insight related to the effects of a bimodal degradation profile on ECM evolution and to elucidate its advantages in PEG-based chondrocyte carriers by examining a narrow compositional range of copolymer gels synthesized from PEG-DM and PEG-LA-DM macromers. The compositions were selected such that the fraction of fast-degrading crosslinks narrowly spans the reverse gel point, measured by gel degradation in the absence of chondrocytes. Through this analysis, the goal was to maximize the gel's compressive modulus of elasticity while maintaining the desired chondrocyte behavior during neocartilage development by using copolymer gels rather than gels made from only the fast-degrading PEG-LA-DM macromer. Because the reverse gel point corresponds to a region of rapid and dramatic changes with respect to gel properties, this narrow compositional space actually elucidates a wide range of gel properties. An additional aim was to further investigate whether copolymer gels have specific advantages, especially with respect to ECM distribution and biomechanical properties over time, over homopolymer gels synthesized from the very slowly degrading PEG-DM macromer alone.

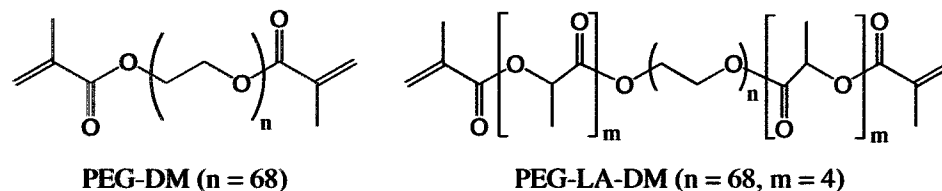


Figure 2. Macromers used to synthesize the gels analyzed in this study, poly(ethylene glycol) dimethacrylate (PEG-DM) and poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) dimethacrylate (PEG-LA-DM).

MATERIALS AND METHODS

Isolation of chondrocytes

Chondrocytes were isolated from the femoral-patellar groove and femoral condyles of knee joints harvested from a young calf (Research 87, Marlboro, MA). Cartilage blocks were excised under aseptic conditions, diced into small pieces, and digested on an orbital shaker at 37°C with the use of a solution of 0.2% collagenase type II (Worthington, Lakewood, NJ) and 5% fetal bovine serum (Gibco) in Dulbecco's modified eagle medium (DMEM, Gibco) without additives. After a digestion period of 15–17 h, the solution was centrifuged at 1200 rpm for 10 min; the supernatant was aspirated off; the cells were resuspended in warm phosphate-buffered saline (PBS, Gibco, pH 7.4) supplemented with 1% penicillin/streptomycin (P/S, Gibco) and 0.02% ethylenediaminetetraacetic acid (EDTA, Aldrich); this cell suspension was then filtered through a 100- μ m cell strainer. Cells were then centrifuged and resuspended in PBS with 1% P/S two additional times. Cells were counted and the viable fraction was determined with the use of trypan blue staining and a hemacytometer.

Preparation of hydrogels

Linear poly(ethylene glycol) (Fluka) with an average molecular weight of approximately 3000 Da was used to synthesize poly(ethylene glycol) dimethacrylate (PEG-DM), as well as a triblock copolymer, poly(lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid) dimethacrylate (PEG-LA-DM) as described previously.¹⁶ Macromer structures are shown in Figure 2. Although the PEG-DM macromers form hydrogel crosslinks that are nondegradable on the time scale of the experiments performed in this work (≤ 8 weeks), the lactic acid repeat units of the PEG-LA-DM macromers are hydrolytically degraded under culture conditions and completely degrade on the time scale of these experiments. NMR analysis of the PEG-LA-DM molecules revealed an average of four lactic acid repeat units per side of the PEG core molecule.

To prepare gels, the individual macromers or mixtures of the two were dissolved in sterile PBS to a final concentration of 10% by weight. Each gel fabricated from comonomer mixtures is identified in terms of the mol % of PEG-DM macromer and mol % PEG-LA-DM macromer. For example, hydrogels with 19 mol % PEG-DM and 81 mol % PEG-LA-DM are referred to as 19:81 gels. The UV

photoinitiator, 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone (D2959, Ciba-Geigy, Ardsley, NY), was added to a final concentration of 0.05% by weight. The resulting solution was sterilized by filtration through a 0.2- μ m syringe filter.

Encapsulation of chondrocytes

Chondrocytes were added to sterile macromer/initiator solutions to a final seeding density of 75 million cells per milliliter. Individual 40- μ L aliquots of the resulting suspensions were polymerized under 365-nm UV light for 10 min at an intensity of approximately 10 mW/cm². Following polymerization, the constructs were incubated in untreated 24-well plates at 37°C and 5% CO₂ in a humid environment. Constructs were cultured statically in Dulbecco's modified eagle medium (DMEM, Gibco) supplemented with 1% P/S, 10 mM HEPES (Gibco), 0.1 mM MEM nonessential amino acids (Gibco), 0.4 mM L-Proline, 0.05 mg/mL L-Ascorbic acid, 0.5 μ g/mL Fungizone (Gibco), and 10% fetal bovine serum (Gibco). Constructs received 2 mL of fresh media every 2–3 days.

Hydrogel degradation

Copolymer hydrogels, in the absence of cells, were prepared from 10 wt % macromer solutions consisting of 19, 21, 23, and 25 mol % PEG-DM macromer. The hydrogels were degraded for a period of up to 2 weeks at 37°C in the same culture media described above. During this degradation period, samples were removed every 1–2 days, freeze-dried for 24 h, and weighed to determine the mass loss to that point. Percent mass loss was determined by comparison to the average dry mass of hydrogels with the same composition freeze-dried immediately after polymerization. A sample size of three was used.

Biochemical analysis

Constructs were freeze-dried for 24 h and digested with a papain solution for 18 h at 60°C. The papain was in aqueous solution consisting of 125 μ g/mL papain (Worthington), 10 mM L-cysteine (Aldrich), 100 mM phosphate, and 10 mM EDTA at a pH of 6.3. Total GAG content was measured with

the use of a dimethylmethylene blue assay.¹⁷ Total collagen content was determined by a hydroxyproline assay,¹⁸ which measures 10% of the total collagen.¹⁹ DNA content was measured by Hoechst 33258 (Polysciences, Inc., Warrington, PA) and related to cell number by dividing DNA content by 7.7 pg per chondrocyte.²⁰ GAG and total collagen content were reported as values per chondrocyte to account for any differences in cell proliferation with gel composition. A sample size of three was used.

Histology and immunohistochemistry

Constructs were fixed in 10% formalin for 18–24 h, dehydrated, paraffin-embedded, and microtomed into 8- μ m-thick sections. Sections were stained without further treatment with the use of fast green and safranin-O, which stains GAGs red, or Masson's trichrome method, which stains collagen blue. Sections designated for immunohistochemical staining were treated with a pepsin solution [1 mg/mL pepsin (Sigma) in Tris HCl, pH 2.0] for 15 min at room temperature. After treatment with pepsin, sections were incubated with primary antibody for either type I collagen (Sigma) or type II collagen (Research Diagnostics, Inc., Flanders, NJ) followed by treatment with a biotin-avidin immunoperoxidase kit (Vectastain Elite ABC, Vector Labs, Burlingame, CA) and visualization with Vector NovaRED (Vector Labs). Native bovine articular cartilage was stained as a positive control for type II collagen, and additional samples were stained without primary antibody as a negative control for each construct.

Mechanical testing

The compressive modulus of elasticity of polymer/cell constructs was measured for both homopolymer compositions and a 23:77 copolymer after *in vitro* culture periods of 1, 3, 6, and 8 weeks. Measurements were made with the use of a dynamic mechanical analyzer (DMA-7, PerkinElmer, Wellesley, MA) in unconfined compression at room temperature. Samples were initially unloaded, and then subjected to a static load that increased at a rate of 50 mN/min. The compressive modulus was determined by analyzing the linear region of the stress versus strain curve on samples at low deformation (<10% strain). A sample size of 3–5 was used.

Statistical analysis

Statistical analysis was performed with the use of single-factor analysis of variance with a confidence interval of 0.05. All values in this text are reported as the average plus or minus one standard deviation.

RESULTS AND DISCUSSION

Understanding the physical and biochemical consequences of gel degradation profiles on encapsulated

chondrocytes is critically important when attempting to regenerate cartilaginous tissue with the use of cell-laden hydrogel scaffolds. In this study, a narrow range of copolymer hydrogels was synthesized from degrading (PEG-LA-DM) and nondegrading (PEG-DM) macromers, focused on a region near the reverse gel point in the polymer erosion profile. The first part of this study consisted of degradation of these gels in cell-culture media and examination of the mass-loss profiles. In the second part of this study, chondrocytes were encapsulated in these gels and the resulting neocartilaginous tissue was examined biochemically, histologically, mechanically, and immunohistochemically. Homopolymer gels made from PEG-LA-DM or PEG-DM served as controls for the encapsulation experiments.

Degradation of copolymer hydrogels

Copolymer hydrogels without cells were synthesized by photopolymerization of 10 wt% macromer solutions. The copolymer networks were fabricated from comonomer solutions containing 19, 21, 23, or 25 mol% PEG-DM, with the balance being PEG-LA-DM. Results of the degradation mass-loss studies are shown in Figure 1. In an ideal gel, two nondegradable crosslinks must exist per kinetic chain to prevent reverse gelation. If the kinetic chains are on average 100 repeat units long, a 2:98 ratio of PEG-DM:PEG-LA-DM would be near this critical point of preventing complete degradation. However, because of numerous nonidealities and shorter kinetic chains that result when polymerizing these high-molecular-weight macromers in 10% solutions, it was found that a ratio close to 20:80 is near this critical point. Thus, a narrow compositional range was selected based on this proximity to the reverse gel point, and a variety of degradation profiles were found to exist with gels fabricated at these ratios. The gels incorporating the least amount of PEG-DM macromer, 19:81 gels, reached the reverse gel point and completely degraded after 11 days. On the other end of this narrow range, 25:75 gels appeared to lose mass at a similar rate for the first 7 days; this rate then begins to level off as degradation moves into a region of very slow degradation of the PEG crosslinks without LA (nondegradable on the time scale and conditions of this experiment). The initial burst of mass loss shown in the experimental data between days 0 and 2 is the loss of soluble unreacted monomer. The model used to calculate the degradation profiles shown as lines in Figure 1 do not take this loss into account.

Between the two copolymer composition extremes, a distribution of gel degradation profiles exist. However, it is important to note that the presence of chon-

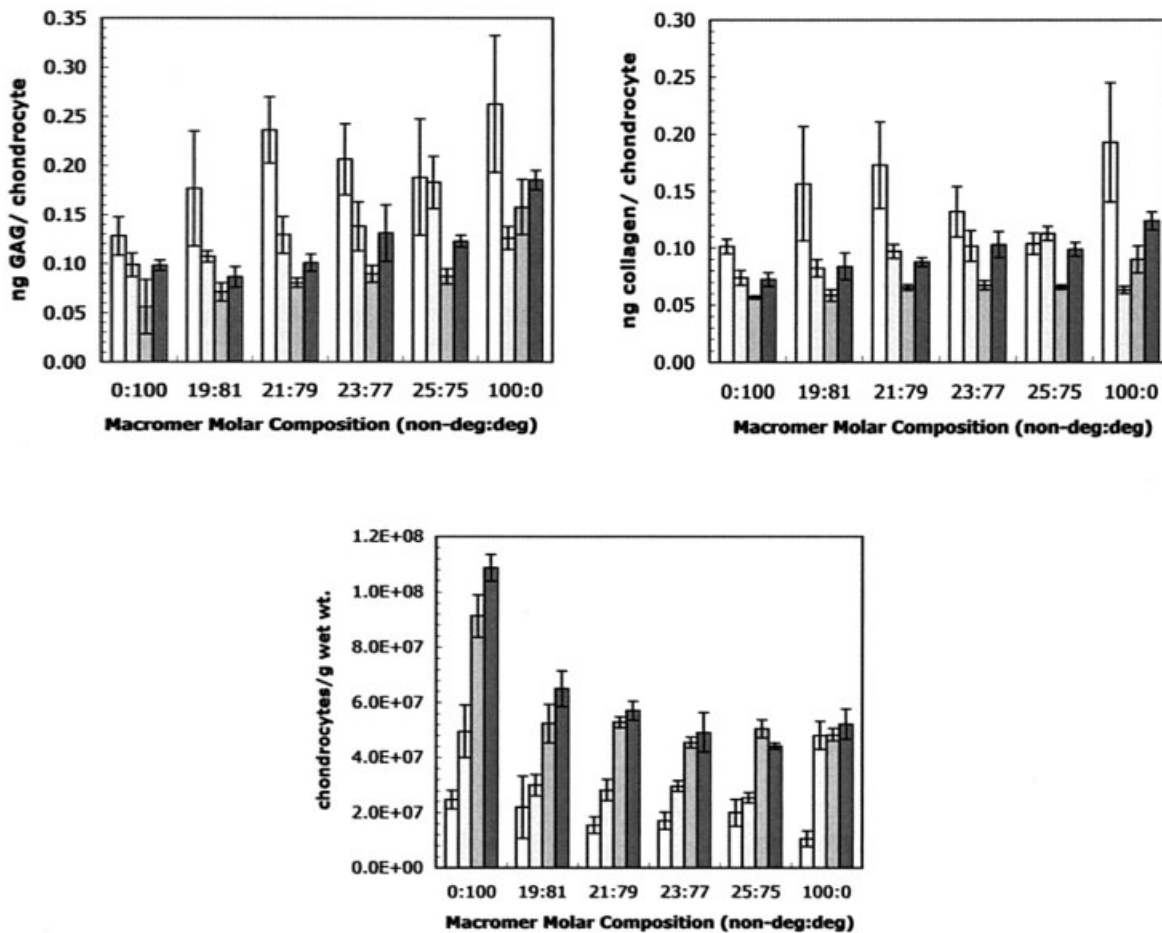


Figure 3. GAG, total collagen, and cell content as a function of culture time and gel composition. Data for each composition are shown at time points of 1 week, white bars; 5 weeks, light gray bars; 6 weeks, dark gray bars; and 8 weeks, black bars.

drocytes tends to slow down the degradation process because the cells are producing an ECM as the crosslinks are being cleaved. Thus, a composite network evolves with time, which includes the newly secreted ECM network distributed throughout the resorbing synthetic network. This compositional range was examined further because it appears to produce gels very near the reverse gel point, and with encapsulated cells, these gels may likely have a desirable bimodal degradation profile. The first part erodes quickly, as the degradable crosslinks cleave and produce a highly swollen gel, and then mass loss essentially stops for the time scale of these encapsulation experiments.

Encapsulation experiments

Chondrocytes were encapsulated in homopolymer gels made from PEG-LA-DM or PEG-DM, as well as copolymer gels with the same compositions that were used in the degradation studies. Constructs were removed from culture after 1, 2, 3, 4, 5, 6, and 8 weeks

for biochemical analysis and compared as a function of copolymer composition. Results of this analysis are shown in Figure 3. Data from 2-, 3-, and 4-week time points are not shown because they do not show statistically significant differences among the homopolymers and varying copolymer compositions. Although the encapsulated chondrocytes may be slightly more active in producing GAGs and collagen in 100:0 gels, it is difficult to glean a trend from these data, especially when comparing among only the copolymer gels. Thus, analysis of the biochemical data might lead one to believe that the ECM composition and amount are relatively unaffected by the gel composition. However, an interesting aspect of the biochemical analysis in Figure 3 is the chondrocyte proliferation data. Chondrocytes do not generally proliferate at a very high rate in three-dimensional culture,²¹ but the cells in the 0:100 constructs showed much higher proliferation than in any of the copolymer gels or the 100:0 constructs in the final 3 weeks of this study. A high rate of proliferation is one characteristic of dedifferentiated chondrocytes that is expressed when chondrocytes are cultured in monolayers.²¹

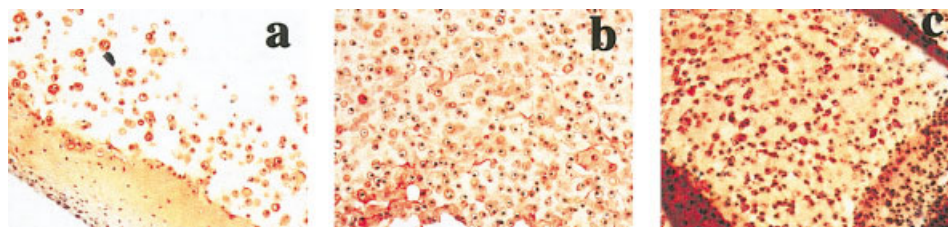


Figure 4. Histological staining of GAGs as a function of hydrogel composition after a period of 4 weeks of *in vitro* culture. Safranin-O stains proteoglycans red. Micrographs (a–c) are 0:100, 23:77, and 100:0 constructs, respectively. Area in the bottom left of (a) is a surface artifact present in all compositions. Dark areas in (c) are histological artifacts resulting from tissue section folding and do not represent areas of higher proteoglycan content.

Constructs were examined histologically after 4 and 8 weeks of culture to examine the proteoglycan and collagen distribution. Figure 4 shows the proteoglycan staining at four weeks in the 23:77 experimental copolymer gel, as well as both homopolymer control gels. As anticipated, major defects were present in the 0:100 control constructs [Fig. 4(a)]. Because this gel was comprised completely of degradable PEG-LA crosslinks, the gel clearly degraded too quickly and left major holes in the developing tissue at 4 weeks. Minor defects persist in the copolymer sample, but the construct appears to retain most of its structure. No defects associated with gel degradation appear to be present in the gel made from completely nondegrading macromer [Fig. 4(c)]. In general, the copolymer gels appear similar, and all gels maintained macroscopic integrity during neocartilage development (data not shown). Though the constructs with a higher fraction of nondegradable crosslinks were slightly more

homogeneous, all of the copolymer gels appeared to maintain a critical amount of structural integrity while also allowing distribution of proteoglycans throughout the developing neotissue. The remaining data comparisons are made among one copolymer composition (23:77 constructs) and the two control conditions (0:100 fast-degrading and 100:0 nondegrading constructs) to give insight related to the advantages and effects of a bimodal degradation profile on ECM evolution.

Histological stains of constructs at 4 and 8 weeks for the two control conditions and one copolymer gel are shown in Figure 5. Figure 5(a–c)] shows the GAG distribution at 8 weeks. Interestingly, the 0:100 constructs no longer show the defects present at 4 weeks [Fig. 4(a)], and the neotissue formed in this gel appears to be uniformly distributed with GAGs. The copolymer and the 100:0 constructs also show a similar, and relatively uniform, distribution of GAGs at 8 weeks. This distribution of GAGs is

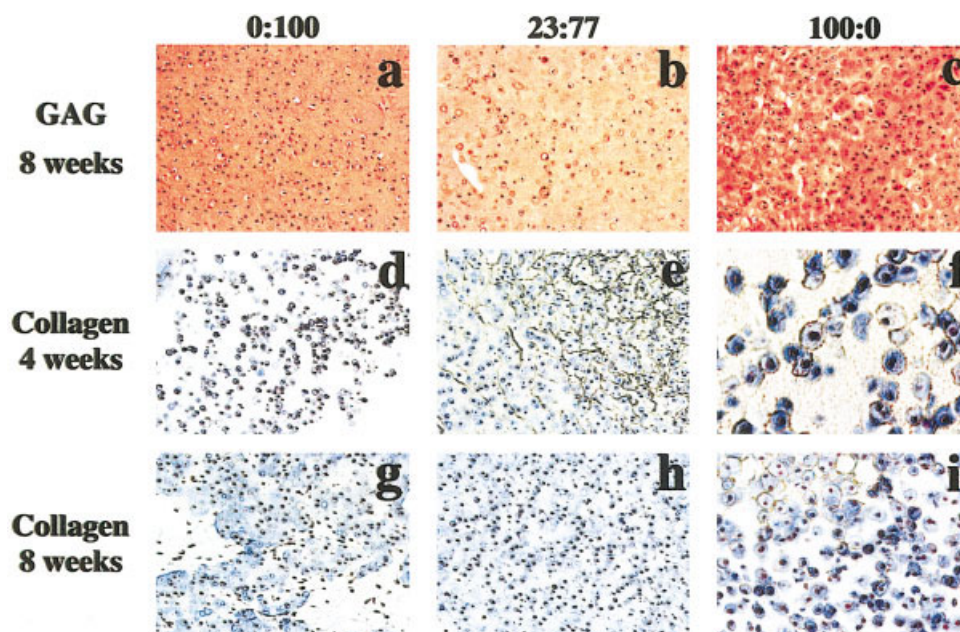


Figure 5. Histological staining for GAGs where safranin-O stains proteoglycans red (a–c), and for collagen where Masson's trichrome method stains collagen blue (d–i). Original magnification of (a–e) and (g–h) is 100 \times , (f) is 400 \times , and (i) is 200 \times . Light gray colors in (d–i) are processing artifacts.

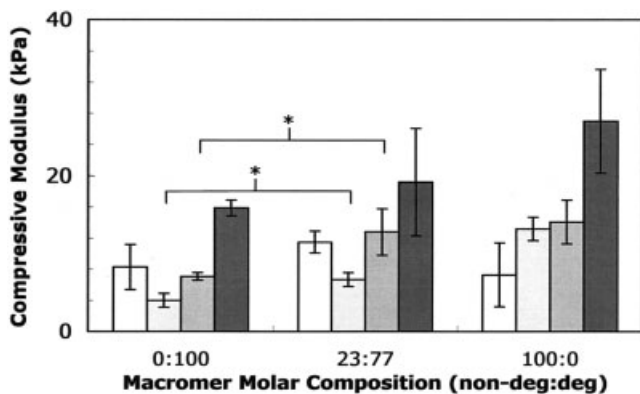


Figure 6. Compressive modulus of developing constructs as a function of time and gel composition. Data for each composition are shown at time points of 1 week, white bars; 5 weeks, light gray bars; 6 weeks, dark gray bars; and 8 weeks, black bars. $*p < 0.05$

encouraging, but a great deal of the strength of native cartilage is derived from a network-like structure of intertwined insoluble collagen.² Clearly, this cannot form if all the collagen being produced by the chondrocytes is trapped in the local region of the cell. Although collagen was localized to the pericellular region in the 100:0 constructs [Fig. 5(f,i)], this was not the case with either the copolymer or 0:100 constructs [Fig. 5(d,e,g,h)]. In addition, it appears that after 8 weeks, the chondrocytes in the 0:100 constructs filled in the major defects that were present at 4 weeks. Further examination shows that the cells in these constructs have filled in these holes by flattening out and entering a proliferative mode. This flattened shape is most apparent in large regions with low collagen content but can also be seen in some cells that appear to be surrounded by normal, rounded chondrocytes. This change in cell shape is another indicator of chondrocyte dedifferentiation and corroborates the chondrocyte proliferation data shown in Figure 3, discussed previously.

Histological analysis of the major defects formed in 0:100 constructs was supported by mechanical testing of constructs after 1, 3, 6, and 8 weeks of *in vitro* culture. The results of these compression tests are shown in Figure 6. It is interesting to note the profiles for the different compositions in Figure 6. In both constructs that contained fast-degrading crosslinks (0:100 and 23:77), the compressive modulus first decreased and then began to increase with additional culture time. This indicates that the hydrogel scaffold initially degraded faster than the encapsulated cells could produce ECM. At some point between 1 and 6 weeks, all of the fast-degrading crosslinks were cleaved and the constructs were being supported either by the ECM that had been secreted by the cells (0:100 gels) or by a combination of ECM and slow-degrading crosslinks (23:77 gels). As shown in Figure

6, the 23:77 gels had significantly higher compressive modulus than the 0:100 gels after 3 and 6 weeks of *in vitro* culture. This is likely due to the presence of slow-degrading crosslinks in the 23:77 gels that did not exist in the 0:100 gels. The constructs with a bimodal degradation profile thus maintained a mechanical advantage over the 0:100 constructs during the early stages of neotissue development.

The profile of compressive modulus over time in the 100:0 constructs is also worth noting. These gels did not show any initial decrease that would have been due to hydrogel degradation, and actually show a significant increase in compressive modulus over the timescale of this experiment. This increase is likely a result of charge interactions among distributed GAGs, and also between the GAGs and the aqueous environment. It is unlikely that collagen content played a major role in the compressive modulus of the 100:0 gels because histological analysis showed collagen to be primarily localized to the pericellular region [Fig. 5(f,i)]. Although one result of the slow-degrading hydrogel network in the 100:0 gels was localization of collagen to the pericellular region, another result was high retention of differentiated chondrocyte morphology.

Dedifferentiation of chondrocytes in these gels is a major concern because it also means that the cells may be producing large amounts of type I collagen instead of type II collagen,^{22,23} which would lead to fibrocartilage (nonarticular) with inferior mechanical properties. Immunohistochemistry was performed on 0:100, 23:77, and 100:0 constructs after 8 weeks to assess collagen type, and the results are shown in Figure 7. The left column of micrographs in Figure 7 shows type II collagen found near cells with a rounded morphology that appear to be embedded in neocartilage but also shows significant type I collagen production in 0:100 gels. By comparison, chondrocytes in the 23:77 copolymer appear to have produced significantly less type I collagen and still manage to show reasonable distribution of type II collagen. The micrographs in the right column of Figure 7 show almost exclusive staining for type II collagen in the 100:0 homopolymer gels, but it is clear that nearly all of the collagen in these gels is localized to the pericellular region. Thus, it appears that when these gels degrade too quickly, the composition of the ECM is clearly shifted to favor type I collagen production. However, when the encapsulated chondrocytes continue to be surrounded by even a minimal amount of intact PEG crosslinks, the cells maintain the correct morphology and clearly have a better chance of producing the desired collagen type. The addition of a second, slower mode of degradation may therefore improve the macroscopic distribution of type II collagen by decreasing or eliminating defects that fill with proliferating

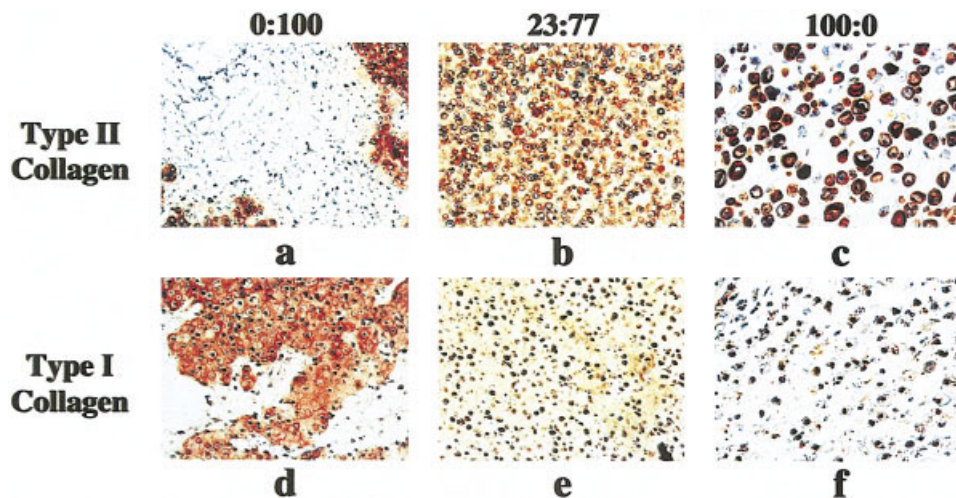


Figure 7. Immunohistochemical staining of constructs for type I and II collagen after a period of 8 weeks of *in vitro* culture. Vector NovaRED was used to stain antigen red. Original magnification for samples (a,b) and (d,e) is 100 \times , and for samples (c) and (f) it is 200 \times .

cells producing type I collagen. A bimodal degradation profile in chondrocyte cell carriers thus has a likely biochemical advantage over gels with a fast, monomodal degradation profile.

CONCLUSIONS

These studies attempt to elucidate the physical and biochemical advantages of using gels with a bimodal degradation profile for tissue-engineered cartilage applications. Manipulations in a narrow range of copolymer compositions can create bimodal degradation profiles that allow for the distribution of large ECM components while maintaining a minimum, but critical, level of structural integrity. The structural integrity is not only important for the mechanical support of developing tissue but also to maintain the correct phenotype of encapsulated chondrocytes. It has been shown that copolymer constructs have a significantly higher compressive modulus than degrading homopolymer (0:100) constructs during the early stages of neocartilage development. In addition, when compared to fast-degrading homopolymer (0:100) constructs, chondrocytes encapsulated in the copolymer compositions studied did not proliferate as much or form regions of elongated cells producing primarily type I collagen. Also, 23:77 constructs allowed collagen to distribute outside of the pericellular region, which is a clearly preferable result that was not seen in the 100:0 systems. The quality of tissue-engineered cartilage produced in these hydrogel systems will continue to improve with better understanding and thoughtful manipulation of the gel degradation profiles.

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References

1. Martini FH, Timmons MJ. Human anatomy. Upper Saddle River, NJ: Prentice Hall; 1997.
2. Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic creep and stress-relaxation of articular-cartilage in compression—Theory and experiments. *J Biomech Eng Trans ASME* 1980;102: 73-84.
3. Peppas NA, editor. Hydrogels in medicine and pharmacy, volume 2. Boca Raton, FL: CRC Press; 1986.
4. Martens PJ, Bryant SJ, Anseth KS. Tailoring the degradation of hydrogels formed from multivinyl poly(ethylene glycol) and poly(vinyl alcohol) macromers for cartilage tissue engineering. *Biomacromolecules* 2003;4:283-292.
5. Riley SL, Dutt S, de la Torre R, Chen AC, Sah RL, Ratcliffe A. Formulation of PEG-based hydrogels affects tissue-engineered cartilage construct characteristics. *J Mater Sci Mater Med* 2001; 12:983-990.
6. Bryant SJ, Anseth KS. The effects of scaffold thickness on tissue engineered cartilage in photocrosslinked poly(ethylene oxide) hydrogels. *Biomaterials* 2001;22:619-626.
7. Bryant SJ, Anseth KS. Hydrogel properties influence ECM production by chondrocytes photoencapsulated in poly(ethylene glycol) hydrogels. *J Biomed Mater Res* 2002;59:63-72.
8. Martens P, Metters AT, Anseth KS, Bowman CN. A generalized bulk-degradation model for hydrogel networks formed from multivinyl cross-linking molecules. *J Phys Chem B* 2001; 105:5131-5138.
9. Metters AT, Bowman CN, Anseth KS. Verification of scaling laws for degrading PLA-b-PEG-b-PLA hydrogels. *AIChE J* 2001;47:1432-1437.
10. Armstrong CG, Mow VC. Variations in the intrinsic mechanical properties of human articular-cartilage with age, degeneration, and water-content. *J Bone Joint Surg Am* 1982;64:88-94.
11. Bryant SJ, Durand KL, Anseth KS. Degradation kinetics influence ECM production of photoencapsulated chondrocytes in PEG-based hydrogels. *Abstr Pap Am Chem Soc* 2001;222:19.

12. Bryant SJ, Anseth KS. Controlling the spatial distribution of ECM components in degradable PEG hydrogels for tissue engineering cartilage. *J Biomed Mater Res* 2003;64A:70–79.
13. 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.
14. 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.
15. Bryant SJ, Bender, RJ, Durand, KL, Anseth KS. Encapsulating chondrocytes in degrading PEG hydrogels with high modulus: Engineering gel structural changes to facilitate cartilaginous tissue production. *Biotechnol Bioeng* 2003. Forthcoming.
16. 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.
17. 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.
18. Woessner JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. *Arch Biochem Biophys* 1961;93:440–447.
19. Hollander AP, Heathfield TF, Webber C, Iwata Y, Bourne R, Rorabeck C, Poole AR. Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. *J Clin Invest* 1994;93:1722–1732.
20. Kim YJ, Sah RLY, Doong JYH, Grodzinsky AJ. Fluorometric assay of DNA in cartilage explants using Hoechst-33258. *Anal Biochem* 1988;174:168–176.
21. Benya PD, Shaffer JD. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell* 1982;30:215–224.
22. Mayne R, Vail MS, Mayne PM, Miller EJ. Changes in type of collagen synthesized as clones of chick chondrocytes grow and eventually lose division capacity. *Proc Natl Acad Sci USA* 1976;73:1674–1678.
23. Vondermark K, Gauss V, Vondermark H, Muller P. Relationship between cell-shape and type of collagen synthesized as chondrocytes lose their cartilage phenotype in culture. *Nature* 1977;267:531–532.