

Formulation of PEG-based hydrogels affects tissue-engineered cartilage construct characteristics

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The limited supply of cartilage tissue with appropriate sizes and shapes needed for reconstruction and repair has stimulated research in the area of hydrogels as scaffolds for cartilage tissue engineering. In this study we demonstrate that poly(ethylene glycol) (PEG)-based semi-interpenetrating (sIPN) network hydrogels, made with a crosslinkable poly(ethylene glycol)-dimethacrylate (PEGDM) component and a non-crosslinkable interpenetration poly(ethylene oxide) (PEO) component, and seeded with chondrocytes support cartilage construct growth having nominal thicknesses of 6 mm and relatively uniform safranin-O stained matrix when cultured statically, unlike constructs grown with prefabricated macroporous scaffolds. Even though changing the molecular weight of the PEO from 100 to 20 kDa reduces the viscosity of the precursor polymer solution, we have demonstrated that it does not appear to affect the histological or biochemical characteristics of cartilaginous constructs. Extracellular matrix (ECM) accumulation and the spatial uniformity of the ECM deposited by the embedded chondrocytes decreased, and hydrogel compressive properties increased, as the ratio of the PEGDM:PEO in the hydrogel formulation increased (from 30:70 to 100:0 PEGDM:PEO). Total collagen and glycosaminoglycan contents per dry weight were highest using the 30:70 PEGDM:PEO formulation ($24.4 \pm 3.5\%$ and $7.1 \pm 0.9\%$, respectively). The highest equilibrium compressive modulus was obtained using the 100:0 PEGDM:PEO formulation (0.32 ± 0.07 MPa), which is similar to the compressive modulus of native articular cartilage. These results suggest that the versatility of PEG-based sIPN hydrogels makes them an attractive scaffold for tissue engineering of cartilage.

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Introduction

Once damaged, cartilage has a limited ability to repair [1]. Reconstruction of damaged cartilage tissue often requires large and specific shapes of tissue which are frequently not available. Applications of biomaterials, tissue engineering technologies, and other cell-based therapies have begun to address some of these limitations; unfortunately, current technologies in clinical use and those in advanced research and development programs are unable to provide viable tissue with the optimal size, shape, and mechanical properties appropriate for many of these clinical needs. Estimates report that articular cartilage thickness can exceed 6 mm in the patella and tibial plateau [2, 3]. Significant problems that tissue engineers face when attempting to culture cartilage constructs having thickness' greater than 2 mm are the development of methods to uniformly seed prefabricated macroporous scaffolds and overcoming diffusion limita-

tions [4, 5]. Fortunately, it has been recognized that hydrogel-based scaffolds may overcome many of these limitations [6–8].

The potential advantages of using a hydrogel as a matrix for tissue engineering are many. First, because of their high water content and low coefficient of friction, hydrogels are attractively similar to that of cartilage [9]. In fact, articular cartilage is a natural composite hydrogel of collagen, chondroitin sulfate, and other polysaccharides. Second, the hydrogel matrix may enhance the chondrocytes' function by maintaining their spherical shape [10]. Third, the cellular incorporation/embedding method may offer an improved system for cell seeding by increasing cell seeding yields, allow for a more uniform initial distribution of cells throughout the hydrogel matrix as compared to prefabricated macroporous scaffolds, and facilitate the seeding of different cell types into isolated regions of prefabricated scaffolds by

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using the hydrogel as a cell carrier for the seeding and essentially the creation of multi-scaffold and multi-tissue composites. Fourth, the *in situ* crosslinking method used to fabricate the hydrogel matrix should facilitate the formation of large and abstract-shaped constructs. Fifth, maintenance of mechanical properties over culture time may be achieved by tailoring the hydrogel composition to achieve a balance between the degradation rate or mechanical properties and the deposition rate of extracellular matrix (ECM) by the embedded cells. Sixth, the hydrogel may be impermeable to the migration of immunological cells and protect embedded cells from attack by the host cells [11]. Seventh, the ability to photopolymerize the hydrogel *in situ* will allow for minimally-invasive delivery options.

The benefit of hydrogels in retaining the spherical shape of chondrocytes and maintaining their differentiated function has already been demonstrated [12]. This ability of hydrogels to mimic the three-dimensional structure of tissue has prompted researchers to perform cellular response studies on chondrocytes that are embedded in alginate and other hydrogels [13, 14]. Alginate [15], collagen [16], and hyaluronic acid [17] hydrogels have also been shown to support the engineering of cartilaginous tissue. Synthetic hydrogels offer more precise control over degradation rates and crosslinking densities or mechanical properties than their biological counterparts, without the immunological concerns for the animal derived materials. In addition, the PEG-based hydrogels in this study have previously been found to be impermeable to large proteins (22–45 kDa) [18] and are therefore likely to be impermeable to immunological cells.

Besides other biological applications, poly(ethylene glycol) and poly(ethylene oxide) macromers have been tethered to drugs to extend the drug's circulatory lifetimes [19], and as such have had a long history of biocompatibility. Preliminary studies have already indicated that chondrocytes embedded in PEG-based semi-interpenetrating (sIPN) hydrogels can produce hyaline-like cartilage tissue [6, 20] and can be delivered and polymerized under the skin with minimal invasiveness [21]. This PEG-based sIPN hydrogel formulation contains a crosslinkable PEG-dimethacrylate (PEGDM) and a poly(ethylene oxide) (PEO) polymer component having a large molecular weight (100 kDa) which makes the solution very viscous and is meant to eventually diffuse out of the gel to create space for tissue development. Some applications may require a less viscous hydrogel solution, which can be achieved by using a lower molecular weight (MW) of PEO. A lower MW PEO should enhance diffusion of the PEO from the gel and also overcome potential bodily elution problems, since the maximum molecular weight that will allow renal excretion in human beings is approximately 20–30 kDa or below [22]. A simple change in the molecular weight of the PEO component or the ratio of PEGDM to PEO components may affect the hydrogel crosslinking density or the configuration of the microporous space and thus can, in principle, be used to change the kinetics of cellular growth, matrix deposition, and mechanical strength development.

Therefore, our objective in this study was to determine

how hydrogel formulation affects matrix deposition by embedded chondrocytes with the ultimate goal of producing cartilage tissue of biologically relevant size, shape, and mechanical properties. More specifically, the objectives of this work were to: (1) determine if cartilage constructs of significant size can be produced using the PEG-based hydrogel as a scaffold; (2) determine if the PEG-based hydrogel can be formulated with a polyethylene (PEO) component having a MW of 20 kDa; (3) determine how the ratio of the two components (PEGDM and PEO) affects the amount and uniformity of matrix deposited in the hydrogel, and the hydrogel's compressive properties.

Materials and methods

Cell isolation

Bovine and ovine chondrocytes were isolated from the patellar groove and condyles of three-week-old animals (Research 87, Marlboro, MA and local abattoir, Valley Center, CA, respectively) [23]. Briefly, the cartilage tissue was minced and digested with agitation overnight in 0.2% collagenase (Worthington Biochemicals Corp., Lakewood, NJ) in high glucose Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Grand Island, NY) with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and other additives described elsewhere [23]. Single cells were obtained from the resulting digest by passing through a 70 μm cell strainer (Becton Dickinson, Franklin Lakes, NJ). The cells were then resuspended in medium, rinsed in PBS, and pelleted (240 g for 12 min).

Hydrogel preparation and photopolymerization

Poly(ethylene glycol)-dimethacrylate (PEGDM) (MW 3400, Shearwater Polymers, Hansville, AL) and poly(ethylene oxide) (PEO) of MW 20 kDa (Polysciences, Warrington, PA) or 100 kDa (Aldrich, Milwaukee, WI) were combined at various weight ratios and dissolved in PBS (Life technologies) containing 25 $\mu\text{g}/\text{ml}$ gentamicin sulfate (Life technologies) to form a 20% (w/v) solution. The photoinitiator 1-cyclohexyl phenyl ketone (HPK; Aldrich) was added to the polymer solution to make a final initiator concentration of 0.01% (w/v). The polymer solution was then pipetted to a pellet of bovine or ovine chondrocytes and mixed thoroughly to make a final concentration of 50×10^6 cells/ml of hydrogel. For hydrogel formulation experiments, constructs approximately 10 mm in diameter and 2 mm thick were produced by aliquoting approximately 300 μl of the polymer/cell solution into each well of a 48-well plate and placed under a UVA lamp (365 nm) for 3–5 min, delivering approximately 3 mW/cm^2 for photopolymerization. Large hydrogels were created by photopolymerizing the precursor solution in the wells of a 24-well plate.

Cell-embedded hydrogel culture

The resulting cell-embedded hydrogels were removed from the wells with a spatula and placed in the wells of 6-well plates containing 10 ml of complete media [DMEM

with 10% FBS, and 50 μ /ml L-ascorbic acid (J. T. Baker, Phillipsburg, NJ). Hydrogels were incubated statically for 4–6 weeks at 37 °C in a humidified (5% CO₂) environment and the medium was changed biweekly.

Compositional assays

Each hydrogel construct was weighed wet and divided into approximately equal halves. One half was processed for histology (see below), while the other half was used for biochemical analysis after weighing wet and then drying using a speed-vac. Dried hydrogel constructs were weighed and papain digested [24] and assayed for DNA, S-GAG, and collagen. DNA was measured fluorometrically with the Hoechst dye 33258 using a modification of the method of Le Barca and converted to cell number by using the conversion factor 7.7 pg DNA/cell [24]. Total sulfated-glycosaminoglycan (S-GAG) content was determined spectroscopically using the dye 1, 9-dimethylmethylene blue [25]. Collagen was assayed by hydrolyzing the papain digest using the technique of heating with an equal volume of 12 M HCl at 107 °C for 18 h, and dried. The hydrolysates were assayed for hydroxyproline content using a colorimetric procedure adapted for use with a microtiter plate [26]. Biochemical results were either expressed as a percent of construct dry weight (dw) or normalized to the number of cells found in the hydrogel constructs.

Histology

Hydrogel constructs were processed by standard histological methods described previously [27]. Briefly, constructs were fixed in 10% formalin, dehydrated in graded concentrations of ethanol to 100%, embedded in paraffin, and sectioned 4–5 μ m thick with a microtome. The sections were then deparaffinized, rehydrated and stained with safranin-O, and visualized by light microscopy.

Biomechanical assay

A 6.35 mm diameter disk was cut from the center of the constructs with a circular punch. The thickness of each disk was measured at four points distributed approximately equally around the edge of the samples and one point in the center of the sample using a current sensing micrometer. Samples were tested in confined compression by applying successive ramp compressions of 15%, 30%, and 45% of the specimen thickness at a strain rate of 0.038%/s [28]. A minimum stress-relaxation was allowed for 20 min at each strain level giving attainment of approximate mechanical equilibrium. The stress-strain data were fit to a finite deformation relationship [29] to obtain an estimate of the confined compression modulus, H_{A0} .

Statistical analysis

One-way analysis of variance (ANOVA), using a commercially available statistical software, Sigma Stat, was used to determine whether significant differences existed between the results of constructs generated with the various formulations. Post hoc Tukey testing or

Dunn's method (for data sets that failed the normality or equal variance testing) was used for subsequent pairwise comparisons.

Results

Large cartilage constructs

Large cartilage constructs (in diameter and depth) were grown using the hydrogel with a PEGDM : PEO ratio of 40 : 60. Ovine chondrocytes were embedded at 50×10^6 cells/mL in the PEG-based sIPN hydrogel by photocrosslinking the cell-containing precursor solution within the wells of a 24-well plate (inner diameter approximately 16 mm). The resulting dimensions of the hydrogel constructs after four weeks of culture were approximately 17 mm in diameter and 6 mm thick. It is apparent from the safranin-O stained histological section that the cells were able to synthesize a proteoglycan-rich matrix (red staining) that was relatively uniform throughout this entire large construct (Fig. 1).

Molecular weight of PEO

Using a lower MW of PEO (20 kDa) in the hydrogel formulation (PEGDM : PEO of 40 : 60) resulted in a much less viscous polymer solution than when the 100 kDa PEO was used, thereby enhancing the ease of handling of the polymer solution prior to photocrosslinking. The spatial distribution of the safranin-O stained matrix appears similarly uniform in hydrogels produced with the 100 kDa and 20 kDa PEO (Fig. 2A and 2B). Quantitative results for the amount of extracellular matrix molecules deposited by the embedded chondrocytes revealed similar quantities of sulfated glycosaminoglycans and collagen in the two hydrogel formulations ($p = 0.625$ and $p = 0.660$, respectively) (Fig. 2c). The results indicate that changing the molecular weight of the PEO component from 100 kDa to 20 kDa did not affect the histological or biochemical characteristics of cartilage constructs produced by the two-component PEG-based hydrogel.

PEGDM : PEO ratio

Changes in the ratio of PEGDM : PEO considerably affected the spatial deposition of the ECM in hydrogels produced using PEO of MW 20 kDa and 50×10^6 bovine chondrocytes per ml of hydrogel (Fig. 3). A trend of decreasing matrix uniformity with increasing composition of PEGDM in the hydrogel formulation was observed. A high magnification view of the central region of the 20 : 80 PEGDM : PEO construct reveals a uniform distribution of safranin-O (Fig. 3F). The hydrogel formulated with no PEO component (having a component ratio of 100 : 0 PEGDM : PEO) produced constructs lacking a uniform distribution of safranin-O stained matrix throughout the hydrogel (Figs 3E and 3G). It appeared as though proteoglycans were being deposited by the bovine chondrocytes in the 100 : 0 PEGDM : PEO hydrogels, but these large molecules could not diffuse beyond the cells' pericellular region as can be seen by a halo of red staining around each cell and the absence of staining between cells (Fig. 3G).

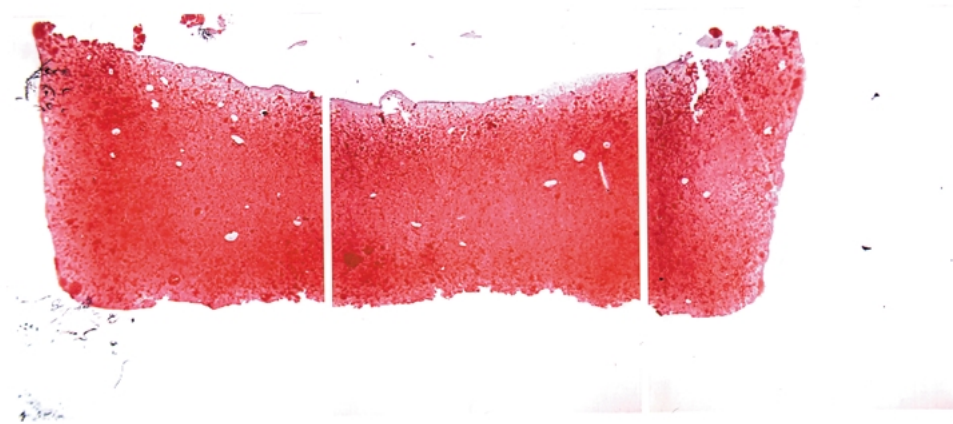
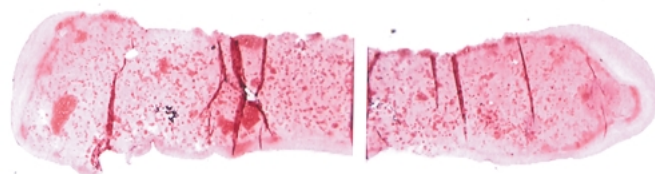


Figure 1 Representative safranin-O stained histological section of a cartilage construct having dimensions of approximately 17 mm diameter \times 6 mm thick shown at magnification of 1.25 \times . Hydrogels were made with a component ratio of 40:60 PEGDM:PEO (PEO MW 100 kDa) polymerized with 50E6 cell/mL of ovine chondrocytes, and cultured for four weeks. The vertical white lines in the photograph are a result of the limitations in the image capturing software.

A



B

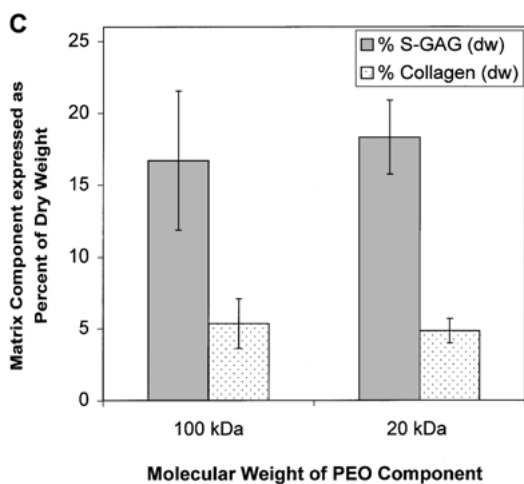


Figure 2 PEG-based hydrogel formulated with a PEGDM:PEO ratio of 40:60 were photocrosslinked with 50E6 cells/mL bovine chondrocytes and cultured for six weeks. Safranin-O stained sections (1.25 \times) of formulations using PEO of MW 100 kDa (A) or 20 kDa (B) show similar distribution of deposited proteoglycan matrix (approximate dimensions of 10 mm diameter \times 2 mm thick). Biochemical results (C) reveal insignificant differences in the amount of ECM deposited in the hydrogels formulated with the two different sized PEO polymers. Means \pm standard deviation (SD), $n = 3$.

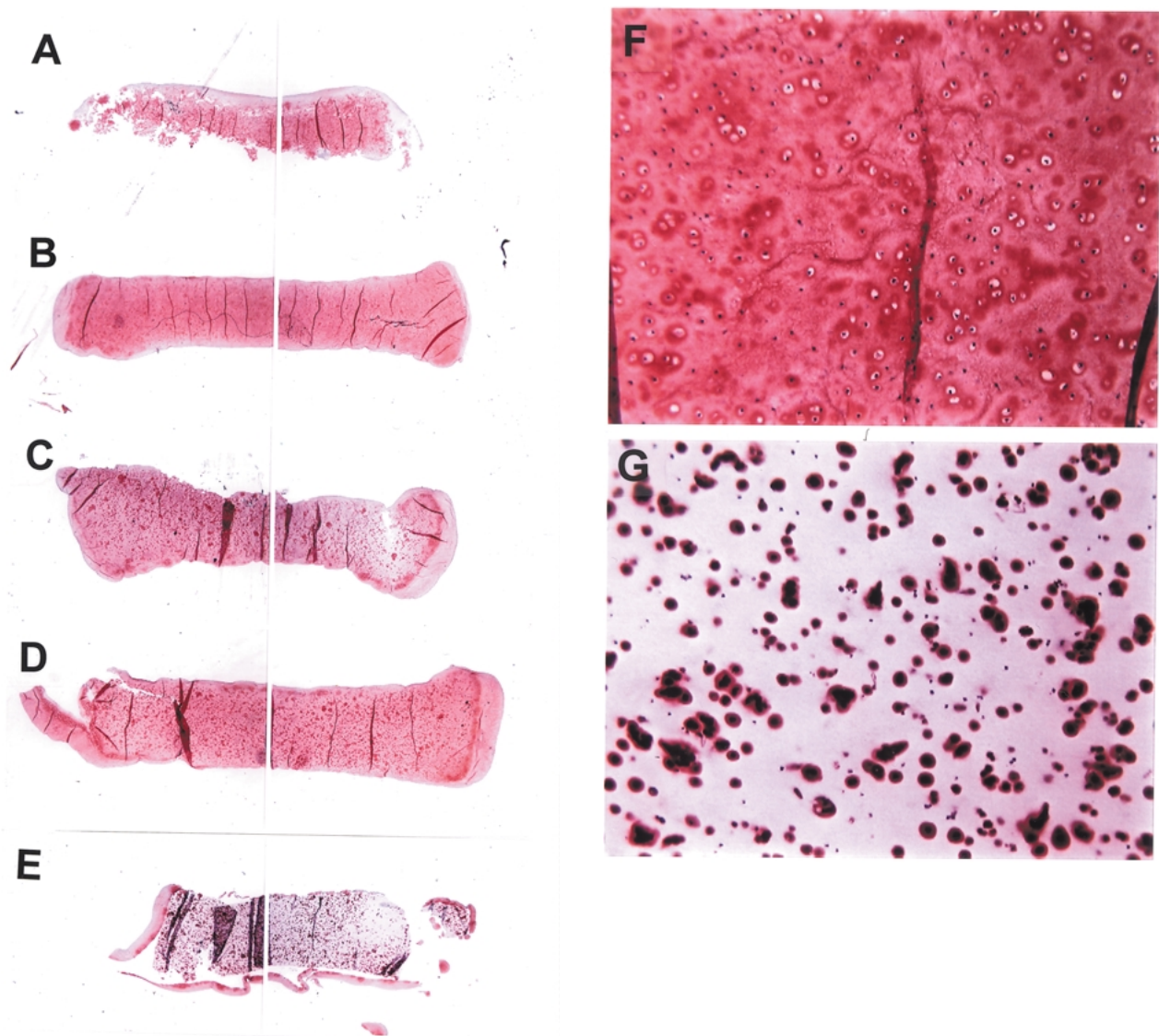


Figure 3 Histological safranin-O stained sections of hydrogels made with PEO MW of 20kDa and PEGDM:PEO ratios of 20:80 (A), 30:70 (B), 40:60 (C), 50:50(D), 100:0 (E), 20:80 (F), and 100:0 (G). Images in panels A–E are shown at 1.25 \times , while magnifications for panels F and G are 10 \times . Hydrogels were embedded with bovine chondrocytes at a concentration of 50E6 cells/mL and they were cultured for six weeks.

It was found that the percentage of water (w/w) in the swollen constructs changed significantly in response to the PEGDM:PEO ratio ($p < 0.001$). Cell content in the hydrogels decreased as the percent of PEGDM in the formulation increased. Constructs generated with a 100% PEGDM resulted in fewer cells/dry weight than construct made with 20% and 30% PEGDM (of the total hydrogel polymer weight), ($p < 0.05$) (Fig 4). With the exception of the construct generated with 20% PEGDM, both percentage of S-GAG and percentage of collagen accumulation per dry weight decreased with increasing PEGDM content. Constructs made with 30% PEGDM contained greater amounts of %S-GAG(dw) than constructs made with 20%, 50%, and 100% ($p = 0.003$, $p = 0.008$, and $p = 0.017$, respectively). Similarly, constructs generated with 30% PEGDM had greater amounts of %collagen(dw) than constructs made with 40%, 50%, and 100% PEGDM ($p < 0.001$, $p = 0.037$, and $p = 0.023$, respectively). Additionally, constructs made with 100% PEGDM also contained significantly less collagen than constructs generated with 20%, 40%, and 50% PEGDM ($p < 0.001$, $p = 0.003$, and $p = 0.014$, respectively).

When the biochemical results were normalized on a per cell basis, the amount of GAG/cell increased significantly with increase in PEGDM concentration in the hydrogel formulation (Fig. 5). However, the effect of this on collagen deposition per cell was small. More specifically, lower S-GAG/cell production was found in the hydrogel containing 20% PEGDM than hydrogels containing 30%, 40%, and 100% PEGDM ($p = 0.009$, $p = 0.013$, and $p < 0.001$, respectively). Furthermore, constructs generated with 100% PEGDM had greater amounts of S-GAG/cell than hydrogel constructs made with 50% of the total polymer as PEGDM ($p = 0.006$). The only statistically significant differences in collagen production was found for constructs produced with 30% PEGDM, which resulted in a smaller amount of collagen/cell than constructs produced using 100% PEGDM ($p < 0.05$).

An opposite trend to the matrix uniformity was found for the hydrogel's compressive modulus, with the equilibrium compressive modulus increasing as the amount of PEGDM or compositional ratio increased for hydrogels polymerized without cells ($p = 0.0016$) (Table D). In effect, the hydrogel formulated with 100% PEGDM

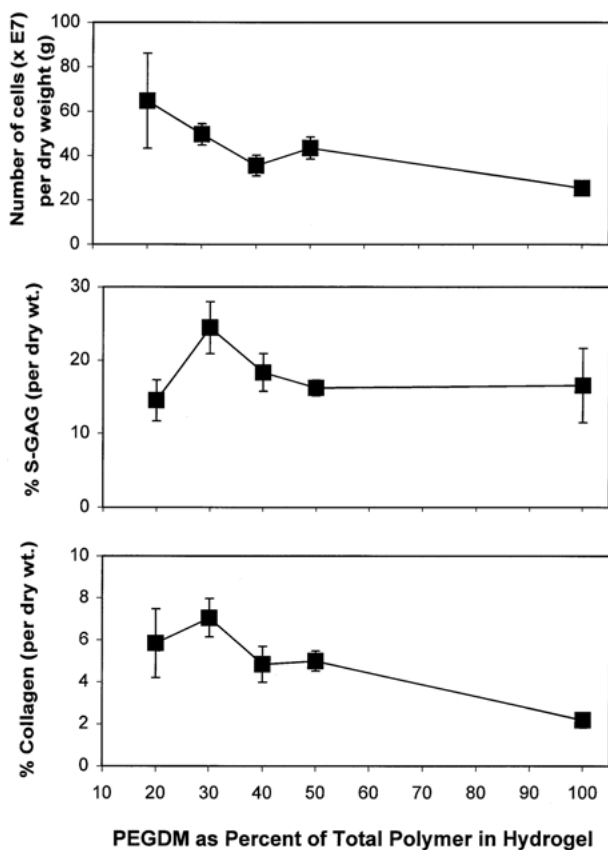


Figure 4 Extracellular matrix production normalized per dry weight for hydrogels made with increasing percentages of PEGDM in the hydrogel polymer formulation. Hydrogels were produced with PEO of MW 20kDa, embedded with bovine cells, and cultured for six weeks. Means \pm SD, $n = 3-5$.

yielded an equilibrium compressive modulus similar to that reported for full-thickness bovine articular cartilage (BAC) obtained from the patellofemoral groove [28].

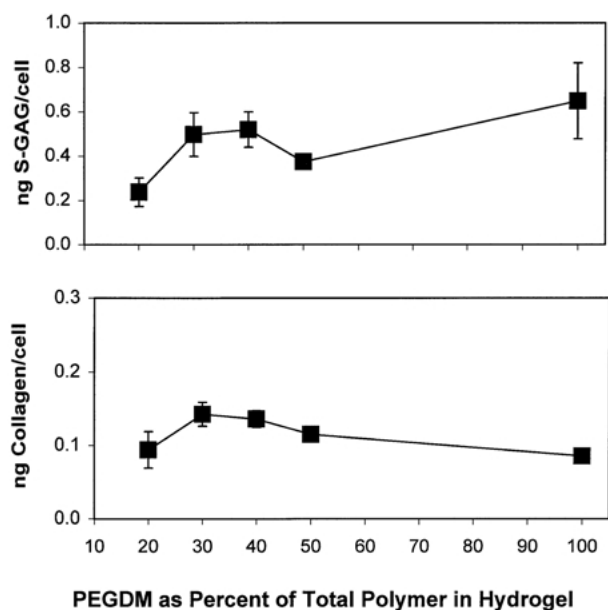


Figure 5 Extracellular matrix production in nanograms normalized per cell for hydrogels made with increasing percentages of PEGDM in the hydrogel polymer formulation. Hydrogels were produced with PEO of MW 20kDa, embedded with bovine cells, and cultured for six weeks. Means \pm SD, $n = 3-5$.

TABLE I Equilibrium compressive modulus (H_{A0}) for hydrogels formulated using PEO of MW 100kDa and without cells. Means \pm SD, $n = 3$. For comparison, literature values of H_{A0} for BAC is also listed

Sample	H_{A0}
40 : 60 PEGDM : PEO Hydrogel	0.02 ± 0.00 Mpa
100 : 0 PEGDM : PEO Hydrogel	0.32 ± 0.07 Mpa
Bovine articular cartilage	$0.38^{[28]}$

Discussion

Chondrocytes from various sources (ovine articular, bovine articular, and bovine septal (results not shown)) can be cultured in the PEG-based hydrogel scaffolds and produce a cartilaginous matrix. The PEG-based hydrogel has been found to support the survival of chondrocytes and the deposition of a cartilage-like matrix uniformly throughout constructs having a nominal dimension of 6 mm. A more extensive investigation on chondrocyte survival in PEG-based hydrogels of varying thickness' from 2 to 8 mm by Bryant *et al.* [30] has also demonstrated that chondrocyte viability is readily maintained in constructs up to 8 mm thick. This group has also shown that the biochemical content does not vary significantly with hydrogel construct thickness or spatial position in the construct, as our histological findings suggest. The lack of a "necrotic core" or lack of a central region devoid of significant cartilage-like matrix in these hydrogel constructs is worthy to note for such a large construct. This is likely due to the uniform cell seeding that is achieved in the hydrogels, but may also be due to the cell's limited ability to proliferate excessively in the confined space of the hydrogel matrix, subsequently preventing the over production of ECM molecules in the exterior region of the constructs and creation of a diffusion barrier to nutrients. In contrast, the non-uniform spatial distribution of cartilage-like ECM that appears in prefabricated macroporous scaffolds that are cultured statically may be due to either a non-uniform cell seeding or over-proliferation and matrix deposition by the cells in the exterior of the constructs and adjacent to the medium [5]. It is believed that chondrogenesis is initiated in the periphery of these constructs due to the increased cell-to-cell contact, which has been shown to play a key role in initiating the differentiation cascade. Consequently, it may be responsible for the higher deposition rates of GAG and collagen type II in the exterior regions of these constructs [4, 31] thereby creating a diffusion barrier to nutrients.

We have found that cartilaginous constructs with a uniformly distributed matrix rich in sulfated-proteoglycans are produced with the PEG-based hydrogel using either the 20 kDa or 100 kDa PEO component and resulted in no significant differences in the amount of ECM deposited in the two formulations. These results suggest that the MW of the interpenetrating component does not affect the microporous space or crosslinking density in the hydrogel enough to affect the spatial distribution and amount of deposited extracellular matrix molecules for these constructs. It also allows flexibility in the viscosity of the polymer solution prior to UV polymerization. The viscous solution, formed when

using the PEO of MW 100 kDa, can be easily shaped under the skin prior to UV polymerization as demonstrated by Elisseff *et al.* [21] and should be applicable for cosmetic applications. On the other hand, the PEO of MW 20 kDa results in a free flowing liquid that is easily mixed and molded *in situ* or preformed for other orthopedic and craniofacial applications.

The ratio of the PEGDM to the PEO in these semi-interpenetrating networks is effectively controlling the amount of crosslinkable polymer in the hydrogel, since the PEO does not participate in the crosslinking and can eventually diffuse out of the hydrogel. As previously reported, as the polymer volume fraction of the swollen gels increases (or in our case as the component ratio or percent of the crosslinkable PEGDM component increases), the average molecular weight between crosslinks decreases (i.e. the crosslinking density increases) [32,33]. This also relates to the hydrogel mesh size, where increases in polymer volume fraction lead to decreases in mesh size [34]. Effectively, the crosslinkable polymer volume fraction controls the permeability of the hydrogel and diffusion of matrix molecules through the hydrogel, as demonstrated by Cruise *et al.*, with reduced diffusion of biological molecules with increasing concentrations of PEG-diacrylate in the hydrogel precursor solution [18].

In view of previously reported observations of hydrogel microarchitecture, the differences we have observed in spatial uniformity and total accumulation of matrix molecules may be explained by a decrease in hydrogel permeability for increasing PEGDM concentrations in the formulation. Therefore, it is not surprising that as the PEGDM:PEO ratio increased the spatial uniformity of proteoglycan matrix deposition decreased. Sulfated proteoglycans are large molecules (> 500 kDa) and when aggregated they can reach a MW as large as 100 000 kDa. Consequently, they may not be able to diffuse through the hydrogel beyond the cell's pericellular region for the highly crosslinked hydrogels. Cruise *et al.* estimated a mesh size of 22 Å for similar hydrogels prepared with 10% PEG-diacrylate of MW 4K and found that they were impermeable to proteins with a size equal to or larger than myoglobin (22 kDa) [18]. Our hydrogels are formulated with a PEGDM of MW 3.4 kDa and the 50:50 formulation essentially has a final concentration of PEGDM of 10% in the precursor solution. In addition, considering that the S-GAG production per cell is greatest for the 100:0 PEGDM:PEO hydrogel and the spaces between the cells did not stain appreciably with safranin-O, the matrix surrounding the cells in this hydrogel formulation is likely to be more densely packed. The matrix surrounding the cells in 100:0 PEGDM:PEO (Fig. 3G) also appears to have stained more intensely than the matrix surrounding the cells in the other formulations.

Extracellular matrix accumulation was normalized to the dry weight of the hydrogel constructs because the percentage of water in the hydrogels was found to decrease over increasing PEGDM concentration ($p=0.007$). Due to the large effective radius of the 20 kDa PEO (90.5 Å) [33], we do not expect the PEO to diffuse quickly from the hydrogels and thus, normalization of the ECM accumulation to dry weight should be

appropriate. The decrease in hydrogel permeability over increasing concentrations of the PEGDM component seemed to have affected the ability of the cells to proliferate in the hydrogel matrix, because we observed a decrease in cell number over increasing PEGDM concentration in the hydrogel. This in turn affected the total accumulation of the matrix molecules in the hydrogels. An exception to the decreasing matrix accumulation for increasing PEGDM:PEO ratio trends was our results for the hydrogel formulated with 20% PEGDM, in which we found a decrease in matrix accumulation compared to the 30:70 PEGDM:PEO hydrogels (Fig. 4). The probable reason for this lower matrix production may be explained by the well-known competing effects of cell proliferation and matrix deposition by cells. The extent of chondrocyte proliferation in the 20:80 PEGDM:PEO constructs was likely so great that the cells were not in a matrix production phase. The very high permeability of these hydrogels may also lead to, not only to diffusion of matrix molecules away from the cells, but out from the hydrogel itself.

Similar decreases in proteoglycan accumulation with increases in PEGDM:PEO ratios from 10:90 to 40:60 were found by Elisseff *et al.* when the hydrogels were injected subcutaneously in athymic mice and transdermally photopolymerized [21]. However, this group did not observe a statistically significant decrease in cellular and collagen content for the various formulations. The cause of these somewhat contradictory results is not clear, but may be related to the differences in the boundary conditions and other environmental factors influencing the results for these *in vivo* vs. *in vitro* studies.

Maximum ECM content was found in the 30:70 PEGDM:PEO hydrogel, resulting in total S-GAG and collagen values per dry weight of $24.4 \pm 3.5\%$ and $7.1 \pm 0.9\%$ respectively. When compared to the $44 \pm 20\%$ and $31 \pm 4\%$ S-GAG(dw) and collagen(dw), respectively, previously measured in-house for bovine articular cartilage, we find that our cell-embedded hydrogels have not yet reached native values.

As expected, the compressive moduli of the hydrogels increased with increasing amounts of the PEGDM. The 20:80 PEGDM:PEO hydrogel readily fell apart when handled while the 100:0 hydrogel held together very well and resulted in an equilibrium compressive modulus similar to native bovine articular cartilage [28]. As predicted by rubber elasticity theory, the compressive modulus of a swollen hydrogel is proportional to the crosslinking density [35]. It should be noted that our compressive results are for hydrogels photocrosslinked without cells and we would expect the compressive properties to decrease slightly with their addition, since cellular volume is not negligible and will decrease the final concentration of PEGDM in the hydrogel. Conceivably, if the hydrogel were formulated to degrade over the appropriate time, the degrading polymer would relinquish space that would simultaneously be filled in with ECM deposited by the cells. It may thus be possible to formulate a hydrogel construct that is initially as stiff as native cartilage at the onset, and that can maintain its compressive properties over time, finally resulting in a completely natural tissue.

As mentioned earlier, the ideal scaffold for tissue engineering is one that is degradable so that it acts as a temporary matrix for the organization of transplanted cells until deposition of the ECM by the cells forms a natural tissue. The next steps in this research are to explore a degradable derivative of the PEGDM and the effect of cell seeding density on matrix deposition. Combining this information with an understanding of how the MW of the PEO and the ratio of the crosslinking and non-crosslinking components can be varied to affect the matrix deposition and mechanical properties, we aim to develop appropriate tissue-engineered hydrogel constructs for a variety of indications.

Conclusion

The PEG-based hydrogel is a promising material for the tissue engineering of cartilage tissue. The ease with which cells can be embedded in the gel, the versatility of the material with respect to its viscosity prior to polymerization, and its mechanical properties following UV polymerization, makes the hydrogel appropriate for multiple indications. We have found that a cartilaginous matrix is produced in the hydrogel when either a 20 kDa or a 100 kDa PEO is used in the formulation. The ratio of the crosslinkable to non-crosslinkable components in the hydrogel affects both the uniformity of matrix deposition and the mechanical properties of the construct. Future work in optimizing the cell seeding density and degradation rate of a degradable hydrogel for enhanced matrix deposition and mechanical properties should result in a desirable formulation not only for cartilage applications but possibly for other tissue types as well.

Acknowledgments

The authors would like to thank Rebecca Calzo and Maria (Chelo) Abarro for the histological processing.

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Received 14 May
and accepted 21 May 2001