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The role of hydrogel structure and dynamic loading on chondrocyte gene expression and matrix formation

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Abstract

Crosslinked poly(ethylene glycol) (PEG) hydrogels are attractive scaffolds for cartilage tissue engineering because of their ability to mimic the aqueous environment and mechanical properties of native cartilage. In this study, hydrogel crosslinking density was varied to study the influence of gel structure and the application of dynamic loading (continuous, 1 Hz, 15% amplitude strain) on chondrocyte gene expression over ~1 week culture. Gene expression was quantified using real time RT-PCR for collagen II and aggrecan, the major cartilage extracellular matrix (ECM) components, and collagen I, an indicator of chondrocyte de-differentiation. When chondrocytes were encapsulated in PEG gels with low or high crosslinking, a high collagen II expression compared to collagen I expression (1,000 or 100,000:1, respectively) indicated the native chondrocyte phenotype was retained. In the absence of loading, relative gene expression for collagen II and aggrecan was significantly higher (e.g., 2-fold and 4-fold, respectively, day 7) in the low crosslinked gels compared to gels with higher crosslinking. Dynamic loading, however, showed little effect on ECM gene expression in both crosslinked systems. To better understand the cellular environment, ECM production was qualitatively assessed using an *in situ* immunofluorescent technique and standard histology. A pericellular matrix (PCM) was observed as early as day 3 post-encapsulation and the degree of formation was dependent on gel crosslinking. These results suggest the PCM may protect the cells from sensing the applied loads. This study demonstrates that gel structure has a profound effect on chondrocyte gene expression, while dynamic loading has much less of an effect at early culture times.

Keywords

Cartilage tissue engineering; chondrocyte; poly(ethylene glycol); hydrogel; gene expression; mechanical loading

1. Introduction

Articular cartilage provides a load bearing cushion between articulating bones and a nearly frictionless surface for smooth joint movement. Because the tissue has limited ability to self-repair, damage to cartilage can lead to severe pain and reduced joint mobility. Therefore, strategies have been developed to encapsulate cells into three-dimensional (3-D) scaffolds in an attempt to regenerate healthy functional tissues. However, a main challenge in cartilage

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engineering is regenerating tissue with the appropriate structure and mechanical integrity such that the new tissue can function long-term in the joint (Darling and Athanasiou 2003).

Mechanical loading has been identified as an important regulator of cartilage cells (i.e., chondrocytes) and overall tissue quality (Grodzinsky et al. 2000; Guilak et al. 2001; Li et al. 2001). The role of mechanical loading on chondrocyte function has been examined in cartilage explants (Kim et al. 1995; Davisson et al. 2002), high density cell pellets (Graff et al. 2003), and 3-D scaffolds (Mauck et al. 2000; Bryant et al. 2004b; Hunter et al. 2004; Kisiday et al. 2004; Schmidt et al. 2006). Studies have shown that mechanical stimulation can enhance tissue production (Mauck et al. 2002; Lee et al. 2003), or lead to increased catabolic activity and decreased tissue production (Bonassar et al. 2000; Upton et al. 2003; Bryant et al. 2004b; Hunter et al. 2004). For example, when a 1 Hz unconfined dynamic strain of 15% was applied to chondrocytes encapsulated in two different hydrogels for 48 hours, proteoglycan synthesis increased by 40% in agarose (Lee and Bader 1997), but decreased by 10% in PEG gels (Bryant et al. 2004b). These contradictory results indicate that the 3-D environment surrounding the cells plays an important role in translating the biomechanical signals to the cells.

Photopolymerized poly(ethylene glycol) (PEG) hydrogels have shown tremendous promise as chondrocyte carriers for cartilage regeneration (Elisseeff et al. 2000; Nguyen and West 2002; Bryant et al. 2003). The mild photopolymerization conditions allow for *in vivo* scaffold formation (Elisseeff et al. 1999), while hydrogels in general provide an aqueous-rich medium for solute diffusion and cell support. One of the advantages of synthetic systems is the ability to control the final structure, which influences many of the macroscopic gel properties (Elisseeff et al. 2000; Riley et al. 2001; Bryant et al. 2003). When encapsulated in PEG hydrogels with compressive moduli (i.e., 260-900 kPa) similar to that of human articular cartilage (i.e., 790 kPa $\pm$ 360 (Armstrong and Mow 1982)), chondrocytes remain viable and synthesize cartilage specific ECM (Bryant and Anseth 2002). Although these systems have been well characterized as potential scaffolds for cartilage regeneration, only a few studies have examined cell response within these scaffolds under dynamic loading (Bryant et al. 2004b; Schmidt et al. 2006).

Bryant *et al.* demonstrated that when chondrocytes are encapsulated in PEG hydrogels and subjected to compressive strains, the crosslinking density dictates the level of cell deformation (Bryant et al. 2004a), one mechanism by which chondrocytes sense mechanical forces (Guilak et al. 1995). When adult bovine articular chondrocytes were encapsulated in PEG hydrogels and subjected to dynamic loading conditions for 2 days, cell proliferation and proteoglycan synthesis were inhibited and this inhibition was greater in gels with higher crosslinking (Bryant et al. 2004b). This study illustrated that the combination of gel crosslinking and loading impact tissue growth.

Our long-term goal is to design biodegradable PEG hydrogels that when combined with mechanical loading promote functional cartilage growth. Towards this goal, the present study investigates the role of gel crosslinking and dynamic loading on gene regulation of and early tissue formation by immature chondrocytes encapsulated in PEG gels at early culture times (~1 week). We hypothesize that crosslinking density and dynamic loading will dramatically affect chondrocyte response (via gene expression). In an effort to elucidate the effects of gel structure on early chondrocyte response, PEG gels were chosen, which do not exhibit temporal changes in the gel structure over the course of the experiment. In addition, we chose a continuous, dynamic loading regime based on previous studies, which found that continuous loading at physiological levels enhanced tissue deposition compared to unloaded constructs (Lee and Bader 1997; Davisson et al. 2002; Lee et al. 2003). Intermittent loading regimes have also been examined (Mauck et al. 2000; Chowdhury et al. 2003) and will be explored in future studies.

2. Materials and Methods

2.1 Hydrogel formation

Poly(ethylene glycol) dimethacrylate (PEGDM) macromer was synthesized by reacting methacryloyl chloride to PEG (Fluka, MW=3kDa) in the presence of triethylamine in dichloromethane for 24 hrs at 4°C and purified by precipitations in ethyl ether (Sawhney et al. 1993). The percent methacrylation was 90-95% and purity was >99% as determined by ¹H NMR (Varian YVR-500S). Hydrogel cylinders (5mm × 5 mm) were fabricated by dissolving 10 or 20% (w/w) PEGDM in phosphate buffered saline (PBS, pH 7.4) with cytocompatible photoinitiator concentrations of 0.05 or 0.0125% (w/w) 1-[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (Ciba Specialty Chemical), respectively, and exposing to 365 nm light (6 mW/cm²) for 10 minutes (Bryant et al. 2000).

2.2 Hydrogel Characterization

Cell-free hydrogels (n=4) were swollen in PBS+1% penicillin-streptomycin (PBS+P/S) for 48 hours, weighed to determine the equilibrium swollen mass (M_S) and lyophilized to determine the dry polymer mass (M_D). The volumetric swelling ratio, Q , was calculated from the equilibrium mass swelling ratio (M_S/M_D) and gel crosslinking density (ρ_x) was determined using a modified version of the Flory-Rehner equation neglecting chain ends (Bryant et al. 2004b). Tangent modulus was determined on swollen constructs by applying a constant strain rate under unconfined compression (0.5 mm/min) (MTS Synergie 100, 10N).

2.3 Chondrocyte isolation and encapsulation

Cartilage was harvested from the patellar-femoral groove of 1-3 week old calves (n=2) (Research 87) within 24 hours of slaughter. The tissue was digested in 500 units/mL collagenase II (BD Worthington) in Dulbecco's minimal essential medium (DMEM, Invitrogen) supplemented with 5% fetal bovine serum (FBS, Invitrogen) for 16 hours at 37°C. Isolated chondrocytes were washed and resuspended in chondrocyte media (DMEM supplemented with 20% FBS (v/v), 0.04 mM L-proline, 50 mg/L L-ascorbic acid, 10 mM HEPES buffer, 0.1 M MEM-nonessential amino acids, 1% penicillin-streptomycin, 0.5 µg/mL fungizone, and 20 µg/mL gentamicin (Invitrogen)). Chondrocytes were added to sterile macromer/photoinitiator solutions at 50×10^6 cells/mL and photopolymerized as described above. Cell-hydrogel constructs were pre-conditioned under free swelling conditions for 48 hours at 37°C and 5% CO₂ in chondrocyte medium prior to loading in the loading apparatus.

2.4 Mechanical Stimulation

Multiple custom-built loading apparatuses (Fig. 1), which fit into a standard incubator, were used and are described elsewhere (Villanueva et al. 2008). Each apparatus holds a standard 24-well tissue culture plate in which each well is associated with an impermeable loading platen, of which 12 samples are subject to continuous, unconfined dynamic 15% strain at 1 Hz, applied from 0 to 750 µm in a sinusoidal waveform, while 12 samples serve as unloaded controls. Gels were submersed in 2 mL of chondrocyte media, refreshed every 48 hours. After the pre-conditioning period (labeled time 0), samples were then loaded into the apparatus for 1, 3, 5, and 7 days. Cell viability was qualitatively assessed using a live/dead assay (Invitrogen) post-encapsulation and after 7 days of loading. Cell viability was high with no observable differences temporally or between the 10% and 20% gels.

2.5 Gene Expression

Loaded and control constructs were removed from the bioreactor at specific time points (n=3 per condition) and immediately frozen in liquid nitrogen. RNA was extracted by TRI Reagent (Sigma) (Chomczynski 1993) and reverse transcribed using the iScript cDNA Synthesis Kit

(Biorad). Real-time RT-PCR (Bio-Rad iCycler4) was performed using custom designed and validated sense and anti-sense primers, which are described elsewhere (Nicodemus et al. 2007), and iQ™ SYBR® Green Supermix (Bio-Rad). Data are presented as

$$\text{Relative Expression} = \frac{E_{(\text{HKG})}^{C_{T(\text{HKG})}(\text{sample})}}{E_{(\text{GOI})}^{C_{T(\text{GOI})}(\text{sample})}} \text{ or as Normalized Expression} = \frac{E_{(\text{GOI})}^{\Delta C_{T(\text{GOI})}(\text{calibrator-sample})}}{E_{(\text{HKG})}^{\Delta C_{T(\text{HKG})}(\text{calibrator-sample})}}$$

where E is the efficiency of the gene amplification, GOI is the gene of interest, HKG is the housekeeping gene, and C_T is the cycle threshold point. For normalized gene expression, the loaded samples were normalized to their respective unloaded controls (referred to as calibrator). Efficiency was determined using cDNA dilutions over 4 orders of magnitude, and ranged from 88-110%. L30, a mitochondrial ribosomal protein, was used as the HKG (Galois et al. 2006) because it was stable throughout the experiment (unpublished data).

2.6 Expression and Visualization of Extracellular Matrix Molecules

Loaded and control constructs were removed from the bioreactor at specific time points (n=3 per condition) and cut in half along their horizontal axis. Half was used for histology and half was used for immunofluorescent staining. For the *in situ* immunofluorescent technique, construct halves were cut again along their vertical axis and placed in PBS supplements with 0.5 Units/mL Chondroitinase ABC (Sigma) and 1% BSA (Sigma) to expose the chondroitin-6-sulfate epitope. Samples were treated for one hour with anti-chondroitin-6-sulfate (clone MK-302, Chemicon) (1:50) in DMEM+20% FBS, rinsed twice with Earle's Balanced Salt Solution (EBSS, Gibco) and placed for one hour in DMEM+20% FBS with goat anti-mouse IgG labeled with Alexa Fluor 546 (1:20) (Invitrogen). Live cells were counterstained with 5 μ M calcein AM (Invitrogen). Images were captured in 3 different regions of interest (middle of gel) for a total of 15 cells per condition using confocal microscopy (Zeiss LSM 5 Pascal). The remaining hydrogel half was fixed in paraformaldehyde, dehydrated, embedded in paraffin, sectioned (10 μ m), and stained for negatively charged glycosaminoglycan (GAGs) molecules using Safranin-O/Fast Green and the cell nuclei were counterstained using hemotoxylin.

2.7 Statistical Analysis

Data are represented as mean $\pm$ standard deviation. Relative and normalized gene expression values were analyzed using one-way analysis of variance (ANOVA) and Tukey post-hoc to determine the statistical significance as a function of culture time for control and dynamically loaded samples in both crosslinked systems. Multiple factor ANOVA was performed using a general linear model with Tukey multiple comparisons to determine the significance of culture time, gel crosslinking and loading and their interactions on gene expression. A confidence level of 0.05 was considered significant.

3. Results

Hydrogels were fabricated from 10% and 20% (w/w) PEGDM to form low and high crosslinked gels, respectively and their macroscopic properties are listed in Table 1. Doubling the macromer concentration resulted in an increase in gel crosslinking density, a 48% decrease in equilibrium swelling ratio, and 850% increase in compressive modulus.

Gene expression for collagen II and aggrecan, two common ECM molecules of hyaline cartilage, and collagen I, a chondrocyte de-differentiation indicator, were analyzed as a function

of gel crosslinking density in the absence of loading (Fig. 2). Collagen I was expressed 1,000 and 100,000 times less than collagen II in the low and high crosslinked gels, respectively, indicating that the native chondrocyte phenotype was maintained. Aggrecan expression was significantly less compared to collagen II for each crosslinked system over the culture period. All genes were significantly down-regulated in high crosslinked gels compared to low crosslinked gels ($p < 0.001$).

Gene expression was also analyzed as a function of dynamic loading and gel crosslinking (Fig. 3a-c). Similar to the unloaded samples, all genes were expressed higher in the low crosslinked gels compared to the high crosslinked gels over the course of the experiment ($p < 0.001$). Interestingly, a down-regulation of collagen II and aggrecan was observed at day 3 in the low crosslinked gels. However by day 5, expression levels recovered to levels in the pre-conditioned samples. In the higher crosslinked gels under dynamic load, no statistically significant temporal differences in gene regulation for collagen II and aggrecan were observed.

To isolate the effects of loading on chondrocyte response, gene expression of the dynamically loaded hydrogels were normalized to their respective unloaded samples at each time point (Fig. 3d-f). In the low crosslinked gels at day 3, loading resulted in a 4-fold down-regulation in collagen II and a 2-fold down-regulation of aggrecan. By day 7, there were no differences in gene expression between the unloaded and loaded gels. In the higher crosslinked gels, no significant differences were observed due to loading.

A multiple factor ANOVA revealed that crosslinking density affected aggrecan and collagen I and II ($p < 0.001$) expression, culture time had a significant affect on collagen I ($p < 0.01$) and aggrecan ($p < 0.05$) expression, but no affect on collagen II expression ($p = 0.09$), and dynamic loading had no significant effect on the genes examined. A significant interaction was noted between culture time and crosslinking density for all genes examined ($p < 0.01$).

Based on our findings that the application of dynamic loading had little effect on the expression ($p > 0.05$) of the genes examined here, we hypothesized that the formation of a pericellular matrix (PCM) may protect the cells from the biomechanical environment. We assessed the formation of the PCM through staining for proteoglycans using two approaches: a live *in situ* immunofluorescent approach (Knight et al. 1998) and standard histology. The former approach allows for real-time imaging of live cells and their surrounding matrix within the hydrogel in the hydrated state, while the latter approach necessitates fixation of the specimen.

The presence of a PCM, as determined by positive staining for chondroitin-6 sulfate, in the unloaded, low crosslinked gels was observed as early as day 1 by immunofluorescent staining (Fig. 4). By day 3 a uniform PCM with a mean thickness of $5.7 \pm 1.1 \mu\text{m}$ surrounded most of the cells and diffusion of proteoglycans (PGs) away from the cells was visualized by day 5. In contrast, the loaded samples showed fewer cells with a PCM at day 1 but by day 3, a loosely ill-defined PCM was present. In the high crosslinked gels, no differences were observed in PCM formation between the unloaded and loaded gels over time. The mean PCM thickness was $2.9 \pm 1.0 \mu\text{m}$. (Fig. 4) with no evidence of PG diffusion in the extracellular space.

Representative histological micrographs for the low and high crosslinked gels at day 1 and day 7 are shown in Fig. 5, where negatively charged proteoglycans are stained red. At day 1, little staining was present in the ECM of both gels. With increased culture time, dark staining was present throughout the construct in the low crosslinked gels indicating that proteoglycans had diffused into the extracellular space (Fig. 5b,f). However, in the higher crosslinked gels, proteoglycans were restricted to the immediate vicinity surrounding the cells with no staining present in the bulk hydrogel (Fig. 5d,h). There were no differences observed as a function of loading. It is important to note that our studies are restricted to following development of proteoglycans, specifically associated with chondroitin-6-sulfate.

4. Discussion

When isolated chondrocytes are encapsulated in PEG hydrogels, they begin synthesizing tissue-specific molecules that form a PCM and eventually diffuse into the extracellular space to form a macroscopic tissue. Previous studies have demonstrated that increasing crosslinking density of PEG hydrogels results in decreased diffusion of ECM molecules through the hydrogel (Bryant and Anseth 2002), which is supported further by the observations seen here (Fig. 5). As a result, matrix components are entrapped and restricted to the pericellular regions in gels with high crosslinking. Here, we demonstrate that crosslinking density impacts the evolution of the PCM, resulting in a thin and dense PCM in gels with high crosslinking (Fig 4i-p) and a thick and diffuse PCM in gels with low crosslinking (Fig. 4a-h). These differences in PCM development, that result due to differences in gel crosslinking, are likely to impact both the biochemical and biomechanical environment surrounding the chondrocytes (Guilak et al. 2006).

In cartilage, chondrocytes interact with their surrounding environment to receive biochemical cues from the PCM and ECM. These interactions are essential in the regulation of tissue development and maintenance (Adams and Watt 1993; Rosso et al. 2004). In this study, we demonstrate that, in the absence of loading, the PCM forms within 3 days post cell encapsulation regardless of the gel structure (Fig 4a,i), but that the physical structure of the PCM is highly dependent on the gel crosslinking. Interestingly, gene expression of collagen I and II and aggrecan was significantly reduced in gels that exhibited a thin, dense PCM, associated with the high crosslinked gel, compared to gels exhibiting a thick, diffuse PCM, associated with the low crosslinked gel. These results suggest a potential biochemical regulation of the cells possibly through differences in the physiochemical nature of the PCM (e.g., matrix diffusion limitations in the higher crosslinked gels may lead to differences in PCM composition) or through differences in the diffusivity properties of the PEG hydrogel (Lu and Anseth 2000), which may impact diffusion of growth factors or other signaling molecules involved in gene expression.

Furthermore, assessment of temporal changes in ECM gene expression within the low crosslinked gels suggests a potential link to matrix development through outside-in signaling. Specifically, our studies illustrate a down regulation in collagen II and aggrecan gene expression at day 5 in the control samples and at day 3 in the loaded samples, but by day 7 gene expression levels returned to their previous values. It is possible that the formation of the PCM and/or the initial evolution of ECM may regulate gene expression through changes in cell-matrix interactions (Boudreau et al. 1995; Larson et al. 2002) and/or through binding, sequestering and/or activation of signaling molecules important in gene regulation (Behonick and Werb 2003). As matrix molecules, such as aggrecan, are deposited into the PCM they undergo modification before depositing into the ECM regions (Sandy et al. 1989). This process may alter the biochemical composition of the PCM as tissue initially forms. Together these changes may influence the biochemical regulation of the cells (Guilak et al. 2006). Interestingly, a down-regulation was not observed in gels with higher crosslinking in which matrix diffusion was restricted. Additional studies are needed to elucidate the exact mechanisms of gel structure and PCM development on chondrocyte gene regulation.

In addition to biochemical regulation, the PCM is likely to have an important biomechanical function in chondrocytes. Previous studies have shown that when the PCM forms around chondrocytes encapsulated in PEG or agarose hydrogels, the PCM provides a mechanically protective barrier around the cells, reducing cellular strain (Knight et al. 1998; Knight et al. 2002; Bryant et al. 2004a). For example, when chondrocyte-laden PEG hydrogels of different crosslinking densities were subjected to compressive strains, cell deformation was highest in gels with the highest crosslinking density, but as the PCM developed cell strain was

significantly reduced. This reduction was more significant in gels with higher crosslinking density indicating a stiffer PCM (Bryant et al. 2004a). These studies demonstrate that evolution of the PCM directly influences the degree of cell deformation. In this study, dynamic loading hindered PCM development in the low crosslinked gels during the first day of loading, but did not affect PCM development in the high crosslinked gels. It is possible that initially (in the absence of a PCM) the higher levels of cellular strain imparted on the cells by the higher crosslinked gel results in a greater need for a protective PCM. Alternatively, loading may enhance diffusion of matrix molecules away from the cells in the low crosslinked gels delaying the formation of the PCM, while in the high crosslinked gels matrix deposition is restricted to the pericellular regions. Once the PCM forms, it appears to protect the cells from the applied loads and not affect gene regulation, although it is possible that loading may have influenced protein synthesis and deposition, which was not studied here.

Our findings suggest that mechanical loading influences PCM formation during initial culture periods and has only a minor impact on gene expression. Crosslinking density, however, plays a major role in PCM evolution and development and gene regulation. The formation of the PCM may be the initial innate function of isolated chondrocytes (Chang and Poole 1997), and that changes in the PCM structure and/or composition may significantly impact chondrocyte gene expression (Zhang et al. 2006; Kanbe et al. 2007). Additional studies are necessary to elucidate the PCM composition and to better understand the impact of “outside-in” signaling on chondrocyte function during early tissue development. Furthermore, diffusivity properties of the hydrogel must also be considered in examining cell carriers with different crosslinking densities. The question remains as to the long-term effects of this early gene regulation by gel crosslinking density and PCM formation. A recent study by Lima *et al.* suggested that delayed loading may be more influential in controlling cell response long-term than loading immediately (Lima et al. 2007). The pre-conditioning time period used here (48 hours) may need to be extended to enable sufficient tissue to develop and aid in cellular acclimatization to their new environment prior to loading.

In summary, we demonstrate that simple changes in the gel structure affect chondrocyte gene expression, PCM formation and matrix evolution. Dynamic loading may influence chondrocyte response at very early time points where limited PCM and ECM has been produced. Our results indicate that loading affects PCM formation in the low crosslinked gels; although we did not capture changes in the high crosslinked gels at the time points investigated. Despite initial changes in gene regulation, continuous dynamic loading at 1 Hz and 15% strain interestingly, did not affect ECM expression as the conclusion of this experiment. Matrix synthesis, however, may be affected over long-term culture. We are currently investigating delayed and/or intermittent loading regimes.

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Figure 1.

Custom built mechanical loading bioreactor (a) that utilizes a stepper motion and lifting mechanism (b) connected to a loading platform that is equipped with impermeable loading pins sitting above a standard 24-well tissue culture plate (c). A total of 12 samples are subjected to unconfined compressive strains and the remaining 12 samples serve as unloaded controls where the pins rest on the constructs. Motion is open-loop controlled with a resolution of 1 μm using a unique “elbow” type bend that translates stepper motor motion into axial displacement, which is measured using the built-in LVDT (Villanueva et al. 2008).

Figure 2.

Relative gene expression for collagen I (a), collagen II (b), and aggrecan (c) compared to the housekeeping gene L30 for the low and high crosslinked PEG hydrogels in the absence of loading. Asterisks above a column indicate a significant difference between the low and high crosslinked gels at a given time point. * $p < 0.05$, ** $p < 0.01$.

LEGEND: Day 0 (pre-conditioned) ■, Day 1 ■, Day 3 ■, Day 5 ■, Day 7 □.

Figure 3.

Relative gene expression for collagen I (a), collagen II (b), and aggrecan (c) compared to the housekeeping gene (HKG) L30 for the low and high crosslinked PEG hydrogels subject to dynamic loading at 1.0 Hz dynamic with 15% strain. Normalized expression for collagen I (d), collagen II (e), and aggrecan (f) in loaded samples compared to unloaded samples at the same time point for the low and high crosslinked PEG hydrogels. For panels a-c, asterisks above a column indicate a significant difference between the low and high crosslinked gels at a given time point. For panels d-f, asterisks above a column illustrate a significant difference between loaded and unloaded samples at a given time point and gel crosslinking. * $p < 0.05$, ** $p < 0.01$.

Figure 4.

Laser scanning confocal microscopy images of articular chondrocytes encapsulated in low (a-h) and high (i-p) crosslinked hydrogels. The PCM was stained red for proteoglycans (using anti-chondroitin-6-sulfate) and the cytosol of live chondrocytes stained green. Hydrogels were cut in half prior to staining to capture matrix formation in the central regions of the gel. Images were acquired at the edge of the cut surface, however, due to limited diffusion of the antibody, the PCM in many cases was void of a live cell. However, high cell viability was observed just past the cut surface, but beyond the limit of antibody diffusion. Due to these limitations, day 1 images were taken approximately one cell layer deeper than other images. Images were taken at day 1 (a, e, i, m), day 3 (b, f, j, n), day 5 (c, g, k, o), and day 7 (d, h, l, p) for both unloaded controls (a-d, i-l) and hydrogels subjected to dynamic loading at 1.0 Hz dynamic with 15% strain. (e-h, m-p). Original magnification is 400x. For the inset image, original magnification was enlarged 4x. Scale bar represents 20 μm .

Figure 5.

Histological images of articular chondrocytes encapsulated in low (a, b, e, f) and high (d, g, h) PEG hydrogels. Images were taken at day 1 (a, e, c, g) and day 7 (b, d, f, h) for both unstrained controls (a-d) and under dynamic 15% strain at 1 Hz (e-h). Proteoglycans were stained red using Safranin O/Fast green. Original magnification is 400x. For the inset image, original magnification was enlarged 4x. Scale bar represents 20 μm . Symbol (*) represents extracellular regions that stained positive for sulfated glycosaminoglycans, where symbol (+) represents regions that had little to no staining for sulfated glycosaminoglycans.

Table 1

Properties of PEG hydrogels

Hydrogel	PEGDM % (w/w)	Crosslinking Density (mol/L)	Swelling Ratio (Q)	Water Content (%)	Compressive Modulus (kPa)
LOW CROSSLINKED	10	0.10 ± 0.007	12.0 ± 0.4	91.7 ± 0.3	70 ± 10
HIGH CROSSLINKED	20	0.40 ± 0.016	6.3 ± 0.1	84.2 ± 0.3	600 ± 60