Tuning Heterogeneities into Photoclickable Synthetic Hydrogels for Improved Cartilage Tissue Engineering

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Statement of Purpose: Hydrogels are promising for *in situ* delivery of chondrocytes (i.e., cartilage cells) and cartilage tissue regeneration. However, tuning degradation is challenging because newly secreted extracellular matrix (ECM) molecules cannot diffuse through the crosslinked mesh until the hydrogel network reaches reverse gelation, but reverse gelation causes a loss of mechanical integrity. We have developed a computational model that predicts when cells are homogeneously distributed in a hydrogel with uniform crosslinks and which degrades by bulk degradation, it is not possible to achieve ECM elaboration without complete loss of mechanical integrity. However, we and others have reported ECM growth in hydrolytically-labile hydrogels. These observations point to the presence of spatial heterogeneities in the hydrogel construct. This study uses a combined experimental and computational approach to identify potential mechanisms that contribute to spatial heterogeneities. Once identified, these heterogeneities can be manipulated to improve ECM growth and overall construct mechanical properties.

Methods: Bovine chondrocytes were used as a model cell. Freshly isolated articular chondrocytes (1-3 week old calves) were encapsulated in photoclickable hydrogels. Hydrogels were prepared from either 10 wt% PEG-CAP-NB (8-arm$_{20k}$PEG-caprolactone$_{1.26}$-norbornene) and PEG$_{1k}$dithiol at 1:1 thiol:ene ratio or from 9 wt% PEG-norbornene (8-arm$_{20k}$PEG-NB), MMP-2 sensitive CVPLSLYSGC at 1:1 thiol:ene ratio, and 1 wt% chondroitin sulfate (ChS) under cytocompatible photoinitiating conditions to produce hydrogels sensitive to hydrolysis or enzymes, respectively. Chondrocytes were encapsulated at 50, 100, or 150 million (M) cells/mL and cultured either under free swelling conditions or under dynamic compressive loading (5% strain, 1 Hz, 1hr/day). Hydrolytically-sensitive hydrogels were cultured in chondrocyte growth medium with 10% FBS. Enzyme-sensitive hydrogels were cultured in defined medium (DMEM/F12 supplemented with 1% ITS+ Premix and 5 ng/mL TGFβ3). Freshly isolated chondrocytes were also incubated with fluorescently-labeled PEG$_{1k}$dithiol followed by centrifugation washes and assessed by flow cytometry. Cell-laden constructs were assessed for compressive modulus, viability, DNA, sulfated glycosaminoglycan (sGAG), total collagen (hydroxyproline), enzyme activity, ECM deposition (immunohistochemistry) for collagen II and aggrecan over time. A multiscale computational approach[1] that spans three characteristic length scales was used to understand the coupled effects of hydrogel degradation and ECM elaboration.

Results: Two observations where made when chondrocytes were encapsulated in hydrolytically-sensitive hydrogels. First, the cells were not evenly distributed throughout the crosslinked network with more cell clusters appearing at higher seeding densities (Fig. 1A). Second, the compressive modulus of the cell-laden hydrogels decreased significantly with increasing cell seeding density (Fig. 1B). The decrease in modulus could not be explained solely by the presence of softer (~1 kPa modulus) cells. Previous studies have indicated that chondrocytes interact with radicals during photoencapsulation; thus potentially lowering crosslinking efficiency around the cell[2]. We further demonstrate here that chondrocytes interact with PEG-dithiol and thus may further lower crosslink efficiency. By introducing cell clusters and spatial variations in crosslinking into the computational model, we are able to describe the drop in mechanical properties.

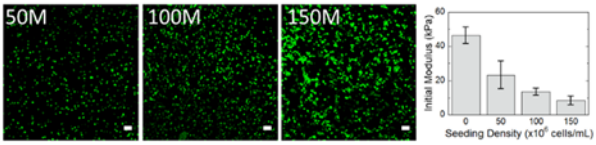

Figure 1. Chondrocytes were encapsulated in hydrolytically-sensitive hydrogels at 50, 100, and 150 million cells/ml. Representative confocal microscopy images 24 hours post-encapsulation (live cells=green; dead cells=red; scale bar=50 μm. Initial compressive modulus (E_o) of cell-laden hydrogels (n=3, mean and error bars represent standard deviation.

These heterogeneities help to explain the observed ECM growth and construct mechanical properties (Fig. 2). At week 4 with low encapsulation density (50M), some constructs had degraded while others had not, resulting in a decreased modulus. With higher cell encapsulation densities (100&150M), constructs have degraded and formed connected matrices of new tissue concomitant with higher modulus. Computational results support these phenomenon, but only with spatial heterogeneities.

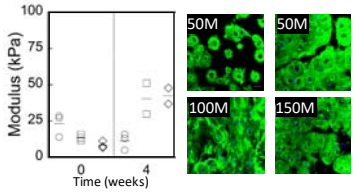

Figure 2. Modulus of cell-laden hydrogels at week 0 and 4. Spatial distribution of collagen II (green) and nuclei (blue) at week 4 for cells encapsuated at 50, 100, 150 million (M) cells/ml.

We also identified spatial heterogeneities in MMP-2 sensitive hydrogels evident by cell clustering from confocal microscopy images of live cells in the MMP-2 sensitive hydrogel at 100M cell density. We are currently assessing ECM growth and modulus under loading.

Conclusions: Spatial heterogeneities in the form of cell clustering and variations in hydrogel crosslink density were identified for chondrocytes photoencapsulated in synthetic-based degradable hydrogels. These hetero-geneities are necessary to promote ECM growth and minimize loss of mechanical properties. This knowledge can be used to develop novel strategies that induce spatial heterogeneities to improve cartilage tissue engineering.

References: [1] Akalp A. Soft Matter 2016;12:7505-7520. [2] Farnsworth N. Osteoarthritis Cartilage 2012;20:1326-1335.

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