Cell-mediated degradable hydrogels tailored to adult cells for cartilage tissue engineering

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Statement of Purpose: Photopolymerizable and degradable poly(ethylene glycol) (PEG) hydrogels are promising in situ cell carriers for cartilage tissue engineering. Degradation is particularly important to provide space for extracellular matrix deposition and macroscopic tissue growth, yet matching degradation with neotissue deposition is challenging in part due to variability that exists among donors (e.g., due to age). Cell-mediated enzymatic degradation may offer the ability to spatio-temporally match neotissue deposition. This research set out to characterize the catabolic activity of cartilage cells (chondrocytes) as a function of donor age and then to use this knowledge to design more appropriate enzymatically-degradable hydrogels for cartilage tissue engineering. Specifically, enzymes that degrade aggrecan (matrix metalloproteinases (MMPs) and aggrecanases) were investigated because aggrecan has a relatively high turn over in cartilage. It was hypothesized that hydrogels tailored to the enzyme activity of chondrocytes would exhibit increased matrix production and more extensive spatial deposition.

Methods: Catabolic activity was investigated using a simple hydrolytically degradable hydrogel, which was formed by photopolymerizing in the presence of a photoinitiator a 15 wt% solution of oligo(lactic acid)-PEG_{4600}-oligo(lactic acid) dimethacrylate with an average of 2-3 lactic acid repeats per side. Juvenile (1 mo) and adult (1-2 yr) primary bovine articular chondrocytes were encapsulated in hydrogels at $20x10^6$ cells/mL and cultured through 4 weeks. Catabolic activity was assessed by Western blot analysis of degraded protein by probing with primary antibodies against aggrecan neoepitopes ARG and FFGV (MDBioproducts). Enzymatically-degradable hydrogels were formed by photopolymerizing a 10 wt% solution of PEG_{3400}-[peptide]-PEG_{3400} diacrylate. Peptide sequences were GTEGE-ARGSK, GIPEN-FFGVK, and scrambled sequences of each, synthesized on an ABI peptide synthesizer and purified by HPLC. PEG macromer was formed by reacting monoacrylate-PEG_{3400}-succinimidyl valerate (Laysan Bio) with peptides. Adult bovine chondrocytes were encapsulated at $20x10^6$ cells/mL and cultured in serum-free medium containing hgDMEM, 1% ITS+premix, 50 µg/ml L-ascorbic acid 2-phosphate, 40 µg/ml L-proline, 0.1 µM dexamethasone, 110 µg/ml sodium pyruvate, and antibiotics. Paraffin-embedded sections were probed with anti-collagen II.

Results: Western blot analysis of juvenile and adult chondrocytes revealed that aggrecan catabolism is elevated by adult cells in a 3D environment (Figure 1). While the MMP-generated FFGV fragment, which is seen primarily in normal tissue homeostasis[1], was present in constructs with both juvenile and adult cells, the aggrecanase-generated ARG fragment, which is more abundant in osteoarthritis[2], was only detected in hydrogels with adult cells.

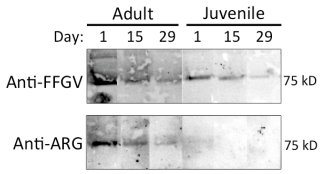

Figure 1. Western blot detection of aggrecan fragments cleaved by MMPs (anti-FFGV), or aggrecanases (anti-ARG), in cell-laden hydrogels as a function of cell age and culture time.

Immunohistochemical analysis of adult cells encapsulated in enzyme-degradable hydrogels (Figure 2) revealed that collagen deposition is increased in degradable hydrogels, and that the choice of degradable sequence affects the spatial deposition of collagen II.

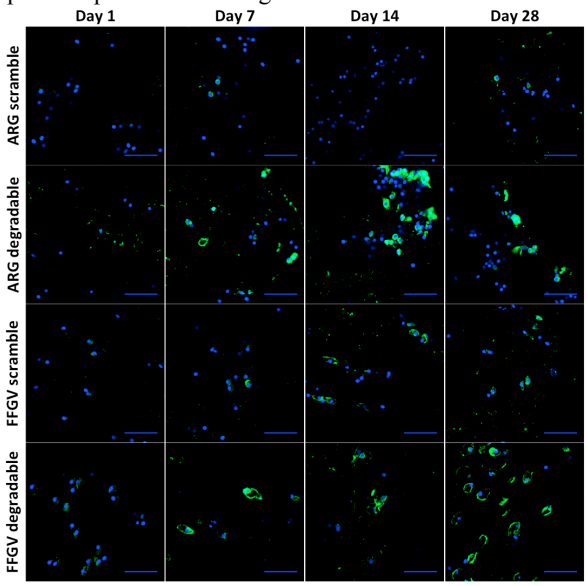

Figure 2. Collagen II (green) deposition in degradable or non-degradable (scrambled sequence) constructs. Nuclei are counterstained blue with DAPI. Scale bars represent 50 µm.

Conclusions: This work demonstrates the design of enzyme-sensitive hydrogels for cartilage engineering, which are tailored to the activity of the cell. We first verified elevated catabolic activity in adult chondrocytes, and the identified degradation sites on aggrecan were incorporated into the design of enzymatically-sensitive hydrogel crosslinks. Preliminary analysis suggests that enzymatically-sensitive hydrogels improve tissue deposition, and that the choice of degradable sequence may influence neotissue morphology. Further studies are underway to investigate behavior of juvenile cells in enzyme-sensitive hydrogels and to characterize gel degradation. This information will be used to further refine a bioresponsive hydrogel environment that encourages tissue elaboration by adult chondrocytes.

References: 1) Mok SS. J Biol Chem. 1994;269:33021-7. 2) Lohmander LS. Arthritis Rheum. 1993;36:1214-22.

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