Characterization of Aggrecan Retention in Fumarate-Based Hydrogels for Orthopaedic Tissue Engineering

Kelly S. Brink, Jeremy J. Lim, Jason D. Weaver, Derek M. Doroski, Johnna S. Temenoff
Coulter Department of Biomedical Engineering, Georgia Tech and Emory University, Atlanta, GA, USA

Introduction: Our laboratory is investigating a novel hydrogel system based on oligo(poly(ethylene glycol) fumarate) (OPF) as a carrier for ligament fibroblasts and marrow stromal cells (MSCs) to aid regeneration of orthopaedic tissues. Aggrecan is a large proteoglycan found in the extracellular matrix (ECM) of articular cartilage and fibrocartilaginous tissues such as the menisci and ligament insertion points (Waggett AD. Matrix Biol. 1998;16:457-70). Recent evidence suggests that the presence of this molecule can increase the production of cartilaginous ECM by dermal fibroblasts (French, MM. Ann Biomed Eng. 2004;32:50-6). In order to elucidate the effects of aggrecan on cells relevant to both cartilage and fibrocartilage tissues, first, the morphology of bovine ligament fibroblasts and MSCs on aggrecan-coated plates was examined *in vitro*. Subsequently, aggrecan was non-covalently incorporated into OPF biohybrid scaffolds and its retention within these hydrogels was monitored over one month *in vitro*.

Methods: For the cell culture studies, aggrecan (Sigma) was suspended in phosphate buffered saline (PBS), placed in 24-well tissue culture plates and allowed to evaporate in a sterile environment. Bovine MSCs and ligament fibroblasts were then plated at $4.4x10^4$, $8.8\ x10^4$, and $2.0\ x10^5$ cells/well and wells were monitored via light microscopy over ~2 weeks for changes in morphology as compared to controls plated at the same density on standard tissue culture poly(styrene) surfaces.

For the formation of the biohybrid hydrogels, OPF 10K (original poly(ethylene glycol) (PEG) nominal Mn ~10,000) was combined with a PEG-diacrylate crosslinker (PEG-DA, nominal Mn 3,400) in the ratio 1 OPF: 1 PEG-DA by weight (~20% of total initial hydrogel weight was polymer). Aggrecan was then included at 0 μg/mL polymer solution (control), 100 μg/mL or 250 μg/mL. The radical initiator pair TEMED and ammonium persulfate (0.018M) was added to form hydrogels 6 mm dia X 1 mm thick before swelling. These samples were placed on a shaker table for 29 days in PBS at 37°C. The PBS (750 μL/well) was completely changed every other day, collected and frozen for further analysis. At 1, 7, 15, 21 and 29 days, the hydrogels were assayed for aggrecan content. For all samples, the dimethylmethylene blue (DMMB) assay was employed (absorbance at 520 nm recorded). For the hydrogels, statistics were performed on raw absorbance values as no standards were available.

Results and Discussion: In the cell culture studies on the aggrecan-treated wells, cells formed dense aggregates as early as 1 day after seeding, regardless of plating density or cell type (arrow in Fig. 1). No such aggregation was observed in control wells. Further staining is required to determine if these striking changes in morphology affect the production of ECM molecules by these cells.

For the aggrecan-OPF hydrogel studies, data in Fig. 2 is normalized to absorbance of the 0 μg/mL hydrogel (dashed line) at each time point (through Day 15 shown). Absorbance values were found to be significantly higher for the 250 (0.64 ± 0.04) and 100 μg/mL samples (0.54 ± 0.04) than the control (0.42 ± 0.02) at Day 1. At Day 7, absorbance of the 250 μg/mL hydrogel only (0.63 ± 0.02) was greater than the control. By Day 15, there was no significant difference in absorbance for any hydrogel type, suggesting a loss of aggrecan from the hydrogels over this time period. Supernatants for each hydrogel type were analyzed for each of the time periods depicted and confirmed low levels of continuous aggrecan release over this time course (data not shown).

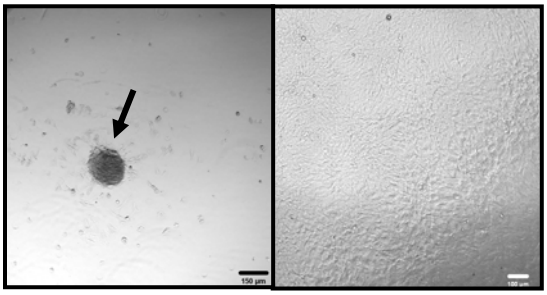

Figure 1. MSCs (p3, $2.0\ x10^5$ cells/well) at 6 days after plating on aggrecan coated wells (left) or standard tissue culture surfaces (right). Scale bar is 150 μm in left/100 μm in right image (n = 3).

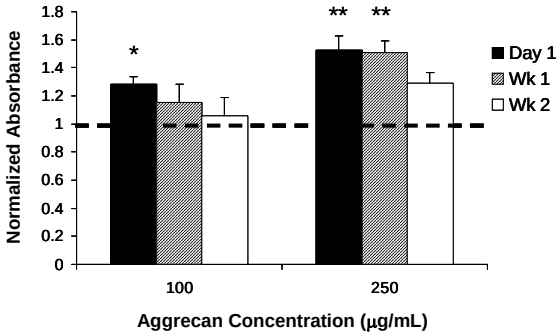

Figure 2. Normalized absorbance values for aggrecan-OPF hydrogels. * indicates signficantly greater than control, ** indicates significantly greater than control and 100 μg/mL hydrogels ($p<0.05$, n = 3-4 ± SD).

Conclusions: The results indicate that adsorbed aggrecan has a marked effect on morphology of both MSCs and ligament fibroblasts. Additionally, by altering parameters such as the amount of aggrecan loaded into the biohybrid OPF hydrogels, the length of time that the molecule is retained in the matrix can be controlled. Thus, this system may provide a unique means to examine effects of dose and length of exposure to aggrecan, as well as method of molecule presentation (within a hydrophilic hydrogel matrix vs. on a hydrophobic tissue culture surface) on morphology and ECM production by both differentiated and undifferentiated cell types.

Acknowledgements: Arthritis Foundation Investigator Award, NSF Graduate Fellowship to KSB