Hyaluronic Acid Interferes with Insulin-like Growth Factor-1 Signaling Among Alginate Embedded Chondrocytes

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Statement of Purpose: Articular cartilage has a difficult ability to heal itself. In order to aid chondrocytes to re-grow fully functional articular cartilage, we decided to study chondrocyte signaling in a 3-D environment specifically alginate hydrogels. While there are a slew of biomolecules that are vital for chondrocytes, an important anabolic growth factor that has been limited in study is insulin-like growth factor-1 (IGF-1). Construct properties were altered to better understand upstream IGF-1 signaling molecule expression such as IGF-1, IGF-1 receptor (IGF-1R), and IGF-1 binding protein (IGFBP-3). Previous research has shown that increasing cell density resulted in increased IGF-1 expression.[1] Additionally by increasing alginate concentration, IGF-1 expression was found to be further upregulated.[1] By delivering exogenous IGF-1, chondrocytes hindered their expression of IGF-1 but increased their expression of IGF-1R while showing no changes in expression for IGFBP-3.[2] These results indicated that endogenous expression can be changed by altering construct properties. Our goal for this study was to investigate how extracellular matrix (ECM) molecules alter chondrocyte endogenous signaling. Hyaluronic acid (HA) is an ECM protein that is readily found in cartilage. Specifically, in this experiment we wanted to observe how HA alters IGF-1 delivery and expression of upstream IGF-1 signaling molecules.

Methods: Two percent w/v alginate solution was mixed with varying amounts of HA (0, 0.05, 0.5, and 5 mg/mL) at a cell density of 100,000 chondrocytes per bead. Alginate/HA hydrogels were formed by Ca^{2+} crosslinking and incubated in media containing 10% FBS. *In vitro* and *in vivo* experiments were done. For the *in vitro* work, media was changed continuously with 100 ng/ml IGF-1 over a period of 8 days. Safranin-O/Fast Green staining was done. RT-PCR was analyzed at day 1, 4, and 8 for CD44 (receptor for HA), IGF-1, IGF-1R, & IGFBP-3. For the *in vivo* work, constructs were pre-incubated with IGF-1 (5 ug) and then subcutaneously implanted into SCID mice. Immunohistochemistry for IGF-1, type II collagen, and type I collagen was analyzed for constructs at day 7, 14, and 21.

Results: Safranin-O/Fast Green staining indicated entrapment of HA. CD44 expression was the highest for HA groups. HA was able to entrap free IGF-1 in alginate hydrogels (Figure 1). Increasing HA led to decreased expression of IGF-1 (Figure 2). IGF-1R expression was upregulated for the HA groups compared to the control case (Figure 3). HA did not alter IGFBP-3 expression. *In vivo*, IGF-1 staining decreased with increasing HA concentration (Figure 4). Presence of HA and/or IGF-1 did not alter type II collagen expression. Increasing HA resulted in higher type I collagen staining (Figure 4). IGF-1 delivery reversed the affects of HA by decreasing type I collagen staining (Figure 4).

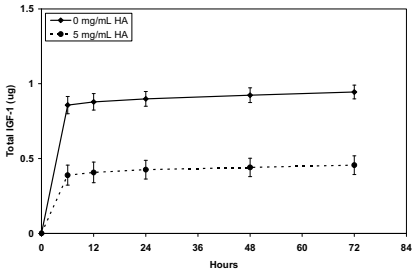

Figure 1: IGF-1 Diffusion in Hydrogels

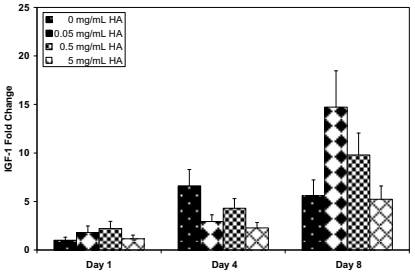

Figure 2: IGF-1 Expression

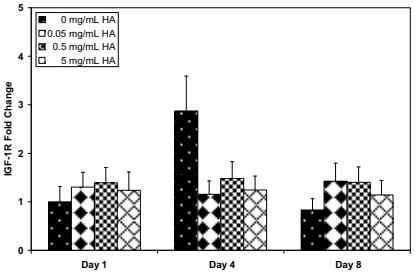

Figure 3: IGF-1R Expression

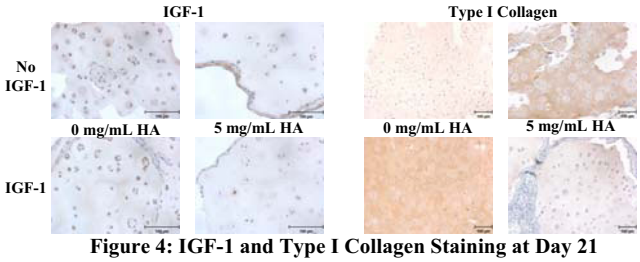

Figure 4: IGF-1 and Type I Collagen Staining at Day 21

Conclusions: Chondrocytes were found to interact with HA by CD44. High retention of IGF-1 in the 5mg/mL HA construct caused chondrocytes to express similar levels of IGF-1 expression as the 0mg/mL HA group. HA interacted with IGF-1R which led to limitations of IGF-1 controlling IGF-1R expression as seen in previous works.[3] *In vivo*, IGF-1 overcame the affects of HA by lowering type I collagen expression. Overall, these results show that HA and IGF-1 both influence each other and therefore chondrocyte expression and function.

References: **1.**Yoon DM, et al. Biomaterials. 2007; 28: 299-306. **2.** Yoon DM, et al. Tissue Engineering Part A; 14(7):1263-73. **3.** Karna, et al. Mol Cell Biochem; 308: 57-64.