Exogenous Insulin-like Growth Factor-1 Delivery Effects on Endogenous Signaling of Encapsulated Chondrocytes in Alginate Hydrogels

Diana M Yoon[1] and John P Fisher[2]
[1]Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD, USA
[2]Fischell Department of Bioengineering, University of Maryland, College Park, MD, USA

Statement of Purpose: A typical strategy to create an engineered articular cartilage construct focuses on the transplantation of encapsulated chondrocytes. Following this approach, preliminary studies from our laboratory have shown that decreasing paracrine signaling distance between chondrocytes within an alginate hydrogel induces increased insulin-like growth factor-1 (IGF-1) expression due to enhanced chondrocyte competition.[1] The objective of this work is to examine the effects of exogenous IGF-1 on chondrocyte phenotype and endogenous IGF-1 expression and function. In particular, we investigate here the effect of varying the concentration of exogenous IGF-1 molecules to encapsulated chondrocytes within alginate hydrogels and observing the effects on endogenous signaling molecules. In order to confirm phenotypic function, type II collagen as well as type I collagen expression are assayed. Furthermore, IGF-1 function is studied by IGF-1, insulin-like growth factor-1receptor (IGF-1R), and insulin-like growth factor-binding protein-3 (IGFBP-3) expression.

Methods: Fresh cartilage was harvested from the metatarsal phanlangeal joint of calves (15-18 wk old). Cartilage was digested in *collagenase P* to isolate chondrocytes, which were encapsulated at a 100,000 cell density in 2.0% w/v alginate beads. Chondrocytes were precultured in media and 10% FBS for 3 days and then treated with varying insulin-like growth factor-1 (IGF-1) concentrations (10, 50, and 100 ng/μL), where media was changed daily. Chondrocytes were isolated for analysis at day 1, 4, and 8, by disrupting the bead. Total RNA was isolated from chondrocytes using an RNeasy Mini Kit (QIAGEN, Valencia, CA) and then reverse transcribed into cDNA. Quantitative RT-PCR was performed by using oligonucleotide primers and Taqman probes (Applied Biosystems, Foster City, CA) that were created for the genes of interest (type II collagen, type I collagen, IGF-1, and IGF-1R) and endogenous gene control (glyceraldehyde 3 phosphate dehydrogenase, GAPDH). Gene expression was analyzed using a relative standard curve method, which was normalized to GAPDH and the calibrator was the group with 10 ng/μL IGF-1 treatment at day 1. Furthermore, protein production was validated by dot blots and biochemical analysis.

Results/Discussion: These initial studies examined the effects of exogenous IGF-1 delivery on endogenous gene expression by encapsulated chondrocytes. Results indicated that chondrocytes maintained a high level of type II collagen expression compared to type I collagen expression with all IGF-1 concentrations, as expected.[2] In regards to IGF-1 expression, chondrocytes were observed to be most affected by exogenous IGF-1 on day 1, with a general trend of decreasing endogenous IGF-1 expression over time for all IGF-1 delivery concentrations (See Figure 1). These expression levels are expected to be due to the abundance of IGF-1 and therefore a limited need for chondrocytes to express IGF-1. However, there is an apparent constant trend of IGF-1R expression over the 8 days (See Figure 2). This data indicates that exogenous IGF-1 allows chondrocytes to continually express IGF-1R. However, the level of IGF-1R expression does not appear to depend on exogenous IGF-1 concentration. Therefore, the data indicates that exogenous IGF-1 delivery down regulates endogenous IGF-1 expression without affecting chondrocyte type II collagen phenotype.

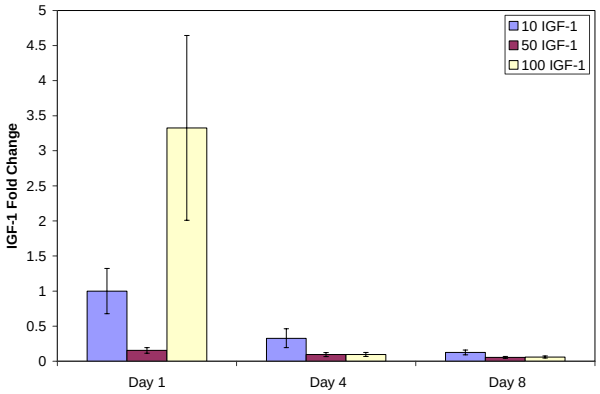

Figure 1. Effect of exogenous IGF-1 delivery on IGF-1 expression

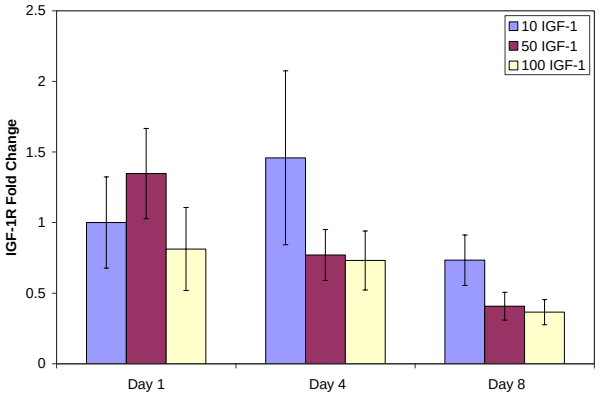

Figure 2. Effect of exogenous IGF-1 delivery on IGF-1R expression

Conclusions: This study investigated the impact of exogenous IGF-1 delivery upon endogenous signaling expression. Preliminary results indicate that incorporating exogenous IGF-1 to encapsulated chondrocytes in an alginate hydrogel leads to low levels of IGF-1 expression with a constant IGF-1R expression over the course of 8 days. Thus exogenous growth factor delivery must be considered, in light of its effect upon endogenous signaling expression.

References:

[1]Yoon, DM, et al., Biomaterials, **28(2)**, 2007, 299-306.
[2]Hauselmann, et al., J. Cell Sci., **107(1)**, 1994, 17-27.