Mesenchymal Stem Cell Chondrogenesis and Chondrocyte Response to Extracellular Matrix Molecules

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Statement of Purpose: Articular cartilage contains three distinct tissue zones: superficial, middle, and deep. Proper tissue function is dependent on healthy tissue of all zones. Due to differences in morphology and protein production, superficial zone chondrocytes are often studied separately from middle and deep zone chondrocytes. (1,2) Clinically, cell source is a major limitation for regenerating damaged articular cartilage. It has been well documented that mesenchymal stem cells can be differentiated into mature chondrocytes. However, phenotype retention of mature chondrocytes remains a challenge for tissue engineers. Here we investigate MSC chondrogensis in alginate hydrogels and the effects of extracellular matrix molecules on zonal chondrocyte phenotype. The ultimate goal of the work is to direct zone specific phenotype retention in chondrocytes differentiated from MSCs.

Methods: Primary bovine cell populations were harvested from 20 week old calves. Bone marrow was harvested from the tibia, and the MSC population was isolated via plastic adhesion to two passages. Superficial zone cartilage was isolated from the femoral condyles, and chondrocytes were isolated following digestion in collagenase P. MSCs were either encapsulated in alginate (experimental group), or plated in monolayer (control group). Control group received growth media and experimental group received chondrogenic media. Superficial zone chondrocytes were in either control alginate or alginate containing the following concentration of matrix molecules: 0.1 mg/mL CS, 1 mg/mL CS, 0.1 mg/mL HA, 2 mg/mL HA, and 5 mg/mL HA. At time points, mRNA isolation was conducted for analysis by qRT-PCR. All data was analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple-comparison test to determine statistical differences. Means and standard deviations are shown on each figure.

Results: Results demonstrate chondrogenesis in MSCs and upregulation of aggrecan mRNA (major matrix proteoglycan) in superficial zone cells. Figure 1 shows MSC gene expression for chondrogenic differentiation markers. Upregulation of type II collagen (100-fold) and sox 9 (2.5-fold) mRNA is seen after 10 days in chondrogenic media. Both sox 9 (chondrogenic transcription factor) and type II collagen (major cartilage matrix component) are indicators of the chondrocyte phenotype. Figure 2 shows superficial zone cell expression of aggrecan mRNA in control alginate, CS-alginate, and HA-alginate. At days 7 and 14 CS-alginate significantly increases aggrecan expression compared to alginate control. Figure 3 confirms cell proliferation and proteoglycan production as seen by clusters of cells (pink) with stain for proteoglycan (light blue) inside the cell clusters.

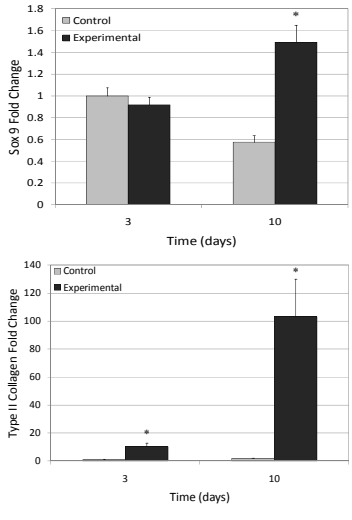

Figure 1: MSC chondrogensis, Sox 9 and type II collagen mRNA fold change respectively. Star indicates statistical significance. (n=3, p<0.5)

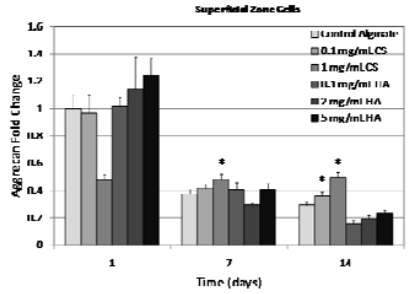

Figure 2: Aggrecan mRNA fold change of superficial zone chondrocytes in alginate containing CS and HA. (n=3, p0.5)

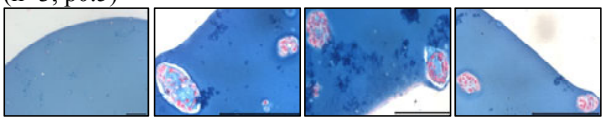

Figure 3: Alcian Blue histological staining of superficial zone cells for proteoglycans. From left: control alginate day 1, control alginate day 7, CS-alginate day 7, and HA-alginate day 7. Scale bars all 100μm.

Conclusions:
Here we have successfully demonstrated upregulation of chondrogenic markers by MSCs in alginate beads, and demonstrated that extracellular matrix mRNA can be stimulated in superficial zone cells through addition of chondroitin sulfate to alginate beads. Thus, providing the hypothesis that CS-alginate may further enhance expression of chondrogenic markers in MSCs during differentiation in alginate beads. Future studies aim to investigate the effects of HA and CS on MSC chondrogensis in alginate beads.

References:

1. Darling, E, et al. J Orthop Res. 2004; 22:1182-7.
2. Zanetti, M, et al. J Cell Biol. 1985; 101:53-9.