**Articular Cartilage Engineering: A comparison of canine and human cells**

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**Statement of Purpose:** Osteoarthritis (OA) is a multifactorial disease characterized by progressive degeneration of the articular cartilage. OA affects more than twenty million Americans, and costs the U.S. over sixty billion dollars a year. While there are surgical techniques to replace damaged cartilage with synthetic material, no treatment effectively regenerates cartilage. Recently, strategies to engineer articular cartilage *in vitro* using adult stem cells have been developed. Adipose derived mesenchymal stem cells (ADSC) can be isolated from adults and utilized to engineer tissues. In order to induce chondrogenic differentiation in these stem cells a signal protein, such as transforming growth factor-ß3 (TGF-ß3) or bone morphogenic protein 6 (BMP-6) can be utilized. The goal of the current project was to compare *in vitro* chondrogenesis in both canine and human models by examining stem cell differentiation and tissue matrix production.

**Methods:** All methods were approved by the appropriate University of Arizona regulatory bodies. Canine adipose tissue was collected post-mortem after dogs were sacrificed for other studies. Human adipose tissue discards were collected from orthopaedic surgeries and cells were purchased (Lonza Scientific Inc, Walkersville, MD). Stem cells were isolated from adipose tissue using a collagenase type I digestion protocol. The cells were expanded in flat culture through passage 4 (Figure 1). Then, 1 million cell pellets were made and assigned a treatment group (control, 10ng/ml TGF-ß3, or 500ng/ml BMP-6). Cell pellets were harvested on days 0, 7, 14, or 28 and a biochemical analysis was performed using RT-PCR to determine if chondrogenesis had taken place. The following markers were examined: Dynactin, GAPDH, Collagen I, Collagen II, Aggrecan, Sox 9, CD10, and CD13. PCR data was analyzed using the $2^{-\Delta\Delta Ct}$ method.

**Results:** Canine cells showed increased expression of collagen type II and a decreased expression of collagen type I in all treatment groups (Figure 2). The expression of Aggrecan also increased over time. Sox 9, an early marker of chondrogenesis, increased in all groups but was highly expressed in the two growth factor treatment groups. CD13 decreased in all groups but was still present.

In the human cell model, collagen type II was detected but shows a slight decrease in gene expression by day 28. Collagen type I was not detected in any human sample. Aggrecan was undetectable early, but shows an increase over time. Sox 9 was detected early, but was highly variable and decreased over time. CD13was consistently detected with an increase over time.

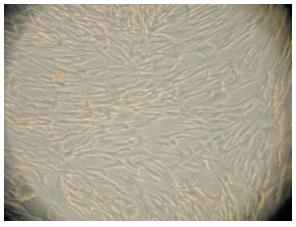

Figure 1: ADSC cells grown in flat culture until confluent

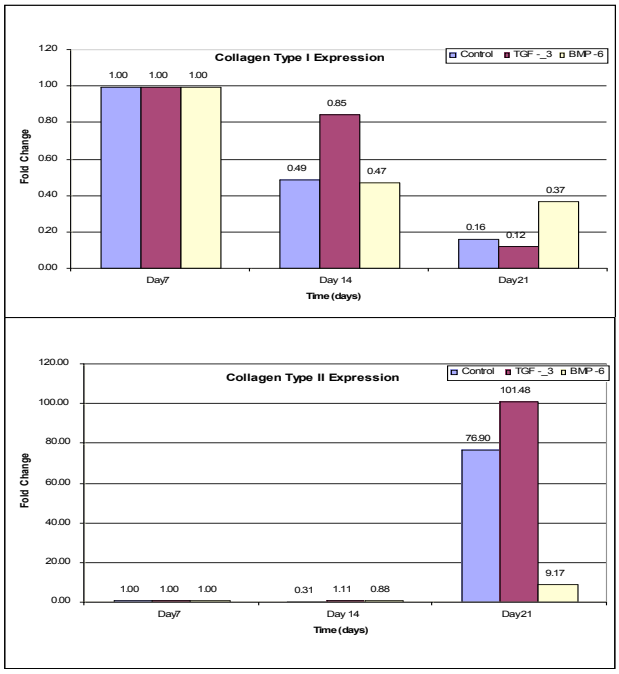


Figure 2: Canine ADSC collagen type I (top) and collagen type II (bottom) expression over 21 days. Similar trends are seen in the human model.

**Conclusions:** The increase in collagen type II, aggrecan, and Sox 9 suggest that chondrogenesis is taking place in both the canine and human models. The presence of collagen type I in the canine model suggests that these cells are more differentiated upon pelleting, possibly from expansion in flat culture. However, the increase in collagen type II and decrease in collagen type I shows that the canine cells will respond to treatment with growth factors and begin to form the correct extracellular matrix for articular cartilage. The appearance of CD13, a stem cell marker, in both models at day 28 suggests that some cells remain undifferentiated.

The similarities in trends for chondrogenesis of both models suggest that data obtained from our canine model is applicable in a human model. Once the tissue culture procedure is optimized in canine and human cells, the tissue constructs will be tested *in vivo* by placing cartilage that was grown on a sensate scaffold, originally developed for a dog, into a joint. Prior *in vivo* results demonstrated a hyaline-like cartilage formation at a 6 month time point. Additional shorter time points will provide a plan to test cells in a patient model.

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