Dedifferentiation of Chondrocytes *in vitro* by treatment with bFGF

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The field of tissue engineering is motivated by an increasing need for tissue replacement within patients. Its goal is to promote regeneration of tissue within patients who cannot re-grow lost or diseased tissue through natural processes or by donation. Cartilage engineering has a variety of applications including therapies in both bone and cartilage trauma and disease. Integral to the task of bone and cartilage engineering is the selection of the correct growth factors to induce specific conformational changes in the cells. We are particularly interested in engineering cartilage tissue in order to study the factors that cause chondrocytes to dedifferentiate. It is known that chondrocytes grown in monolayer culture tend to dedifferentiate into fibroblasts due to crowding. We intended to provoke the same response in 3-D culture by inducing the chondrocytes to overgrow by treating cells with basic fibroblast growth factor (bFGF) which has been shown to cause chondrocyte dedifferentiation in monolayer. (de Haart, 1999) We grew one sample of chondrocytes in Sigma Aldrich standard chondrogenic medium (250 cps at 2%, high M) with 5 ng/μL bFGF and the other without. Both samples were grown at an initial density of 5 million cells/mL. Chondrocytes, upon dedifferentiation are known to produce greater quantities of collagen I (CNI) as compared to collagen II (CNII) than they would normally. By assaying the amounts of collagen I and collagen II that the two samples generated, we expected to find that the chondrocytes grown with bFGF would have a lower CNII/CNI ratio than our control, indicating loss of the chondrocytic phenotype and therefore their dedifferentiation.

Analysis of the transcriptional activity of collagen I and collagen II by RT-PCR (using ~80 ng of RNA) reveals that the ratio of collagen I to collagen II did decrease in chondrocytes treated with bFGF when compared to the control. (Figure 1, Table 1) Similar results were seen in the protein expression of each collagen by analysis with ELISA. (Table 1) However, while the ELISA assay indicated a drop in the collagen II to collagen I ratio after treatment with bFGF, the actual amount of collagen II was reported to be less than 0 ng/mL. In effect, this most likely means that there was no collagen II present and the negative value was simply due to background noise. Since the amount of collagen II in the control was also very low, but happened to be greater than 0, we should consider the possibility that practically no collagen II was produced in either experiment. If this is the case, we must then wonder why, in all samples, collagen I is produced in much higher quantities than collagen II. This result was not expected since chondrocytes normally produce higher quantities of collagen II rather than I. While we expected to see the drop in ratio of CNII/CNI with the addition of bFGF, we did not expect to see a greater amount of collagen I in any sample. In light of this unexpected outcome, we are skeptical to accept any of our data as completely valid and question whether we had enough sample to accurately carry out either assay.

Further research is needed to determine the particular effects of bFGF on chondrocyte dedifferentiation. From cell counts early on in the experiment and right before RT-PCR and ELISA, we know that there was a major decrease in cell population (from ~5 million cells/mL to ~50,000 cells/mL in both samples treated with and without bFGF). This indicates a need for increased cell volume when measuring protein and mRNA amounts, as verified by the low signals achieved in both assays. In the ELISA assay we want to increase the range of our standards to include lower ranges for detection of smaller amounts of collagen. For this

experiment, all of our ELISA results were below the range of our standards. Overall, we cannot reject or accept our hypothesis that bFGF will cause chondrocytes to dedifferentiate to fibroblasts due to lack of quality data. In the future, we would like to stress the importance of more frequent cell density checks to confirm that there is an adequate amount of cellular material with which to assay for CNI and CNII. We believe that it is important to continue to study the effects of growth factors and other stimuli on collagen and bone tissue constructs as we perfect cultivation *in vitro* with an eye toward adapting these techniques to become therapeutic.

De Haart, Mirjam et al. "Optimization of chondrocyte expansion in culture : Effect of TGF-2, bFGF and L-ascorbic acid on bovine articular chondrocytes." Acta Orthopaedica 70.1 (1999): 55-61.

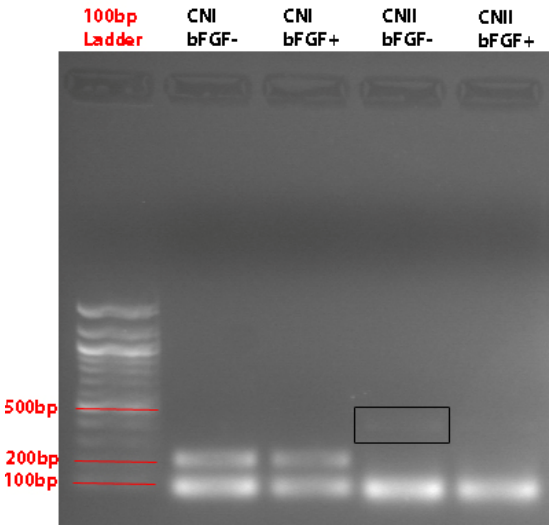

Figure 1. RT-PCR of amplified Collagen I and Collagen II transcription products. RT-PCR was used to determine the relative expression of CNI and CNII as compared to GAPDH (lowest band in all lanes). Band intensity was analyzed using ImageJ and the expression ratios recorded in Table 1. CNII bands are barely visible (one inside the black square), while CNI bands are dark for both samples. This does not fit our hypothesis that CNII should be produces in larger quantities in both samples.

Table 1. Collagen Ratios from RT-PCR and ELISA

RT-PCR	
-bFGF CNII/CNI	.170
+bFGF CNII/CNI	.014
ELISA	
-bFGF CNII/CNI	.070
+bFGF CNII/CNI	0*

***** the actual value for +bFGF CNII/CNI from ELISA was less than 0, however we treated this as 0 for purposes of analysis. See discussion for further explanation.