Effective Concentration of Growth Factors, TGF-β1 and Dexamethasone, for Bovine Stem Cell Chondrogenesis in Collagen II–Alginate Bead Scaffolds

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Introduction

The field of tissue engineering seeks to repair damaged tissues via insertion of a construct containing relevant combinations of porous scaffolding, mature or progenitor cells, and cytokines, often soluble growth factors, into the disease or wound site. One promising application of tissue engineering is the regeneration of cartilage lost in such diseases as osteoarthritis, which affects 20 million individuals in the United States alone. Increasing the number of chondrocytes, cartilage-producing cells, in cartilage-deficient joints would reverse the effects of the disease. This research investigates the role of growth factors, TGF-β1 and Dexamethasone, in the differentiation of stem cells into chondrocytes in collagen II-alginate bead scaffolds. Collagen II, TGF-β1, and Dexamethasone had previously been implicated in the chondrogenesis of stem cells by Bosnakovski et al. In this research, bovine stem cells were grown in alginate scaffolds containing collagen II and TGF-β1 and Dexamethasone (C1:1), collagen II and 1/5th the amount of TGF-β1 and Dexamethasone (C1:5), a collagen II no cell control, and an alginate only control. Cells exposed to the high amounts of TGF-β1 and Dexamethasone were expected to differentiate most significantly, as evidenced by the highest production of collagen II. Cells exposed to only low levels of TGF-β1 and Dexamethasone are expected to differentiate the least.

Results

Alginate beads were made using Sigma Aldrich alginate (1.3 ml at 1.0%) and collagen II (500 μl of .3% collagen II) in 200 μl HEPES buffer. Each sample contained 2.5x10^6 stem cells in approximately 20 alginate beads. The stem cell samples were exposed to either 10 ng/mL TGF-β1 and 100nM Dexamethasone (C1:1) or 2 ng/mL TGF-β1 and 20nM Dexamethasone (C1:5). A no cell alginate-collagen II sample and a no cell alginate sample were created. Cells were grown for 11 days before cell lysates were harvested for RT-PCR and ELISA analysis. Cells were imaged on the seventh day.

Cell Numbers and Viability

Cells grown in alginate-collagen beads with 10 ng/mL TGF-β1 and 100nM Dexamethasone (C1:1) numbered 3.6 x10^5 cells on the harvest day. Cells exposed to one-fifth the amount of growth factor (C1:5) numbered 2.6 x10^5 cells. Image analysis of cell pictures taken on day seven of cell incubation revealed the viability of the cells was 74%. Despite some cell death, it was expected that samples would produce sufficient amounts of RNA.

RT-PCR analysis demonstrates partial chondrogenesis

C1:1 yielded 120 μg/ml of RNA with a 260/280 absorbance ratio of 1.88. C1:5 gave 68 μg/ml of RNA with a 260/280 absorbance ratio of 1.76. The RNA was sufficiently concentrated and pure to add the recommended 100 ng of RNA to the PCR reaction. The results of the RT-PCR reaction are summarized in Figure 1. Gels displaying RT-PCR results were analyzed using ImageJ software to measure band fluorescence. It was found that C1:1 cells had a similar ratio

of collagenII/collagenI mRNA production (.177) as chondrocytes (.323). The collagenII/collagenI ratio of C1:5 cells ($1.81x10^{-05}$) is comparable to that of stem cells ($2.30x10^{-09}$), demonstrating the importance of growth factors TGF-β1 and Dexamethasone in chondrogenesis.

ELISA assay reveals collagen II protein production correlates with growth factor concentration. An indirect ELISA was used to assay collagen I and collagen II concentrations in C1:1, C1:5, the collagen II-alginate sample, and the alginate only sample (See Figure 2 for results). Collagen I expression inversely correlated with concentration of growth factor. Samples exposed to 10 ng/mL TGF-β1 and 100nM Dexamethasone demonstrated lower collagen I expression than cells exposed to 2 ng/mL TGF-β1 and 20nM Dexamethasone, indicative of chondrogenesis. Collagen II-alginate and Alginate only samples had background levels of absorbance. Both were significantly below detection limit. The ELISA of collagen II production showed higher levels of collagen II in cells exposed to greater amounts of growth factor (C1:1). The no-cell collagen II-alginate samples had higher collagen II concentrations than either cell sample. The Alginate only sample, again, was below the detection limit. The ratio of collagen II protein to collagen I protein was 1.28 in cells grown in 10 ng/mL TGF-β1 and 100nM Dexamethasone and .79 in cells grown in 2 ng/mL TGF-β1 and 20nM Dexamethasone.

Discussion

RT-PCR analysis and ELISA assay demonstrate the role of growth factors in chondrogenesis. Growth factor concentration correlates positively with collagen II mRNA and protein expression, and by extension, chondrogenesis. The higher ratio of collagenII/collagenI demonstrated in the ELISA is likely the result of having collagen II present in the alginate beads. That the no cell collagen II-alginate samples showed measurably higher concentrations of collagen II protein than the two cell samples that we know to be producing collagen II (from the RT-PCR analysis) is curious. This could be due to an error in the making of the collagen II-alginate no cell control. The concentration of collagen between control and experimental samples could have differed due to experimenter error. Alternatively, the collagen II-alginate control was made three days after the experimental samples, and may have lacked the necessary time to allow for collagen II degradation. An additional explanation may be that proteases from the cells degraded collagen II in the experimental samples.

Future work. The ELISA was sufficiently sensitive to detect the two types of collagen in our experimental samples. Had our experimental samples' collagen levels fallen below the limit of detection, a more sensitive assay such as a sandwich ELISA with polyclonal bottom antibodies would have been of use.

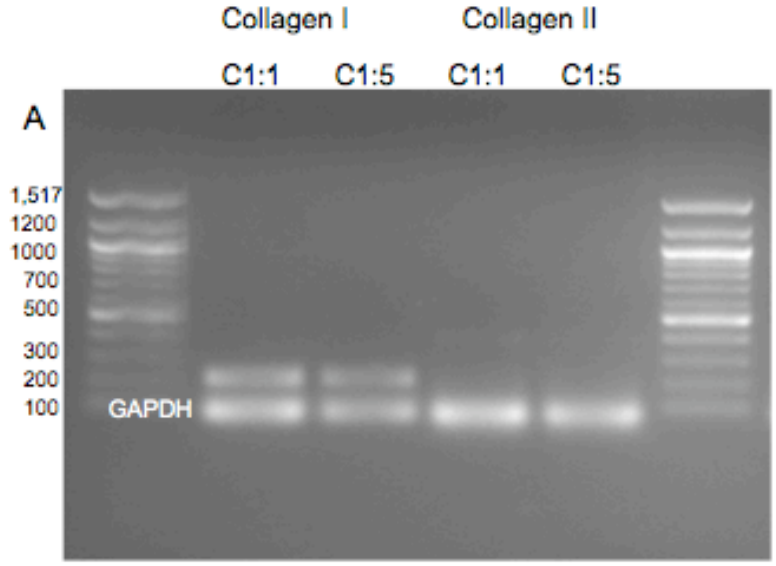
This research's results could be validated through replication. It would be helpful to investigate the significantly higher collagen II concentration of the no cell collagen II-alginate sample demonstrated in the ELISA assay. Replication of the experiment with the control samples made contemporarily with the experimental samples is necessary. Replication of this experiment with increasing concentrations of TGF-β1 and Dexamethasone could potentially discover the maximum effective concentration of the two growth factors, after which higher concentrations produce no discernable effect. The developments of such standards in the field of tissue engineering are vital for the speedy development of clinical applications and for the advancement of tissue engineering as an engineering discipline.

Citations

Shiel Jr., William C. MD, FACP, FACR. "Osteoarthritis (OA or Degenerative Arthritis)." <http://www.medicinenet.com/osteoarthritis/ article.htm>

Bosnakovski et al. "Chondrogenic Differentiation of Bovine Bone Marrow Mesenchymal Stem Cells (MSCs) in Different Hydrogels: Influence of Collagen Type II Extracellular Matrix on MSC Chondrogeneis." *Biotechnology and Bioengineering.* Vol. 93, No. 6, 20 April 2006.

Figures



B

	CollagenII/ CollagenI
C1:1	0.177
C1:5	1.81×10^{-05}
Chondrocytes	0.324
Stem Cells	2.30×10^{-09}

Figure 1 **Reverse Transcriptase PCR demonstrates chondrogenesis.** The mRNA isolated from stem cells grown in collagen alginate beads and growth factors or a 1:5 dilution of growth factors was converted to cDNA. The PCR reactions were run with primers for collagen I or collagen II, and the housekeeping gene, GAPDH. Band intensities were background subtracted and normalized compared to GAPDH expression. C1:1, or the cell sample exposed to 10 ng/mL TGF-beta1 and 100nM Dexamethasone,demonstrated a ratio of collagen II to collagen I most similar to chondrocytes. C1:5, exposed to 2 ng/mL TGF-beta1 and 20nM Dexamethasone, showed a collagen II/collagen I ratio more similar to stem cells.

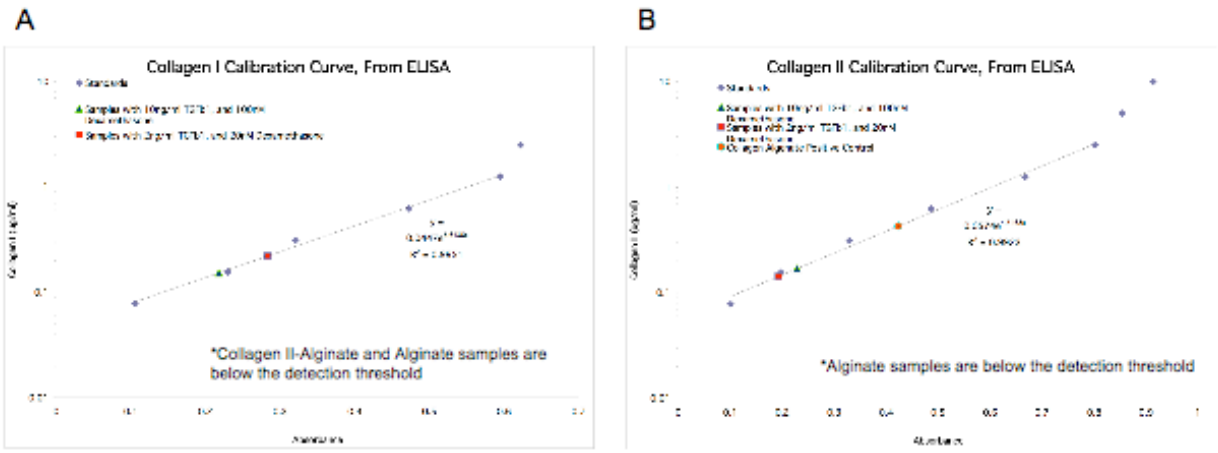


Figure 2 Collagen I and II expression differs in cells exposed to varying growth factor concentrations. A) Samples exposed to 10 ng/mL TGF-beta1 and 100nM Dexamethasone demonstrated lower Collagen I expression than cells exposed to 2 ng/mL TGF-beta1 and 20nM Dexamethasone, indicative of chondrogenesis. Collagen II-Alginate and Alginate only samples had background levels of absorbance only and were below detection limit. B) Growth factor concentration correlates with Collagen II expression, and by extrapolation, chondrogenesis. Collagen II-Alginate samples, interestingly, had higher Collagen II concentrations than either cell sample. The Alginate only sample, again, was below the detection limit.