In Vitro Response of Human Chondrocytes to a Combination of Growth Factors and a Proteinase Inhibitor: Implications for Intervertebral Disc Regeneration

INTRODUCTION:

Patients with disc herniation are indicated for a number of surgical and non-surgical treatment options. Discectomy, a common procedure to alleviate the painful effects of disc herniation, is associated with a re-herniation rate of 10-15%. It is thought that this is due to poor healing of the annulus fibrosus. Inherent low vascularity of intervertebral disc tissue, in addition to the already decreased structural integrity of the herniated tissue, results in an inadequate healing response following discectomy. Recently, transforming growth factor beta 3 (TGF-β3) and bone morphogenetic protein 4 (BMP-4) have shown to differentiate mesenchymal stem cells into a lineage similar to that of fibrocartilagenous tissue. It is, therefore, hypothesized that the treatment of chondrocytes with the aforementioned anabolic growth factors will upregulate phenotypic expression indicative of fibrocartilagenous regeneration. In addition, the inhibition of molecules responsible for the degeneration of extracellular matrix (ECM) macromolecules is hypothesized to aid in the overall healing response. Tissue inhibitor of matrix metalloproteinase 2 (TIMP-2) is a proteinase inhibitor which downregulates the expression of matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9), molecules responsible for the degeneration of important macromolecules. The combination of both anabolic and catabolic inhibitor cytokines is hypothesized to have a synergistic response in fibrocartilagenous healing. The goal of this study was to quantify the response of chondrocytes to combination of growth factors and the proteinase inhibitor to establish a benchmark in intervertebral disc tissue engineering.

METHODS:

Normal human articular chondrocytes (nHAC-Kn) were cultured in culture medium (DMEM/F12, 10% FBS, Pen/Strep, ascorbic acid at 0.05mg/mL) and subsequently exposed to growth factor combinations in the following groups:

1) TGF-β3 (100ng/mL) 2) BMP-4 (100ng/mL) 3) TIMP-2 (100ng/mL) 4) TGF-β3 + BMP-4 5) TGF-β3 + TIMP-2 6) BMP-4 + TIMP-2 7) TGF-β3 + BMP-4 + TIMP-2

Concentrations of growth factors were based on previously-documented ED50s. Growth factors were replenished together with fresh culture media every second day. Expressions of Collagen 1a1 (Col 1a1), Collagen 2a1 (Col 2a1), Collagen 10a1 (Col10a1) and Aggrecan (ACAN) were assessed using quantitative real-time PCR (QRT-PCR) from RNA at 24 hrs, 72 hrs, and 7 days. Glyceraldehyde phosphate dehydrogenase (GAPDH) served as the endogenous control gene. Immunocytochemistry (ICC) staining of Type 1 Collagen, Type II Collagen, and Aggrecan was performed on cells treated with TGF-β3 only and TGF-β3 + BMP-4 + TIMP-2 at 72 hrs. GAGs were quantified using the DMMB assay at 24 hrs, 72 hrs, and 7 days. All results reflect the mean of n=3 experiments.

RESULTS:

Marked upregulation of Col1a1, Col2a1, and Col10a1 was noted due to treatment with TGF-β3 (Group 1), TGF-β3 + BMP-4 (Group 4), TGF-β3 + TIMP-2 (Group 5) and TGF-β3 + BMP-4 + TIMP-2 (Group 7), most significantly at 72 hrs. Treatment with BMP-4 only (Group 2) or TIMP-2 only (Group 3) induced no significant response.

DISCUSSION:

The intervertebral disc is a fibrocartilagenous structure composed of several types of collagen, water, and proteoglycans. Poor vascularity and adverse biomechanical loading lead to a damaged extracellular matrix and lower chondrocyte viability, both of which are involved in a seemingly self-perpetuating and irreversible cycle of tissue degeneration. The regeneration of the intervertebral disc can be modulated by the delivery of certain growth factors. It was our group’s aim to characterize the cellular response to this delivery by using an applicable cell line. Due to the favorable results illustrated above, future studies involve the use of a novel hydrogel system to more effectively deliver the blend of growth factors shown to be most effective (i.e. the anabolic group (Group 4) and the combination group (Group 7)). Conclusively, the use of human annulus fibrosus and nucleus pulposus cells will model the native tissue response more closely.