Tendon Gene Therapy Modulates the Local Repair Environment in the Shoulder

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Introduction: Rotator cuff tears are a common soft tissue injury of the musculoskeletal system. These tears heal by formation of inferior repair tissue, which may lead to severe joint dysfunction. A controversy exists over whether rotator cuff tendons heal after surgery. The endogenous healing is poor or insufficient in most rotator cuff tears and especially in large tears. A method for augmenting the endogenous healing process could be of significant clinical value. The specific aim of this study was to determine if local delivery of an anabolic growth factor using a novel, combined tissue engineering gene therapy approach results in improved healing of rotator cuff tendon defects compared to suture repaired controls.

This study contained two-phases; the in vitro phase demonstrated the feasibility of using a tissue engineered gene therapy platform for tendon repair. Rat tendon fibroblasts (RTFs) were transduced with either platelet-derived growth factor-β [PDGF-β] or insulin like growth factor-1 [IGF-1]. Confirmation of active peptide was assessed, and then ability of the peptide to up regulate metabolism in an adjacent, local environment was demonstrated. The in vivo phase evaluated a new cell-polymer construct with RTFs transduced with PDGF-β or IGF-1 genes for its ability to augment tendon repair in a rat shows tendon fibroblast (RTF) model of rotator cuff injury.

Methods: Phase I, in vitro model: Adult male Sprague-Dawley rats were used to isolate tendons from the shoulder complex and tendon fibroblasts were initiated in culture by explant outgrowth. Explants were fed Dulbecco’s modified eagle medium (DME) supplemented with 10% fetal calf serum. After RTFs were seeded and cultured, they were transduced with the genes for either PDGF-β or IGF-1 by retroviral vectors. The cells containing the active genes were selected by infection with a neomycin resistance gene added to the construct. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and Enzyme Linked Immunosorbant Assay (ELISA) were used for positive confirmation of gene expression. To test whether IGF-1 gene transduced RTF cells modulate the metabolism of surrounding tissue, the following was performed: RTF cells were seeded onto a biocompatible polymer scaffold composed of polyglycolic acid and cultured for five days to assemble a tissue engineered tendon construct. This technique was then repeated to assemble constructs containing the PDGF-β gene transduced RTFs. The two different constructs were cultured in the same tissue culture well but separated by a membrane which allowed diffusion of soluble factors. The tested group configurations included single construct [RTF0; Control 1], non-transduced RTF [RTF/RTF; Control 2], and RTF+IGF-1 [experimental]. Constructs were then allowed to incubate in culture for 24 or 48 hours and then pulse labeled with tritiated proline [H-3H] and tritiated thymidine [H3T] to assess collagen and DNA synthesis. The constructs were harvested individually and scintillation counted to determine the rate of synthesis in each RTF construct.

Phase II, in vivo model: Adult male Sprague-Dawley RTFs were isolated, cultured, and transduced with genes for either IGF-1 or PDGF-β by retroviral vectors. After selection and expansion, the transduced RTFs were seeded onto a polymer scaffold to further cultured. Rotator cuffs of rats were transected surgically and allowed to undergo an inflammatory phase for two weeks at which time they were then re-operated on to repair the original tear. Repair included standard suture realignment as a control or suture repair with the addition of a novel, combined tissue engineering gene therapy approach results in improved healing of rotator cuff tendon defects compared to suture repaired controls.

Results: Phase I, in vitro model: Rat tendon fibroblasts were easily cultured, readily transduced and selected with the IGF-1 and PDGF-β genes. They demonstrated expression of the gene and active peptide via ELISA and RT-PCR analysis (Fig.1). RTF cells rapidly attached to polymer scaffolds and formed highly cellular tissue constructs within the polyglycolic acid (PGA) scaffolds by five days post-seeding. RTF constructs incubated alone exhibited a baseline level of collagen synthesis [control 1] and were not significantly stimulated by placement of a similar RTF construct [control 2]. By 24 hours, PDGF-β transduced cells stimulated adjacent RTF cells to increase collagen synthesis by 300% (Fig.2); however, DNA synthesis was not significantly increased. IGF-1 increased collagen synthesis by 28% and DNA synthesis by almost 100% by 24-hours exposure (Fig.3). At 48-hours, the trends for increased collagen synthesis for PDGF-β and IGF-1 continued. In addition, PDGF-β transduced tendon cells resulted in a 300% increase in DNA synthesis, and IGF-1 maintained a 28% increase in DNA synthesis.

Discussion: The in vitro study demonstrates tendon fibroblasts can be tissue engineered to deliver therapeutic peptides to local environments to stimulate a repair response. The in vivo study demonstrates the efficacy of a new type of bioactive implant for repair of rotator cuff injuries. The current in vivo model closely mimics the clinical sequela of tear, inflammation, and repair. The goal of this work is to develop a bioactive patch capable of accelerating rotator cuff repair and modulating the quality of the repair tissue. Initial work has focused on IGF-1 and PDGF-β, however, other growth factors with different actions on tendon cells are being explored.