Characterization of Bovine Type I Collagen Matrix Combined with Recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) for Rotator Cuff Repair

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Statement of Purpose: Detachment of one or more tendons from the rotator cuff is a common injury of the shoulder, particularly among athletes. Unfortunately, current techniques fall short of producing an enduring repair with some studies citing failure rates as high as 94% [1]. We propose a matrix with the putative ability to promote healing at the insertion site of the rotator cuff tendons. A candidate bovine type I collagen matrix (Integra LifeSciences Corporation, NJ), combined with recombinant human platelet-derived growth factor (rhPDGF-BB), may be a suitable substrate for tendon-bone tissue repair [2, 3]. The goals these studies were to: (1) evaluate the biocompatibility of the collagen matrix in combination with rhPDGF-BB, (2) determine rhPDGF-BB release kinetics from the collagen matrix and (3) determine the stability and the bioactivity of rhPDGF-BB eluted from the collagen matrix.

Methods: *Cytocompatibility.* Primary ovine tenocytes were seeded onto collagen discs (8 mm) and cultured in 2% fetal bovine serum (FBS) alone or in combination with rhPDGF-BB (30 ng/ml, BioMimetic Therapeutics, Inc). On days 2, 4, and 6, triplicate samples were processed for scanning electron microscopy (SEM) and histology. Analyses of cell proliferation were performed using a well-characterized Adenosine 5'-triphosphate (ATP) assay.

rhPDGF-BB release kinetics. Collagen discs (8 mm) were hydrated respectively with 50 µg, 15 µg, and 7.5 µg of rhPDGF-BB and were allowed to saturate at RT for 10 min. The samples were then eluted in 2ml of Elution Buffer (MEM medium containing 2% FBS) at 37°C on a shaker. The same amount of rhPDGF-BB at three doses was added to 2 ml Elution Buffer to serve as baseline controls. At 1, 8, and 24 hr, a complete medium change was conducted and the eluates were analyzed for rhPDGF-BB concentration using an ELISA (PDGF-BB Quantikine assay, R&D Systems).

rhPDGF-BB stability after elution from collagen matrix. Collagen discs (8 mm) were hydrated respectively with 100 µg, 30 µg, and 15 µg of rhPDGF-BB and allowed to saturate at RT for 10 min. The samples were then eluted in 0.4 ml of Elution Buffer (0.3125 M NaCl in 20 mM sodium acetate, pH 6.0) at 37°C on a shaker. The same amount of rhPDGF-BB at three doses was added to the 0.4 ml Elution Buffer to serve as baseline controls. After 1 hr incubation, the eluates were collected for reversed phase and size exclusion high performance liquid chromatography (HPLC).

rhPDGF-BB bioactivity following elution from collagen matrix. Collagen discs (8 mm) were hydrated respectively with 100 µg, 30 µg, and 15 µg of rhPDGF-BB and allowed to saturate at RT for 10 min. The samples were then eluted in 0.4 ml of Elution Buffer (MEM medium containing 2% FBS) at 37°C on a shaker. The same amount of rhPDGF-BB at three concentrations was added to the 0.4 ml Elution Buffer to serve as baseline controls. After 1 hr incubation, the eluates were loaded into 96-well plates, followed by the addition of MG-63 osteosarcoma cells at a density of 20,000 cells per well. After 65 ± 2 hr culture, an alkaline phosphatase bioassay was conducted.

Biocompatibility. The rhPDGF-BB hydrated collagen pads (1×1cm) were surgically implanted on the decorticated femur in rabbits and tied with 5-0 silk suture through the hole in femur. At 1, 2, and 3 weeks post surgery, 4 animals were euthanized and the samples were collected for histological assessment.

RESULTS: *Cytocompatibility.* The tenocytes attached well to the surface of collagen matrix as demonstrated by Scanning Electron Microscopy. Histological assessment showed that cells migrated and evenly distributed throughout the collagen matrix. The ATP assay demonstrated an increase in cell proliferation in the presence of rhPDGF-BB.

rhPDGF-BB release kinetics. Collagen matrix combined with rhPDGF-BB exhibited a bolus release (77%- 81% mean cumulative recoveries) of rhPDGF-BB by 1 hr in all dose groups (50 µg, 15 µg, and 7.5 µg). Greater than 92% rhPDGF-BB was released by 24h in each dose group. A comparable rhPDGF-BB release profile from collagen matrix was observed across all dose groups and time points under the conditions used in this study.

rhPDGF-BB stability after released from collagen matrix. No significant changes in the sequence or modification of amino acid residues in rhPDGF-BB were observed following its interaction with the collagen matrix.

rhPDGF-BB bioactivity after released from collagen matrix. The combination of the collagen matrix with rhPDGF-BB did not impact the biological potency of rhPDGF-BB as compared to rhPDGF-BB controls following the dose response (100 µg, 30 µg, and 15 µg).

Biocompatibility and biodegradation. Results demonstrated that the collagen matrix exhibited minimal inflammatory response. In addition, most of the matrix was degraded by 3 weeks post-implantation.

CONCLUSION: There are several important conclusions from this study: (1) rhPDGF-BB increased tenocyte proliferation; (2) Collagen matrix exhibited a bolus release of rhPDGF-BB and did not hold on to the anabolic for a long period of time (3) rhPDGF-BB was stable and bioactive upon its release from the collagen matrix Thus, the bovine type I collagen matrix in combination with rhPDGF-BB may be a suitable matrix for application in the treatment of sports medicine injuries.

References: 1. Galatz, L.M., et al., J Bone Joint Surg Am, 86-A, 2004; 2. Deuel, T. F., et al., Annu Rev Med, 42, 1991; 3. Robson, M. C., et al., Lancet, 339, 1992.