Release and Biopotency of Recombinant Human Platelet-Derived Growth Factor-BB Combined with a Collagen Matrix for Rotator Cuff Repair
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Statement of Purpose: Detachment of one or more tendons of the rotator cuff is an exceptionally 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 fibrous collagen matrix (Kensey Nash Corporation), combined with recombinant human platelet-derived growth factor (rhPDGF-BB), may be a suitable substrate for tendon-bone tissue repair2,3.

The goal of this study was to evaluate the fine structure of four collagen matrices made using different concentrations of collagen (4.5%, 5%, 6%, 7%), rhPDGF-BB release kinetics for each collagen matrix, and the bioactivity of rhPDGF-BB eluted from the matrices.

Methods: 1. Fine structure of the collagen matrices. Dry collagen matrices (4.5%, 5%, 6%, and 7%) were punched to make 5 mm disks after flushing with liquid nitrogen. Disks were mounted on a stub in three different orientations (top up, bottom up, and side up), coated with gold-palladium, and examined by scanning electron microscopy (SEM).

2. RhPDGF-BB release kinetics. Collagen matrices (5 mm disks) were impaled on a 27½G needle, hydrated with 40 µl of 0.3 mg/ml rhPDGF-BB, incubated for 10 min at room temperature, and 1.5 ml elution buffer (MEM containing 2% fetal bovine serum) was added to elute the rhPDGF-BB in a 2 ml microtube. Triplicate samples were used for each measurement. Control samples consisted of adding 40 µl of rhPDGF-BB to 1.5 ml of elution buffer. The samples were shaken on an orbital shaker in a 37°C incubator. At 1hr, 8hr, and 24hr, the samples were removed from each tube and stored at 2-8°C. An equal volume of fresh elution buffer was added to each tube. The stored samples were assessed for rhPDGF-BB using the DuoSet ELISA (R & D System) kit according to the manufacturer’s instruction.

3. RhPDGF-BB biopotency. Samples were prepared following a modification of the protocol described above. The elution buffer was changed to DMEM containing 2% calf serum. Duplicate samples for each material taken at the one hour time point were used. The concentration of rhPDGF-BB was determined by DuoSet ELISA assay, and the results were used as a reference for diluting the samples to 1 µg/ml. RhPDGF-BB at 0.3 mg/ml was used as reference standard and applied to all plates. Each sample was loaded into a 96-well microtiter plate (black wall and clear bottom) using a starting concentration of 1 µg/ml and then was serially diluted 1.667-fold across the same row. Approximately 10⁴ NIH 3T3 cells were added to each well except for the last column on each plate, which was used as blank control. After 48 hours culture, bromodeoxyuridine (BrdU) label was added to the plate. After another 24 hours culture, a BrdU cell proliferation assay was conducted according to the kit manufacturer’s instruction.

Results: 1. Fine structure of collagen matrices. The SEM images revealed that there were open pores on the surface of the lower density (4.5% and 5%) collagen matrices and a dense lamina with smaller pores on the surface of the higher density (6% and 7%) collagen matrices. Each of the matrices appeared to be porous based on SEM images of longitudinal slices.

2. RhPDGF-BB release kinetics. Collagen matrices with lower densities (4.5% and 5%) released rhPDGF-BB with similar kinetics compared to higher densities (6% and 7%) (Figure 1). The release kinetics consisted of an initial rapid bolus release of rhPDGF-BB in the first hour followed by a slower phase of release between one and 24 hour measurements.

Figure 1. The percentage of rhPDGF-BB released from collagen matrices over 24 hours.

3. RhPDGF-BB biopotency. RhPDGF-BB released from each matrix measured in a NIH3T3 cell proliferation assay demonstrated that the biological activity of the released protein was conserved for all four matrices analyzed.

Conclusions: 1 All four matrices evaluated by SEM were found to be porous. 2. RhPDGF-BB release kinetics from the four collagen matrices were characterized by an initial rapid, bolus release in the first hour accompanied by a slower phase of release over the following 23 hours of the study. The release kinetics were similar for each of matrices tested. 3. RhPDGF-BB biopotency study confirmed that the protein retains its biological activity after being released from the different densities of collagen matrices.