

# Effect of rhPDGF-BB-Coated Sutures on Tendon Healing in a Rat Model: A Histological and Biomechanical Study



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RESULTS

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# INTRODUCTION

Delivering growth factors to the site of injury using growth factorcoated sutures has been recently investigated as a means to augment tissue repair [1]. This is a practical approach, as sutures are the method of choice for most orthopaedic surgeons for soft tissue repairs. Growth factor-coated sutures in tendon repair have the potential to accelerate healing in vivo, thereby improving the outcome of the repair. In particular, platelet-derived growth factor-BB (PDGF-BB) is a well characterized wound healing protein known to be chemotactic and mitogenic for cells of mesenchymal origin, including tenocytes, and is known to improve healing when applied to animal models of tendon injury [2,3]. The aim of this study was to compare the quality of tendons repaired with sutures coated with recombinant human PDGF-BB (rhPDGF-BB). We hypothesized that (1) the amount of rhPDGF-BB coated onto sutures could be varied by changing the initial concentration of the coating solution, and (2) increasing doses of rhPDGF-BB could would result in improved tendon healing, assessed histologically and biomechanically relative to buffer-coated suture repairs.

### In Vitro Analysis:



#### **Biomechanical Analysis:**

Table 1: Summary of Biomechanical Data.						
Group	Initial rhPDGF-BB Coating Conc	Peak Load (N)	Stiffness (N/mm)	CSA (cm <sup>2</sup> )	Peak Stress (MPa)	Modulus (MPa)
1	0 mg/mL	22.1 1.8	6.7 0.4	0.21 0.03	1.0 0.1	3.5 0.4
2	0.3 mg/mL	31.6 3.5	8.9 0.8	0.23 0.04	1.4 0.1	4.4 0.4
3	1.0 mg/mL	28.0 3.7	8.0 1.0	0.17 0.02^	1.9 0.2*	4.9 0.7
4	10.0 mg/mL	27.6 1.8	7.9 0.7	0.14 0.05#	2.1 0.2#	7.2 1.5+
+: Indicates significant difference compared to Group 1, Group 2, and Group 3						

# METHODS

Suture Coating: Four groups of 4-0 Vicryl sutures (Ethicon) were coated using a dip-coating process, as described previously[1], with: (1) 20 mM sodium acetate buffer (carrier control),

(2) 0.3 mg/ml rhPDGF-BB in buffer,

- (3) 1.0 mg/ml rhPDGF-BB in buffer,
- (4) 10.0 mg/ml rhPDGF-BB in buffer.

# Sutures were trimmed to 15 cm lengths for use *in vivo* with the

Figure 1: The *in vitro* release profile for the (A) amount of rhPDGF-BB released at each time point and (B) cumulative rhPDGF-BB released over 48 hours (mean SEM). The (C) *in vivo* dose was estimated using the cumulative rhPDGF-BB released at 48 hours and the (D) implanted suture length.

- There was a bolus release of rhPDGF-BB from all groups at 1 hour, followed by a continuous gradual release over the remaining 47 hours (Figure 1A).
- The cumulative rhPDGF-BB eluted off of the sutures after 48

#: Indicates significant difference compared to Group 1 and Group 2

\*: Indicates significant difference compared to Group 1 ^: Indicates significant difference compared to Group 2

 No significant differences were noted for the structural properties (peak load and stiffness), but the means for the rhPDGF-BB groups were increased, on average, relative to the control group.

The cross-sectional area was significantly decreased in:

- Group 4 relative to Group 1 and 2
- Group 3 relative to Group 2.
- There was a significant increase in the peak stress in:
  - Group 4 relative to Group 1 and 2
  - Group 3 relative to Group 1

The elastic modulus was significantly increased in Group 4 compared to all other groups.

remaining lengths used for *in vitro* analysis.

*In Vitro Analysis:* Coated sutures (n=5/group) were placed in elution buffer (MEM with 2% FBS, 1% penicillin-streptomycin, 1% L-Glutamine, 1% HEPES) and agitated at 37°C. The buffer was fully exchanged at 1, 6, 24, and 48 hours. The rhPDGF-BB released at each time point was determined using a PDGF-BB ELISA (human PDGF-BB DuoSet, R&D Systems).

*Surgical Procedure:* Achilles tendons of Sprague-Dawley rats [350-400 gm] were transected mid-substance. Each animal was randomized in a blinded fashion to one of the four treatment groups. The repair was performed using a modified Mason-Allen stitch and a simple interrupted stitch. After 4 weeks the rats were sacrificed and tendons, including a bone block from the calcaneus and the proximal gastroc-soleus muscle complex, were harvested. The specimens were randomly assigned to biomechanical analysis (n=7/group, fresh-frozen) or histological analysis (n=4/group, formalin-fixed).

*Histology:* Histology sections from each specimen were stained with Mallory's trichrome or picrosirius red. Slides were then imaged and assessed qualitatively for collagen organization/alignment.

- hours (Figure 1B) and the *in vivo* delivered dose (Figure 1C) were logarithmically proportional to the initial coating concentration.
- There were no differences among the implanted suture length for all four groups (Figure 1D).

## Histology:



### DISCUSSION

The amount of rhPDGF-BB coated onto sutures was varied by increasing the concentration of the initial coating solution, allowing multiple doses to be delivered *in vivo*.

Relative to control, rhPDGF-BB-coated sutures increased the mean values for peak load (25-43%, p=0.16) and stiffness (18-33%, p=0.21). The observed differences did not reach significance, potentially due to the sample size used in this study.

These results demonstrate that rhPDGF-BB-coated sutures were able to improve the material properties (ultimate tensile stress, Young's modulus) of repaired tendons in a positive dosedependent fashion, with the most pronounced difference in the highest rhPDGF-BB dose group (initial coating concentration of 10.0 mg/ml).

 The differences in the cross-sectional area and the material biomechanical properties suggests there was a remodeling of the tissue which resulted in a better quality repair.

Qualitative assessment of collagen organization/alignment

**Biomechanical Testing:** Uniaxial tensile biomechanical analysis was performed. Samples were pre-loaded (1 N) and cross-sectional area (CSA) and length were measured. The tendon was then subjected to tensile extension at a strain rate of 0.25%/sec until failure. The resulting load and extension data were collected and analyzed to determine peak load, stiffness, peak stress, and elastic modulus from the load-displacement or stress-strain curves.

**Statistical Analysis:** Statistical analysis on the biomechanical parameters was performed using a one-way ANOVA with a Fisher's LSD post-hoc test. Data are presented as Mean SEM and significance was determined at p < 0.05.

Figure 2: Representative picrosirius red stained bright field (A-D) or polarized light (E-H) images taken at the repair site (10x). Dark regions (arrows) under polarized light indicate a decrease in collagen organization/alignment. (A,E) Group 1, (B,F) Group 2, (C,G) Group 3, (D,H) Group 4.

 Qualitative assessment indicated a trend towards more organized/aligned collagen in the rhPDGF-BB groups (Figure 2). indicated that the rhPDGF-BB groups appeared more normal compared to the buffer-coated controls, which is consistent with the biomechanical results.

 This study demonstrated promising results for the use of rhPDGF-BB-coated sutures to improve the function of repaired Achilles tendons.



[1] Dines+, J Shoulder Elbow Surg 2007; 16:215S-221S, [2]
Thomopoulos+, J Orthop Res 2007; 25:1358-1368, [3]
Thomopoulos+, J Orthop Res 2009; 27(9):1209-1215.