Rotator Cuff Repair in an Ovine Model using a Combination Product Comprised of rhPDGF-BB and a Type I Bovine Collagen Matrix

[2]Dines, J S; [1]Hee, C K; [1]Wisner-Lynch, L A; [1]Roden, C M; [1]Aguiar, D J; [2]Dines, D M; [3]Turner, A S; [3]Ruehlman, D L; [1]Kestler, H K; [1]Lynch, S E; [3]McGilvray, K C; [3]Lyons, A S; [3]Puttlitz, C M; [3]Santoni, B G

[1]BioMimetic Therapeutics, Franklin, TN; [2]Hospital for Special Surgery, New York, NY; [3]Colorado State University, Fort Collins, CO; chee@biomimetics.com

INTRODUCTION

Optimal healing of rotator cuff injuries involves reinsertion of the tendon into bone at the original site of attachment. Studies have reported a relatively high incidence of failure regarding rotator cuff repair [1,2], which has been suggested to result from poor tendon tissue quality and tendon-to-bone healing. Addition of growth factors at the time of surgery may augment tendon to bone healing of these injuries, thereby reducing the incidence of re-tears. 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 osteoblast and tenocytes, and has been shown to improve healing when applied to animal models of tendon injury [3-5]. We hypothesized that the application of rhPDGF-BB, combined with a type I bovine collagen matrix as an interpositional graft at the site of tendon repair, would improve rotator cuff repair in an ovine model.

METHODS

Treatment Groups: 60 skeletally mature ewes (3.5+ years) were distributed among five treatment groups (n=12/group);

Group 1: Suture only repair (No test article)
Group 2: Collagen+0 µg rhPDGF-BB (sodium acetate buffer)
Group 3: Collagen+75 µg rhPDGF-BB
Group 4: Collagen+150 µg rhPDGF-BB
Group 5: Collagen+500 µg rhPDGF-BB

Surgical Procedure: The infraspinatus tendon was surgically exposed and sharply detached from the humeral head [5,6]. The tendon footprint was decorticated and three perforations were made into the bone to induce bleeding. The test articles were placed as an interpositional graft between the tendon and the bone. Two sutures were passed through the tendon using a Mason-Allen technique and the tendon was secured to the humeral head through a single-row repair consisting of 3 bone tunnels. The surgical site was closed using standard procedure and the sheep were allowed to ambulate normally. Animals were sacrificed 12 weeks post-surgery.

Outcome Measures:

▪ **Histologic:** Decalcified specimens (n=3/group) were embedded in paraffin, with sections taken from the central region of the infraspinatus-humerus repair site. Sections were stained with hematoxylin and eosin.

▪ **Histopathology:** Sections were evaluated using a semi-quantitative scoring system (Table 1) assessing the quality of the reparative/healing tissue at the tendon-bone interface, including vascularity, presence of inflammatory cells, collagen orientation/fiber density, and presence of Sharpey's fibers at the insertion site.

Table 1: Summary of Histological Scoring Scale.

Characteristic	Grading	Score
Vascularity	None	0
	Some	1
	Abundant	2
Inflammatory Cells	None	0
	Some	1
	Many	2
Collagen Fiber Orientation	None	0
	Some	1
	Mostly	2
	Completely	3
Collagen Fiber Density	Low	1
	Medium	2
	High	3
Bone-Tendon Interface (Interdigitation)	0% Tendon-Bone Attachment Area Integrated	0
	25%	1
	50%	2
	75%	3

▪ **Biomechanical Testing:** Specimens (n=9/group) were pulled at an approximate angle of 135° relative to the long axis of the humerus (Figure 1). Specimens underwent preconditioning (10-50 N, 0.25 Hz, 60 cycles) followed by a load-to-failure ramp (1 mm/s). Displacement was tracked using three reflective markers. Quasi-static stiffness, ultimate load at failure, elongation, energy to failure, and the failure mode were determined.

Figure 1: Specimen testing configuration.

Statistical Analysis: A one-way ANOVA and post-hoc Fisher's LSD test were performed to identify significant differences in continuous biomechanical parameters among treatment groups. No statistics were performed on the histopathological scores due to the small sample size. Significance was set at $p \leq 0.05$. Biomechanical data are shown as mean ± SEM, histopathological scores are shown as median (range) of the average of 5 sections/animal.

RESULTS

Table 2: Summary of Histopathology Scores

Treatment	Vascularity	Inflammatory Cells	Collagen Fiber		Interdigitation
			Orientation	Density	
Suture Only	2 (2-2)	1.0 (1.0-1.0)	1.2 (1.0-1.4)	2.0 (1.0-2.2)	0.5 (0.2-0.5)
Collagen + 0 µg rhPDGF-BB	2 (2-2)	1.0 (1.0-1.5)	1.3 (1.0-1.9)	1.6 (1.5-2.3)	0.3 (0-0.5)
Collagen + 75µg rhPDGF-BB	2 (2-2)	0.9 (0.6-1.0)	1.5 (1.3-2.4)	2.5(2.0-2.5)	1.9 (1.0-2.0)
Collagen + 150µg rhPDGF-BB	2 (2-2)	0.8 (0.7-1.2)	1.7 (1.7-2.0)	2.0 (1.9-2.5)	1.0 (0.1-3.0)
Collagen + 500µg rhPDGF-BB	2 (2-2)	1.0 (1.0-1.0)	1.0 (1.0-1.1)	1.2 (0-1.4)	0.1 (0.1-0.3)

Data shown as median (range)

Histological Results:

- Tendon retraction was observed in all groups, with fibrous repair tissue spanning the gap between the native tendon and bone.
- No histopathological differences were noted in the assessment of inflammatory cells or vascularity (Table 2).
- The scores for collagen fiber orientation and fiber density were improved, on average, in the 75µg rhPDGF-BB and 150µg rhPDGF-BB groups (Table 2).
- Histologic sections of the 75 µg and 150 µg rhPDGF-BB groups displayed increased interdigitation of the repair tissue with the bone (Figure 2D,E & Table 2).

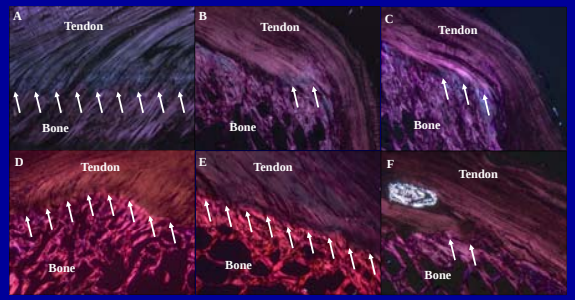

Figure 2: Polarized light images of H&E stained sections show regions of interdigitation (arrows) of repair tendon with bone at the insertion site (20x).
A: Native Tendon, **B**: Suture Only, **C**: Collagen+0 µg rhPDGF-BB, **D**: Collagen+75 µg rhPDGF-BB, **E**: Collagen+150 µg rhPDGF-BB **F**: Collagen+500 µg rhPDGF-BB

Confidential Draft

Table 3: Summary of Biomechanical Data.

Treatment	n	Ultimate Load (N)	Stiffness (N/mm)	Energy to Failure (J)	Peak Elongation (mm)
Suture Only	9	910.4 ± 156.1	131.4 ± 14.2	3.9 ± 0.9	9.0 ± 0.6
Collagen + 0 µg rhPDGF-BB	9	1120.4 ± 157.4	157.3 ± 19.0	5.8 ± 1.4	10.2 ± 1.0
Collagen + 75µg rhPDGF-BB	9	1490.5 ± 224.5#	134.4 ± 15.5	10.5 ± 2.0+	15.2 ± 1.0*
Collagen + 150µg rhPDGF-BB	9	1486.6 ± 229.0#	147.5 ± 13.7	9.8 ± 2.2#	15.4 ± 2.4*
Collagen + 500µg rhPDGF-BB	9	677.8 ± 105.9	104.3 ± 21.4	2.9 ± 0.6	11.5 ± 1.3

#: Indicates significant difference compared to Suture only and Collagen+500 µg rhPDGF-BB
*: Indicates significant difference compared to Suture only and Collagen+0 µg rhPDGF-BB
+: Indicates significant difference compared to Suture only, Collagen+0 µg rhPDGF-BB, and Collagen+500 µg rhPDGF-BB

Biomechanical Results:

- Ultimate load at failure (Table 3) was significantly increased in the 75 µg and 150 µg rhPDGF-BB groups relative to the suture only control (64% and 63%, respectively) and the 500 µg rhPDGF-BB group (120% and 119.3%, respectively).
- Energy to failure (Table 3) was significantly increased in the 75 and 150 µg rhPDGF-BB groups compared to the suture only groups and 500 µg rhPDGF-BB groups. Additionally, the 75 µg group was significantly increased relative to the 0 µg rhPDGF-BB group.
- Peak elongation (Table 3) was significantly increased in the 75 µg rhPDGF-BB and 150 µg rhPDGF-BB groups relative to the suture only control (69% and 71%, respectively) and the 0 µg rhPDGF-BB group (49% and 51%, respectively).
- All specimens in the Suture only, 0 µg rhPDGF-BB group and 500 µg rhPDGF-BB group failed in the repair tissue (Table 4). The 75 µg rhPDGF-BB (66.7%) and 150 µg rhPDGF-BB (55.6%) each had specimens that failed with some bony avulsion.

Table 4: Summary of Failure Locations.

Treatment	Total (n)	Failure in Repair Tissue (n)	Failure with Bony Avulsion (n)
Suture Only	9	9	0
Collagen + 0 µg rhPDGF-BB	9	9	0
Collagen + 75µg rhPDGF-BB	9	3	6
Collagen + 150µg rhPDGF-BB	9	4	5
Collagen + 500µg rhPDGF-BB	9	9	0

DISCUSSION

- RhPDGF-BB, combined with a type I bovine collagen matrix, significantly enhanced repair in a dose-dependent manner, as compared to standard of care treatment (Suture only).
- Biomechanical properties of the repair tissue were significantly enhanced in the low- and mid-dose groups; whereas, the high dose group did not result in enhanced biomechanical properties relative to the suture only repair, indicating that lower doses of rhPDGF-BB are sufficient for rotator cuff repair.
- The ultimate load in the low- and mid-dose treatment groups is comparable to other studies using a biological or matrix augmentation of rotator cuff repair in this ovine model [6,7].
- Histology outcomes, including collagen fiber orientation and density and tendon-to-bone integration, were consistent with the observed biomechanical properties and the location of the biomechanical failure.
- The combination of rhPDGF-BB and a type I collagen matrix may have promise as a therapeutic treatment for clinical augmentation of rotator cuff repair.

REFERENCES

[1] Galatz+, JBJS 2004; 86:219-224, [2] Cole+, Arthroscopy 2007; 23(6):662-669, [3] Thomopoulos+, J Orthop Res 2007; 25:1358-1368, [4] Thomopoulos+, J Orthop Res 2009; 27(9):1209-1215, [5] Hee+, AOSSM Poster #13 [6] Schlegel+, Am J Sports Med 2006; 34(2): 275-280, [7] Seeherman+, J Bone Joint Surg Am 2008; 90:2206-2219.