

SUPRASPINATUS TENDON STRUCTURAL AND MECHANICAL PROPERTIES IN A CHRONIC ROTATOR CUFF TEAR ANIMAL MODEL

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INTRODUCTION

Rotator cuff tears of the shoulder are a common cause of pain and disability and surgical repair of these injuries is plagued with recurrent tear rates ranging from 20-70% [1]. The time from onset of injury to time of surgical repair is one important factor in repair outcome [2]. Animal models have recently been developed to address hypotheses related to rotator cuff tendon to bone injury and repair [3]. However, these studies model an acute injury and repair condition, while the most common clinical presentation is that of chronic or acute on chronic injuries. The healing pathway and potential in these two situations may be fundamentally different. Therefore, the objective of this study is to develop an animal model of chronic rotator cuff tendon tears that would be useful in studying this common clinical problem. In this study, we hypothesize that increased time post-injury will result in increased detrimental changes to supraspinatus tendon organizational and mechanical properties.

MATERIALS AND METHODS

Fifty-one Sprague-Dawley rats were operated upon bilaterally to fully detach the supraspinatus tendon from its insertion site on the humerus. To model a chronic tendon tear, the tendon was allowed to freely retract without repair creating a gap approximately 4mm from its insertion. Rats were sacrificed at one, two, four, eight, or sixteen weeks post-injury. Nine uninjured rats served as normal, un-operated controls. The University of Pennsylvania IACUC approved this study.

Histologic sections, stained with H&E, from the left shoulders of 3 animals in each group (except two weeks) were evaluated blindly for cell number, vascularity, and organization. For organizational analysis, collagen fiber orientations, coefficient of variation (COV), and entropy (H) were determined using a quantitative polarized light microscopy method [4]. Fiber distributions were compared using the Kolmogorov-Smirnov test and COV and H were compared using an ANOVA followed by Fisher's post-hoc test.

Biomechanical testing was performed on the right shoulders of each animal in the control (n=9), one (n=10), two (n=11), four (n=10), eight (n=10), and sixteen (n=10) week injury groups. Supraspinatus

tendons were dissected free from surrounding tissue and prepared for mechanical testing. The width and thickness were measured as previously described to determine cross-sectional area [4]. For biomechanical testing, specimens were immersed in a 39°C PBS bath and preconditioned. A stress relaxation experiment was then performed by elongating the specimen to a strain of 3% at a rate of 50%/s followed by a 600sec relaxation period. Ramp to failure was then applied at a rate of 0.3%/s. Local tissue strain was measured optically. Peak and equilibrium load and stress were determined from the stress relaxation curve for each specimen. Load ratio was calculated as the ratio of the equilibrium to peak values. Stiffness and modulus were calculated using linear regression from the linear region of the load-displacement and stress-strain curves. Maximum load and stress were determined from the load-displacement and stress-strain

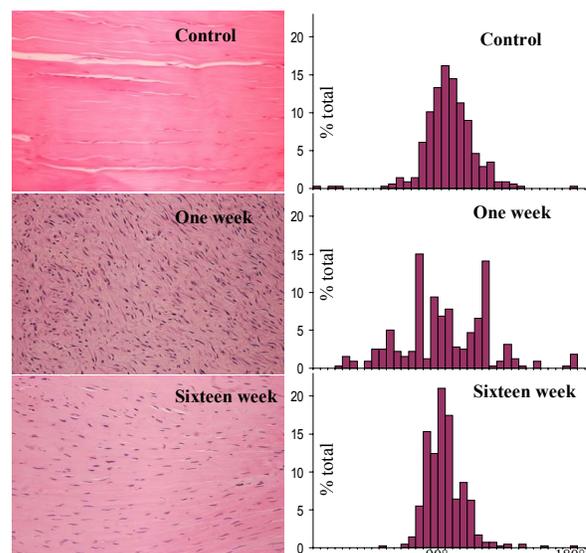


Fig 1: Typical Histology

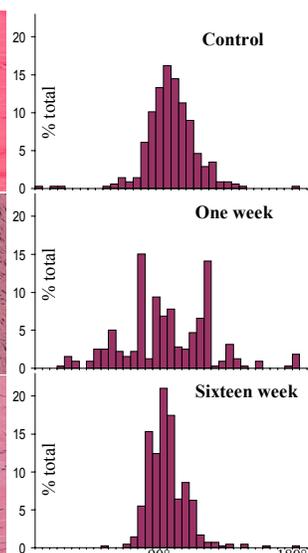


Fig 2: Fiber Distributions

curves. Between group differences were evaluated with one way ANOVA followed by a Fisher's post-hoc test.

RESULTS

Histologically, a dramatic increase in cellularity and vascularity, coupled with a decrease in organization, was seen in all injured specimens relative to uninjured control. Cellularity, vascularity, and organization improved with time but not to near normal levels (Fig 1). Organizationally, the collagen fiber distributions at one and sixteen weeks post-injury was statistically different from the control (Fig 2). Furthermore, there was a statistically significant increase in COV at one week compared to all other groups (Fig 3; Table).

Biomechanically (Figs 4, 5; Table), both the elastic (stiffness and max load) and viscoelastic (peak load, equilibrium load, and load ratio) structural properties initially decreased relative to control and increased with time from injury. Surprisingly, the max load for the eight and sixteen week injury groups was significantly increased relative to control. Interestingly, the load ratio was significantly decreased for the one and two week injury groups relative to control indicating a decrease in viscoelastic properties at those time points (Fig 4). The elastic (max stress and modulus) and viscoelastic (peak and equilibrium stress) material properties showed a trend similar to the structural properties. Unexpectedly, the modulus was significantly increased for the sixteen week injury group relative to all other groups (Fig 5). The area was increased relative to control for all injury groups except one week.

DISCUSSION

This study presents a new animal model for the investigation of chronic rotator cuff tendon tears. We used this model to investigate the organizational and mechanical changes that occur with time as a result of complete tendon detachment. We found that the histologic, organizational, and biomechanical properties followed a similar trend with a dramatic response early followed by a progressive improvement over time.

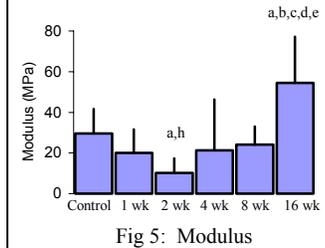
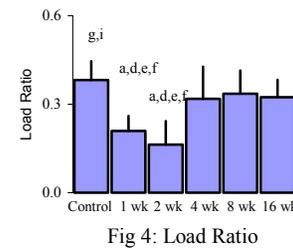
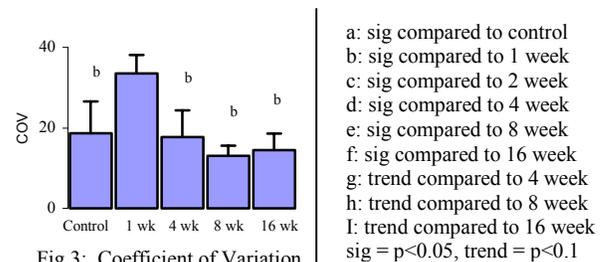
The detached tendons in this model had similar gross and histologic characteristics to chronic rotator cuff tendon tears in humans [5]. There was an increase in cellularity accompanied by disorganized granulation tissue with adhesions to surrounding tissue similar to the human situation. However, unlike human tendon tears, the space between the insertion site and the tendon stump was filled with disorganized scar tissue.

As anticipated, tendon detachment initially resulted in a dramatic decrease in mechanical properties but, surprisingly, the mechanical properties increased with time and the modulus and max load at the longest time point increased relative to control. Adhesions to surrounding tissues and an increase in the amount of tissue may partially explain the findings of this study. While the tendon is initially

completely unloaded, adhesions and connections to adjacent tissues and structures may form over time, causing the tendon to bear load fostering an active remodeling response.

Interestingly, our quantitative collagen fiber orientation results provide critical corroborating support to the mechanical property data. Furthermore, we observed a similar trend in muscle fiber size in this model with a dramatic decrease initially followed by an increase with time (unpublished data). The mechanical results are also consistent with clinical perceptions of a "stiff" tendon at the time of surgical repair for chronic rotator cuff tears in humans. Finally, based on quantitative intraoperative measures, the stiffness and tension of the musculotendinous unit in chronic rotator cuff tears has been shown to increase [6], which is also supportive of our findings.

Based on similarities in histology and mechanical properties, the chronic rotator cuff tendon tear animal model presented here is promising. In future studies, the mechanisms by which these changes occur and the effect of increased tension at the time of repair will be investigated.



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Table: Tendon mechanical and organizational properties (mean \pm sd, * $p < 0.05$)

	Elastic Properties					Viscoelastic Properties					Polarized Light Data	
	Area* (mm ²)	Max Load*(N)	Stiffness* (N/mm)	Max Stress* (MPa)	Modulus (MPa)	Peak Load*(N)	Equilibrium Load* (N)	Load Ratio*	Peak Stress* (MPa)	Equilibrium Stress*(MPa)	Coefficient of Variation*	Entropy
Control	1.0 \pm 0.4	5.5 \pm 2.1	8.6 \pm 3.5	7.7 \pm 4.5	29.6 \pm 12.0	1.0 \pm 0.2	0.37 \pm 0.13	0.38 \pm 0.06	1.2 \pm 0.6	0.47 \pm 0.24	18.7 \pm 7.9	3.3 \pm 0.5
1 week	1.6 \pm 0.6	3.3 \pm 1.2	3.1 \pm 1.2	2.0 \pm 1.2	20.0 \pm 11.6	0.4 \pm 0.1	0.10 \pm 0.03	0.21 \pm 0.05	0.3 \pm 0.1	0.06 \pm 0.02	33.5 \pm 4.6	3.0 \pm 0.5
2 week	3.0 \pm 1.7	3.7 \pm 1.9	3.8 \pm 1.5	1.4 \pm 1.0	10.1 \pm 7.3	0.5 \pm 0.2	0.09 \pm 0.05	0.16 \pm 0.08	0.2 \pm 0.2	0.04 \pm 0.04	-----	-----
4 week	2.1 \pm 0.9	7.4 \pm 4.5	6.3 \pm 3.5	3.6 \pm 2.5	21.3 \pm 25.1	0.6 \pm 0.3	0.17 \pm 0.07	0.32 \pm 0.11	0.3 \pm 0.2	0.08 \pm 0.04	17.8 \pm 6.7	3.1 \pm 0.6
8 week	2.2 \pm 0.9	10.8 \pm 5.0	8.0 \pm 2.2	7.5 \pm 6.8	24.2 \pm 8.9	0.8 \pm 0.5	0.26 \pm 0.13	0.34 \pm 0.08	0.4 \pm 0.3	0.14 \pm 0.12	13.0 \pm 2.6	2.8 \pm 0.5
16 week	1.8 \pm 1.0	12.5 \pm 9.3	8.2 \pm 4.2	6.2 \pm 3.8	54.5 \pm 22.7	0.8 \pm 0.3	0.22 \pm 0.10	0.32 \pm 0.06	0.6 \pm 0.5	0.18 \pm 0.14	14.5 \pm 4.1	2.9 \pm 0.4