Achilles Tendon Mechanical and Compositional Properties Differ Drastically in Early Healing Between Repaired and Non-Repaired Tendons

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Introduction

The decision to surgically repair Achilles tendons following rupture remains controversial[1]. Although operative treatment has been believed to result in superior Achilles function and lower re-rupture rates compared to conservative (non-operative) management[2], there is inadequate scientific evidence to support this belief[3,4]. Recent work has identified superior mechanical properties with conservative management at 3- and 6-weeks post-injury in rodents[5,6]. However, the immediate mechanical, structural, and histological changes during healing that drive these later healing responses remained unknown. Therefore, the purpose of this study was to evaluate the early tendon healing response by directly comparing repaired versus non-repaired Achilles tendons at 1-week post-injury. We hypothesized that non-repaired tendons would have superior mechanical, structural, and histological properties compared to repaired tendons at 1-week post-injury.

Materials and Methods

Study Design

Sprague Dawley rats (n = 36) at 16-weeks of age were used (IACUC approved). Animals received 2 weeks of treadmill exercise training (up to 60 minutes at 10m/min) prior to a complete blunt transection of the right Achilles tendon[5,6]. Animals were then randomized into repaired (Urbaniak variant of the Kessler) and non-repaired groups. Injured hind limbs were immobilized in plantarflexion. *Ex vivo* Assays

1-week post-injury, animals were euthanized and the Achilles tendon-foot complex was carefully removed *en bloc*. Achilles tendons were then finely dissected, cross sectional area measured, and secured in fixtures. Tendons were then loaded at 1N in a PBS bath while a series of sagittal B-mode high frequency ultrasound images (HFUS) were acquired (Vevo 2100, MS550D; VisualSonics) (n =1 0/group)[5]. Tendons (n = 10/group) were then mechanically tested through: stress relaxation (6% strain), a low-load dynamic frequency sweep (0.1 to 10 Hz), and constant strain rate until ultimate failure (Instron Electropuls 3000) [5]. An additional set of tendons was used for histological and immunohistochemical (IHC) analysis (n=8/group). Sagittal sections (7 μm) were collected and stained with Hematoxylin-Eosin (H&E), as well as with Safranin-O and Fast Green (SAF-O). Sections were also stained for collagen types I and III, with proteins visualized using 3,3' Diaminobenzidine.

Analysis

Achilles tendon relaxation, dynamic modulus (|E*|), tanδ, and toe and linear moduli were computed from mechanical data. Echogenicity and collagen fiber alignment were evaluated from the HFUS images at the injury site[7]. Three blinded graders independently evaluated cell density, nuclear shape, and SAF-O staining at the injury site. IHC was analyzed for % area stained with ImageJ (NIH, v1.48). T-tests were used to compare mechanical and structural properties and Mann-Whitney U-tests were used to compare histological scoring ($\alpha = 0.05$).

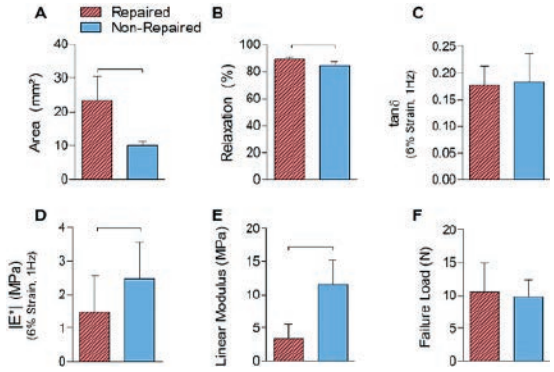

Figure 1. Mechanical Properties. Repaired tendons had elevated **(A)** tendon cross sectional area and **(B)** percent relaxation, but decreased **(D)** IE*I and **(E)** linear modulus compared to non-repaired tendons. No differences in **(C)** tano or **(F)** failure load existed between groups. Data are presented as mean and standard deviation, and statistical significance is indicated with lines ($p < 0.05$).

Results

Repaired tendons had a larger cross sectional area compared to non-repaired tendons (Figure 1A). Low strain viscoelastic testing revealed that percent relaxation (Figure 1B) was greater in repaired compared to non-repaired tendons, but there were no differences in tanδ (Figure 1C). |E*| and linear modulus (Figure 1D,E) were both decreased in repaired tendons compared to

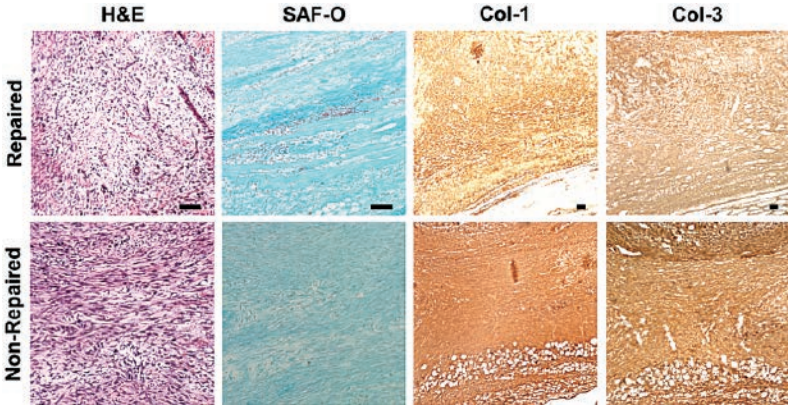

Figure 2. Histological and Immunohistochemical Properties. Repaired and non-repaired tendons were sectioned and stained with H&E, SAF-O, collagen type-I, and collagen type-III. Nuclei in repaired tendons were more round than in non-repaired tendons when assessed at the injury region (midsubstance). Non-repaired tendons had a trend for increased cellularity compared to repaired tendons. No differences in SAF-O, collagen type-I, or collagen type-III staining were observed. Scale bar = 100 μm.

non-repaired tendons, but there were no differences in failure load (Figure 1F). Histological evaluation (Figure 2) found that repaired tendons contained nuclei that were more rounded in shape compared to non-repaired tendons. Additionally, there was a trend for increased cellularity in non-repaired tendons. No differences in SAF-O staining, collagen type-I, or collagen type-III staining were found. No differences in echogenicity or collagen fiber alignment were detected at the injury site (not shown).

Discussion

This study investigated the effects of surgical treatment on Achilles tendon healing at 1-week post-injury in rodents. Overall, we observed large changes in material properties between groups at 1-week post-injury. In contrast, a previous study[8] found no differences in function or mechanics between repaired and non-repaired tendons 15-days post injury. This previous study did not control for the resting position of the ankle, which could reduce the potential mechanical benefits of conservative treatment.The role of mechanical loading has been shown to be a very sensitive factor for Achilles healing[9]. Mild tendon loading protection induced through Botox and limb suspension can modulate tendon material properties. Regarding histological findings, we observed increased cellularity and collagen disorganization at 1-week post-injury, similar to that found at later time points post-injury[5,6]. These changes notably continued later into healing[5,6], suggesting that disorganized matrix rich in collagen types-I and type–III is deposited early and propagates throughout tendon healing. Unlike tenocytes in uninjured tendon that display a spindle-like shape[10], cells in healing tendons had a more round morphology. Although material properties were superior in non-repaired tendons, no differences were observed in collagen staining or fiber alignment, which suggests that other changes in tissue composition may drive these mechanical responses. Future studies will examine additional protein and molecular changes due to surgical treatment.

Conclusions

Given the controversy between surgical or non-surgical treatments for Achilles rupture, well controlled basic science studies are necessary to evaluate tissue healing. This study identified numerous mechanical and cell morphological differences between repaired and non-repaired tendons as early as 1-week post-injury. This data may also provide a foundation for the differential healing response due to surgical treatment observed at later time points.

Acknowledgements

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