Noninvasive Quantification of Tendon Biomechanics: Shear Wave Elastography Imaging in a Porcine Tendinopathy Model

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Introduction

Tendons transmit contractile forces from muscle to bone and also stabilize joints with which they are mechanically associated. Structurally, normal tendons are composed of dense, fibrous connective tissue with an extracellular matrix (ECM) composed of parallel-arranged collagen type I fibers. Tendinopathy involves tendon structural damage. Histologically, the ECM of tendinopathic tissues exhibit a reduced total collagen content and an elevation in the percentage of denatured collagen. Currently, tendinopathy management is guided by traditional ultrasonography, which can only provide morphological assessment of the injured tendon but does not quantify biomechanics. Biomechanic measures, such as tendon elasticity, would serve as a more direct biomarker in guiding the decision to undergo aggressive surgical intervention vs. conservative physical therapy. Shear wave elastography (SWE) imaging is an ultrasound-based elastography technique for the noninvasive quantification of tendon biomechanics. SWE tracks shear wave propagation through tendon tissue and calculates shear wave speed (SWS). SWS is proportional to the tissue elastic modulus and may predict tissue strength, an indicator of the likelihood of rupture, which may be superior to morphological observations in guiding treatment approaches.

Objective

To investigate the potential for SWE to delineate regions of tendon structural damage, based on spatial variations in SWS, in a porcine tendinopathy model.

Methods

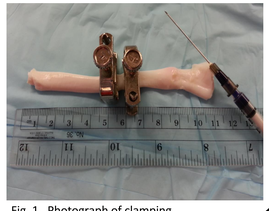
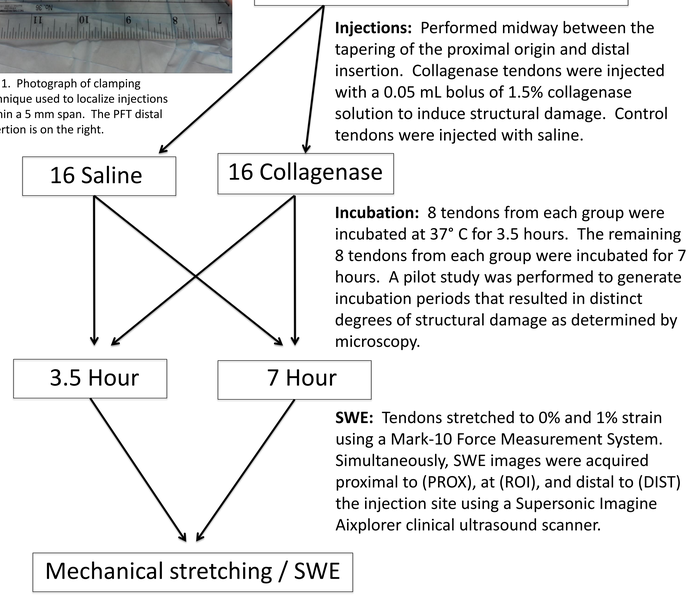


Fig. 1. Photograph of clamping technique used to localize injections within a 5 mm span. The PFT distal insertion is on the right.



Injections: Performed midway between the tapering of the proximal origin and distal insertion. Collagenase tendons were injected with a 0.05 mL bolus of 1.5% collagenase solution to induce structural damage. Control tendons were injected with saline.

Incubation: 8 tendons from each group were incubated at 37° C for 3.5 hours. The remaining 8 tendons from each group were incubated for 7 hours. A pilot study was performed to generate incubation periods that resulted in distinct degrees of structural damage as determined by microscopy.

SWE: Tendons stretched to 0% and 1% strain using a Mark-10 Force Measurement System. Simultaneously, SWE images were acquired proximal to (PROX), at (ROI), and distal to (DIST) the injection site using a Supersonic Imagine Aixplorer clinical ultrasound scanner.

Methods (Cont.)

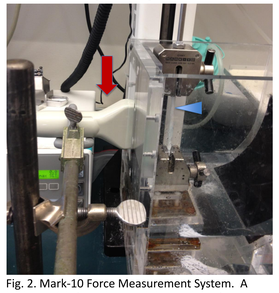


Fig. 2. Mark-10 Force Measurement System. A bath was fabricated around the wedge grips so that tendon (arrowhead) could be bathed in saline during testing. Ultrasound transducer (arrow).

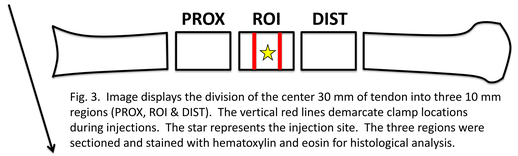


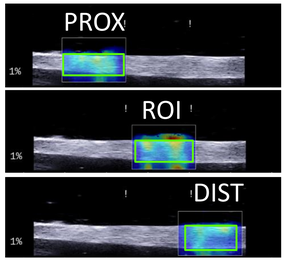
Fig. 3. Image displays the division of the center 30 mm of tendon into three 10 mm regions (PROX, ROI & DIST). The vertical red lines demarcate clamp locations during injections. The star represents the injection site. The three regions were sectioned and stained with hematoxylin and eosin for histological analysis.

Sectioning / H&E staining

Microscopy: Performed to assess validate clamping method in inducing focal structural degradation. Microscopic tissue integrity was quantified along the length of the tendon.

Microscopy / Analysis

Results



SWS calculations: A previously developed MATLAB function was used to circumscribe rectangular regions of the tendon within each acquired SWE image. The function calculated the average SWS within the rectangle. Average SWS calculations were performed in this manner for the SWE images captured at the three locations (PROX, DIST & ROI) for each tendon.

Fig. 4. Three panels depicting SWE images acquired for tendon 18JUNE13-2-7HR-COLL at 1% strain. This tendon was injected with collagenase and incubated for 7 hours. The top panel shows the image captured PROX to the injection site and the accompanying rectangular circumscription in which the average SWS was calculated using the MATLAB function. The middle panel shows the image captured at the injection site (ROI) and the bottom panel shows the image captured DIST to the injection site.

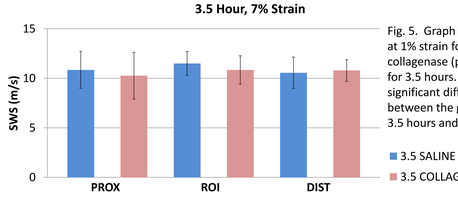


Fig. 5. Graph portraying the average SWS at 1% strain for the 8 saline (blue) and 8 collagenase (pink) that were incubated for 3.5 hours. There are no statistically significant differences in average SWS between the groups at any location for 3.5 hours and 1% strain ($p < 0.05$).

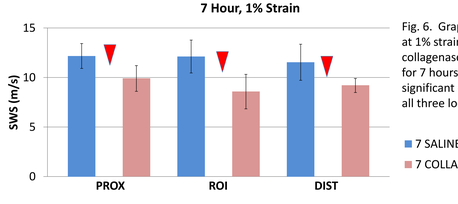


Fig. 6. Graph portraying the average SWS at 1% strain for the 8 saline (blue) and 8 collagenase (pink) that were incubated for 7 hours. There are statistically significant differences in average SWS at all three locations (red arrowheads).

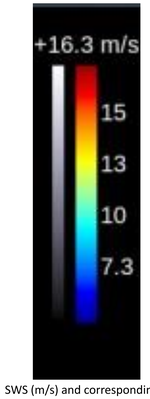


Fig. 7. SWS (m/s) and corresponding coloration spectrum. SWE images of highly elastic tendon regions exhibit a greater proportion of red coloration.

Average SWS results: There were significant differences in SWS (saline > collagenase) at 1% strain and 7 hours incubation for all three locations (PROX p=0.0031, ROI p=0.001, DIST p=0.0043). There were also significant differences at 0% strain and 7 hours, but only at (p=0.0005), and distal to (p=0.0035), the injection site (not shown). No statistically significant differences were observed for 3.5 hours incubation, at 0% or 1% strain.

Results (Cont.)

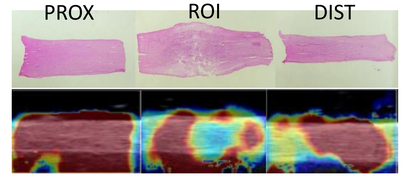


Fig. 8. Images depicting the spatial correlation between structural degradation in gross tissue sections (top panel) and SWS in SWE images (bottom panel) at all three regions (PROX far left) for tendon 31MAY13-8-7HR-COLL. This tendon was injected with collagenase and incubated for 7 hours. Grossly visible collagen degradation can be observed in the ROI tissue section. In the corresponding SWE image, there is a central focus of reduced SWS. In SWE images, a spectrum of coloration from blue to red corresponds to increasing SWS (Fig. 7).

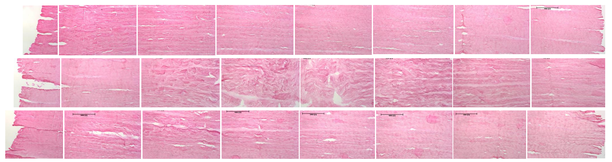


Fig. 9. Compiled microscopic (4X mag.) images captured along the length of the PROX, ROI, and DIST sections for tendon 31MAY13-8-7HR-COLL. In this manner, microscopic images were captured for many of the tendons so that subsequent color thresholding could be performed in an attempt to quantify collagen degradation.

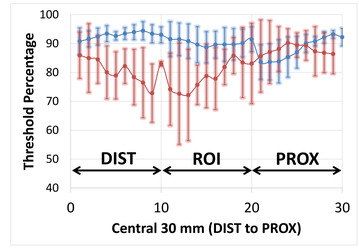


Fig. 10. Results from color thresholding, performed using ImageJ, on 6 collagenase tendons incubated for 7 hours (pink) and 6 saline tendons incubated for 7 hours (blue). The x-axis corresponds to tendon regions (PROX = 30-20 mm, ROI = 20-10 mm, DIST = 10-0 mm). Each point represents an average threshold percentage (n=6) for microscopic images captured at that location along the length of the tendon. The percentage of an image that reaches a user-designated threshold should correspond to the collagen content of that image.

Quantification of tissue degradation: It was expected that collagen content, determined by thresholding, would be similar between saline and collagenase tendons at corresponding locations along the PROX and DIST regions. Significant differences were expected along the center of the ROI region (where injections were originally performed) with collagenase tendons exhibiting less collagen content than saline tendons. From Fig. 10 it can be concluded that collagenase injections using the clamping method didn't result in completely localized structural degradation. The 7 hour collagenase tendons analyzed using color thresholding demonstrated less collagen content in the DIST region as well as the ROI region.

Conclusion

Collagenase-mediated tendon structural damage does appear to convey decreased SWS, proportional to tissue elasticity, on images captured via SWE when *ex vivo* tendons are incubated for 7 hours. These findings suggest that SWE may be a useful tool for predicting ultimate tissue strength in tendinopathic tissues.

Future Direction

Injection method: Alternative approaches to improve collagenase localization should be investigated. Tighter clamping is a possible modification to the method utilized in this study but care must be taken to avoid insulting the tissue integrity by the clamps themselves.

SWE image analysis: The regional rectangular circumscription method used with the MATLAB function to generate average SWS within the ROI region did not purely isolate the clamped injection site and, instead, included regions proximal and distal to where degradation was intended to occur. Alternative approaches to reproducibly and objectively circumscribe the clamped region should be investigated.

Pull-to-failure testing: Should be performed to correlate decreased SWS, and therefore elasticity, with decreased forces at failure.