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BIOMECHANICS OF MUSCLE-TENDON JUNCTION AND TENDON-BONE INSERTIONS

Sandhya Chandrasekaran (1), Ashley J. Saltzman (2), HY Shadow Huang (3)

(1) Mechanical and Aerospace Engineering
North Carolina State University
Raleigh, NC, USA

(2) Mechanical and Aerospace Engineering
North Carolina State University
Raleigh, NC, USA

(3) Mechanical and Aerospace Engineering
North Carolina State University
Raleigh, NC, USA

INTRODUCTION

The muscle-tendon-bone unit transmits contractile loads, developed in the muscle, through the tendon as a tensile load, into the bone to produce motion. The myotendinous junction (MTJ) connects the muscle to the tendon through a series of interdigitated extensions of the microstructure. The last collagen fibrils of the tendon extend into invaginations in the myofibers of the muscle and transmit loads through lateral contractions [1, 2]. The tendon-to-bone insertion is a functionally graded tissue that varies in collagen types, collagen orientation, and mineralization [3-8].

The tendon is a hierarchical tissue starting at the nanoscale with collagen molecules which aggregate into microfibrils which aggregate into fibrils which form fibers which collect to form fascicles which aggregate to form the tendon [8]. It is composed of collagen, proteoglycans, glycoproteins, water and cells [9]. The study of the muscle-tendon-bone unit is of importance because injuries of these tissues are common and they tend to heal slowly. Additionally, biomechanical properties are not restored to their original value after healing [9].Most studies focus on the uniaxial tendon properties aligned with the primary load direction. There are few studies that consider the biaxial properties of the tendon [10, 11]. However, these studies consider one longitudinal and one transverse direction. To the best of the authors' knowledge, no previous studies have been conducted on the biomechanical properties of a cross-section of the tendon.

In this study we report stress-strain data for the tendon cross-section in various locations and relate the findings back to the biochemical composition.

METHODS

Porcine forelimbs of large sows were obtained from the local abattoir. The skin and fatty tissue surrounding the muscle-tendon-bone unit was carefully cleared away from the deep digital flexor tendon of the third digit. The tendon is removed via a cut near the tendon-to-bone insertion and a cut near the muscle-tendon junction (MTJ) (tendon branches for other digits are severed near the main tendon stem).

Biaxial Testing From each of these tendons, three cross-sections for biaxial testing were removed with two razor blades fixed together. One cross-section is extracted from the tendon proximal to the junction of flexor tendons (muscle-end, n=8), one cross-section from the middle of the third digit tendon (mid-tendon, n=9), and one cross-section from the distal end of the removed sample (bone-end) (n=6). Samples that were not large enough to mount in the testing apparatus were thrown out. The average thickness of the cross-sections was 0.79±0.20 mm. After removal from the forelimb, all cross-sections were stored in Hanks' Balanced Salt Solution (HBSS) at 4° C between dissection and testing.

The BioTester 5000 (CellScale Biomaterials Testing, Waterloo, ON, Canada) biaxial testing apparatus, equipped with a 0.5 N load cell in each axis of loading, was used to measure the force and displacement of the tissue samples. The built-in CCD camera is synchronized with the load cells and actuators and collected 1280 x 960 px images at a rate of 1 Hz. Force and displacement data were collected at a rate of 5 Hz and were used to compute stress and strain data. Each tested sample was mounted to the BioTester with four BioRakes each consisting of five tungsten tines spanning 4 mm in width. Testing was performed in a room temperature bath of HBSS. The dorsopalmar axis of the tendon cross-section was aligned with the x-axis of the BioTester and the mediolateral axis was aligned with the y-axis. A pre-load of 10mN was applied in both axes and this state was defined as the zero strain state. Each sample was pre-conditioned equibiaxially with five successive triangular waveform to 10% true strain at a medium strain rate of 2%/s [12]. After pre-conditioning samples were rested at zero strain for 30 seconds to allow the tendon to relax. This rest duration was determined during preliminary testing to allow the tendon samples to return to a zero stress state. Finally, each sample was strained equibiaxially at 2%/s to a peak of 20% true train. Stress and strain curves are prepared from the collected force and displacement data. These curves are broken into three strain regions: 0-7.5% (low strain), 7.5-15% (medium strain), and 15-20% (high strain). An effective elastic modulus in each strain region was computed. **Statistical Analysis** All data are presented as the means ± the standard deviations. Differences in collagen concentration are tested using a single factor ANOVA. A Bonferroni multiple comparisons test with $p < 0.05$ was performed to compare the elastic modulus of the tendon samples between location and direction to determine if there is a variation in biomechanical property along the length of the tendon or any anisotropy within the cross-section. All statistical analysis was conducted using GLM in SAS 9.4 (SAS Institute Inc, Cary, NC, USA). A value of $p < 0.05$ was considered significant.

RESULTS

The biaxial testing results for the three locations (bone end, mid tendon, and muscle end) were used to produce stress versus strain curves presented in **Figure 1**. Maximum stresses at 20% true strain for the muscle end were 35.2±34.2 kPa in the x-direction and 36.9±51.9 kPa in the y-direction, for the mid tendon were 83.8±34.4 kPa in the x-direction and 92.0±52.1 kPa in the y-direction, and for the bone end were 142.2±85.7 kPa in the x-direction and 110.6±77.6 kPa in the y-direction. From these data, effective elastic moduli were computed for each of the previously mentioned strain ranges. Using a Bonferroni multiple comparisons test with $p < 0.05$, it was determined that the bone end of the tendon is stiffer in all regions of strain than the muscle end of the tendon. No differences between the bone end of the tendon and the mid tendon were detected. And the muscle end of the tendon was only more compliant in the larger strain range (15-20%). No differences were detected between the x- and y-direction stiffnesses in any strain range or location.

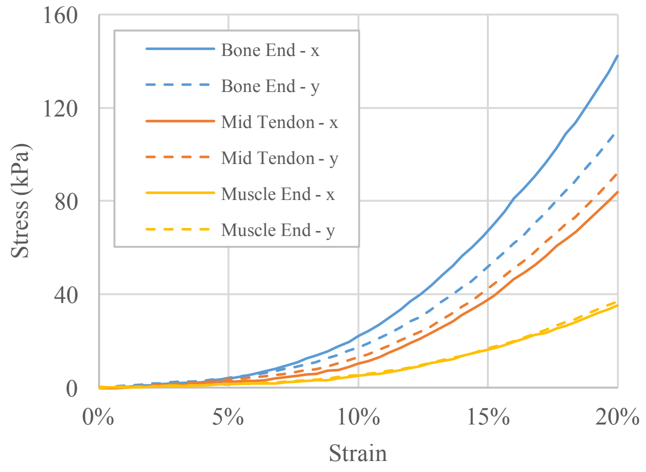

Figure 1: Average stress versus strain response for tendon cross-sections collected from the bone end (blue, n=6), the mid tendon (red, n=9) and the muscle end (purple, n=8). The tendon cross-section tends to be more stiff toward the bone end and more compliant toward the muscle end. No dramatic transverse anisotropy is apparent.

CONCLUSION

The current study, to the best of the authors' knowledge, is the first to provide stress-strain data for the cross-section of the tendon. This equibiaxial testing was performed at three locations along the porcine deep digital flexor tendon and a variation in stiffness was observed. Additionally, we report the collagen concentration in these same three regions, however, observed no variation. The result of this study will be furthered by expanding the locations and orientations tested along the muscle-tendon-bone unit in an effort to improve the understanding of its unique structure and to aid in tissue engineering repair techniques.

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