Effect of Intermolecular Crosslinking on the Mechanics of Tendon Fibrils and Fascicles

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INTRODUCTION: The collagen molecule is the main building block of connective tissues. Molecules are connected laterally by intermolecular crosslinks1. When collagenous tissues are overloaded in tension, collagen molecules can unravel, resulting in molecular denaturation. However, the role of crosslinks in resisting tissue damage is unclear2. Molecular dynamics simulations have shown that crosslinks can transfer load between adjacent collagen molecules during axial extension3. However, this load transfer mechanism and its role in the damage of collagen molecule assemblies has not been experimentally demonstrated. Hierarchical levels of organization between the collagen molecule and whole tissue further complicate the cascade of load transfer, so multiple organizational levels need to be tested. The objective of this study was to discover the mechanical effect of increased crosslinking during tensile overload of both tendon fascicles and fibrils to explore the role of crosslinks in tendon mechanics. We hypothesized that increased crosslinking would increase the strength and stiffness at both the fibril and fascicle level of tendons thus altering the damage and yield behavior.

METHODS: To increase crosslinking in tendon, the low-toxicity chemical genipin was used to form covalent intermolecular crosslinks with amine groups in collagen residues.4 Tendon fascicles were isolated from frozen male Sprague-Dawley rat tails older than 12 weeks. Fascicles were divided into two groups. The control group was soaked in 1X PBS, while the treated group was soaked in 0.625% w/v genipin solution in 1X PBS.

Intact control and treated fascicles were soaked for 2 hours at room temperature and then underwent either differential scanning calorimetry (DSC) or uniaxial tensile testing (UTT) (n=3 for all 4 groups). For DSC, fascicles were blotted dry and placed in hermetically sealed pans. The DSC tests included one heating cycle from 20-90°C and one cooling cycle back to 20°C at 10°C/min. Temperature at onset of denaturation and peak temperature were quantified, along with curve width and curve area. For UTT, fascicle cross-sectional area (CSA) was measured using a custom rotating laser system. Fascicles were then preloaded to 0.03N and stretched to failure at a rate of 0.5%/s.

Fascicles in each group were soaked for 1 hour at room temperature and then mechanically agitated to isolate individual collagen fibrils. Custom MEMS devices were used to stretch individual collagen fibrils in tension to failure at a strain rate of 0.5-1 %/s (n=6 control, n=7 treated). Fibrils were mounted on custom MEMS devices and adhered using UV-curing epoxy drops. Each MEMS device was placed on a piezoelectric actuator and immersed in PBS. Elongation was applied at a constant strain rate of 0.5-1%/s until failure and images of the MEMS device and fibril were acquired at 1 Hz. Images were processed to obtain the displacement of a force-gauge beam integrated with the MEMS device. Beam displacement was converted to applied force using the results of a finite element model of the MEMS device. To calculate fibril CSA, scanning electron microscopy was used to measure diameter of an unstretched region of each fibril. For both UTT and MEMS tensile testing, CSA and gauge length were used to obtain stress-strain curves from force-elongation curves. The yield point was defined as the stress and strain when modulus was reduced by 50%. Data were analyzed using independent t-tests (α=0.05).

RESULTS: For DSC, onset temperature (p=0.003), area (p=0.002), and width (p<0.001) were significantly higher for treated samples. Peak temperature trended higher for treated samples but was not significant. Genipin treatment produced changes in the mechanical behavior of both fascicles and fibrils that were readily apparent from the stress-strain curves (Fig. 1). Fascicles treated with genipin sustained significantly higher yield stress (p=0.016), yield strain (p=0.007) and peak stress (p=0.024) (Fig. 2). At the fibril level, genipin treatment resulted in higher yield stress and peak stress than control fascicles (p<0.001), but there was no effect of treatment on yield strain. Unlike fascicle level tests, treated fibrils sustained higher peak modulus (p<0.001) but did not differ in peak strain or yield strain (Fig. 3).

DISCUSSION: Increased onset temperature, area, and width confirmed that genipin crosslinking produced a more thermally stable, structurally uniform tissue. Increased fibril modulus yield stress demonstrates that increased intermolecular crosslinking improves load transfer between collagen molecules. Improved load sharing between collagen molecules allows fibrils and fascicles to sustain higher forces and stresses before yielding and failing. Interestingly, the increased modulus of fibrils did not translate into increased modulus of fascicles. This suggests that relative sliding above the fibrillar level, i.e. between fibers, is unaffected by genipin treatment.

SIGNIFICANCE/CLINICAL RELEVANCE: Collagen crosslinking occurs during tendon development, and it is altered due to both injury and disease. Crosslinking via exogenous methods such as genipin treatment is often used to improve the mechanical properties of engineered replacement tissues. Treatments or therapies that target intermolecular crosslinking via exogenous or endogenous approaches could be used to repair damaged tendons in situ.


Figure 1. Fibril stress-strain curves for control and genipin treated groups.

Figure 2. Fascicle testing. Peak stress (a), yield stress (d), and yield strain (e) were higher for genipin-treated samples. Strain at peak stress (b), peak modulus (c), and CSA (f) did not differ between groups. Data points with mean ± SEM. *p < 0.05.

Figure 3. Fibril testing. Peak stress (a), peak modulus (c), and yield stress (d) were higher for genipin-treated samples. Strain at peak stress (b), yield strain (e), and CSA (f) did not differ between groups. Data points with mean ± SEM. *p < 0.05.