TENSILE TESTING OF SINGLE COLLAGEN FIBRILS FROM ACHILLES TENDONS FROM AN OSTEOGENESIS IMPERFECTA MOUSE MODEL

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Introduction

The homozygous *oim* mouse model of osteogenesis imperfecta forms homotrimeric collagen type I molecules, which assemble into fragile bone tissue [1]. Counterintuitively, it has been shown that individual collagen fibrils from *oim/oim* (severe OI type III) tail tendons have higher ultimate stress compared to wild-type (WT) fibrils [2]. However, collagen fibril mechanics are in part steered by the prevalence of enzymatic and non-enzymatic crosslinks between collagen molecules [3], and elevated non-enzymatic crosslinks have been reported in the bones of the *oim* mouse [1], compared to WT.

So far, only fibrils from the positional tail tendons were investigated from *oim/oim* and littermate WT mice. No data exists on fibrils from energy-storing tendons. To fill this gap, here we report the mechanical properties of *oim/oim* and WT fibrils obtained from Achilles tendons.

Methods

Achilles tendons of two wild-type (B6C3Fe-a/aCol1a2$^{+/+}$) and two *oim/oim* (B6C3Fe-a/aCol1a2 $^{oim/oim}$) mice were dissected and spread out on a glass slide to expose isolated collagen fibrils. Collagen fibrils (n=8 for *oim/oim*, n=5 for WT) of lengths of about 100 µm were tested to rupture at a strain rate of 5%/s. The tests were conducted in phosphate buffered saline (PBS, pH 7.4), at room temperature, using the NanoTens device [4]. In the NanoTens, fibrils are reversibly attached to a microgripper mounted on an interferometric force probe, which is actuated to conduct the tensile tests.

Fibril diameters were determined by means of atomic force microscopy (AFM) (NanoWizard Ultraspeed A, JPK, Germany) in Quantitative Imaging™ mode in PBS. Collagen fibrils are identified prior to tensile tests via their characteristic D-banding by imaging in air in contact mode. Force-displacement data were transformed into engineering stress and strain data using the initial cross-section determined via AFM and length determined via optical microscopy. Tangent tensile modulus was calculated as the smoothed derivative of engineering stress vs. engineering strain.

Stress-strain curves were classified as showing 3-phase behavior if, after the initial increase of tensile modulus and its consecutive post-yield decrease, another pronounced increase was observed before rupture.

Fibril cross-sectional areas, ultimate stresses and strains, and tensile modulus values were not normally distributed. Hence, groups were compared by a Mann-Whitney-U-Test and reported as median and quartiles.

Results

Generally, samples of both groups, WT and *oim/oim* fibrils exhibited three-phase behavior, with a strain stiffening before rupture (see. Fig.1).

Geometrically, no significant differences were detected in dry fibril cross-sectional area, wet fibril cross-sectional area, or swelling ratio, between the tested *oim/oim* and WT fibrils. WT fibrils showed no significant difference in maximum tensile modulus before yield (*oim/oim*: 0.63 GPa (0.58- 1.24 GPa), WT: 0.98 GPa (0.72-1.45 GPa)). Ultimate strength for WT fibrils (0.32 GPa (0.34- 0.25 GPa)) was significantly higher than for *oim/oim* (0.14 GPa (0.08-0.26 GPa)). There was no significant difference in ultimate strains.

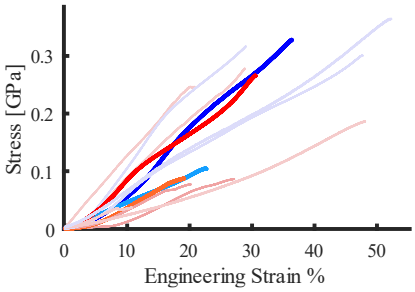

Figure 1. *Stress-strain curves (oim/oim red, WT blue), with highlighted exemplary curves for 2-phase (oim/oim orange, WT turquoise) and 3-phase behavior.*

Discussion

Swelling ratios, tensile moduli and ultimate stresses measured in the present study were within the range of values previously reported for mouse tail tendons.

The non-significant differences observed between the *oim/oim* and WT Achilles tendons in the swelling ratio and tensile modulus are in contrast with results previously found for tail tendon collagen fibrils [5]. This indicates that possibly different crosslink profiles exist in tendons according to their anatomical function, as well as in bone. This motivates further studies considering crosslink content analysis in *oim/oim* and WT tendons and demineralized bones in parallel to tensile testing of fibrils obtained from these tissues.

References

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