Effects of Hydration on Nanoscale Morphology and Mechanics of Individual Type I Collagen Fibrils in the Brtl Mouse Model of Osteogenesis Imperfecta

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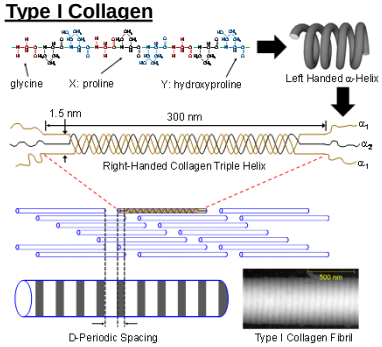
INTRODUCTION

Type I Collagen



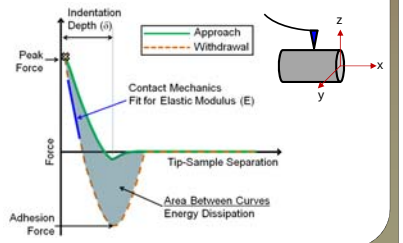
- Collagen exists with a distribution of D-period spacings which shifts with disease
  - Previous atomic force microscopy (AFM) work done in dry bone
- Is D-period related to mechanical function?
- Do hydration or disease play a role?

Osteogenesis Imperfecta (OI)

- Point mutations in collagen result in helical glycine substitutions
- Δ in α chain structure lead to decreased molecular collagen quality
- What are the nanoscale ramifications?
- Brtl/+ mouse: cysteine substituted for glycine at 349 position of α helix

Mechanical Indentation Using AFM

- Probe pushed into surface to a known load



HYPOTHESIS

Nanoscale morphology and mechanical integrity of individual Type I collagen fibrils vary as a function of OI disease state and hydration

MATERIALS AND METHODS

Animals and Sample Preparation

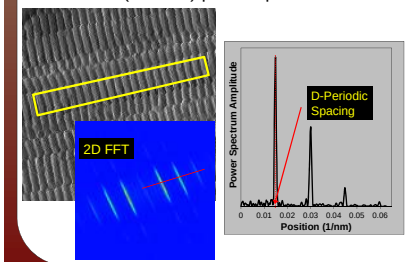
- WT and Brtl/+ male mice, Sv129/CD-1/C57BL/6S strain at 6 months of age (n=4)
- Individual tendon fibers removed from each tail and placed in PBS.
- Fiber rinsed in water, deposited on glass, gently flattened and imaged/indented

AFM Imaging and Indentation

- 3 locations in each of 2-3 fibers per animal
- Wet (tip radius ~2 nm, k = 0.7 N/m, α = 17.5°)
  - Indented to 20 nN: 4-5 indents per fibril
- Dry (tip radius ~8 nm, k = 40 N/m)
  - Indented to 50 nN: 4-5 indents per fibril
- All probes calibrated prior to indenting

Morphological Analysis

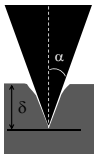
- D-periodic spacing from 2D Fast Fourier Transform (2D FFT) power spectrum



Mechanical Analysis

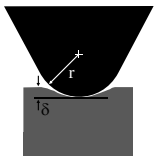
- Indentation modulus (E_s): curve fitting the middle 50% of unloading curve
  - Wet: Sneddon – indent depth > tip radius
  - Dry: Hertz – indent depth < tip radius

Sneddon Model



$$F = \frac{2}{\pi} \cdot \frac{E_s}{1-\nu_s^2} \cdot \tan\alpha \cdot \delta^2$$

Hertz Model



$$F = \frac{4}{3} \cdot \frac{E_s}{1-\nu_s^2} \cdot \sqrt{r} \cdot \delta^{\frac{3}{2}}$$

- Poisson's ratio (ν_s): assumed to be 0.35
- Other properties directly from curves

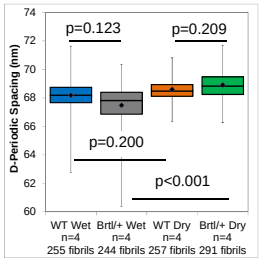
Statistical Analysis

- WT vs. Brtl in wet or dry conditions
- D-spacing: Sample values averaged; compared with Student's t-test (n=4)
- Morphology population distributions: Kolmogorov-Smirnov (K-S) test
- Mechanical Properties:
  - Average in each sample (n = 4; t-test)
  - Average from each fibril (KS test)
  - All indents in group (KS test)

RESULTS

Morphology Measures

- No mean differences in D-Spacing, but significant disease-induced shifts in wet and dry samples

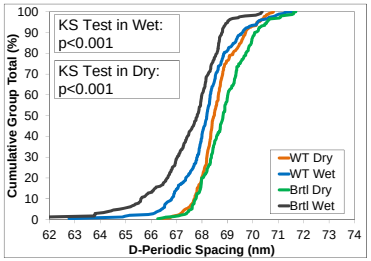


WT Wet: 68.2 ± 1.2 nm

Brtl/+ Wet: 67.5 ± 1.4 nm

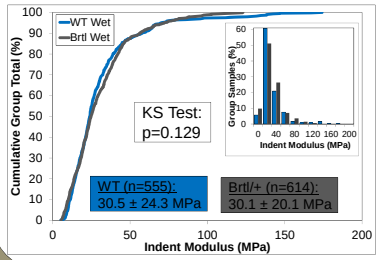
WT Dry: 68.6 ± 0.8 nm

Brtl/+ Dry: 68.9 ± 1.0 nm

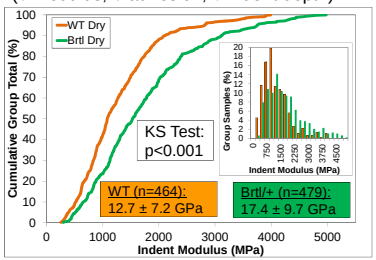


Mechanical Properties

- Wet: No mean mechanical differences
- Wet: No differences when all fibrils grouped



- Dry: No mean mechanical differences
- Dry: Significant mean and distribution differences when all fibrils grouped (↑ modulus, ↑ adhesion, ↓ indent depth)



DISCUSSION

Phenotypic changes in collagen fibril ultrastructure were detected in tendon

- Wet: population distribution of D-spacings was shifted ↓ in Brtl/+, but was not accompanied by nanoscale mechanical changes.
- Dry: population of D-spacings was shifted ↑ in Brtl/+, and was accompanied by significantly ↑ modulus, ↑ adhesion and ↓ indentation depth

Hydration dependence in D-spacing phenotype: differential changes with drying

- 0.4 nm increase in WT (p=0.200) versus a 1.4 nm increase in Brtl D-Spacing (p<0.001)
- May indicate an alteration of the internal structure of Brtl/+ fibrils?
  - Water may facilitate bridging - compresses structure creating shorter-spaced fibrils.
  - Altered Brtl structure: fibrils may be more easily compressed shifting wet population ↓ vs. WT
  - Water and bridging lost with drying: compressed fibrils relax/expand. Fibrils initially more compressed expand more easily causing a loss of fibrils with shorter spacing.
- Poisson Effect: shrinkage in the z-direction with drying should be accompanied by expansion in the y and x directions (x is axial direction leading to increased D spacing when dried)

Hydration dependence in mechanical phenotype

- No wet phenotype vs. significant dry phenotype: related to altered internal structure of Brtl fibrils?
- Water may bridge damaged structure and carry load as a fibril is indented, masking differences.
- As water is lost, the damaged structure collapses resulting in mechanical property changes.

Phenotypic differences in collagen morphology and mechanical properties exist as a function of disease state and tissue hydration.

Dehydration and other manipulations cause artifacts in biological samples which require water, a factor which must be considered for studies at any length scale in collagen-based tissues.