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
- Points mutations in collagen result in helical glycine substitutions
- α chain structure lead to decreased molecular collagen quality
- What are the nanoscale ramifications?

Mechanical Indentation Using AFM
- Probe pushed into surface to a known load

RESULTS

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

Osteogenesis Imperfecta (OI)
- No mean differences in D-Spacing, but significant disease-induced shifts in wet and dry samples

Type I Collagen
- Previous atomic force microscopy (AFM) work done in dry bone
- Is D-period related to mechanical function?
- Do hydration or disease play a role?

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, S129/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 25 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 (Ei): curve fitting the middle 50% of unloading curve
- Wet: Sneddon – indent depth > tip radius
- Dry: Hertz – indent depth < tip radius

DISCUSSION

Phenotypic changes in collagen fibril ultrastructure were detected in tendon
- Wet: population distribution of D-spacings was shifted up in Brtl+, but was not accompanied by nanoscale mechanical changes.
- Dry: population of D-spacings was shifted up in Brtl/+ and was accompanied by significantly up modulus, adhesion and indent depth

Hydration dependence in D-spacing phenotype: differential changes with drying
- 0.4 nm increase in WT (p<0.001) 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 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.