Multiscale Phenotypic Analysis of Osteogenesis Imperfecta in Murine Bone

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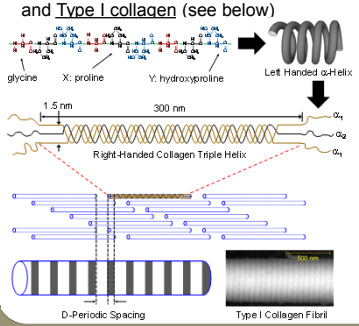
INTRODUCTION

Bone and Type I Collagen

- Bone's hierarchical structure spans 10 orders of magnitude in length
- Fundamental constituents: hydroxyapatite and Type I collagen (see below)



Osteogenesis Imperfecta (OI)

- Mutations in collagen compromise triple helix structure and quality
- Detrimental impacts across length scales
- oim mouse: mutation in the gene encoding collagen α2 chain (proα2(I))
- oim/oim: produce only α1 homotrimers
- oim/+: heterotrimers and α1 homotrimers

Multiscale Analysis

- Studies at various lengths scales have been performed in oim/+ and oim/oim mice
- Limited work performed at multiple scales within same study
- **Goal**: perform multiscale analyses in bone from oim/+ and oim/oim, maintaining samples as close to physiological conditions as possible

HYPOTHESIS

An inability to properly form and organize the collagen matrix in oim mice directly impacts the formation and performance of mineral, leading to brittle tissue behavior, reduced bone strength and altered structural organization.

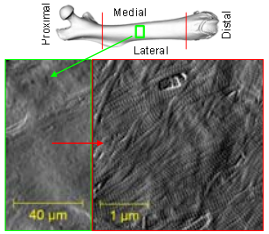
MATERIALS AND METHODS

Animals

- 12 week female mice from Jackson labs: wild type (WT), oim/+ and oim/oim
- Femora used: n=15 per group

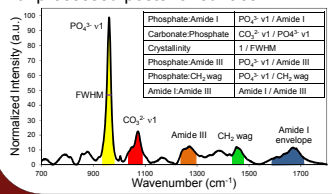
Atomic Force Microscopy (AFM)

- Right femur (n=5) mounted, polished, treated with 0.5M EDTA (pH=8)
- 3 anterior sites per bone, ~55 fibrils per bone analyzed by 2D FFT for D-spacing



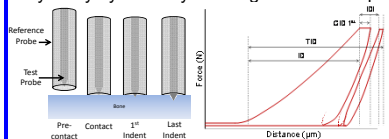
Raman Spectroscopy

- Right femurs (n=5-6): 5 locations along unprocessed posterior surface



Reference Point Indentation (RPI)

- Femora used after Raman – 4 indents at regions corresponding to Raman sites
- Indentation to 2 N for 5 loading cycles.
- Cycle-by-cycle analysis using MATLAB script



Microcomputed Tomography (μCT)

- All left femora, 12 μm voxel size
- Volumetric bone mineral density (vBMD) at 5 posterior locations corresponding to Raman.
- Mid-diaphysis geometry analyzed using a custom MATLAB script
- Distal trabeculae: circular face touching the growth plate and extended 0.5 mm proximal

Whole Bone Mechanics (3 pt Bending)

- Displacement control, 0.3 mm/sec, posterior surface in tension
- Stress/strain from beam bending equations

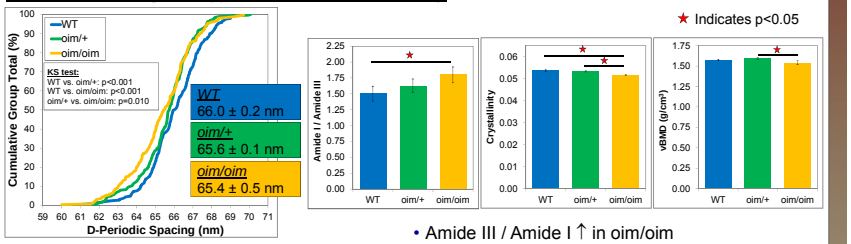
Statistical Analysis

- One-Way ANOVA, Tukey and Dunn's post hoc tests
- Kruskal-Wallis or two-sample t-tests of unequal variance when appropriate
- D-spacing population distributions: Kolmogorov-Smirnov (KS) test

RESULTS AND DISCUSSION

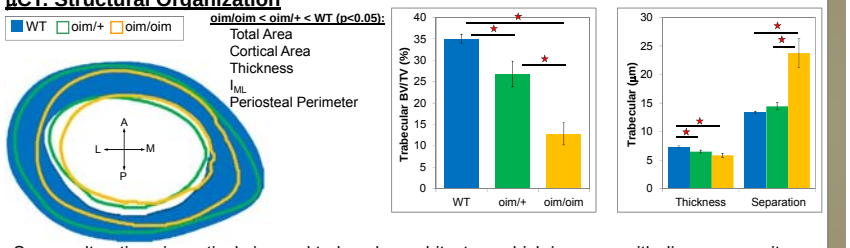
Nanoscale Morphology and Tissue Chemistry

★ Indicates p<0.05



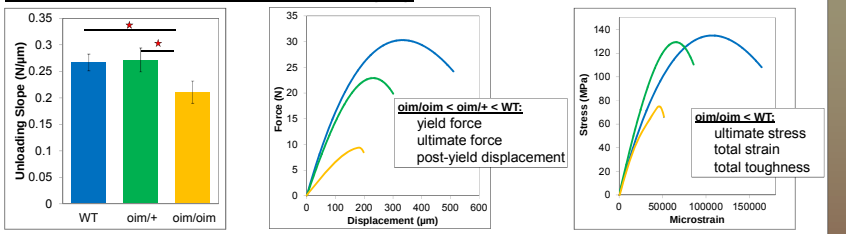
- Mean D-spacing ↓ in oim/+ and oim/oim vs. WT
  - Driven by kinking in tropocollagen molecules?
- D-spacing distributions differed for all group
  - oim/oim shifted ↓ for entire population vs. WT
  - oim/+ intermediate: due to the presence of both heterotrimers and homotrimers?
- Homotrimers may alter crosslinking and stability
- Amide III / Amide I ↑ in oim/oim
  - Amide I band is sensitive to secondary structure of collagen, Amide III is relatively stable
  - Ratio indicative of oim-induced change in collagen alignment and structure, in agreement with AFM data
- Crystallinity and vBMD ↓ in oim/oim
  - Defect in the binding and growth of mineral?
  - Likely caused by noted changes in collagen structure

μCT: Structural Organization



- Severe alterations in cortical size and trabecular architecture which increase with disease severity

Tissue and Structural Mechanical Integrity



- ↑ Indentation distance and ↓ unloading slope in oim/oim
  - Indicate ↓ stiffness and ability to resist damage with loading in oim/oim
  - Trend in unloading slope is similar to modulus from bending test
- Structural stiffness, strength and post-yield behavior ↓ with increasing disease severity
- Tissue properties in oim/oim indicate weak, brittle material
  - ↓ strength, ductility and toughness in oim/oim vs. WT
  - oim/+ modulus ↑ vs. WT and oim/oim
  - ↓ strain and toughness in oim/+ vs. WT
  - No difference in oim/+ strength versus WT

CONCLUSIONS

- OI-induced changes to the collagen triple helix modified the assembly of collagen fibrils.
- Disease-specific shift in D-periodicity caused by α1 homotrimers alters the mineralization process.
- Changes in the collagen/mineral composite impact mechanical properties of the tissue
- Tissue deficiencies and altered structure result in weaker bone with decreased post-yield behavior.

Molecular changes to collagen due to OI altered both the organic and inorganic phases of bone causing defects throughout the bone hierarchy leading to architectural flaws, decreased strength and brittle behavior.