Alterations in Diabetic Bone Revealed by Co-localized Raman and Reference Point Indentation

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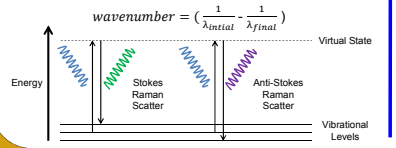
INTRODUCTION

Type 2 Diabetes (T2D)

- Detrimental impacts on multiple systems including the musculoskeletal system
- Chronic hyperglycemia implicated in advanced glycation end product (AGE) formation when reducing sugars react with free amino groups in proteins
- AGEs in Type I collagen may lead to a stiffening of fibrils and decreased toughness in bone

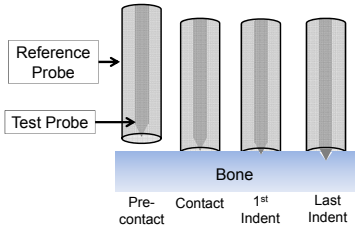
Raman Spectroscopy

- Laser excites chemically bonded electrons and some fall back into different energy state shifting wavelength of scattered light
- Wavenumber of shift is specific to type of bond and chemical composition



Reference Point Indentation (RPI)

- Outer reference probe housing inner test probe makes contact with bone surface
- Test probe cyclically indents bone to investigate mechanical properties at the 10-100 µm scale



Study Contribution

- Detect chemical changes in the bones of a model of T2D with altered collagen morphology and correlate those changes with mechanical data from a RPI system.

HYPOTHESIS

There are differences in the chemical composition of diabetic rat bone compared to control which will correlate with microscale mechanical properties.

MATERIALS AND METHODS

Experimental Groups

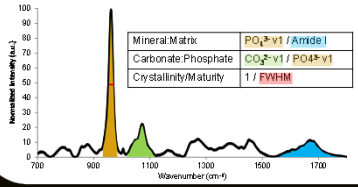
- Zucker diabetic Sprague-Dawley (ZDSD) rats switched to high fat diet at 15 weeks, returned to normal diet at 18 weeks
- Sprague-Dawley control diet (CD) animals remained on normal diet
- Rats sacrificed at 28 weeks (n=4 per group)
- Left tibia harvested, stored at -20°C

Sample Preparation

- 12 mm section of diaphysis surrounding the tibia-fibula junction was removed, mounted posterior side up to a steel disk, and stripped of the periosteum.
- Reference marks on either end of the section allowed for registration.

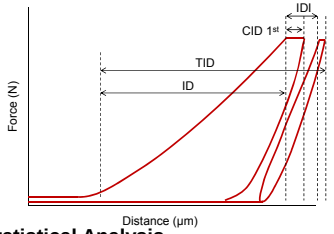
Raman Spectroscopy and Analysis

- 5 locations per sample were imaged
- Gaussian function fit to PO_4^{3-} v1, CO_3^{2-} v1, and Amide I peaks for integrated band area and full width half maximum (FWHM)



RPI and Analysis

- Ten cycles of 5 N at 2 Hz using BioDent
- 1st cycle indentation distance (ID), 1st cycle unloading slope (US 1st), 1st cycle creep indentation distance (CID 1st), total indentation distance (TID), indentation distance increase (IDI), average creep indentation distance (CID), average energy dissipated (ED), average unloading slope (US), and average loading slope (LS) were recorded

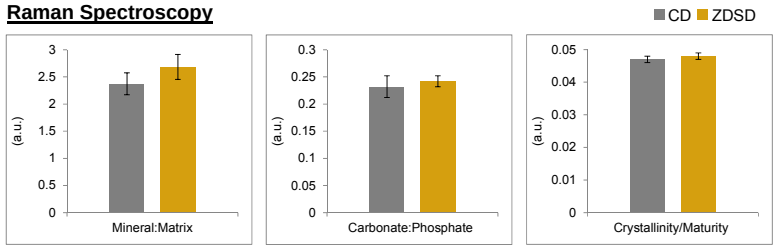


Statistical Analysis

- Raman – 5 samples per group
  - Two-tailed Student's t-test
- RPI – 1 sample per group
  - Percent differences calculated
- Correlation – 10 data points
  - Pearson's Product Moment Correlational Analysis

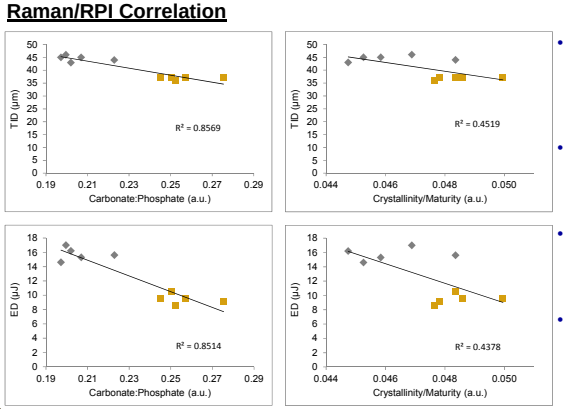
RESULTS

Raman Spectroscopy



Mineral:Matrix	Carbonate:Phosphate	Crystallinity/Maturity
CD: 2.373 ± 0.20	CD: 0.232 ± 0.02	CD: 0.047 ± 0.001
ZDSD: 2.685 ± 0.23	ZDSD: 0.242 ± 0.01	ZDSD: 0.048 ± 0.001
p=0.048	p=0.286	p=0.247

Raman/RPI Correlation



- ID, TID, IDI, ED, and LS all had moderately strong to strong correlations ($p<0.01$) to Carbonate:Phosphate
- TID, ED, US, and LS all had mild to moderate correlations ($p<0.05$) to Crystallinity/Maturity
- ID and IDI had mild marginally significant ($0.05<p<0.1$) correlations to Crystallinity/Maturity
- US had a mild marginally significant ($0.05<p<0.1$) correlations to Mineral:Matrix

DISCUSSION

ZDSD as Model of T2D

- Gradual diet-induced change simulates human adult-onset diabetes
- Bones previously shown to have reduced mineral density and mechanical properties
- Collagen nanoscale changes may be an important contributor to altered mechanics

Raman Spectroscopy Changes

- Mineral:Matrix increased in ZDSD
- Ash fractions performed previously reveal decreased mineralization in ZDSD
- Major reduction in Amide I band in ZDSD
  - Likely presence of increased AGEs in ZDSD could cause the change in Amide I
  - Changes in collagen morphology observed in bone and tendon (ORS2012) could also contribute to Amide I reduction

RPI Differences

- ZDSD vs. CD: IDI, ED, and LS changed by at least 35% (n=1, data not shown)
- Likely increase of AGE-induced crosslinks would create a tougher material at this scale
- Would explain increased LS and decreased IDI and ED

Raman/RPI Correlation

- Significant correlations support the increased utilization of RPI to link mechanics at intermediate scale to chemical composition and/or morphological data
- Carbonate:Phosphate and Crystallinity had directionally matched correlations with LS as only positive correlation supporting validity
- Significance with low sample size compelling
- Future work will expand sample size of RPI groups and quantify AGEs in collagen

ZDSD bone had significantly altered Mineral:Matrix ratio along with indications of a stiffer bone matrix at the microscale with multiple significant correlations between Raman spectroscopy and RPI.