Microscale Intrinsic Property Testing of Lacunae in Trabecular Bone Biopsies of Type 1 Diabetic Women

August 12, 2022

By Maxwyll J. McConnell, University of Nebraska- Lincoln
Under Dr. Wen Qian

Portland State University

NSF

IN OUR GRIT, OUR GLORY™

- A Quick Anatomy Lesson
- Bone Literature Review
- Our Research into Bone Fracturing
- Instrument Overview
- Microscale Investigation of Bones
- Conclusions
- Future Research

Human Bones

Composition

- Bones are comprised mainly of collagen and calcium phosphate

Microstructure

- Bone growth is determined at the central canals
- Bone matrix does not develop uniformly

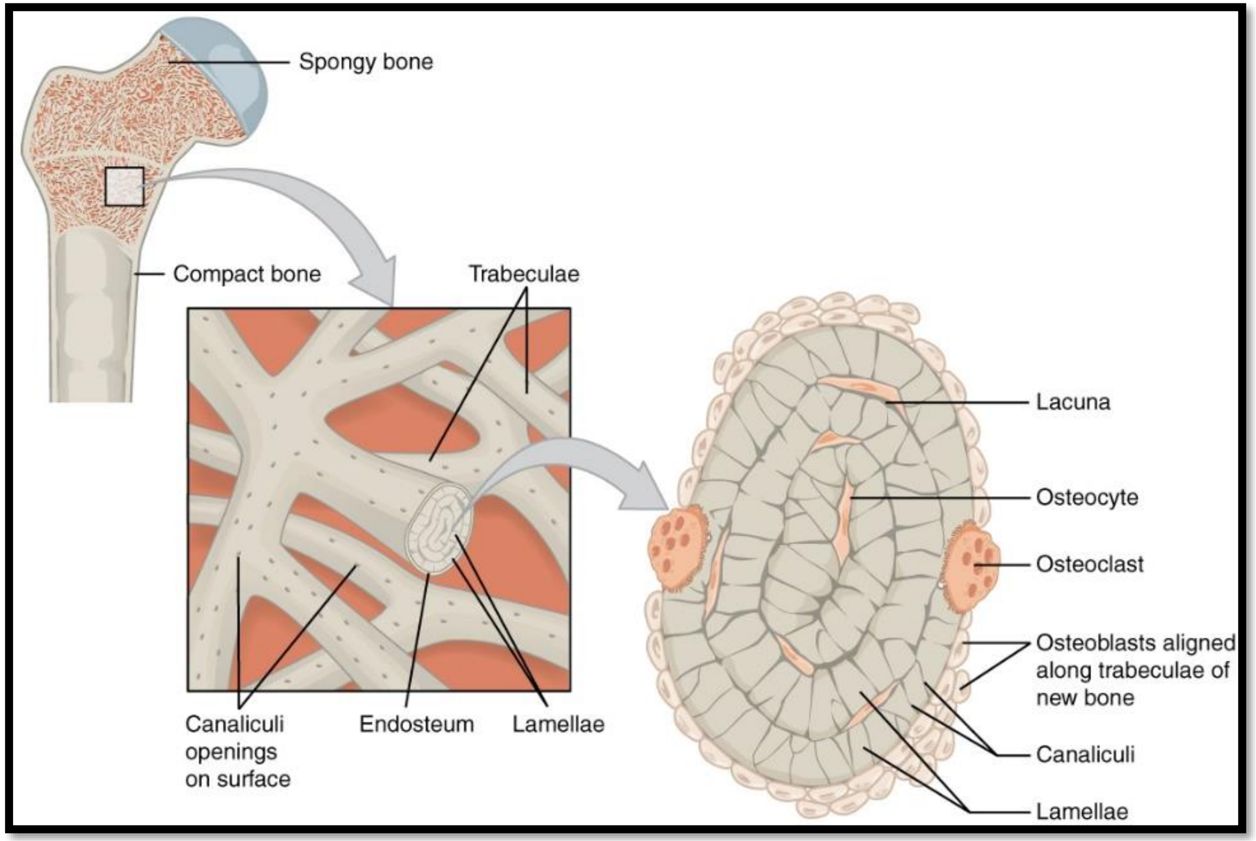


Figure 1: Labeled image of bone tissue structures

Lacunae

Lacuna Definition

- Small cavity within the bone matrix containing an osteocyte

Indications

- A small quantity of lacuna will serve as an indicator that bones are prone to fracturing

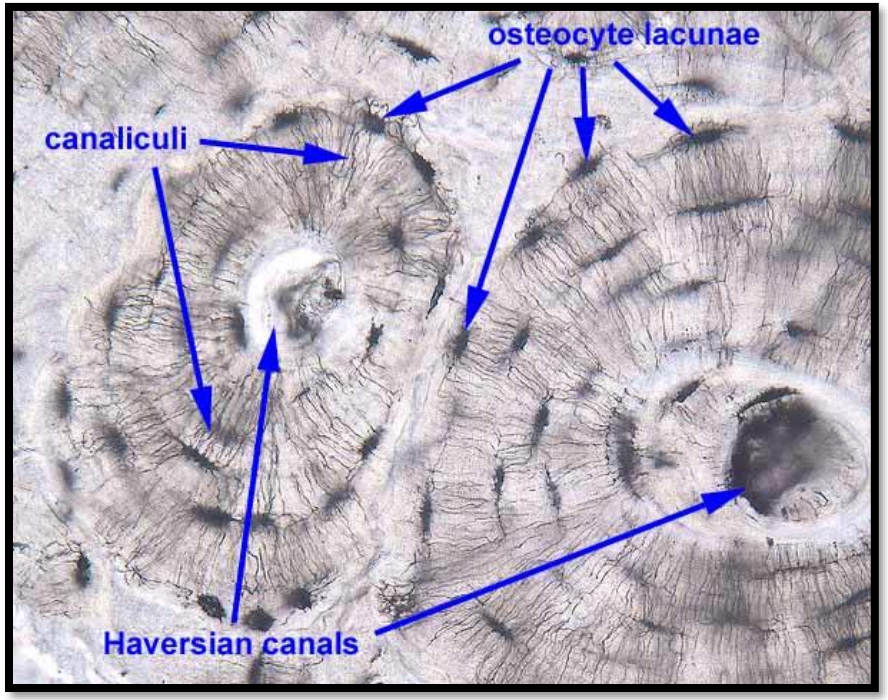


Figure 2: Central Canals and Surrounding Lacunae

Existing Bone Research

Studies have shown that:

- Osteocytes play a key role in the Mechanotransduction and remodeling process of bone tissue
- Postmenopausal and T1D women who have experienced a bone fracture have reduced bone quality
- Higher density of lacunae, and a higher percentage of mineralized osteocyte lacunae contribute to a higher bone tissue hardness

Challenges, Motivations and Goals

Challenges

- Natural problems occur when dealing with biomaterials (heterogeneity)
- The direct role of osteocytes in modulating bone matrix remains largely unknown

Motivations

- There has never been a microscale observation comparing near-lacunar areas to far-lacunar areas in T1Ds
- Additional information with regards to the intrinsic material strength properties of bone tissue is needed for better treatments

Goals

- Develop a method of study to aid in differentiating the probability of bone fracturing in T1D women
- Determine whether a correlation between the distance from a lacunar site and the hardness and modulus of bone tissue exists

Sample Preparation

- Bone biopsies were taken from the iliac crest of type 1 diabetic and non-diabetic women
- A Longitudinal section of 250 µm thickness was cut from each bone biopsy
- Each slice includes both trabecular and cortical bone, but only trabecular bone was analyzed
- Biopsies were then embedded in clear resin on a glass slide
- Sample surfaces were polished with a series of sandpaper and buffing solutions ending with a 1 µm slurry buff

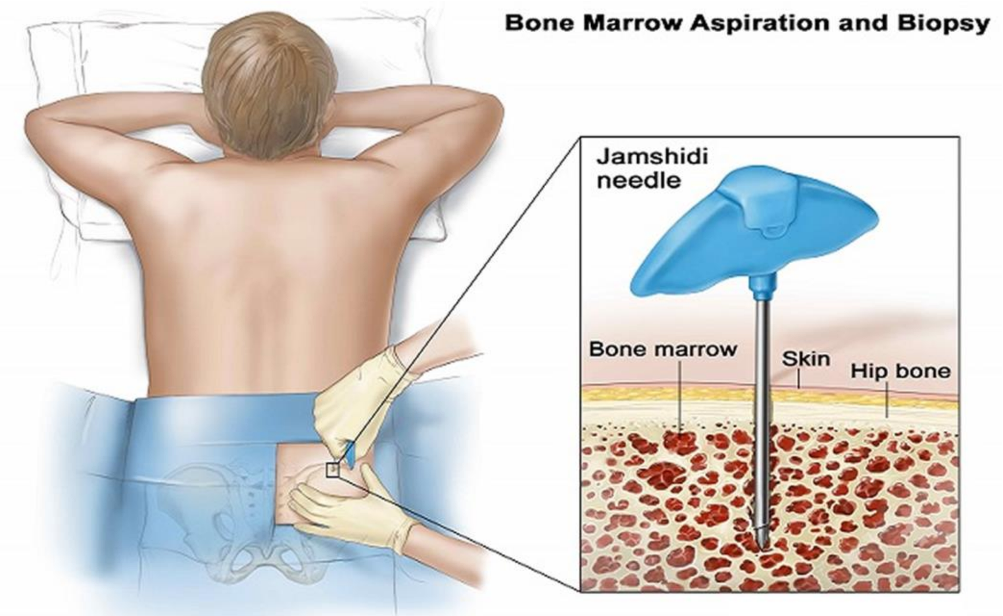


Figure 3: CGI image of bone biopsy acquisition

Laser Microscopy and Nanoindentation

- Laser Scanning Microscopy: A microscopy technique combining features of optical microscopes, roughness gauges, laser profilometers, and scanning electron microscopes

- Laser Microscopy is a wide scale tool that can generate images up to 28,800x magnification with a large depth of field

- Nanoindentation is among the most important tools for the assessment of the mechanical properties of bone tissue

- Nanoindentation utilizes a small diamond tip pressed into the material surface, and a high accuracy transducer measures the force feedback, allowing for hardness and modulus values to be obtained

"Keyence laser scanning microscope VK-X200K," *Keyence laser scanning microscope VK-X200K | College of Engineering | University of Nebraska–Lincoln*. [Online]. Available: https://engineering.unl.edu/nercf/keyence-laser-scanning-microscope-vk-x200k/. [Accessed: 06-Aug-2022].

"TI 950 TriboIndenter user Manual - University of Nebraska–Lincoln." [Online].Available:https://engineering.unl.edu/downloads/files/UserManual-HystrionTriboIndenter-sm.pdf. [Accessed: 06-Aug-2022].

Keyence Confocal Scanning Laser Microscope

The VK-X200K Series combines features of an optical microscope, roughness gauge, laser profilometer, and scanning electron microscope, our laser scanning confocal microscope performs non-contact surface profile, surface roughness, and thickness measurements without the need for sample preparation.

- **Up to 28,800x magnification**
- **.5 nm Z-axis resolution on almost any material**
- **High-resolution, large depth-of-field observation**
- **Profile and roughness measurements with zero sample preparation**
- **Measures thickness and uniformity of clear layers**
- **No data loss – even on steep angles**
- **3D Color imaging**

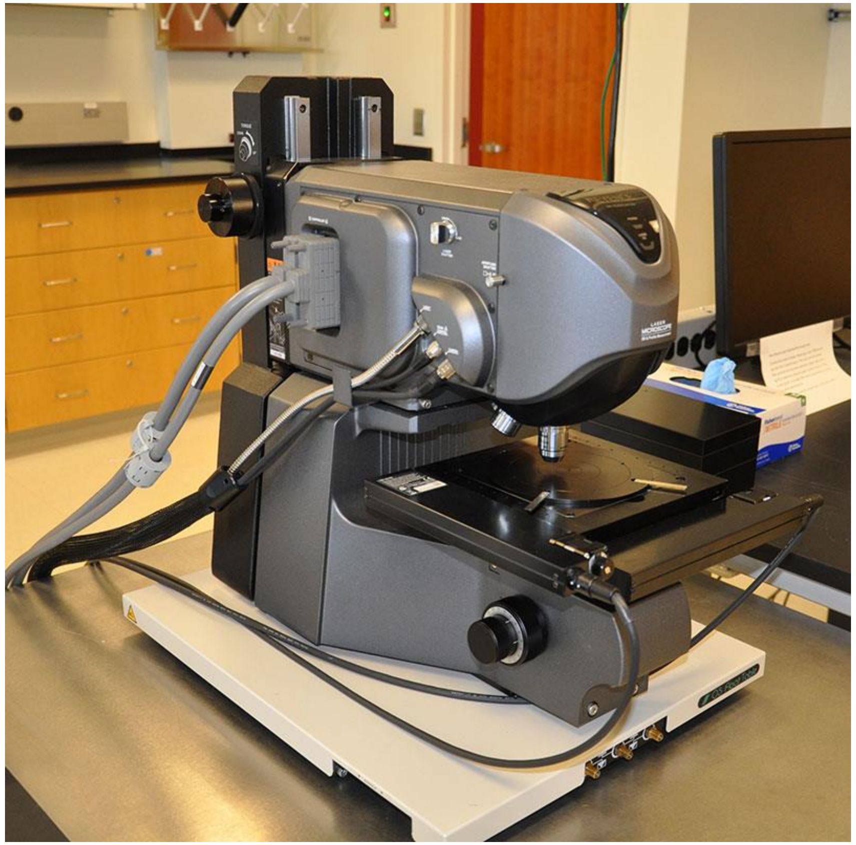


Figure 4: Keyence Confocal Scanning Laser Microscope

Hysitron TI 950 Triboindenter

Microscale Investigation of Bones

The TI 950 combines Hysitron's patented three plate capacitive transducer* technology with state-of-the-art control technology to achieve unmatched performance in nanomechanical characterization. The system features a sub 30 nN force noise floor, ultra-fast feedback control, user-definable data acquisition rates up to 30 kHz, the widest range of nanomechanical testing techniques, and the ability to test with various Hysitron heads seamlessly.

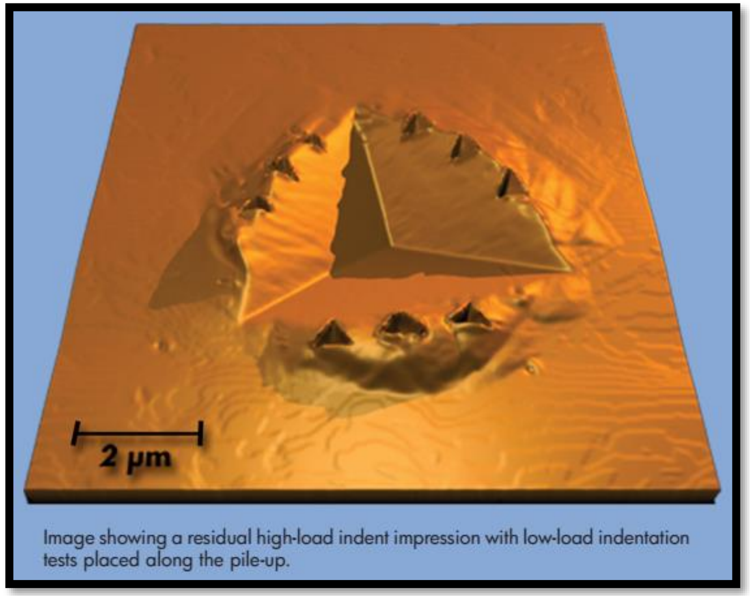


Figure 5: CGI image of nanoindentation

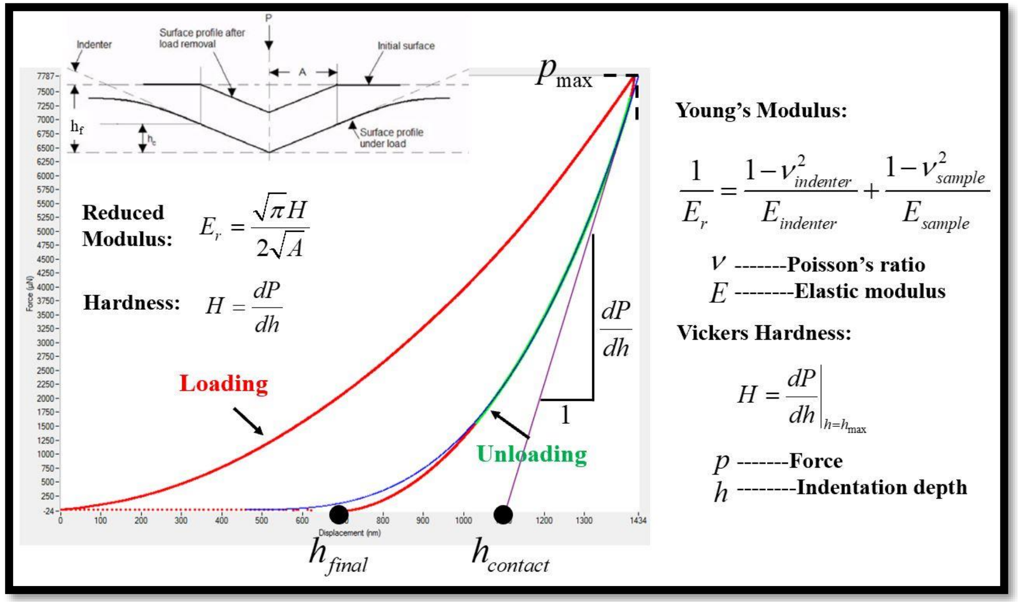


Figure 6: Force-Displacement curve

Microscale Investigation of Bones

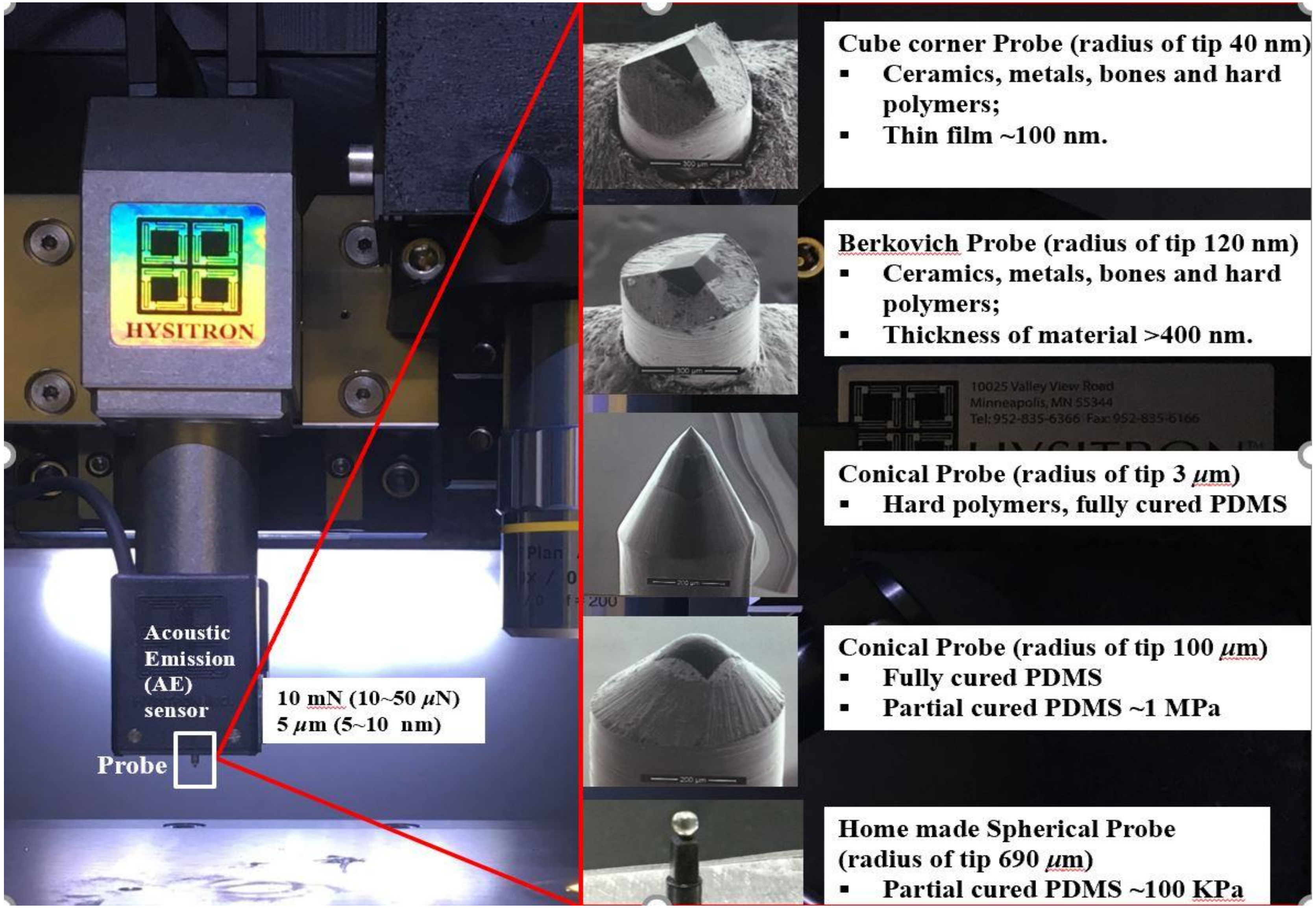
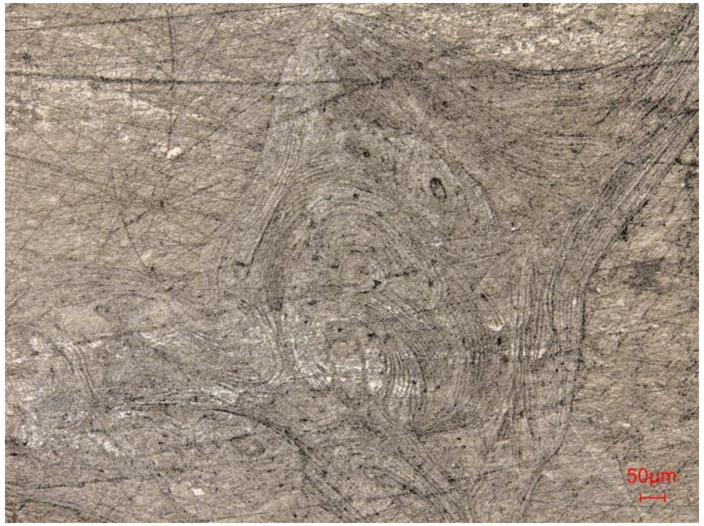
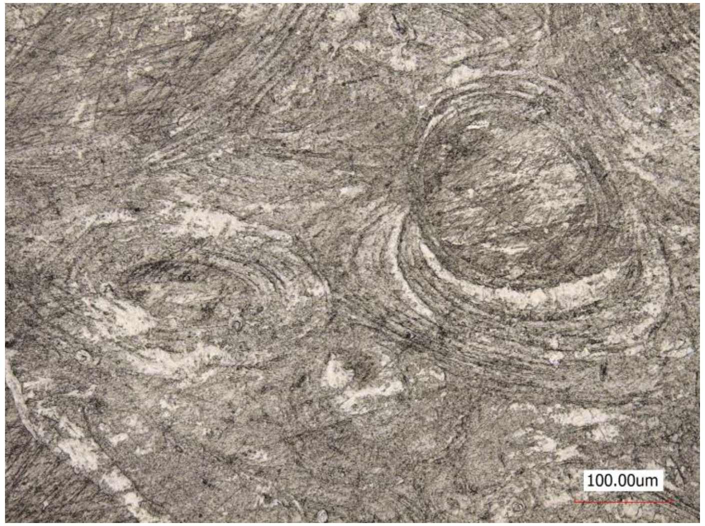


Figure 7: Common nano-indenter probes

Keyence Data

- The scanning laser microscope provided the first image of the lacuna and the surrounding structure.
- The surrounding bone features were utilized to relocate the same lacuna on the Nanoindenter

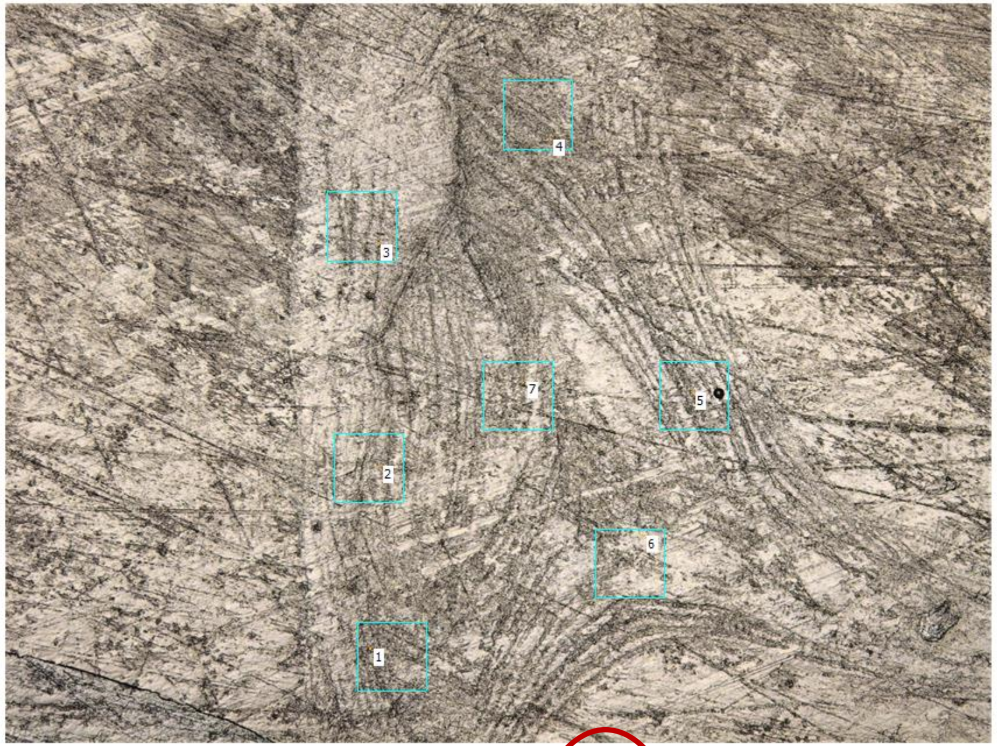




Figures 8-10: Images taken by laser microscope of central canals and the local lacuna

Roughness Measurement

- Roughness Data provided by Keyence laser microscope
- Ra is the arithmetic average roughness for the length of measurement performed
- 49 total roughness areas tested
- 50µm x 50µm measurement areas
- Total roughness average: 1.32µm



	Rp	Rv	Rz	Ra	Rq	Rsk	Rku
Seg.1	4.82um	4.72um	9.53um	1.20um	1.50um	-0.0793	2.7236
Seg.2	3.30um	3.82um	7.12um	1.02um	1.23um	-0.0761	2.3370
Seg.3	2.72um	2.55um	5.27um	0.61um	0.75um	-0.0955	2.7687
Seg.4	2.41um	2.47um	4.88um	0.56um	0.70um	0.1021	2.8448
Seg.5	3.15um	2.65um	5.80um	0.58um	0.73um	0.0227	3.1461
Seg.6	2.90um	3.50um	6.40um	0.75um	0.93um	-0.2702	2.8412
Seg.7	2.52um	2.81um	5.33um	0.58um	0.72um	-0.0776	2.8230

Figure 11: Roughness analysis showing sample areas and table with corresponding Ra values

Nanoindentation Data

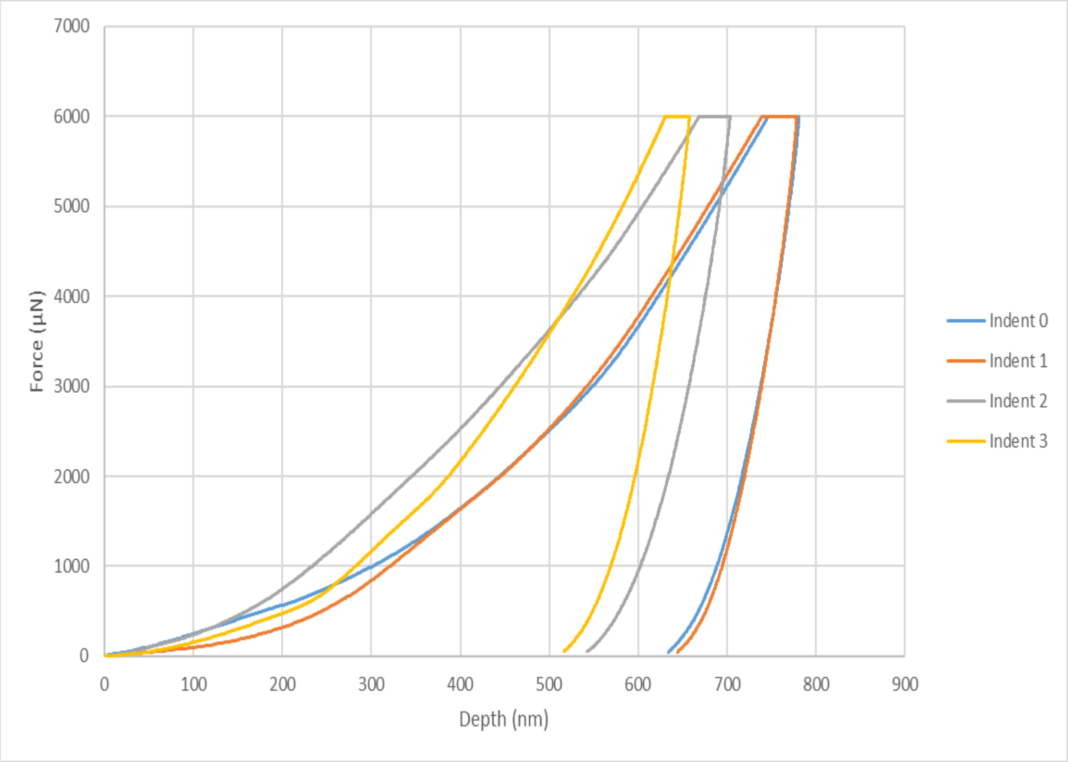


Figure 12: Near-Indentation Force-Displacement Curves

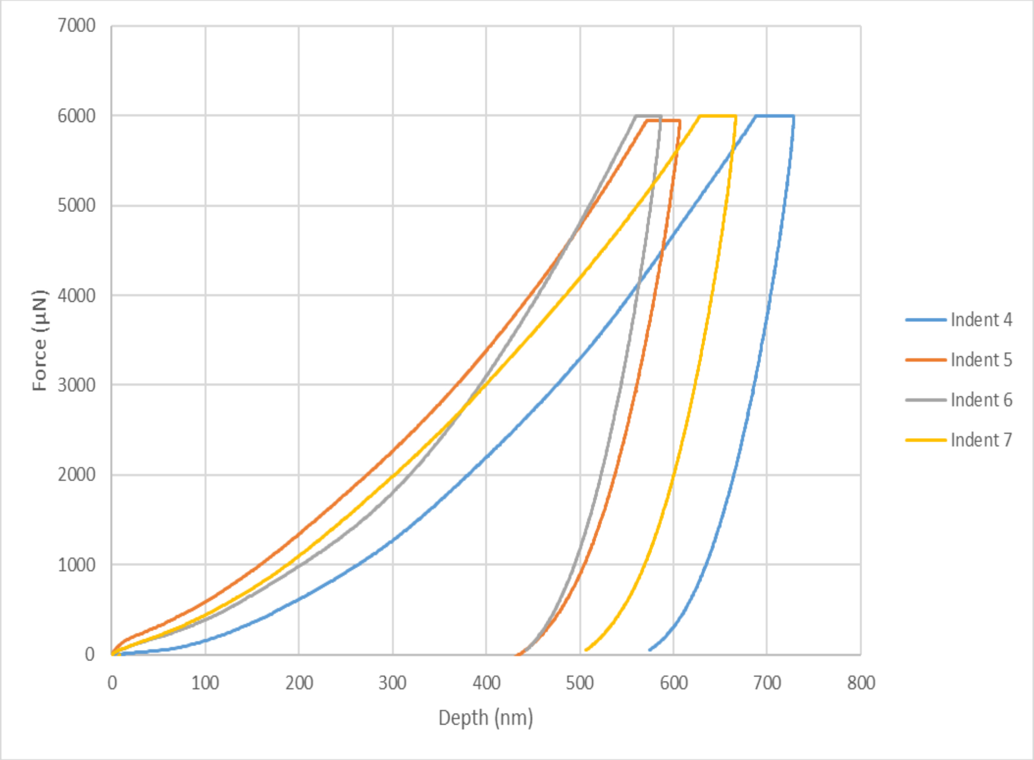


Figure 13: Far-Indentation Force-Displacement Curves

Post-Indentation Images

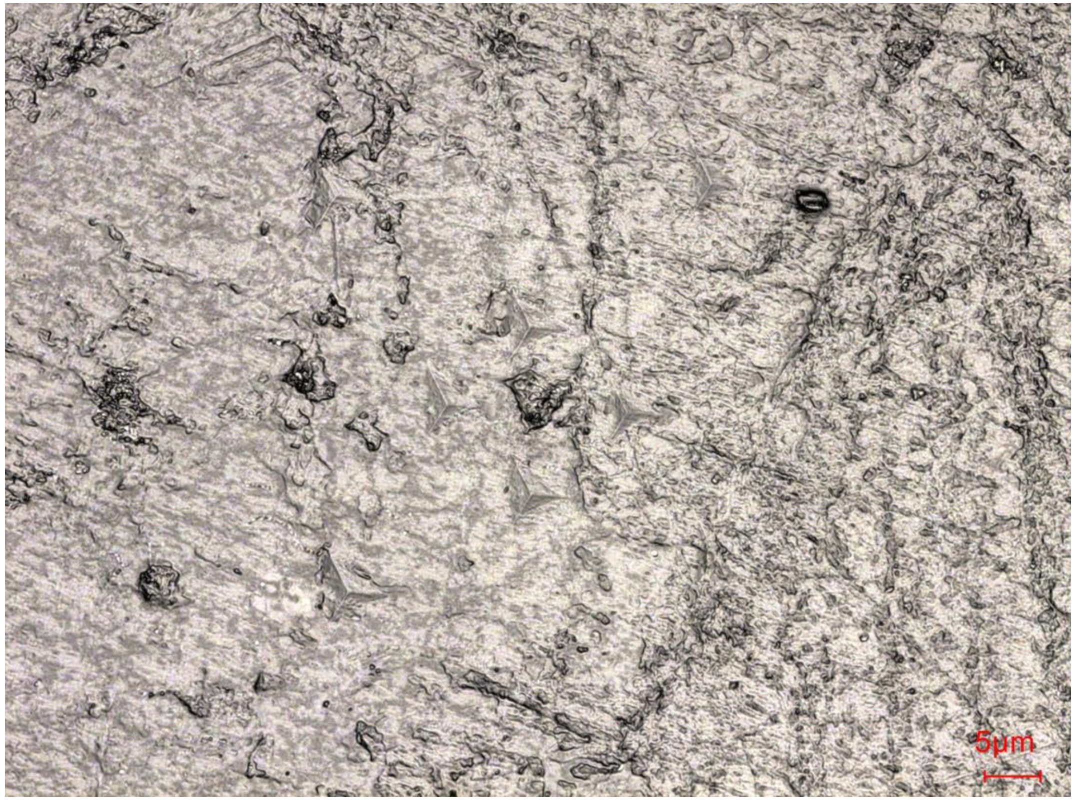


Figure 14: 150x laser scanning image following nanoindentation

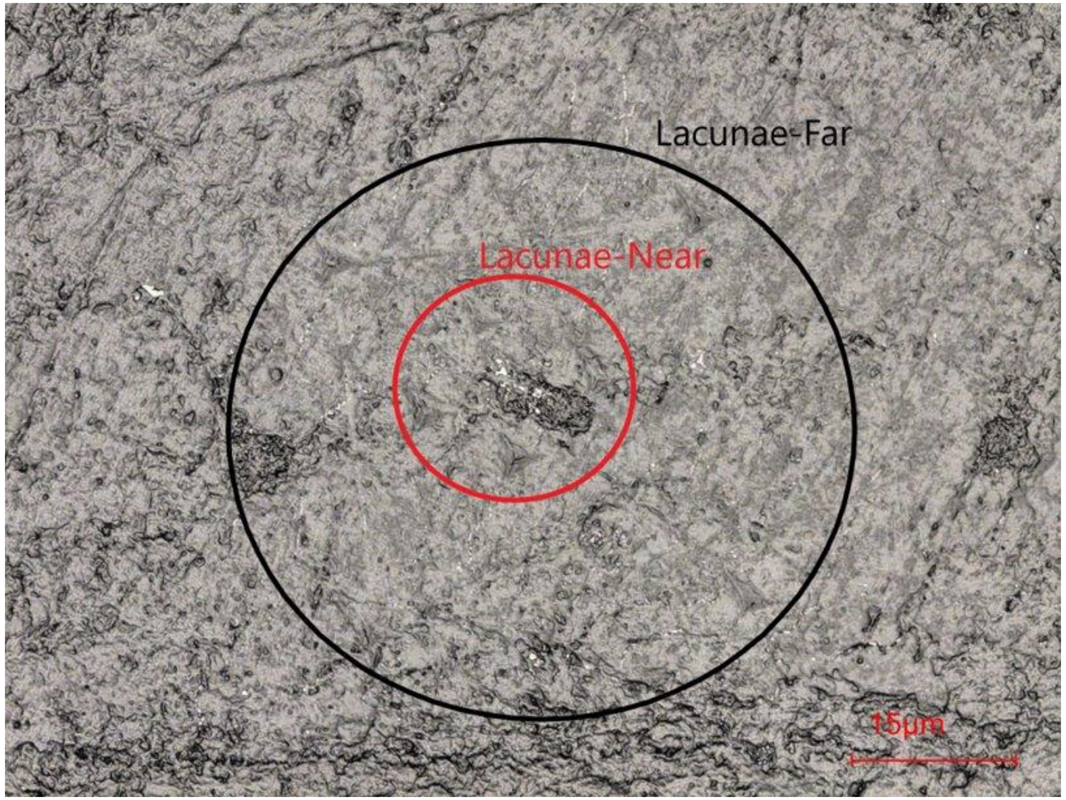


Figure 15: 150x laser scanning image following indentation with labeled near & far lacunar areas

Statistical Data Analysis

		t	df	Sig. (2-tailed)
Pair 1	LN Er - LF Er	-1.097	46	.278
Pair 2	LN H - LF H	-.625	46	.535

- With p-values > .05, there is no statistically significant difference between lacunar-near and lacunar-far regions' modulus or hardness values

Figure 16: SPSS data analysis of the modulus and hardness values. Pair 1 is a paired t-test between the lacunae-near and lacunae-far modulus values. Pair 2 is a paired t-test between the lacunae-near and lacunae-far hardness values. Sig. (2-tailed) represents the p-value of the respective test.

End of presentation

Summary

- We implemented two different scientific instruments, including a laser scanning microscope and a quasi-static Nanoindenter with the objective of characterizing material properties near and far from lacunae in trabecular bone biopsies from T1D women
- No statistically significant difference was found in either the modulus or hardness values when comparing the near and far values

Ongoing work

- Additional extensive data analyses are required to provide more convincing evidence of altered lacunar characteristic and changes in peri-lacunar bone as mechanisms related to T1D women and fragility fractures

From us

- We will continue this research by improving the smoothness and number of samples to derive a more complete and consistent understanding of bone tissue

Thank You

- Dr. Jun Jiao
- Dr. Erik Sanchez
- Dr. Wen Qian
- Dr. Mohammed P Akhter
- Portland State University
- Osteoporosis Research Center, Creighton University
- All the Women Who Donated Bones to Aid In Our Research

This Research Was Funded By

- National Science Foundation #1851851
- Nebraska Research Initiative

References

1. Ego S, Pierre DD. Bone Quality – The material and Structural Basis of Bone Strength and Fragility. N Engl J Med 2006;354:2250-61.
2. VV Zhukouskaya, C Eller-Vainicher, AP Shepelkevich, Y Dydyshko, E Cairoli, I Chiodini. Bone health in type 1 diabetes: focus on evaluation and treatment in clinical practice. J Endocrinol Invest 2015 Sep;38(9):941-50.
3. Wen Q, Roman S, Joseph AT, Sue PB, Joan ML, Robert RR, Mohammed PA. A pilot study on the nanoscale properties of bone tissue near lacunae in fracturing women. Bone Reports 2022 July 16;17:101604.
4. Koji I, Tomoyasu F, Takashi N, Takuma K, Noriko H, Takeshi Y, Katsunori I, Tsutomu H. Type 1 diabetes patients have lower strength in femoral bone determined by quantitative computed tomography: A cross-sectional study. J Diabetes Investig 2015 Nov;6(6):726-33.
5. Laura AGA, Mohammed PA, Andjela D, Robert RR. Trabecular bone nhistomorphometry in humans with Type 1 Diabetes Mellitus. Bone 2012 Jan;50(1):91-6.
6. Charlotte V, Wout P, Loic B, Stefan G, Hans-Georg Z, Samyah S, Jean-Marc K, Bruno L. Cortical Bone Size Deficit in Adult Patients With Type 1 Diabetes Mellitus. J Clin Endocrinol Metab 2017 Aug 1;102(8):2887-2895.
7. Naiemh A, Christie M, John EF, John M, Robert SL, John M, Martin M, Russell D, Derek G, Gerald AM, MG Shaikh, Colim GP, SF Ahmed. Deficits in Trabecular Bone Microarchitecture in Young Women With Type 1 Diabetes Mellitus. J Bone Miner Res 2015 Aug;30(8):1386-93.
8. Farlay D, Armas LA, Gineyts E, Akher MP, Recker RR, Boivin G. Nonenzymatic Glycation and Degree of Mineralization Are Higher in Bone From Fractured Patients With Type 1 Diabetes Mellitus. J Bone Miner Res 2016 January;31(1):190-195.
9. Shah VN, Shah CS, Snell-Bergeon JK. Type 1 diabetes and risk of fracture: meta-analysis and review of the literature. Diabet Med. 2015 Sep;32(9):1134-42.
10. Mariano EV, Luciana MS, Carola BB, Angela MU. Influence of short-term diabetes on osteocytic lacunae of alveolar bone. A histomorphometric study. Acta Odontol Latinoam 2006;19(1):23-8.
11. P Vestergaard. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. Osteoporos Int 2007 Apr;18(4):427-44.
12. Benjamin JP, Philip AY, Mohammed PA, Robert RR, Joseph AT. Intrinsic material properties of trabecular bone by nanoindentation testing of biopsies taken from healthy women before and after menopause. Calcif Tissue Int. 2012 April;90(4):286-93.
13. S Vennin, A Desyatova, JA Turner, PA Watson, JM Lappe, RR Recker, MP Akhter. Intrinsic material property differences in bone tissue from patients suffering low-trauma osteoporotic fractures, compared to matched non-fracturing women. Bone 2017 Apr;97:233-242.
14. Adolfo DP, Roberto G, Xavier N, Enric C, Maria JP, Leonardo M, Connor R, Daniel B, James CW, Alexander P, Davis B, Kurt JK, Robert OR, Paul KH. Microindentation for in vivo measurement of bone tissue mechanical properties in humans. J Bone Miner Res 2010 Aug;25(8):1877-85.
15. Mohammed PA, DB Kimmel, JM Lappe, RR Recker. Effect of Macroanatomic Bone Type and Estrogen Loss on Osteocyte Lacunar Properties in Healthy Adult Women. Calcif Tissue Int 2017 Jun;100(6):619-630.
16. "TI 950 TriboIndenter user Manual - University of Nebraska–Lincoln." [Online].Available:https://engineering.unl.edu/downloads/files/UserManual-HystrionTriboIndenter-sm.pdf. [Accessed: 06-Aug-2022].
17. "Keyence laser scanning microscope VK-X200K," *Keyence laser scanning microscope VK-X200K | College of Engineering | University of Nebraska–Lincoln*. [Online]. Available: https://engineering.unl.edu/nercf/keyence-laser-scanning-microscope-vk-x200k/. [Accessed: 06-Aug-2022].
18. Oliver, W. C., and G. M. Pharr. "An Improved Technique for Determining Hardness and Elastic Modulus Using Load and Displacement Sensing Indentation Experiments." Journal of Materials Research 7, no. 6 (June 1992): 1564–83. https://doi.org/10.1557/JMR.1992.1564.
19. Pharr, G. M., W. C. Oliver, and F. R. Brotzen. "On the Generality of the Relationship among Contact Stiffness, Contact Area, and Elastic Modulus during Indentation." Journal of Materials Research 7, no. 3 (March 1992): 613–17. https://doi.org/10.1557/JMR.1992.0613.