APPLICATION NOTE

Anisotropy of Bone Lamella

Introduction

Scanning nanoindentation, a technique pioneered by Hysitron, combines scanning probe imaging (SPM) with nanoindentation. The complimentary techniques are combined onto a single instrument platform to provide test placement accuracy that cannot be matched using nanoindentation with optical microscopy alone. The technique has been enthusiastically adopted by the biomaterials community across the globe. Topographical images of the sample surface reveal features too fine to observe optically and permit placement of nanomechanical tests on specific elements of hierarchical structures, making scanning nanoindentation the ideal tool for research into the mechanics of small-scale biological structures.

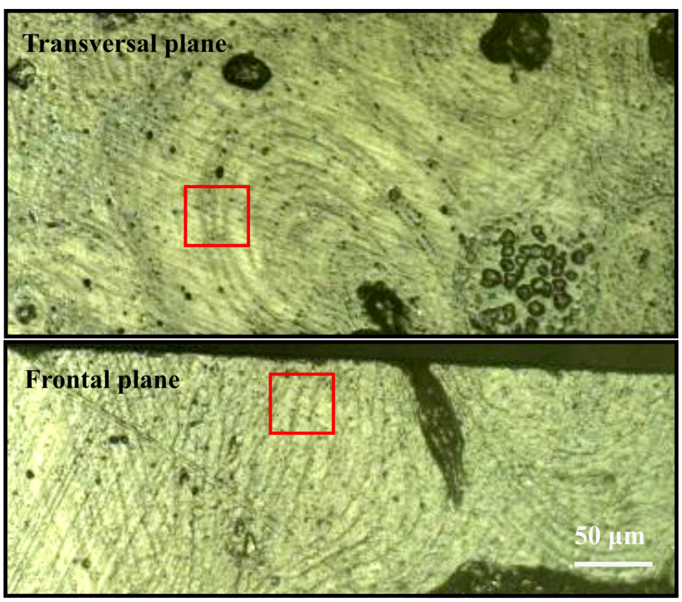

Figure 1: **TriboIndenter**'s microscopy images of two polished perpendicular plains. Red squares indicate regions where in-situ SPM imaging was used for testing individual osteonal lamella.

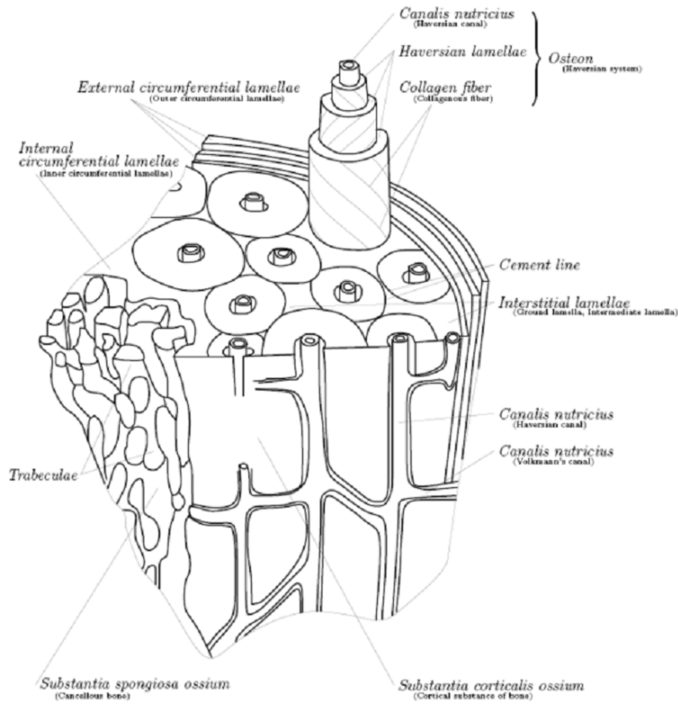

Figure 2: Schematics of hierarchical structure of cortical bone [1].

Composed of mineralized collagen fibrils packed into layered lamellae that form concentric rings around an osteon, bone is a prototypical example of hierarchical structure (Figure 2). Knowledge of the anisotropic elastic properties of osteons and osteonal lamellae provides a better understanding of various pathophysiological conditions such as aging, osteoporosis, or osteoarthritis. Successfully modeling the mechanics of bone requires accurate knowledge of the mechanical properties of individual components within its complex structure. Further, the anisotropic nature of the structures requires measurement along multiple directions to fully describe the mechanical properties.

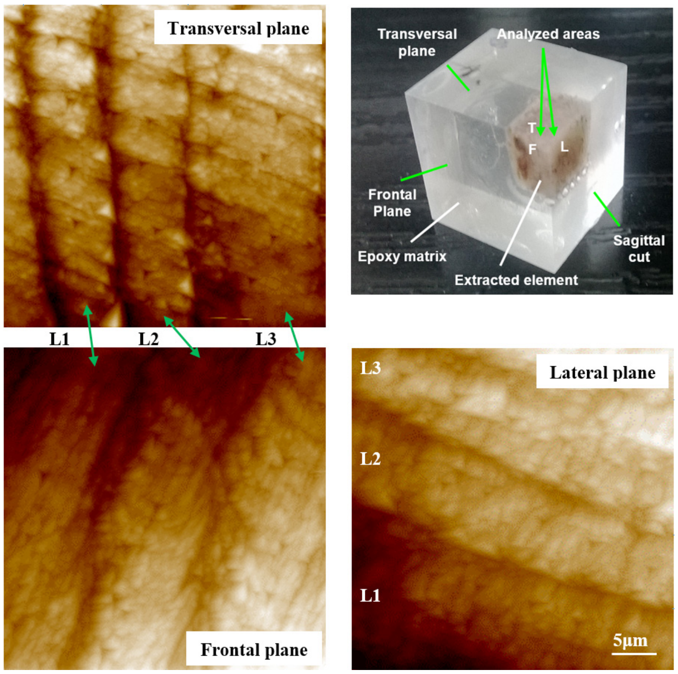

Figure 3: Cortical bone sample was embedded in epoxy resin and cut in a cube. Three perpendicular sample planes were polished afterwards. In-situ SPM topography images (35x35µm) of the lamellar structure of bone osteon from three perpendicular sections. Triangular residual imprints from Berkovich indenter confirms precise positioning within single lamella.

Procedure

A Hysitron **TI 950 TriboIndenter®** nanomechanical test instrument was used to perform scanning nanoindentation tests and measure the mechanical response of a single osteonal lamella along three perpendicular planes. In-situ SPM imaging was first used to visualize the surface topography and select a lamella of interest. Figure 3 shows topographical images of the surface collected using SPM.

Nanoindentation tests were placed precisely atop selected locations in the SPM image to measure mechanical properties of desired locations. The indents were performed to a depth of roughly 200nm and gave the elastic modulus of the lamella at each location. SPM imaging and indentation testing were performed using the same probe without ever leaving the surface, ensuring the highest possible test placement accuracy.

Results

Elastic properties were measured along the three perpendicular planes (transversal, frontal and sagittal) of the extracted element of human humeral cortical bone (Figure 3).

Direction	E_r [GPa]	Std [GPa]	E_s [GPa]
Transversal – L1	22.1	2.5	20.2
Frontal – L1	15.6	2.7	14.2
Sagittal – L1	12.5	1.4	11.3
Transversal – L2	23.5	2.9	21.5
Frontal – L2	14.8	0.8	13.5
Sagittal – L2	16.1	2.3	14.7
Transversal – L3	24.1	1.0	22.1
Frontal – L3	16.2	0.7	14.7
Sagittal – L3	14.1	1.2	12.8

Table 1: Average elastic properties of the measured lamellae. E_r – measured reduced elastic modulus; E_s – elastic (Young's) modulus calculated from E_r; std – standard deviation of measurements. Lamellae 1-3 correspond to the positions in (Figure 3).

Indentation tests were performed on three neighboring lamellae within each plane. Average measurements of reduced elastic moduli E_r and the corresponding elastic moduli E_s are shown in Table 1. The highest stiffness was measured along the transversal plane in the range of E_sT = 20.2 – 22.1 GPa. The modulus measurements from the frontal and sagittal plane were significantly lower: E_sF = 13.5-14.7 GPa and E_sS = 11.3-14.7 GPa, respectively. There were no substantial differences among the elastic properties of three measured osteonal lamellae.

Conclusions

The Hysitron TI 950 provides the necessary measurement sensitivity and test placement accuracy to perform a series of nanoindentation measurements within single bone lamella. Results from three orthogonal directions show that the lamella's elasticity is strongly anisotropic, with the directionality controlled primarily by collagen orientation. The results in Table 1 may further be used as inputs to derive the full stiffness tensor of the anisotropic lamella [1][2]. The results may also be used to develop and validate Hierarchical modeling approaches [1] which are used to understand and predict the mechanical properties of hierarchical structures.

References

1. Elastic Properties of Human Osteon and Osteonal Lamella Computed by a Bidirectional Micromechanical Model and Validated by Nanoindentation, R. Korsa, J. Lukes, J. Sepitka, T. Mares , Journal of Biomechanical Engineering, *in press*, 2015.
2. Nanoindentation of Porcine Bone Lamellae, J. Lukes, S. Otahal, Comput Methods Biomech Biomed Engin, Vol. 12, 177-178, 2009.

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