HETEROGENEOUS ORTHOTROPIC ELASTICTY ABOUT A NUTRIENT FORAMEN VIA MICROINDENTATION

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ABSTRACT
We determined the spatial distribution of orthotropic engineering constants about a nutrient foramen using the combined techniques of microindentation, polarized microscopy, and elasticity transformation. One goal of this estimation was to provide an independent verification of our related work[2] that relied upon more laborious techniques, and we found variations in the constants that were quite similar to our previous findings.[2] We reached our second goal of developing a methodology for mapping the spatial variation in orthotropic elasticity at a fine resolution in bone.

INTRODUCTION
Microindentation is a testing technique more suited for probing heterogeneous materials such as bone than conventional techniques that rely on larger specimens. Macroscopic testing cloaks spatial variations in mechanical properties that exist in bone and that play an important role in its function and form. A mature technique, microindentation has been given new consideration for bone[7] and for synthetic heterogeneous materials.[6,9] Its potential for mapping variations in a set of anisotropic properties, as opposed to a single one, has not been explored. We used it to map such a set.

The Knoop microindenter is ideal for exploring mechanical anisotropy: it is a diamond pyramid that creates an indentation with one longer diagonal D (**Figure 1**). The pyramid angles and D (μm)are used to compute the Knoop microhardness H_K (kg/mm^2) from

$$H_K = 2{,}000\frac{\tan(172.5°/2)}{\tan(130°/2)}\frac{f}{D^2} \approx 14{,}229\frac{f}{D^2} \quad (1)$$

where f is the indentation mass (g), and the tangent function arguments are the pyramid half angles. Typically, H_K < 100 kg/mm^2 in bone. If the material possesses mechanical anisotropy, the H_K reflects that: the H_K changes depending on the indenter orientation. The extreme aspect ratio of the Knoop indenter is such that it is generally assumed that the hardness reflects properties normal to the long diagonal.

METHODS
The overall flow to this study is as follows. Knoop microindentation tests were performed at an angle θ_i (i = 1,2,3) between the long diagonal direction x and the mediolateral direction X on each of three specimens (**Figure 1**). The H_K values were determined and converted to Vickers hardness H_V values using a correlation we developed. The H_V values were converted to elastic moduli $E(\theta_i + 90^o)$ using an existing regression relation.[1] The principal material angle θ_P at the region of microindentation was determined from the osteon trajectory there. This angle was used to transform the three moduli (along with an assumed Poisson's ratio ν_{LT}) into the orthotropic engineering constants E_L, E_T, and G_{LT} in the principal material directions L and T.

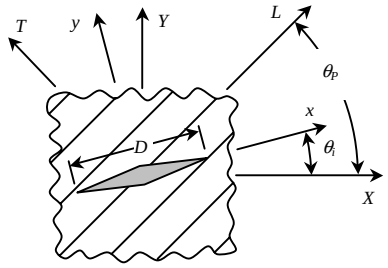

Figure 1. Schematic of Knoop microindentation.

An equine third metacarpus (MC3) was harvested from a skeletally mature standardbred with no bone abnormalities and whose death was unrelated to this work. All work was approved by our Institutional Animal Care and Use Committee. The palmar aspect was sectioned into four 26 mm wide by 47 mm long by 200 μm thick longitudinal specimens with the foramen centrally located. The foramen was 1.6 mm wide (mediolateral dimension) and 3.9 mm long; its centerline was normal to the cortex. One specimen was used for determining osteon trajectories about the foramen; the other three were used for microindentation testing. Four transverse specimens were prepared from the dorsal aspect for H_K and H_V correlations. Each specimen was mounted to a glass slide with double stick tape and polished on both sides to a final thickness of 100 μm.

Microindentation tests were performed using Knoop and Vickers indenters. The microindenter is equipped with a reflected light microscope to measure the indentation size; microindentation locations are determined by stage mounted digital micrometers. A load mass of 0.05 kg and a 10 s dwell time was used in all tests. All specimens were wetted throughout testing with calcium buffered[3] gentamycin doped saline with intermittent blotting for indentation measurements. The orientation θ_i of the long diagonal of the Knoop indenter on each specimen containing the foramen was either 0º, 30º, or 90º from the mediolateral direction. Up to more than 482 microindentation tests were performed on each specimen. Radial basis functions were used to interpolate between converted moduli so that each was available for elasticity transformation at every (*X*,*Y*) location. This was done because it is impossible to perform a microindentation test at the exact same (*X*,*Y*) location from one specimen to the next.

The dorsal transverse specimens were used to develop the conversion from H_K to H_V. This region of the MC3 was chosen because it seems reasonable that this is a region of elastic transverse isotropy; if true, H_K values should be independent of orientation for indentations into this transverse plane. The specimens were partially demineralized for various time lengths with hydrochloric acid (HCl) at room temperature while agitating to produce a wide range on mineral content so that hardness would vary considerably. For consistent results, microindentations were performed in the same region of each specimen for each indenter and with random orientations of each indenter.

We used results of a previous study that correlated tensile elastic modulus (GPa) with Vickers microindentation hardness (MPa) for variously mineralized mammalian tissues.[1] These tissues included long bones and teeth so as to capture a wide range of mineral densities and moduli. That correlation was

$$E(\theta_i + 90°) = 0.36H_V + 0.58 \quad (R^2 = 93\%) \tag{2}$$

The longitudinal trajectories of the osteons about the foramen were quantified using techniques of polarizing light microscopy and image analysis. All microscopy work was performed with a polarizing light microscope, a RGB CCD video camera, a frame grabbing card, and a workstation. Using an adaptation[2] of the Johanssen equation of optics,[4,5] the birefringence of collagen was exploited to determine the osteon trajectories θ_P about the foramen from

$$\theta_P \sim \varphi + \frac{1}{2}\sin^{-1}\sqrt{I} \tag{3}$$

where I is the transmitted light intensity measured at each rotation angle φ of a "filter pack" (polarizer and analyzer locked together at 90º between them). Each image was acquired for several rotations of the filter pack and averaged over a 0.04 mm^2 area. The principal material angle θ_P is the rotation angle at maximum light intensity. A 530 nm retardation plate was inserted into the light path to generate interference colors,[8] so as to distinguish between the fast and slow axes of the collagen fibers (*T* and *L* directions).

The elastic modulus $E(\theta_i + 90º)$ in the *y* direction normal to the long diagonal can be expressed as a function of four principle engineering constants and the angle θ_i using elasticity transformation:

$$\frac{1}{E(\theta_i + 90°)} = \frac{\sin^4(\theta_P - \theta_i) - 2\nu_{LT}\cos^2(\theta_P - \theta_i)\sin^2(\theta_P - \theta_i)}{E_L} + \frac{\cos^4(\theta_P - \theta_i)}{E_T} + \frac{\cos^2(\theta_P - \theta_i)\sin^2(\theta_P - \theta_i)}{G_{LT}} \tag{4}$$

The set of three **Equations (4)** at every (*X*,*Y*) and an assumed $\nu_{LT} = 0.3$ provide a set of equations to solve for the other three orthotropic principle engineering constants in the *LT* plane.

RESULTS

Microindentations with the Knoop (kg/mm^2) and Vickers (kg/mm^2) indenters yielded the linear conversion

$$H_V = 0.86H_K + 0.097 \quad (R^2 = 97\%) \tag{5}$$

Furthermore, H_K was found not to depend on indenter orientation, thus confirming transverse isotropy in the dorsal cortex. The osteon trajectories were generally parallel to the longitudinal axis of the MC3 except for in the immediate vicinity of the foramen, where they were tangential to it. Heterogeneity was most pronounced for E_L (**Figure 2**), ranging from 6 GPa near the foramen to 16 GPa further away. We found a compliant region next to the foramen and increasing stiffness mediolaterally. The range on E_T was nearly the same, but the distribution was more homogeneous. Little variation was found in G_{LT}. except for isolated regions near the foramen.

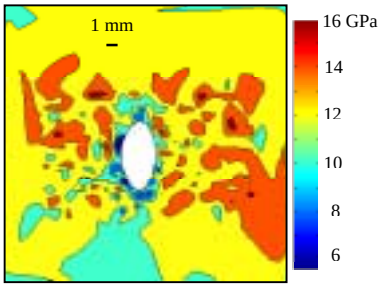

Figure 2. Spatial variation in longitudinal elastic modulus.

DISCUSSION

We found excellent agreement between the microindentation derived maps of elasticity and those of our previous work.[2] Previously, we analyzed literally thousands of images and relied upon an elasticity algorithm based on multiple works to create such maps. We are using this microindentation methodology to explore other foramen and to populate heterogeneous models of skeletal elements.

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