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Technical Note

The elastic properties of trabecular and cortical bone tissues are similar: results from two microscopic measurement techniques

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Abstract

Acoustic microscopy (30–60 μm resolution) and nanoindentation (1–5 μm resolution) are techniques that can be used to evaluate the elastic properties of human bone at a microstructural level. The goals of the current study were (1) to measure and compare the Young's moduli of trabecular and cortical bone tissues from a common human donor, and (2) to compare the Young's moduli of bone tissue measured using acoustic microscopy to those measured using nanoindentation. The Young's modulus of cortical bone in the longitudinal direction was about 40% greater than ($p < 0.01$) the Young's modulus in the transverse direction. The Young's modulus of trabecular bone tissue was slightly higher than the transverse Young's modulus of cortical bone, but substantially lower than the longitudinal Young's modulus of cortical bone. These findings were consistent for both measurement methods and suggest that elasticity of trabecular tissue is within the range of that of cortical bone tissue. The calculation of Young's modulus using nanoindentation assumes that the material is elastically isotropic. The current results, i.e., the average anisotropy ratio (E_L/E_T) for cortical bone determined by nanoindentation was similar to that determined by the acoustic microscope, suggest that this assumption does not limit nanoindentation as a technique for measurement of Young's modulus in anisotropic bone. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Accurate measurement of the intrinsic stiffness or Young's modulus of bone tissues is important now with the growing capabilities for tissue-level computational stress analysis. The reported Young's moduli for cortical bone tissue have been shown consistently to be about 20–22 GPa along the axis of long bone and about 12–14 GPa transversely (Ashman et al., 1984; Yoon and Katz, 1976), while the reported Young's moduli for trabecular bone tissue range from 1 to over 20 GPa (Ashman and Rho, 1988; Choi et al., 1990; Choi and Goldstein, 1992; Guo and Goldstein, 1997; Ko et al., 1995; Kuhn et al., 1989; Mente and Lewis, 1989; Rho et al., 1993; Runckle and Pugh, 1975; Ryan and Williams, 1989; Townsend et al., 1975; Williams and Lewis, 1982; Williams and Johnson, 1989). The mineral and collagen contents of trabecular and cortical tissues are similar (Gong et al., 1964), so it is unclear why there is so much discrepancy between the measured Young's modulus values for the two tissues. Possibly the difficulty in preparing and mechanically testing specimens from trabeculae has contributed to the variation in measured elastic properties. Newer, microscopic testing techniques, used in the current study, allow measurement of bone elasticity with minimal experimental artifact and provide new information about the Young's moduli of cortical and trabecular bone tissue.

Here we report Young's moduli of cortical and trabecular bone tissue measured using acoustic microscopy, with a resolution of 30–60 μm (Hasegawa et al., 1995; Katz and Meunier, 1993; Shieh et al., 1995; Zimmerman

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et al., 1994), and using a nanoindentation technique, often with a resolution of better than 1 μm (Doerner and Nix, 1986; Oliver and Pharr, 1992; Pharr and Oliver, 1992). The results of these two techniques are compared to help resolve a technical question about the nanoindentation technique. Nanoindentation allows the calculation of Young's modulus of a material under the assumption that the material is elastically isotropic. Bone is elastically anisotropic so the assumption used for Young's modulus measurement by nanoindentation may produce inaccurate results.

The goals of the current study were (1) to measure and compare the Young's moduli of trabecular and cortical bone tissues from a common human donor, and (2) to compare the Young's moduli of bone tissue measured using acoustic microscopy to those measured using nanoindentation.

2. Methods

Samples of human trabecular bone from the distal femur and human cortical bone from the femoral midshaft from a human donor (age 65, male, no history of bone pathology) were prepared for acoustic measurements made at Indiana University and adjacent sections were sent to Oak Ridge National Laboratory for nanoindentation measurements.

2.1. Specimen preparation

A 2 cm block of cancellous bone from the distal femoral condyle and a 1 cm section of cortical bone from the midshaft were removed from the right femur. The bone specimens were dehydrated in ascending grades of ethanol (six changes ranging from 70% to 100% ETOH) and embedded in polymethylmethacrylate (previous experiments showed that plastic embedding does not affect the acoustic measurements in bone (Takano et al., 1996)). Three cubes were cut using a diamond wire saw (Histosaw, Delaware Diamond Knives, Wilmington, DE) under constant lubrication, one from cancellous bone and two from cortical bone, from the embedded specimens. One of the cubes of cortical bone was used to measure elastic properties along the long axis of the bone and the other was used to measure transverse elastic properties. A 500 μm slice was taken from each cube using the diamond wire saw. This slice was used for acoustic velocity measurements, which were made at Indiana University, and the adjacent block was used for nanoindentation measurements, which were made at the Oak Ridge National Laboratory. The x- and y-coordinates marking the location of each acoustic velocity measurement were recorded and sent to the investigators at ORNL to assure that the acoustic and nanoindentation measurements were made at the same locations on the bone specimen.

2.2. Acoustic measurements

Acoustic velocity measurements were made using a scanning acoustic microscope (UH3, Olympus, Japan) by a method described previously (Hasegawa et al., 1995). The accuracy of the method was verified by measuring the elastic constants of materials with known properties (Fig. 1). A 50 MHz transducer (V-390, Panametrics, Waltham, MA) was used to generate acoustic waves in pulse–echo mode. The 50 MHz lens produced an acoustic beam that was approximately 60 μm in diameter. Specimens were submerged in a water bath at constant temperature (22°C) and a delay time between acoustic waves reflected at the top of the specimen and those reflected from the bottom of the specimen was measured using a digital oscilloscope (TDS 620, Tektronix, Beaverton, OR). The acoustic velocity was calculated as twice the specimen thickness divided by the average delay time. The acoustic velocity was measured in three locations for each bone specimen.

2.3. Density measurement

After acoustic measurement, the plastic was dissolved away from the bone specimens in MMA and densities were obtained using an analytical balance. The specimens were allowed to equilibrate in saline solution. Wet weight was measured after specimens were blotted dry on absorbant paper. Submerged weight was measured during submersion in ethanol. Density was calculated as wet weight divided by tissue volume determined using Archimedes' principle.

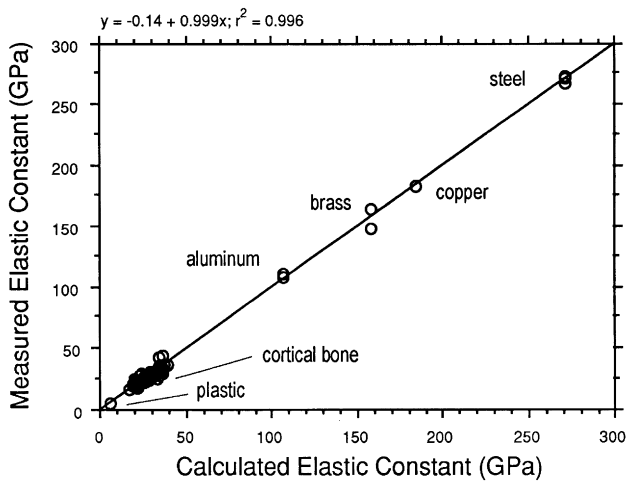

Fig. 1. Verification of the elastic constant measurements using the acoustic microscope. For comparison with measured values, elastic constants of metals and plastics were calculated for mechanical properties provided by the manufacturers. The elastic constants of cortical bone (canine) were measured using a standard technique reported previously (Turner et al., 1995). The slope of the regression line was not significantly different than one ($p > 0.8$, t-test), and the y-intercept was not significantly different than zero ($p > 0.7$, t-test).

2.4. Elastic property calculations

The elastic constant was calculated from the acoustic velocity (v) and density (ρ) as $C = \rho v^2$. Since the acoustic waves propagated in the bulk mode, the elastic constant was not equal to the Young's modulus. For the cortical bone specimens, Young's moduli were calculated from the elastic constants using the following equations:

$$E_{\text{long}} = E_3 = \frac{\Delta}{1 - v_{12}v_{21}} C_{33},$$

$$E_{\text{trans}} = E_2 = \frac{\Delta}{1 - v_{13}v_{31}} C_{22}, \quad (1)$$

where $\Delta = 1 - v_{12}v_{21} - v_{23}v_{32} - v_{31}v_{13} - 2v_{21}v_{32}v_{13}$.

Using values for the Poisson's ratios provided by Ashman et al. (1984), i.e., $v_{12} = 0.376$, $v_{21} = 0.422$, $v_{13} = 0.222$, $v_{31} = 0.371$, $v_{23} = 0.235$, $v_{32} = 0.350$, Eq. (1) becomes

$$E_{\text{long}} = 0.727\, C_{\text{long}}, \qquad E_{\text{trans}} = 0.666\, C_{\text{trans}}, \quad (2)$$

where E is Young's modulus in the axial and transverse directions, respectively. Young's moduli were calculated for trabecular specimens assuming that the trabecular tissue was isotropic and had a Poisson's ratio of 0.3. For isotropic materials, $v_{12} = v_{21} = v_{13} = v_{31} = v_{23} = v_{32}$, so

$$E = 0.743\text{C}. \quad (3)$$

2.5. Nanoindentation measurements

The embedded samples were metallographically polished with abrasive papers of decreasing grit size (600, 800, and 1200 grit), followed polishing by 0.05 μm diamond suspensions. Experiments were performed using a Nanoindenter II (Nano Instruments, Inc., Knoxville, TN). A Berkovich diamond indenter was used for all measurements. A permanent hardness impression was made by driving the indenter into the specimen to a depth of 100 nm at a constant loading rate of 12.5 μN/s, holding this load for 10 s, and unloading to 15% of the peak load at a rate equal to half that used during loading. This procedure was repeated to depths of 300 and 1000 nm at loading rates of 75 and 750 μN/s, respectively, without withdrawing the indenter from the specimen surface. Elastic moduli reported in this paper were determined from indentation load–displacement data obtained at the 1000 nm depth. The contact stiffness was measured from the load–displacement data as the slope of the upper portion of the unloading curve (Fig. 2). Young's modulus was calculated from the contact stiffness (S) by

$$S = 2\pi^{-1/2} \left(\frac{1 - v_{\text{b}}^2}{E_{\text{b}}} + \frac{1 - v_{\text{i}}^2}{E_{\text{i}}} \right)^{-1} A^{1/2}, \quad (4)$$

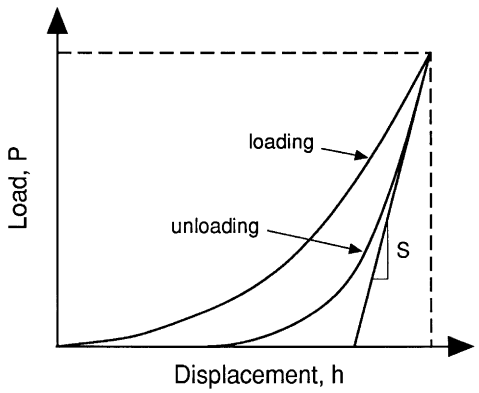

Fig. 2. For the nanoindentation tests, Young's modulus was calculated from contact stiffness (S). The contact stiffness was measured from the load–displacement data as the slope of the upper portion of the unloading curve (Rho et al., 1997).

where E and v are Young's modulus and Poisson's ratio, respectively, for the bone (b) and indenter (i), and A is the contact area (Oliver and Pharr, 1992). For the diamond indenter, $E = 1141$ GPa, and $v_{\text{i}} = 0.07$; v_{b} for bone was assumed to be 0.3. The contact area was estimated directly from the indentation load–displacement curve using a method described elsewhere (Oliver and Pharr, 1992). Twenty indentations were made at each location (i.e. within the 50–100 μm region) for the cortical bone, and 10 indentations were made for each location (within a 30–50 μm region) for the trabecular bone, for a total of 150 indentations.

2.6. Statistical methods

Young's modulus values for cortical and trabecular bone were compared using a one-way analysis of variance with a Fisher's least significant difference test for pairwise comparisons.

3. Results

The Young's modulus of cortical bone in the longitudinal/axial direction (E_{L}) was about 40% greater than ($p < 0.01$) the Young's modulus in the transverse direction (E_{T}) (Table 1). The Young's modulus of trabecular bone tissue was slightly higher than the transverse Young's modulus of cortical bone, but substantially lower than ($p < 0.01$) the longitudinal Young's modulus of cortical bone. The Young's modulus of trabecular tissue measured acoustically was not significantly different than the average modulus $(E_{\text{L}} + E_{\text{T}})/2$ of cortical bone, while the nanoindentation measurement of Young's modulus for trabecular tissue was significantly less than the average cortical modulus but not significantly different from the transverse cortical tissue modulus.

Table 1
Young's moduli determined using acoustic and nanoindentation measurement techniques. Data is reported as mean ± S.D. in units of GPa

Specimen	Young's modulus (acoustic)	Young's modulus (nanoindentation)
Trabecular bone	17.50 ± 1.12 ($n = 3$)	18.14 ± 1.7 ($n = 30$)
Cortical bone (transverse)	14.91 ± 0.52 ($n = 3$)	16.58 ± 0.32 ($n = 60$)
Cortical bone (longitudinal)	20.55 ± 0.21 ($n = 3$)	23.45 ± 0.21 ($n = 60$)
Cortical bone (average)	17.73 ± 0.22 ($n = 3$)	20.02 ± 0.27 ($n = 60$)

The nanoindentation technique estimated Young's moduli that were 4–14% greater than those measured using acoustic microscopy. However, the average anisotropy ratio (E_L/E_T) for cortical bone determined by nanoindentation (1.41) was similar to that determined by the acoustic microscope (1.39).

4. Discussion

The Young's modulus values for cortical bone measured using the acoustic microscope were similar to those measured by Ashman et al. (1984) using a continuous wave acoustic technique. Ashman found an average Young's modulus of 13.4 GPa in the transverse direction and 20.0 GPa in the longitudinal direction. Young's moduli measured using nanoindentation techniques were 10–20% higher than those found by Ashman. This discrepancy may have been caused the dehydration of the bone tissue during preparation. Since the specimens used for acoustic measurements were allowed to rehydrate before the measurement was made, it stands to reason that the acoustic Young's moduli would be lower in value compared to the moduli determined by nanoindentation. It is important to note that the average anisotropy ratio determined by nanoindentation was similar to that determined by the acoustic microscope, indicating that the two techniques measured similar structural anisotropy for cortical bone. This indicates that the assumption of tissue isotropy made when calculating Young's moduli from nanoindentation results (Eq. (4)) did not compromise the techniques' ability to measure elasticity of anisotropic bone.

For the calculation of Young's modulus from acoustic or nanoindentation measurement techniques, we estimated Poisson's ratios for the bone tissue (Eqs. (2)–(4)). While the calculation of Young's modulus by nanoindentation was fairly insensitive to the chosen value for Poisson's ratio (varying ν_b from 0.2 to 0.4 changed the measured values of E_b by no more than 8%), the calculation of Young's modulus using acoustic microscopy was sensitive to Poisson's ratio. However, a review of the average elastic constants (C's in Eqs. (1)–(3)) measured by acoustics shows

$$C_{long} = 28.3 \pm 0.3 \text{ GPa}, \qquad C_{trans} = 22.4 \pm 0.8 \text{ GPa},$$

$$C_{trab} = 23.6 \pm 1.5 \text{ GPa}.$$

Clearly the elastic constant for trabecular bone tissue (C_{trab}) falls between the elastic constants for cortical bone tissue (C_{long} and C_{trans}), supporting the conclusion that the elasticity of trabecular bone tissue is similar to that of cortical bone tissue, especially in the transverse direction. Since the elastic constants were derived directly from the measurements of acoustic velocity and bone tissue density, they were not affected by the chosen values for Poisson's ratio. Therefore, it appears that the general findings of this study are not greatly affected by the choice of Poisson's ratio used in the calculations.

There has been considerable controversy about the value for Young's modulus of trabeculae (Guo and Goldstein, 1997). The present study found the trabecular modulus to be approximately equal to, or slightly higher than the Young's modulus of cortical bone in the transverse direction. This value for trabecular modulus is consistent with several reports (Ashman and Rho, 1988; Rho et al., 1993; Townsend et al., 1975), but as much as an order of magnitude higher than values reported by a number of investigators (Choi et al., 1990; Kuhn et al., 1989; Mente and Lewis, 1989; Runkle and Pugh, 1975; Ryan and Williams, 1989). Many of the previous studies used mechanical test methods that suffered from considerable artifact due to small specimen size and irregular geometry. The microstructural measurement techniques reported here are free from such artifact.

In conclusion, we found the Young's modulus of trabecular bone tissue to fall between the average and transverse Young's moduli of cortical bone tissue. This finding suggests that elasticity of trabeculae is within the range of that of cortical bone tissue. The interpretation of the present results should be limited somewhat because they reflect data from only one human subject. Nevertheless, these results are consistent with our previous studies in which the acoustic velocity of trabecular bone tissue from 10 human donors (3663 ± 63 m/s, age = 66 ± 6 yr) fell between the acoustic velocities of cortical bone in the transverse and longitudinal directions (3528 ± 18 m/s, and 3984 ± 17 m/s, $n = 6$, age = 69 ± 6 yr) (Hasegawa et al., 1995; Takano et al., 1996). Therefore, our conclusion that the Young's modulus of trabecular tissue is similar to that of cortical bone tissue appears to be generally applicable.

Furthermore, the results suggest that the nanoindentation technique can be used to measure the elastic properties of anisotropic bone. This result supports previous

work showing that nanoindentation can estimate anisotropic moduli of β-silicon nitride (Hay et al., 1998). With its high resolutation (< 1 μm), nanoindentation has the potential to provide new information about the microstructure properties of bone.

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