Elastic properties of microstructural components of human bone tissue as measured by nanoindentation

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Abstract: The elastic properties of several microstructural components of dry human vertebrae (T-12 and L-1) and tibiae have been investigated in the longitudinal and transverse directions using nanoindentation. The largest Young's modulus was that for the interstitial lamellae in the longitudinal direction (25.7 ± 1.7 GPa). This was followed in decreasing order by osteons in the longitudinal direction (22.4 ± 1.2 GPa), trabeculae in the longitudinal direction (19.4 ± 2.3 GPa), an average over osteons and interstitial lamellae in the transverse direction [16.6 ± 1.1 GPa (it was difficult to microstructurally distinguish osteons from interstitial lamellae in the transverse direction)], and trabeculae in the transverse direction (15.0 ± 2.5 GPa). An ANOVA statistical analysis revealed that the values all are significantly different ($p < 0.05$). Since the elastic moduli in the longitudinal direction are all greater than in the transverse, measurable elastic anisotropies exist in the components. The hardnesses also varied among the microstructural components in the range 0.52–0.74 GPa. © 1999 John Wiley & Sons, Inc. J Biomed Mater Res, 45, 48–54, 1999.

Key words: nanoindentation; elastic modulus; hardness; trabecular bone; osteons; interstitial lamellae; anisotropy

INTRODUCTION

It often is assumed that the elastic properties of single osteons, interstitial lamellae, and individual trabeculae are the same as those measured for macroscopic specimens of cortical bone.[1] However, many recent investigations suggest that the elastic properties of bone tissue at the microstructural level may be very different from those measured in macroscopic tests.[2–9] For example, the elastic modulus of large tensile cortical specimens has been shown to be in the 14–29 GPa range (wet specimen, macrostructural property).[10] That of cortical and trabecular bone obtained from microbending specimens was 5.4 GPa and 4.3 GPa, respectively (wet specimen, microstructural property).[3] That of single osteons with the majority of the lamellar orientation in the longitudinal direction was 12 GPa in tension,[11] 6 GPa in compression,[12] 2 GPa in bending,[13] and 20 GPa in torsion.[14] However, it is unclear whether this difference is due to the testing method or whether it represents a true difference in the properties of the microstructural components.[2–14] The problem of understanding bone elasticity is further complicated by the fact that the elastic properties of microstructural components generally are thought to be anisotropic and often are difficult to measure in specific directions, even when using microbending tests, because of specimen size limitations. For example, individual trabeculae in the transverse direction are less than 500 μm thick. Thus it clearly would be advantageous to explore the elastic properties of bone tissue with a technique that allows for the examination of elastic anisotropy at the microstructural level.

In this regard, one promising new technique is nanoindentation. Nanoindentation recently has been used to measure the elastic properties of human lamellar bone[15] as well as small filler particles in resin composites and other dental restoratives.[16] From analysis of the indentation load-displacement behav-

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ior, it is possible to derive values of the elastic modulus and hardness of the materials. Nanoindentation can be used to probe a surface and map its properties on a spatially resolved basis, often with a resolution of better than 1 μm.[6,17] In a conventional microhardness test, the projected contact area is determined optically by measuring the diagonal lengths after the load is removed. This method is quite satisfactory when there is very little elastic recovery, as is the case for most metals and ceramics.[18] In a material such as bone, however, the projected contact area may not be determined precisely because the hardness impression is altered upon unloading due to elastic recovery. However, nanoindentation does not require visual observation of the indentation to obtain the projected contact area. Because the indenter geometry is known, the projected contact area can be calculated directly from measurements of the indentation depth. Since microstructural features in bone are very small, the nanoindentation technique offers a means by which their elastic properties can be explored. The technique also allows one to examine the properties in different directions and therefore to explore elastic anisotropy even in very small specimens, such as individual trabeculae.

The objective of this study was to use the nanoindentation technique to determine the elastic properties of single osteons, interstitial lamellae, and individual trabeculae of bone tissue at the microstructural level in the longitudinal and transverse directions. The results obtained in this study may be used to assess whether the elastic properties of bone tissue at the microstructural level are significantly different from those measured in macroscopic tests. In addition, anisotropy in the elastic behavior at the microstructural level was examined.

MATERIALS AND METHODS

Two male human cortical tibiae (ages of 60 and 66) were obtained, and four thoracic (T-12) and three lumbar (L-1) vertebrae were obtained from five male and two female frozen, unembalmed human cadavers (mean age of 61, SD 3.4). For the vertebrae, the spinous processes first were removed, and then the vertebral body was cut along the sagittal plane with a low-speed diamond saw (Buehler Isomet 1000, Buehler, Lake Bluff, Illinois) under constant deionized water irrigation to minimize the undesired mineral formation on the surface of the specimen. Sections 3 mm thick containing a trabecular network were obtained from the center of each vertebral body near its midsagittal plane. After removal of the bone marrow with a water jet, the sections were dehydrated in a series of alcohol baths and embedded without vacuum in epoxy resin at room temperature (EPO-THIN™ low viscosity epoxy, Buehler, Lake Bluff, Illinois) to provide support for the porous network. Dehydration was unavoidable for the nanoindentation technique; the consequences of dehydration will be explored in the discussion section. The embedded samples were metallographically polished to produce the smooth surfaces needed for nanoindentation testing. After being ground with silicon carbide abrasive papers of decreasing grit size (600, 800, and 1200 grit) under deionized water, the specimens were polished on microcloths (TEXMET®, Buehler, Lake Bluff, Illinois) with successively finer grades of alumina powder, the finest being 0.05 μm grit. The last polishing step was on plain microcloth under deionized water, and the specimens were cleaned ultrasonically to remove surface debris. The tibia sections were prepared in the same way. Optical micrographs of various sections and components of the bones are given in Figure 1.

All experiments were performed using the Nano Indenter® II (Nano Instruments, Inc., Knoxville, Tennessee) at the Oak Ridge National Laboratory. This fully automated hardness testing system makes small indentations at precise positions on a specimen surface while continuously monitoring the loads and displacements of the indenter with theoretical resolutions of 75 n*N* and 0.04 nm, respectively. The specimens are held on an x-y-z table, the position of which, relative to the microscope or the indenter, is controlled with a joystick. The spatial resolution of the position of the table in the x-y plane is 400 nm. However, the optical microscope used for locating indentation sites may limit the positioning resolution to roughly 1 μm. The apparatus is enclosed in an insulated cabinet to provide thermal stability and suspended on a pneumatic antivibration table to isolate it from external vibrations.

The Oliver–Pharr method for determining the indentation modulus, hardness, and the indenter area function have been well documented[7] and will not be discussed here at length. The quantities of interest include the peak load (P_{max}), the peak displacement (h_{max}), the contact depth (h_c), the contact area (A_c), and the contact stiffness (S). The equations used to compute the hardness (H) and the effective indentation modulus (E_{eff}) from the measured quantities are

$$H = \frac{P_{max}}{A_c} \tag{1}$$

and

$$E_{eff} = \frac{\sqrt{\pi}}{2}\frac{S}{\sqrt{A_c}}. \tag{2}$$

The elastic modulus is derived from stiffness data using Equation (2). E_{eff}, the effective modulus for the indenter–specimen combination, is given by

$$E_{eff} = \left[\frac{1-v_S}{E_S} + \frac{1-v_i}{E_i}\right]^{-1} \tag{3}$$

where ν is Poisson's ratio and the subscripts s and i refer to the sample and the indenter, respectively. The elastic properties of the diamond indenter, ν_i and E_i, are 0.07 and 1140 GPa, respectively. Equation (2) is derived under the assumption that the material is homogeneous and isotropic in its elastic properties. When used to measure the elastic modulus of an anisotropic material, the modulus derived from this equation is an average of the anisotropic elastic constants that is biased toward the modulus in the direction of force

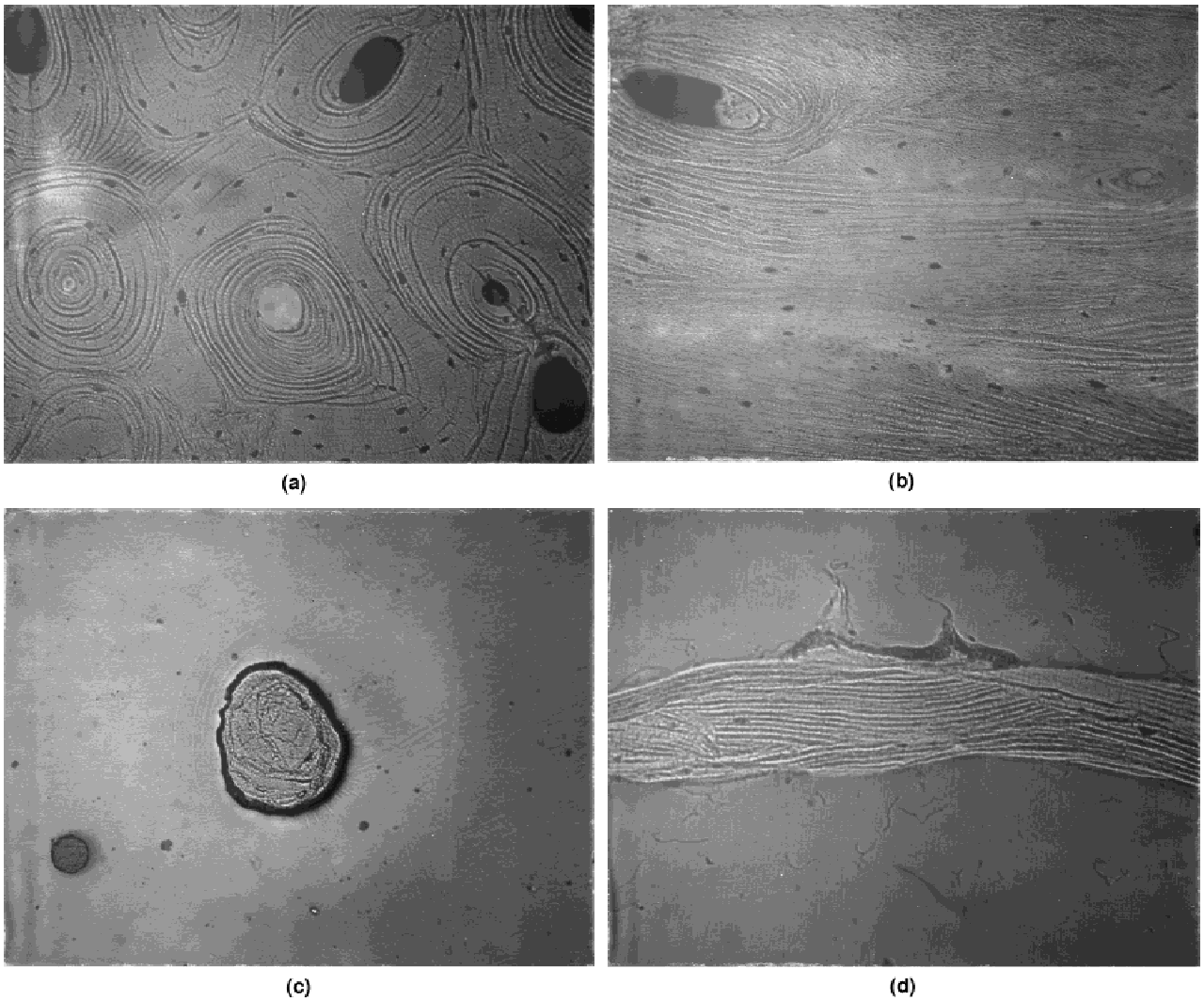

Figure 1. Optical micrographs of bone microstructure (original magnification ×200): (a) transverse cross section of tibial cortical bone; (b) longitudinal cross section of tibial cortical bone (note that it is difficult to differentiate microstructurally between osteons and interstitial lamellae in this longitudinal section); (c) transverse cross section of vertebral trabecular bone; (d) longitudinal cross section of vertebral trabecular bone.

application.[15] Further experiments regarding the determination of the individual anisotropic elastic constants are warranted. However, in this paper only the simple average value is reported.

Fused silica, which exhibits elastic isotropy and has a relatively low modulus-to-hardness ratio, was used to calibrate the tip shape function. The elastic modulus of fused silica was calculated to be 71.8 GPa, which is similar to the known value of 72 GPa.

Each nanoindentation test was conducted to a maximum load of 20 mN at a constant loading rate of 1 mN/s, which produced hardness impressions with depths of about 1 μm. The impression was held for a period of 10 s at this peak load and then unloaded at 85% of the peak load at a rate equal to half that used during loading. At the end of the unloading cycle, the indenter was held on the surface for a period of 100 s to establish the rate of thermal drift in the machine and specimen for correction of the data, and then it was completely withdrawn. The first 50% of the unloading curves were used for calculation of the elastic properties of the entire unloading curve, which is non-linear. Approximately five indentations were made in each microstructural feature. Any indentations close to the mounting resin were removed from the data set to minimize the effects of embedding on the measurements.

A total of 373 indentations were produced in this study. In the vertebrae, three to five indentations were made in 12 separate trabeculae in the longitudinal direction (i.e., perpendicular to the transverse section) and 29 separate trabeculae in the transverse direction. For the tibiae, three to five indentations were made in 15 osteons and 14 interstitial lamellae in the longitudinal direction. In the transverse direction (i.e., perpendicular to the longitudinal section), it was difficult to microstructurally distinguish osteons from interstitial lamellae, so individual measurements were not possible. Consequently, the transverse direction data (Table I) represent an average over all the indentations in the longitudinal section without regard to their exact locations in the two microstructural components. Rather than using pooled data for all the indentations, average values in separate mi-

TABLE I
Mean Elastic Moduli and Hardnesses For the Microstructural Components of Cortical and Trabecular Bone as Measured in This Study

Bone Type	Direction To Be Tested		No. of Subjects	No. or Indentations	No. of Microstructural Components	Elastic Modulus, GPa (SD)	Hardness, GPa (SD)
Cortical bone	Longitudinal	Osteons	2	72	15	22.4 (1.2)	0.617 (0.039)
		Interstitial lamellae	2	58	14	25.7 (1.0)	0.736 (0.044)
	Transverse		2	58	13	16.6 (1.1}	0.564 (0.034)
Trabecular bone	Longitudinal		3	53	12	19.4 (2.3)	0.618 (0.061)
	Transverse		7	132	29	15.0 (2.5)	0.515 (0.082)

Standard deviations are shown in parentheses (SD). The transverse cortical bone data represent an average over all the transverse section indentations without regard to their locations in the two structural components.

crostructural features were used in order to perform statistical analysis. For example, 15 measurements were used for osteons in the longitudinal direction (Table I). Significant differences in the elastic properties of trabeculae, osteons and interstitial lamellae in the longitudinal and transverse direction were analyzed using one-way analysis of variance (ANOVA). Scheffe's test then was employed to find differences between the elastic properties of the microstructural features and the directions tested.

RESULTS

Basic modulus and hardness results are summarized in Table I. The elastic modulus was found to be highest for the interstitial lamellae, intermediate for osteonal lamellae, and lowest for trabecular bone. With regard to direction, moduli determined in the longitudinal direction were always greater than those determined in the transverse direction for a given microstructural constituent. The ratio of elastic moduli in the longitudinal to transverse directions was found to be 1.35 for osteons and 1.29 for trabecular bone. The hardnesses follow in similar order: the ratio of hardness in the longitudinal to transverse direction is 1.10 for osteons and 1.19 for trabeculae.

As shown in Table II, the mean values of the elastic moduli for all of the bone components were found to be statistically different ($p < 0.05$). Therefore, for a given microstructural component there is an anisotropy between the longitudinal and transverse directions. Similar conclusions hold for the hardnesses.

DISCUSSION

The elastic modulus and hardness of osteons, interstitial lamellae, and trabeculae of bone tissue in both the longitudinal and the transverse directions were measured by the nanoindentation technique. The elastic modulus of interstitial lamellae, at least in the longitudinal direction, is significantly greater than that of osteons. This possibly may result from the osteons being newer bone material than the interstitial lamellae since newer bone is known to have a lower mineral content.[19,20] The observation that interstitial lamellae have a higher modulus than osteons is consistent with the work of Evans and Vincentelli,[21] who reported that osteons tend to reduce cortical bone strength and its elastic modulus. While Lipson and Katz[22] did not study microspecimens, they performed extensional wave ultrasonic measurements on small specimens of both bovine femoral plexiform (structurally, some-

TABLE II
Statistically Significant ($p < 0.05$) Results of Scheffe's Test for Differences in Elastic Modulus and Hardness

Parameter	p Elastic Modulus	p Hardness
Longitudinal osteon < longitudinal interstitial	0.0009	<0.0001
Longitudinal osteon > transverse cortical	<0.0001	<0.0001
Longitudinal osteon > longitudinal trabecular	<0.0001	1.000
Transverse cortical < longitudinal interstitial	<0.0001	<0.0001
Transverse cortical = transverse trabecular	0.2462	0.2176
Longitudinal trabecular < longitudinal interstitial	<0.0001	0.0003
Longitudinal trabecular > transverse trabecular	<0.0001	0.0002

The transverse cortical bone data use an average over osteons and interstitial lamellae since it is difficult to distinguish microstructurally osteons from interstitial lamellae in the transverse direction.

what similar to parallel-like interstitial lamellae) and Haversian bone. They found that the elastic moduli in both the longitudinal and the transverse directions were larger by about 20% in plexiform bone than in Haversian bone; the absolute values in the longitudinal direction for both bone types were only about 10% larger than reported here.

The results obtained in this study also have bearing on the issue of whether there are significant differences in the elastic properties of cortical and trabecular bone tissue. Wolff assumed more than a century ago that cortical bone can be modeled as dense cancellous bone.[1] According to Wolff's assumption, individual trabeculae and single osteons should have approximately the same elastic properties. However, investigators have suggested recently that trabecular moduli are substantially lower than cortical moduli.[2–9] The elastic moduli in the longitudinal direction of individual trabeculae and osteons measured in this study were 19.4 ± 2.3 and 22.4 ± 1.2 GPa (dry bone), respectively. However, a commonly used modulus for trabeculae, 5.4 ± 1.3 GPa,[3] (wet bone) is nearly a factor of 4 smaller than that observed here (dry bone); and the 10.7 ± 3.8 GPa modulus observed for single osteons (wet and fully calcified osteons) tested in tension[1] is approximately a factor of 2 smaller than the current measurements suggest.

The elastic moduli measured in this study therefore are higher than the commonly quoted values.[3,10] This difference may be due to drying on the measurement of elastic properties of bone tissue. Because of experimental complications associated with testing in liquid environments and keeping specimens wet during testing, most nanoindentation work to date has focused on completely dried specimens.[15] However, tests recently were conducted to examine the degree to which nanoindentation elastic property measurements are affected by drying.[23] For wet bovine femora, the elastic modulus of interstitial lamellae was found to be 16% higher than for osteons ($E_{interstitial}$ = 25.1 ± 1.6 GPa vs. E_{osteon} = 21.1 ± 2.0 GPa). For dry bovine femora, the same trends were observed, with the elastic modulus of interstitial lamellae being 11% higher ($E_{interstitial}$ = 27.5 ± 1.2 GPa vs. E_{osteon} = 24.4 ± 2.2 GPa). The elastic moduli of interstitial lamellae and osteons thus are increased by drying by approximately 10 and 15%, respectively. The modulus increase observed in the nanoindentation results is qualitatively consistent with that of other measurement techniques although the increase (10–15%) is not quite as large as that observed elsewhere (>20%).[9,24] Assuming that there is approximately a 15% increase in the elastic modulus of bone tissue when it is dried,[23] the longitudinal moduli for the trabeculae and osteons examined in this study after correction for drying would be 16 and 19 GPa, respectively. Although drying increases the moduli of bone specimens, the ordering of the differences due to microstructural type and anisotropy stay the same.

The reason that the elastic moduli measured by the nanoindentation technique are so much larger than those measured using other techniques, such as microbending and micro tensile testing, is not clear and cannot be firmly established without careful, systematic study. However, we wish to point out that nanoindentation elastic modulus measurements are not as sensitive to defects and inhomogeneities in the microstructure as other micromechanical testing techniques since the defects and inhomogeneities can be avoided by careful placement of the indentations. In this context, nanoindentation offers a distinct advantage in the experimental characterization of the elastic properties of bone tissue at the microstructural level. Since the microstructural features of interest in bone, such as osteons and interstitial lamellae, usually have dimensions of at least several microns, their properties conveniently can be probed by nanoindentation using indentations with surface dimensions of approximately one micron. For a Berkovich indenter, an indentation with a surface edge length of 5 μm is about 1 μm deep, and it is exactly at these scales that nanoindentation testing excels. At the smaller ultrastructural level, less than 50 nm, where individual collagen and mineral components are important, bone is inhomogeneous, but since the nanoindentation hardness impressions used to probe the properties of bone at the microstructural level are much larger than this, a continuum analysis may be applied.[25]

A recent study[26] examined the relationship between the elastic modulus measured by nanoindentation using the Oliver–Pharr method and that using the Young's modulus for elastically anisotropic solids. It showed that an important but often overlooked consequence of the Oliver–Pharr method[17] is that the elastic modulus obtained from nanoindentation techniques and the conventional Young's modulus are the same only for elastically isotropic media. For materials with lower symmetry, elastic anisotropy affects indentation measurements. For single crystals of β-silicon nitride with the long axis of the crystal oriented parallel to the c-direction of the hexagonal crystal structure, the elastic modulus obtained from nanoindentation in the longitudinal direction underestimates the single crystal Young's modulus, and in the transverse direction the elastic modulus by nanoindentation overestimates the single crystal Young's modulus. However, Hay et al.[26] found that the elastic modulus obtained from nanoindentation is predominantly controlled by the elastic properties in the indentation direction and only weakly influenced by properties in the transverse direction. Moreover, the elastic moduli in the longitudinal and transverse directions obtained from nanoindentation were found to represent a con-

servative estimate of the lower bounds on the actual elastic anisotropy.[26]

In light of these observations, it is interesting to examine the anisotropy in Young's modulus in the longitudinal and transverse directions of bone. For cortical bone, the moduli of osteons and interstitial lamellae in the longitudinal direction, 22.4 and 25.7 GPa, respectively, are significantly greater than the 16.6 GPa transverse modulus (recall that the transverse modulus is an average over several osteons and interstitial lamellae). For trabecular bone, the modulus in the longitudinal direction, 19.4 GPa, is significantly greater than the transverse modulus, 15.0 GPa. For the sake of comparison, Reilly et al.[10] reported a Young's modulus of 17.9 GPa for macroscopic wet femoral cortical specimens in the longitudinal direction and 10.1 GPa in the transverse direction, giving an anisotropy ratio of 1.77. Assuming that the longitudinal modulus of the cortical bone studied in this investigation is an average of the values for the osteons and interstitial lamellae, the equivalent cortical bone anisotropy ratio as measured here is 1.45. The accuracy of this value is again obscured by the fact that the elastic modulus in a specific direction in an anisotropic material as measured by nanoindentation techniques actually is a composite quantity that depends on all of the elastic constants of the material.[26–28] Unfortunately, without some *a priori* knowledge of the type of elastic anisotropy and an estimate of the associated anisotropic elastic constants, it is difficult to estimate how large the error in the modulus ratio may be. However, assuming that the observation of Hay et al.[26] can be applied to bone, the bone anisotropy ratio would be at least 1.45. Furthermore, the actual elastic modulus of an osteon in the longitudinal direction would be larger than 22 GPa while that in the transverse direction would be smaller than 17 GPa. In the same context, the actual longitudinal elastic modulus of trabecular bone tissue would be larger than 19 GPa while that in the transverse direction would be smaller than 15 GPa. The upper bounds on these properties cannot be estimated since the exact type of elastic anisotropy of bone at the microstructural level is unknown. This problem merits further investigation.

It also should be noted that the elastic moduli of osteons might differ depending on the degree of calcification and orientation of the collagen bundles.[11–14] In this study, it was not determined if the osteons were at the initial stage of calcification or fully calcified, or if successive lamellae in osteons showed differences in collagen bundle arrangement.

CONCLUSIONS

This study demonstrates that the nanoindentation method may offer a valuable tool for gaining insight into the elastic properties of bone tissue at the microstructure level. However, uncertainties in the measurement of bone tissue by nanoindentation, including the influences of elastic anisotropy, need to be addressed further.

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References

1. Wolff J. Das Gesetz der Transformation der Knochen. Berlin: Hirschwald; 1892.
2. Ashman RB, Rho JY. Elastic moduli of trabecular bone material. J Biomech 1988;21:177–181.
3. Choi K, Kuhn JL, Ciarelli MJ, Goldstein SA. The elastic moduli of human subchondral trabecular and cortical bone tissue and the size-dependency of cortical bone modulus. J Biomech 1990; 23:1103–1113.
4. Kuhn JL, Goldstein SA, Choi KW, London M, Feldkamp LA, Matthews LS. Comparison of the trabecular and cortical tissue moduli form human iliac crests. J Orthop Res 1989;7:876–884.
5. Mente PL, Lewis JL. Experimental method for the measurement of the elastic modulus of trabecular bone tissue. J Orthop Res 1989;7:456–461.
6. Rho JY, Ashman RB, Turner CH. Young's modulus of trabecular and cortical bone material: Ultrasonic and microtensile measurements. J Biomech 1993;26(2):111–119.
7. Runkle JC, Pugh J. The micro-mechanics of cancellous bone. Bull Hosp J Dis 1975;36:2–10.
8. Ryan JC, Williams JL. Tensile testing of rod-like trabeculae excised from bovine femoral bone. J Biomech 1989;22:351–355.
9. Townsend PR, Rose RM. Buckling studies of single human trabeculae. J Biomech 1975;8:199–201.
10. Reilly DT, Burstein AH, Frankel VH. The elastic modulus of bone. J Biomech 1974;7:271–275.
11. Ascenzi A, Bonucci E. The tensile properties of single osteons. Anat Rec 1967;158:375–386.
12. Ascenzi A, Bonucci E. The compressive properties of single osteons. Anat Rec 1968;161:377–392.
13. Ascenzi A, Baschieri P, Benvenuti A. The bending properties of single osteons. J Biomech 1990;23:763–771.
14. Ascenzi A, Baschieri P, Benvenuti A. The torsional properties of single selected osteons. J Biomech 1994;27:875–884.
15. Rho JY, Tsui TY, Pharr GM. Elastic properties of human cortical and trabecular lamellar bone measured by nanoindentation. Biomaterials 1997;18(20):1325–1330.
16. Willems G, Celis JP, Lambrechts P, Braem M, Vanherle G. Hardness and Young's modulus determined by nanoindentation technique of filler particles of dental restorative materials compared with human enamel. J Biomed Mater Res 1993;27: 747–755.
17. Oliver WC, Pharr GM. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J Mater Res 1992;7(6):1564–1583.
18. Doerner MF, Nix WD. A method for interpreting the data from

depth-sensing indentation instruments. J Mater Res 1986;1(4): 601–609.
19. Dorlot J-M, L'Esperance G, Meunier A. Characterization of single osteons: Microhardness and mineral content. Trans 32nd Orthop Res Soc 1986;11:330.
20. Katz JL, Meunier A. Scanning acoustic microscope studies of the elastic properties of osteons and osteon lamellae. Trans ASME J Biomech Eng 1993;115:543–548.
21. Evans FG, Vincentelli R. Relations of the compressive properties of human cortical bone to histological structure and calcification. J Biomech 1974;7:1–10.
22. Lipson SF, Katz JL. The relationship between elastic properties and microstructure of bovine cortical bone. J Biomech 1984;17(4):231–240.
23. Rho JY, Pharr GM. Effects of drying on the mechanical properties of bovine femur measured by nanoindentation. J Mater Sci Mater Med 1998; to appear.
24. Evans FG. Mechanical properties of bone. Springfield, Illinois: Charles C. Thomas Publishers; 1973. p 43–55.
25. Tao NJ, Lindsay SM, Lees S. Measuring the microelastic properties of biological material. Biophys J 1992;63:1165–1169.
26. Hay JC, Sun EY, Pharr GM, Bercher PF, Alexander KB. Elastic anisotropy of β-silicon nitride whiskers. J Am Ceram Soc 1998; 81:2661–2669.
27. Vlassak JJ, Nix WD. Indentation modulus of elastically anisotropic half spaces. Philos Mag A 1993;67(5):1045–1056.
28. Vlassak JJ, Nix WD. Measuring the elastic properties of anisotropic materials by means of indentation experiments. J Mech Phys Solids 1994;42(8):1223–1245.