Published in final edited form as:

J Biomech. 2008 ; 41(2): 267–275. doi:10.1016/j.jbiomech.2007.09.019.

Mechanical property determination of bone through nano- and micro-indentation testing and finite element simulation

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Abstract

Measurement of the mechanical properties of bone is important for estimating the stresses and strains exerted at the cellular level due to loading experienced on a macro-scale. Nano- and micro-mechanical properties of bone are also of interest to the pharmaceutical industry when drug therapies have intentional or non-intentional effects on bone mineral content and strength. The interactions that can occur between nano- and micro-indentation creep test condition parameters were considered in this study, and average hardness and elastic modulus were obtained as a function of indentation testing conditions (maximum load, load/unload rate, load-holding time, and indenter shape). The results suggest that bone reveals different mechanical properties when loading increases from the nano- to the micro-scale range (μN to N), which were measured using low- and high-load indentation testing systems. A four-parameter visco-elastic/plastic constitutive model was then applied to simulate the indentation load vs. depth response over both load ranges. Good agreement between the experimental data and finite element model was obtained when simulating the visco-elastic/plastic response of bone. The results highlight the complexity of bone as a biological tissue and the need to understand the impact of testing conditions on the measured results.

Keywords

Nano-indentation; Micro-indentation; Mechanical properties; Bone; Finite element modeling

1. Introduction

An increasing number of measurements of hardness and Young's modulus of bone have been made using different nano-instruments (Turner et al., 1999; Hengsberger et al., 2003; Rho et al., 2002; Rho and Pharr, 1999; Hoffer et al., 2005; Ashman and Rho, 1988; Fan et al., 2002; Garner et al., 2000; Fan and Rho, 2003). However, testing conditions have not been uniform. For example, Turner et al. (1999) indented to a depth of 1000 nm at 750 $\mu\text{N/s}$, an unloading rate of 375 $\mu\text{N/s}$, and a hold time of 10 s. Rho et al. (2002), Rho and Pharr (1999), and Fan and Rho (2003) performed tests using a maximum load of 8 mN, and a 400 $\mu\text{N/s}$ load rate in studying the microstructural elasticity and heterogeneity in human femoral bone. Additional researchers (Hengsberger et al., 2003; Ashman and Rho, 1988; Fan et al., 2002; Garner et al., 2000) utilized other indentation testing conditions. Micro-indentation tests have also been used to probe the micro-scale properties of bone (Weaver, 1966; Hodgkinson et al., 1989; Carlstrom, 1954; Amprino, 1958; Currey and Brear, 1990; Evans et al., 1990; Blackburn

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Conflict of interest

The authors have no personal or financial relationships with other people or organizations who could have inappropriately influenced or biased this work.

et al., 1992; Ko et al., 1995). For example Ko et al. (1995) used micro-indentation to measure the properties of bone in a contact region $150 \times 20 \mu\text{m}$ to quantify the stiffness of a single trabeculae, which was then compared to nanoindentation measurements at a sub-micron level. The effects of testing conditions and micron to sub-micron testing scale property variations, however, were not discussed. Although the ISO has issued a draft international standard, ISO 14577 (Fischer-Cripps, 2002), for instrumented indentation tests, it does not provide details on testing conditions.

This investigation examines the relationship between three testing parameters: maximum load, load/unload rate, and holding time, on Young's modulus and hardness based on nano- and micro-indentation of cortical bone from monkey vertebra. An experimental design matrix was used to investigate the relationship between these parameters and the computed mechanical properties. In addition, indenter geometry effects on Young's modulus, maximum depth, and contact area were investigated using immature bovine cortical bone due to its higher degree of uniformity compared to other bone types. Typical indentation load vs. depth data revealed time-dependent (creep) behavior; therefore, a four-parameter visco-elastic/plastic constitutive model implemented via an axisymmetric finite element simulation was used to simulate the indentation data. The parameters were then correlated with nano- and micro-indentation mechanical property measurements.

2. Materials and methods

2.1. Specimen preparation

Two bone specimens approximately $15 \times 8 \times 2 \text{ mm}$ thick were dissected from monkey vertebra. In this work, sample 1 was placebo-treated, sham-ovariectomized and sample 2 was placebo-treated, ovariectomized, both from female cynomolgus monkeys. Additional details on the monkey specimens may be found elsewhere (Lees et al., 2002). The bovine bone samples were taken from the distal posterior aspect of bovine tibiae of 18 month to 2-year old animals obtained from a local slaughter house (Martin's Meats, Wakarusa, IN). All specimens were dehydrated in a series of alcohol baths and embedded in epoxy resin at room temperature. Both specimens were subjected to the same cleaning and mounting protocol, and polished using silicon carbide abrasive papers and diamond paste to an approximate surface roughness of $0.05 \mu\text{m}$ R_a (center-line-average roughness) necessary for repeatable results.

2.2. Design of experiments

Experimental design methods are widely used in the creation of testing protocols. One of the goals of these methods is to identify the optimum values for the different factors that affect the desired performance characteristic. The major classes of designs typically used in industrial experimentation include: $2^{(k-p)}$ (two-level, multi-factor) designs, screening designs for large numbers of factors, $3^{(k-p)}$ (three-level, multi-factor), central composite (or response surface), Latin square, Taguchi analysis, mixture designs, and special procedures for constructing experiments in constrained experimental regions (Box and Draper, 1987; Montgomery, 1990; Ross, 1988).

Taguchi (Wilkins et al., 1994) designed techniques for performing fractional factorial experiments in the form of orthogonal arrays. Table 1 shows the array for 3 parameters (maximum load, P_{max} , load/unload rate, and hold time) and 5 levels for each parameter; 25 tests total, for the low-load nano-indentation tests. The test matrix for high-load micro-indentation tests is also shown in Table 1. Note that for each matrix test condition, a total of 20 indents were performed with the mean and standard deviation values reported.

Choice of the levels for each parameter was based on a maximum load of 10 mN for the low-load transducer, and 1 N for the high-load transducer, and limiting test times for the indenter system (which affect load/unload rate and hold times). Procedures for estimating the effects of each parameter on the performance characteristic E are sometimes referred to as analysis of means (or ANOM). Suppose that $E_1, E_2, E_3, \dots, E_{25}$ are the results of the experiments. If $\bar{E}_{p1}$ is the performance characteristic (e.g., Young's modulus) averaged over those experiments for which maximum load, $P_{\max}$ is at level 1, $\bar{E}_{p2}$ corresponding to level 2, etc. Thus:

$$\begin{aligned}\bar{E}_{p_{\max 1}} &= (E_1 + E_2 + E_3 + E_4 + E_5)/5, \\ \bar{E}_{p_{\max 2}} &= (E_6 + E_7 + E_8 + E_9 + E_{10})/5, \\ \bar{E}_{p_{\max 3}} &= (E_{11} + E_{12} + E_{13} + E_{14} + E_{15})/5, \\ \bar{E}_{p_{\max 4}} &= (E_{16} + E_{17} + E_{18} + E_{19} + E_{20})/5, \\ \bar{E}_{p_{\max 5}} &= (E_{21} + E_{22} + E_{23} + E_{24} + E_{25})/5.\end{aligned}\quad (1)$$

Similarly, one could have:

$$\bar{E}_{\dot{p}1} = (E_1 + E_6 + E_{11} + E_{16} + E_{21})/5, \quad (2)$$

where $\dot{p}$ represents the loading/unloading rate, etc. Note that since 20 indents were performed for each of the matrix test conditions, the values computed via Eqs. (1) or (2) are calculated from 100 indents total.

Indenter shape is related to the effective strain imposed, which led to the development of Berkovich tip (Tabor, 1951). The Berkovich tip has a fixed strain as long as the indent shape is self-similar. Thus, the tip shape at shallow indentation depths (where the finite tip radius on the order of 100 nm is a significant portion of the contact region) may lead to different results. Therefore, in this portion of the investigation, Berkovich and 1 μm radius 90° cono-spherical tips were used on the bovine cortical bone specimen.

2.3. Nano- and micro-indentation measurements

Nano-indentation experiments were performed using a Hysitron TriboIndenter™ with a 10 mN two-axis capacitive transducer. Micro-indentation experiments were performed using the 3D OmniProbe™ transducer. All measurements employed standard diamond Berkovich or 1 μm radius cono-spherical indenters in the load-control mode. Tip-shape calibration was based on determination of the tip-area function. Indent load was varied from 1000 μN to 100,000 μN for the calibration. The calibration results (reduced modulus, E_r) may be seen in Fig. 1, and approximate known values for fused quartz (69.6 GPa) and single crystal aluminum (75 GPa) reasonably well.

Various methods have been developed to estimate the elastic modulus and hardness of a material (King, 1987; Hainsworth et al., 1996; Cheng and Cheng, 2000; Hay et al., 1998; Field and Swain, 1993) via indentation methods. Here, the method of Oliver and Pharr (1992) was used to determine the reduced Young's modulus E_r and hardness H for each indentation:

$$H = \frac{P_{\max}}{A_c}, \quad (3)$$

$$E_r = \frac{\sqrt{\pi}}{2} \frac{S}{\sqrt{A_c}}. \quad (4)$$

The contact stiffness, S , the slope of the unloading force–displacement curve, is determined by the region between 90% and 40% of the unloading portion of the curve. Here, A_c is the contact area. Young's modulus E of the specimen is then obtained from the relation:

$$\frac{1}{E_r} = \frac{1 - \nu^2}{E} + \frac{1 - \nu_{\text{indenter}}^2}{E_{\text{indenter}}}, \quad (5)$$

where the known Young's modulus and Poisson's ratio of the indenter are 1140 GPa and 0.07, respectively, and the assumed Poisson's ratio for bone is 0.3 (Turner et al., 1999). The hardness, H , and Young's modulus E of bone are then computed.

Indentation testing was performed by viewing the sample surface in the microscope of the nano-indenter and then locating regions on the surface that were more or less defect-free. Testing on or very near Haversian canals or large pores results in very poor data, as expected. These regions were avoided.

2.4. Finite element simulation

The results of the nano-indentation experiments showed that material creep occurs during the load-hold phase. In addition, permanent deformation of the bone is visible at the end of the test. Thus, a specific constitutive material model is required that includes both visco-elastic and plastic behavior. To accomplish that, a four-parameter constitutive model was developed (Ovaert et al., 2003) and implemented as a FORTRAN user-defined subroutine (UMAT) in the AbaqusTM finite element program. The model essentially consists of a linear dashpot in parallel with an elasto-plastic spring, as shown in Fig. 2.

The total stress, σ , is the sum of the stresses in the dashpot, σ_d , and the elasto-plastic spring, σ_s ; while the strains in both cases, ε , are the same:

$$\begin{aligned} \sigma &= \sigma_s + \sigma_d, \\ \varepsilon &= \varepsilon_s + \varepsilon_d. \end{aligned} \quad (6)$$

Here, the subscript 'd' refers to a dashpot and 's' the spring. The equations illustrate that the total tangent stiffness matrix necessary for the finite element simulation may be obtained by adding the dashpot and the elasto-plastic spring stiffness matrices, which may be determined independently. The elasto-plastic spring is modeled using a Ramberg-Osgood relationship:

$$\varepsilon = \sigma_s / E + A (\sigma_s / E)^m \quad \text{or} \quad \varepsilon = \varepsilon_{\text{elastic}} + \varepsilon_{\text{plastic}}. \quad (7)$$

The term on the left represents the elastic strain and the term on the right the plastic strain. Eq. (7) yields one stiffness (modulus) parameter, E (units in Pa), and two dimensionless plasticity constants, A and m . The model thus contains four parameters, with η being the fourth parameter representing the dashpot viscosity (units in Pa · s). Note that an indentation (compression) yield stress may also be estimated by setting the stress in the elasto-plastic spring, σ_s , equal to the

yield stress, Y , and setting the plastic portion of the strain, $\epsilon_{\text{plastic}}$, equal to 0.002 (0.2% offset yield criteria). Solving for Y :

$$Y = E(0.002/A)^{1/m}. \quad (8)$$

Two experimental load vs. deformation curves were used in the analysis, one at low load (500 μN P_{max} , 200 $\mu\text{N/s}$ load/unload rate, 5 s hold time) and the other at high load (100 mN, 50 mN/s, 2 s). An axisymmetric 140.6° conical indenter was used to model the Berkovich indenter since it approximates the Berkovich shape with the same projected area-to-depth ratio. A typical mesh consisted of 910 axisymmetric 4-node elements, and may be seen in Fig. 3. The mesh size used in the low-load simulations was 3 μm high $\times$ 4 μm width, and 30 μm high $\times$ 40 μm wide for the high-load simulations.

3. Results

3.1. Experimental results

From Table 2 and Fig. 4 one can see that average Young's modulus values decreased as the maximum load and load/unload rate increased. Load-hold time, however, had little effect. The magnitude of the variations between bone samples 1 and 2 was also relatively small. In addition, variation within each test grouping (low- vs. high-load conditions) was relatively small. This similarity further suggests that sample preparation and testing method were consistent. Variations in the data and any observable trends tended to fall within the standard deviations for each measurement, thus it is difficult to suggest any trends within the load range groupings.

Average Young's modulus was approximately 17.8 GPa for sample 1 and 17.3 GPa for sample 2 under low-load conditions. In the high-load group, the average values were 12.3 and 12.2 GPa, respectively, a significant decrease. Hardness was also relatively unaffected by test conditions. In Table 2, the average values of Young's modulus vs. maximum load, load/unload rate, and hold time for the low-load and high-load tests were tested for significance using a t -test comparison of sample means for both samples. The same test was applied to the hardness values. In Table 2, the average values of Young's modulus vs. maximum load, load/unload rate, and hold time for the low-load and high-load tests were tested for significance using a t -test comparison of means for both samples. The same test was applied to the hardness values. The variation in Young's modulus values between the low-load and high-load data was significant ($p < 0.05$); whereas it was not significant when tested against the hardness data. The transition from low-load to high-load conditions suggests that load range and the resulting contact area over which it acts have a large effect on the measured properties.

To further investigate the effects of load and contact area on properties, indentation tests were performed on immature bovine cortical bone using both 1 μm (radius) cono-spherical and Berkovich indenters. Table 3 shows the results for maximum applied loads of 10,000 and 100 μN for the 1 μm tip and 10,000 μN for the Berkovich tip. The results again show that Young's modulus values decrease with increasing contact area.

3.2. Simulation results

Fig. 5 shows the effects of simulation parameter values on the load vs. depth relationships in the low-load range from 0 to 500 μN . When decreasing E from 26 to 10 GPa, while fixing η (2.4 GPa $\cdot$ s), A (500), and m (2.6), the load vs. depth curve reflects the increasing compliance of the material by shifting from left to right (decreasing load/unload curve slopes) and achieving a greater indentation depth as expected. Varying η from 9.0 to 0.5 GPa $\cdot$ s with fixed E (17 GPa), A (500), and m (2.6), has the effect of reducing the dashpot viscosity and thus decreasing

creep displacement during the hold segment, as well as decreasing the load/unload curve slopes. Increasing A and reducing m cause an increase in strain/displacement as well. As seen in Fig. 5(a) (the circle symbols), an iterative matching process yields the four parameters: $E = 17$ GPa, $\eta = 2.3$ GPa · s, $A = 500$, and $m = 2.6$, as a reasonable match to the experimental data with the exception of the end of unloading region. Here, the experimental data shows a ‘softening’ as the load returns to zero which may be due to small-scale surface irregularities and/or damage effects in the bone which are a result of the indentation deformation. Application of Eq. (8) to the parameter values above yields $Y = 142.7$ MPa, which is similar to other results for the compressive yield stress of bone (Burstin et al., 1976).

Simulations were performed for the high-load tests, and the trends were similar to those in Fig. 5. As seen in Fig. 6 (the circle symbols), the combination of the four parameters: $E = 12.7$ GPa, $\eta = 0.37$ GPa · s, $A = 1380$, and $m = 2.3$, provides a reasonable match to the high-load experimental data with the exception of the end of unloading region. Application of Eq. (8) to the parameter values above yields $Y = 36.7$ MPa, which is lower than Y computed from the low-load data (142.7 MPa).

4. Discussion

The low-load range test conditions used for the determination of Young’s modulus and hardness are similar to those employed by Turner et al. (1999). They selected a depth of 1000 nm, which required a maximum load of approximately 10 mN, a 375 μ N/s unloading rate, and 10 s hold time. Hengsberger et al. (2003) selected a maximum depth of 900 nm, which required a 10 mN load, followed by a 5 s holding period. Rho et al. (2002) tested at a maximum load of 8 mN, with a load/unload rate of 400 μ N/s. The difference in Young’s modulus and hardness in this range of maximum loads is small. Thus, one might expect only slight variation in bone properties within this nano-indentation scale. However, when utilizing higher loads in the micro-indentation range, there is a noticeable decrease in modulus. Thus, the scale of load, the resulting contact area, and plastic deformation zone influence the values of Young’s modulus. This is not the case, however, with hardness, which does not display any significant dependency on test parameters.

As noted in the literature (Rho and Pharr, 1999; Hoffler et al., 2005), there are differences in the measured modulus and hardness values when testing hydrated vs. dry specimens, thus the measured values presented here would likely decrease if tested under hydrated conditions. It is not clear, however, whether or not the maximum high-load penetration depths encountered in this study (on the order of 3000 nm, 3–5 times larger than in Rho and Hoffler, respectively) would still result in significantly different hydrated vs. dry results; and whether or not the trends observed between hydrated and dry specimens would be similar to those observed in this study. These questions form the basis for future work.

A likely cause for the decrease in Young’s modulus going from low to high load is the fact that contact area and plastic deformation increase significantly at higher loads. Unlike hardened steels whose heterogeneities (carbides) are significantly harder than the surrounding matrix, it is likely that defects (pores, Haversian canals) in bone create a ‘softening’ influence on the load/deformation characteristics. At high loads, this effect is magnified as the physical number of defects and their cumulative effects in the contact increase.

The variation in mechanical properties is important when computing stresses and strains in bone. For example, bone in the region near a metallic implant must be strong enough to withstand the mechanical loads; and at the same time its mechanical attributes must be capable of load transfer from the implant, remodeling, and sustaining itself for long periods of time. In

addition, bone that has lost its flexibility and toughness due to osteoporosis, for example, may reflect those changes via altered mechanical properties.

The finite element modeling reveals additional information on the effects of changes in the parameters obtained from the low-load and high-load simulations. Figs. 7 and 8 show subsurface von-Mises stress contours at maximum load at the end of the hold time, corresponding to the point of maximum indentation depth. Note that the micro-indentation results at high load display a reduced maximum von-Mises stress (830 MPa) vs. the low-load simulation (2400 MPa). This is likely due to the combined effects of changes in the simulation parameters: a decrease in E , a decrease in η , an increase in A , and a decrease in m going from low to high load. Decreasing E and η has the effect of reducing the average slope of the loading curve; and similarly when increasing A and decreasing m . Increasing A and decreasing m also increase the degree of plastic deformation, which reduces the von-Mises stress based on the assumed four-parameter constitutive model. This suggests that the anisotropic nature of bone and/or the nature of its plasticity have a significant effect on the unloading behavior during nano-indentation. Future work will focus on capturing the unloading behavior more accurately, by examining different plastic deformation models that account for compaction and/or variations in tensile vs. compressive mechanical behavior.

The viscosity coefficients determined in this investigation were compared with similar estimates in the literature, though the test methods were different. In Katsamanis and Raftopoulos (1990), η was estimated in the range of 3.7×10^4 Pa · s. Other studies (Tennyson et al., 1972; Lewis and Goldsmith, 1975) show similar or slightly larger values. In these studies, measurements were taken at high strain rates. In Bargren et al. (1974) η was on the order of 4×10^7 Pa · s for dry human bone tested at low dynamic frequencies (7.4 Hz). In this study, values of η range from 3.7×10^8 to 2.3×10^9 Pa · s from quasistatic/creep tests. Thus, it appears that the viscosity of bone increases as the frequency of loading or strain rate decrease. The higher viscosity values may also be due to the difference in loading conditions and contact area in this study. Indentation (compression) is thus likely to produce different results than dynamic tensile or split Hopkinson bar tests.

The Ramberg–Osgood coefficients were also compared to literature results. Hight and Brandeau (1983) modeled the visco-plastic response of bone using a modified form of the Ramberg–Osgood equation, including strain rate effects. Their conclusions noted that Ramberg–Osgood accurately models the stress-strain behavior of bone over a wide range of strain rates. Since indentation testing at different load ranges produces large variations in the plastic zone, it is expected that the Ramberg–Osgood coefficients would vary with the scale of indentation testing, particularly going from the nano- to the micro-indentation range.

The results of the tip study on bovine cortical bone samples show that increasing load and contact area results in a decreased modulus which verifies the results obtained from the simulations. At the same load, the contact area for the Berkovich tip is larger than the $1 \mu\text{m}$ cono-spherical tip and yielded a smaller modulus value. Thus, there appears to be an inverse correlation between contact area and modulus.

Indentation testing is used to determine the effects of drug therapies on bone density and mechanical properties during clinical trials, and obtaining test samples at prescribed time intervals *in vivo* is a straightforward, minor surgical procedure compared to the difficulties with obtaining larger test samples for standard ASTM tensile tests. Therefore, indentation test protocol standardization is a desirable goal for interpretation of indentation test results. Based on these results, a reasonable set of test conditions to use when indentation creep-testing bone with a Berkovich indenter are 10 mN maximum load, 400 mN load/unload rate, and a 10 s hold time.

Acknowledgements

The support of the NIH under Grant R01 AR052008-01A1 is gratefully acknowledged. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. The authors would also like to thank Dr Charles Turner, Departments of Orthopaedic Surgery and Biomedical Engineering, Indiana University, for providing the monkey bone specimens.

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Fig. 1. Tip calibration results on fused quartz and single crystal aluminum.

Fig. 2.
Four-parameter visco-elastic/plastic constitutive model.

Fig. 3. Axisymmetric finite element mesh used in the low-load simulations.

Fig. 4. Young's modulus and hardness data as a function of $P_{\max}$, load/unload rate, and hold time for nano- and micro-indentation tests.

Fig. 5.

Low-load finite element simulation results: (a) varying E with fixed $\eta = 2.4 \text{ GPa} \cdot \text{s}$, $A = 500$, and $m = 2.6$; (b) varying η with fixed $E = 17 \text{ GPa}$, $A = 500$, and $m = 2.6$; (c) varying A with fixed $E = 17 \text{ GPa}$, $\eta = 2.4 \text{ GPa} \cdot \text{s}$, and $m = 2.6$; (d) Varying m with fixed $E = 17 \text{ GPa}$, $\eta = 2.4 \text{ GPa} \cdot \text{s}$, and $A = 500$.

Fig. 6. High-load finite element simulation results, varying E with fixed $\eta = 0.37 \text{ GPa} \cdot \text{s}$, $A = 1380$, and $m = 2.3$.

Fig. 7. Contours of von-Mises stress (MPa) for low-load simulation with $E = 17$ GPa, $\eta = 2.4$ GPa s, $A = 500$, $m = 2.6$, maximum load = 500 μ N.

Fig. 8.

Contours of von-Mises stress (MPa) for high-load simulation with $E = 12.7$ GPa, $\Delta = 0.37$ GPa s, $A = 1380$, $m = 2.4$, maximum load = 100 mN.

Table 1

Test matrices for nano- and micro-indentation tests

Low-load test parameters			
Test#	$P_{\max}$ (μN)	Load/unload rate ($\mu\text{N/s}$)	Hold time (s)
1	100	100	2
2	100	200	3
3	100	300	5
4	100	400	10
5	100	500	15
6	500	100	3
7	500	200	5
8	500	300	10
9	500	400	15
10	500	500	2
11	1000	100	5
12	1000	200	10
13	1000	300	15
14	1000	400	2
15	1000	500	3
16	5000	100	10
17	5000	200	15
18	5000	300	2
19	5000	400	3
20	5000	500	5
21	10 000	100	15
22	10 000	200	2
23	10 000	300	3
24	10 000	400	5
25	10 000	500	10
High-load test parameters			
Test#	$P_{\max}$ (mN)	Load/unload rate (mN/s)	Hold time (s)
1	100	50	2
2	100	100	5
3	100	150	15
4	500	50	5
5	500	100	15
6	500	150	2
7	1000	50	15
8	1000	100	2
9	1000	150	5

Table 2
Young's modulus and hardness data. Standard deviation shown in parentheses

	Sample 1 Average <i>E</i> (GPa)	Sample 2 Average <i>E</i> (GPa)	Sample 1 Average <i>H</i> (GPa)	Sample 2 Average <i>H</i> (GPa)
<i>P</i> _{max} (μN)				
100	17.47 (0.88)	16.47 (0.43)	0.59 (0.04)	0.52 (0.01)
500	18.19 (0.61)	17.27 (0.78)	0.73 (0.04)	0.62 (0.05)
1000	17.53 (1.16)	16.63 (0.21)	0.71 (0.08)	0.61 (0.02)
50,00	17.94 (1.29)	17.68 (0.93)	0.61 (0.03)	0.54 (0.02)
10,000	18.09 (0.76)	18.65 (1.38)	0.59 (0.01)	0.54 (0.02)
*100,000	13.86 (1.53)	14.19 (0.24)	0.63 (0.03)	0.68 (0.02)
*500,000	11.14 (1.24)	11.29 (0.46)	0.57 (0.09)	0.57 (0.03)
*1,000,000	11.87 (0.74)	11.16 (0.27)	0.55 (0.04)	0.55 (0.03)
Load/unload rate (μN/s)				
100	17.20 (0.59)	16.54 (0.42)	0.63 (0.05)	0.57 (0.05)
200	17.14 (0.87)	17.47 (1.69)	0.62 (0.06)	0.54 (0.03)
300	17.90 (0.49)	17.68 (1.23)	0.62 (0.06)	0.57 (0.03)
400	18.38 (1.22)	17.87 (1.06)	0.65 (0.09)	0.58 (0.05)
500	18.58 (0.51)	17.15 (0.66)	0.69 (0.11)	0.57 (0.08)
*50,000	11.38 (0.73)	12.08 (1.67)	0.57 (0.06)	0.60 (0.09)
*100,000	12.64 (2.38)	12.09 (1.79)	0.57 (0.10)	0.60 (0.08)
*1500,00	12.86 (1.51)	12.46 (1.74)	0.61 (0.04)	0.60 (0.06)
Hold time (s)				
2	18.20 (0.34)	17.84 (1.68)	0.68 (0.09)	0.59 (0.08)
3	18.30 (1.36)	17.48 (1.68)	0.67 (0.10)	0.57 (0.04)
5	17.85 (0.74)	17.17 (0.92)	0.63 (0.06)	0.57 (0.04)
10	17.26 (0.78)	16.98 (0.37)	0.62 (0.06)	0.55 (0.04)
15	17.60 (1.11)	17.23 (0.53)	0.63 (0.07)	0.56 (0.05)
*2	12.46 (0.32)	12.34 (1.42)	0.62 (0.03)	0.63 (0.07)
*5	12.38 (2.29)	12.26 (1.62)	0.60 (0.06)	0.60 (0.07)
*15	12.03 (2.21)	12.03 (2.10)	0.53 (0.07)	0.57 (0.08)

Asterisk denotes high-load test results.

Table 3

Average modulus, contact area, and indentation depth for Berkovich and 1 μm cono-spherical tips on immature bovine cortical bone

Indenter type	Average E (GPa)	Contact area (μm^2)	Depth (nm)
Berkovich (10,000 μN P_{max} , 375 mN/s load/unload rate, 5 s hold time)	13.6 (1.7)	28.2 (4.3)	1030 (75.6)
1 μm cono-spherical (10,000 μN P_{max} , 375 mN/s load/unload rate, 5 s hold time)	14.8 (3.4)	21.2 (5.6)	1646 (244)
1 μm cono-spherical (100 μN P_{max} , 10 mN/s load/unload rate, 10 s hold time)	18.5 (3.5)	0.2 (0.03)	100.3 (21)

Standard deviation shown in parentheses..