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In Situ Microindentation for Determining Local Subchondral Bone Compressive Modulus

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Abstract

Background—Alterations to joint tissues, including subchondral bone, occur with osteoarthritis. A microindentation technique was developed to determine the local compressive modulus of subchondral bone. This test in conjunction with a cartilage indentation test at the same location could evaluate changes of these material properties in both tissues. The accuracy of the technique was determined by applying it to materials of known moduli. The technique was then applied to rat tibial plateaus to characterize the local moduli of the subchondral bone.

Method of approach—An established nanoindentation method was adapted to determine the modulus of subchondral bone following penetration of the overlying articular cartilage. Three cycles of repeated loading were applied (2.452 N, 30 s hold). The slope of the load-displacement response during the unloading portion of the third cycle was used to measure the stiffness. Indentation tests were performed on two polyurethane foams and polymethyl-methacrylate for validation (n=15). Regression analysis was used to compare the moduli with reference values. Subchondral bone moduli of tibial plateaus from Sprague-Dawley rats (n=5) were measured for central and posterior locations of medial and lateral compartments. An ANOVA was used to analyze the effects of compartment and test location.

Results—The measured moduli of the validation materials correlated with the reference values ($R^2 = 0.993$, $p = 0.05$). In rat tibial plateaus, the modulus of the posterior location was significantly greater than the center location (4.03 ± 1.00 and 3.35 ± 1.16 GPa respectively, $p = 0.03$). The medial compartment was not different from the lateral compartment.

Conclusions—This method for measuring the subchondral bone in the same location as articular cartilage allows studies of the changes of these material properties with the onset and progression of osteoarthritis.

Keywords

subchondral bone; microindentation; modulus; stiffness; osteoarthritis

Background

Cartilage and subchondral bone health are tightly associated and alterations to both tissues occur with osteoarthritis (OA) [1]. Reduced material density, stiffness and hardness of the subchondral bone have been observed with OA [2,3]. Radin and Rose [4] reported subchondral thickening preceding and associated with cartilage degeneration with OA. Speculation continues regarding the contribution and time course of subchondral bone stiffening or softening and its relationship to cartilage degeneration in the onset and progression of OA [5].

Oliver and Pharr [6] developed a method of measuring material elastic modulus including bone tissue samples by nanoindentation which has subsequently been adapted and widely used in the characterization of small-scale material properties. This method involves measuring the slope (stiffness) of the elastic unloading portion of an indentation test after a series of repeated indentation loadings of the test material. The elastic stiffness and the indenter contact area are used to obtain an effective modulus. The test material modulus is calculated by accounting for the series elasticity of the indenter and the test material. Nanoindentation has been widely used to measure the material properties of trabecular and cortical bone [7]. Hansma *et al.* [8–10] utilize similar techniques to measure bone properties through overlaying soft tissues using an indenter within a cannulated reference probe.

While several techniques have been established to characterize site specific articular cartilage material properties [11], there is not an established *in situ* technique to measure the underlying subchondral bone material properties at the same location, especially in small research animals where excising and machining test specimens is often impractical. This makes it difficult to test hypotheses about changes in cartilage and its underlying subchondral bone. The objective of this study was to develop a microindentation technique to determine the local compressive modulus of subchondral bone *in situ*. Our approach was to evaluate the accuracy of the measurement technique by applying it to homogenous materials of known compressive moduli and then apply the technique to rat tibial plateaus to characterize the distribution of the compressive modulus at different locations on the plateau.

Methods

The Oliver and Pharr [6] method was adapted to measure subchondral bone modulus through the cartilage *in situ*. A custom materials testing system [12] with a stainless steel needle (# 01125, Dyno Merchandise, Pompano Beach, FL USA) with a nominal diameter of 0.90 mm as an indenter was used as the indentation system. The radial profile of the circular cross-section needle along the length of the needle (depth of penetration) was measured from a digital micrograph. The radius of the needle (r) as a function of depth of penetration (h) was fit to the function in Eq. 1

$$r(h)=a_1h+a_2h^{\frac{1}{2}}+a_3h^{\frac{1}{3}}+a_4h^{\frac{1}{4}} \quad (1)$$

The needle indenter profile fit Eq. 1 with a coefficient of determination (R^2) of greater than 0.999. The curve-fit parameters are given in Table 1. A three-point bending test with a straight section of the needle was used to confirm the needle indenter modulus (E_i) was 196.5 GPa. Both the test materials and indenter Poisson's ratios were assumed to be 0.3. The loads on and displacements of the indenter were measured at 128 Hz using a load cell (LGP 310-5, Cooper Instruments, Warrenton, VA USA) and a laser interferometer (Excel Precision, Santa Clara, CA USA). Additional details of the custom materials testing system are given in Roemhildt *et al.* [12].

To initially penetrate the cartilage, the cartilage with subchondral bone is loaded at 0.490 N/s to 2.452 N and then unloaded at the same rate. The location of the subchondral bone surface was defined as the indenter position when the load returned to zero. Due to the viscoelastic nature of bone, this loading was repeated three times with a hold of 30 s at 2.452 N (Fig. 1). The slope of the load-displacement response between 2.403 N and 1.961 N during the unloading portion of the third hold cycle was used to measure the subchondral bone stiffness (Fig. 2).

The compliance of the testing system was measured by testing the needle indenter against a triangular tungsten carbide insert resulting in a mean 2.54 ($SD = 0.94$) μm of displacement at the maximum hold load. This displacement was subtracted from the depth of penetration measurements to correct for system compliance.

Tang *et al.* [13] shows that viscoelastic effects during indentation can be corrected using the change in depth of penetration at the end of the hold, the unloading rate, and the change in load at the end of the hold. Since the custom materials testing system uses load control, the change in load at the end of the hold is essentially zero and was assumed to be zero. The change in depth of penetration at the end of the hold was calculated as the derivative of a nonlinear least squares exponential fit of the displacement data for the last half (nominally 15 s) of the hold evaluated at the end of the hold. This method was used to correct any residual viscoelastic effects.

Rigid polyurethane foams (Pacific Research Laboratories, Vashon, WA USA) with designated densities of 40 lbf/ft^3 and 50 lbf/ft^3 and dental polymethyl-methacrylate (PMMA) (Henry Schein, Melville, NY USA) were used as validation materials. These materials have compression elastic moduli within the range of subchondral bone. The compressive moduli of the 40 lbf/ft^3 and 50 lbf/ft^3 rigid foams are 0.82 and 1.24 GPa , respectively [14]. The compression elastic modulus of the cast PMMA was 2.85 GPa as measured using ASTM D1621. These materials were indentation tested with five trials on each of three consecutive days. Regression analysis was used to compare the mean indentation modulus values with the reference values.

Indentation testing of the subchondral bone was performed on tibial plateau from female Sprague-Dawley rats ($n=5$) greater than four months of age. Following approval by the Institutional Animal Care and Use Committee of the University of Vermont, tibial plateaus from left and right hind legs were excised following euthanasia, wrapped with gauze, soaked in lactated Ringers solution, and stored at -80°C until testing. Specimens were defrosted and attached to aluminum platens with cyanoacrylate adhesive. Central and posterior test locations of medial and lateral compartments were identified under a dissection microscope (Fig. 3). Prior to indentation testing, the surface of the cartilage was aligned normal to the axis of indentation [12]. An ANOVA with blocking on animal was used to analyze the effects of compartment (medial vs. lateral) and test location (central vs. posterior) on the subchondral bone modulus.

Results

The moduli of the validation materials measured by indentation testing were not different by day, so the trials were averaged across days. The moduli (mean $\pm$ standard deviation) for the 40 and 50 lbf/ft^3 rigid foams and the PMMA were $0.70\pm0.15 \text{ GPa}$, $1.56\pm0.31 \text{ GPa}$, and $3.65\pm0.15 \text{ GPa}$, respectively. These values were highly correlated with the reference values ($R^2 = 0.993$, $p = 0.05$, Fig. 4). Indentation testing tended to overestimate the reference moduli with a slope of 1.41 , although this slope was not significantly different from one ($p = 0.18$). The intercept was also not significantly different from zero ($p = 0.37$).

The overall mean subchondral bone modulus for all locations of the rat tibia plateaus was $3.69\pm1.12 \text{ GPa}$. The modulus of the posterior location was significantly greater than the center location (4.03 ± 1.00 and $3.35\pm1.16 \text{ GPa}$ respectively, $p = 0.03$). The modulus of the medial compartment was not different from the lateral compartment (3.86 ± 1.08 and $3.52\pm1.16 \text{ GPa}$ respectively, $p = 0.28$). Using a sample size of five ($n=5$), with $\alpha = 0.05$ and a power of 80% ($\beta = 0.20$), the detectable change in subchondral bone modulus is 1.29 GPa (35.9% of the mean).

The corrections for system compliance and material viscoelasticity after three cycles of loading allow these effects on the measured moduli to be estimated. System compliance reduced the validation materials moduli by a mean of $5.48 \pm 1.96\%$, while reducing the rat subchondral bone moduli by a mean of $2.21 \pm 0.53\%$. Viscoelasticity increased the validation materials moduli by $1.58 \pm 0.68\%$ and the rat subchondral bone moduli by $2.47 \pm 1.13\%$.

Discussion

The microindentation technique presented in this study was validated against three materials with compressive moduli within the range of subchondral bone modulus. This method is able to measure the subchondral bone modulus in the same location as articular cartilage material properties tests in small research animals. This method was also able to detect variations in the compressive moduli of rat tibial plateaus from the central to posterior locations on the plateaus.

The mean rat tibia subchondral bone modulus of 3.6 GPa is very similar to the mean bovine femur subchondral bone modulus of 3.9 GPa measured by Mente and Lewis [15] using three-point bending. However, these subchondral bone moduli are much lower than the moduli of trabecular, cortical and teeth tissue measured by nanoindentation [7].

Variation in the validation data may be due in part to the size of the cellular structures of the foam relative to the diameter of the indentation needle. Even though a large diameter needle was used, the cell structure of the foams may have contributed to the variability of the validation results. The reference value for the PMMA may slightly underestimate the true value since there was some porosity in the PMMA because it was not centrifuged before casting.

There are several sources of variability and assumptions involved in this *in situ* microindentation method. A potential source of variability was the alignment of the indenter with the cartilage surface which may not reflect the orientation of the underlying subchondral bone. The indentation needle not only had to go through the articular cartilage, but also the calcified cartilage. The calcified cartilage modulus is an order of magnitude less than subchondral bone [15], so this would only have a small effect on the subchondral bone modulus measurements. Indentation testing assumes that the material is isotropic, but bone is anisotropic [16]. So, the measured modulus is a composite measure of the anisotropic moduli.

Both system compliance and viscoelasticity had small and counteracting effects (mean effects $< 2.5\%$) on the measured subchondral bone moduli. There was very little viscoelastic effect after the third 30 s hold. While not done here, both system compliance and the remaining viscoelasticity could be ignored to simplify the determination of the subchondral bone modulus without introducing large errors into the measurement.

Combined with existing techniques for determination of articular cartilage material properties, this technique will allow the contribution of both subchondral bone and articular cartilage changes with the onset and progression of OA to be studied. In this study, the compressive modulus of rat tibial plateau changed as a function of location on the plateau and this finding suggests that studies should make site specific measurements and not combine data between different locations.

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Figure 1.

(a) Indentation loads showing the initial penetration of the cartilage with three more cycles with 30 s holds. Also shown are the loads at which the depth of penetration is measured and the range for fitting the elastic unloading. (b) Indentation displacements with the fit of the creep in the last half of the last hold cycle and the depth of penetration.

Figure 2.

Load-displacement response of a rat tibia plateau to cyclic indentation. Horizontal dashed lines show the range of loads for fitting the elastic unloading stiffness. The solid line shows the linear fit to the elastic unloading.

Figure 3.

Axial view of a rat tibia showing the four test sites at the central and posterior locations of the medial and lateral compartments (MC-medial central, MP-medial posterior, LC-lateral central, LP- lateral posterior).

Figure 4. Compressive modulus measured by indentation ($E_{\text{indentation}}$) with reference values ($E_{\text{reference}}$) for two densities of rigid polyurethane foams and a dental polymethyl-methacrylate (PMMA).

Table 1

Curve-fit parameters for the needle indenter radius as a function of penetration depth. See Eq. 1.

Parameter	Value	Units
a_1	0.26912953291966	
a_2	-1.37906254885780	$\mu\text{m}^{1/2}$
a_3	42.59765726135224	$\mu\text{m}^{2/3}$
a_4	-27.53432811067186	$\mu\text{m}^{3/4}$