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Experimental characterization of biphasic materials using rate-controlled Hertzian indentation

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Abstract

This paper describes a new method, based on Hertzian biphasic theory (HBT), to characterize properties of biphasic materials with reduced time demands, increased surface sensitivity, and reduced computational demands compared to the current gold standards. Indentation experiments were conducted at a single location on a representative osteochondral plug to demonstrate and validate the HBT method against two gold standards, linear biphasic theory (LBT) and tension-compression nonlinear biphasic theory (TCN). The 1) aggregate moduli, 2) permeability and 3) tensile moduli from HBT, LBT, and TCN were 1) $H_A=0.47, 0.47, \text{ and } 0.40 \text{ MPa}$, 2) $k=0.0026, 0.0014 \text{ and } 0.0016 \text{ mm}^4/\text{Ns}$, and 3) $E_T=8.7, 0.46, \text{ and } 10.3 \text{ MPa}$, respectively. The results support the HBT method and encourage its use, especially in light of its practical advantages.

Keywords

biphasic; cartilage; indentation; contact mechanics

Introduction

McCutchen first modeled the mechanical response of cartilage to estimate its permeability

[1]. His analytical solution to unconfined compression, $F_p = \frac{\pi \cdot \dot{h} \cdot R^4}{8 \cdot k \cdot h}$, gives considerable insight into the tissue's flow dependent mechanical response and is easy to apply to engineering problems. The solution predicts, for example, that the deformation rate is proportional to the force carried by fluid pressure, which can be no larger than the total applied force. For articular cartilage at physiological conditions, relaxation times on the order of a day are required before strains reach a magnitude for which elastic contact stresses become significant [2]. Because deformation cannot occur instantaneously without an infinite deformation rate and force, the model predicts zero elastic load support and zero friction at the instant contact occurs. By measuring deformation rates of cartilage during

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unconfined compression, McCutchen was able to estimate a radial permeability of $5 \times 10^{-4} \text{ mm}^4/\text{Ns}$.

Agbezuge and Deresiewicz solved the Hertzian contact problem of a hard probe contacting a linear poroelastic half-space under a step load [3]. Unlike McCutchen's work, this solution predicted an instantaneous deformation due to a redistribution of volume that allows deformation without requiring instantaneous fluid flow. At the instant load is applied, it was predicted that the mean fluid pressure is equal to half the mean applied stress and decreases over time at a rate that depends on the permeability, modulus, and contact radius. Initially, the friction force is expected to be half the equilibrium value (since only half the load is supported by frictional contact). Oyen empirically curve-fit this numerical solution and adapted it for characterization of Poisson's ratio, permeability, and aggregate modulus in Hertzian creep testing [4].

Armstrong *et al.* [5] used linear biphasic theory (LBT) to solve the unconfined compression problem previously considered by McCutchen [1]. As the authors note, the nonlinear coupling of fluid flow and elastic deformation creates a very difficult problem that requires numerical solution, even in the simplest loading conditions. This solution also differed fundamentally from McCutchen's solution in its response to an instantaneously applied load due to the coupling of fluid and solid mechanics. In McCutchen's solution, which is mechanically analogous to a spring in parallel with a damper, fluid pressure supports the entire load at the instant load is applied, just as the damper would in the analogous mechanical system. According to Armstrong *et al.*, who used truly unconfined boundary conditions, fluid pressure causes cartilage to expand laterally, enabling a volume conserving deformation without the need for instantaneous flow [5]. Tensile stresses balance the increase in internal pressure, and since the tensile strain is half the compressive strain on average, the fluid pressure is also half the compressive stress. This solution theoretically limits fluid load support and the corresponding friction reduction to 33% [5, 6].

Experimental measurements with cartilage show far greater stresses, fluid load fractions, and frictional reductions. In fact, while attempting to validate the theoretical predictions, Armstrong *et al.* [5] discovered far higher contact stresses than the model allowed. They proposed that adhesion to the loading platen restricted lateral expansion and made the tissue behave more like the confined tissue in McCutchen's model. However, a much more likely explanation, given its low adhesive friction coefficient, is that the stiff internal collagen matrix resists lateral expansion under load as suggested by Soltz and Ateshian [7]. In other words, the high tensile modulus of internal matrix, the rigid (bone) substrate and any other physical impediments to volume-conserving expansion during contact directly increase the tissue's ability to build high fluid pressure; these confining properties are chiefly responsible for the very low friction coefficients observed in practice with this material.

The unconfined compression test presents several experimental challenges for the purposes of materials characterization. First, cartilage has varying thickness across the surface making the boundary conditions difficult to satisfy. Second, it is not possible to make measurements *in-situ* on a surface within a joint. Third, the bulk nature of the test prevents the spatial resolution necessary to probe heterogeneity with location and depth. Mak *et al.* solved LBT

for indentation with a plane-ended porous indenter to address some of these limitations [8]. Later, Mow *et al.* developed the experimental protocol necessary to apply the solution to the characterization of aggregate modulus, Poisson's ratio and permeability [9] using a single indentation creep test. This method has since become one of the gold standards for the characterization of biphasic materials.

The linear plane-ended indentation method has limitations of its own. Firstly, the solution was derived numerically and its use requires access to the custom curve-fitting program developed by the authors. Secondly, the plane-ended contact produces a stress concentrator along the periphery that can damage the surface [10]. Thirdly, the influence of the stress concentrator on the results increases with decreased contact radius, thus limiting spatial resolution. Fourthly, results from the linear biphasic model cannot reproduce the magnitudes of stress observed *in-vivo* (1–5MPa [11]). This is especially important given the fact that permeability is strain-dependent [12] and thus pressure-dependent.

Moore and Burris recently developed an analytical solution to the Hertzian contact problem for biphasic materials to address the limitations of linear theory [13]; here on we call the solution HBT. The solution introduces no stress concentrator and reproduces physiologically consistent pressures. In this paper, we describe how standard indentation measurements can be used to characterize the material properties of articular cartilage and other biphasic materials. The reader is referred to Moore and Burris for a description of the derivation of the model equations [13].

Methods

Theoretical background

Eq. (1) gives the fluid load fraction, F' , as a function of mechanical conditions ($\dot{\delta}$: indentation rate, R : probe radius) and material properties (E_t : tensile modulus, E_{c0} : equilibrium contact modulus, k : permeability) [13]:

$$F' = \frac{E_t}{E_t + E_{c0}} \cdot \frac{\dot{\delta} \cdot R}{\dot{\delta} \cdot R + E_{c0} \cdot k} \quad (1)$$

The first term, $\frac{E_t}{E_t + E_{c0}}$, is an asymptotic limit governed by the resistance to lateral expansion. In the limit of perfect confinement, the asymptote goes to 1. For an unconstrained Hertzian contact, it is governed by tension-compression nonlinearity of the solid matrix [6, 7]. By inspection, linear biphasic materials ($E_t = E_{c0}$) are limited to 50% fluid load support as first shown by Agbezuge and Deresiewicz [3]. Experimentally, it is known that cartilage is capable of much higher levels of fluid load support and friction reduction; this is due to the confining effects of high lateral stiffness of the collagen matrix and the underlying bone.

Eq. (1) assumes a semi-infinite half-space. Although it is common to neglect substrate effects when the indentation depth is less than 10% the layer thickness, the difference in

stiffness, as it turns out, is not negligible. The method for eliminating the substrate effect is addressed in *Methods: Characterization by spherical indentation*.

The second term of (1) describes the rate-driven approach toward the asymptote and is governed by the Peclet number, $Pe = \dot{\delta} \cdot R / (E_{c0} \cdot k)$. Fluid load support is negligible when $Pe \ll 1$, 50% the asymptote when $Pe=1$ and at the asymptote when $Pe \gg 1$ [14]. It is important to recognize here that other forms of Pe apply to other contact configurations; for example, $Pe = V \cdot a / (E_{c0} \cdot k)$ during Hertzian sliding, where V is sliding speed and a is contact radius.

By definition, the fluid load fraction is the ratio of the force carried by fluid pressure, F_P , to the total applied force, F_T . The force carried by fluid pressure is the difference between F_T

and the force carried by the solid, $F_S: F' = \frac{F_P}{F_T} = \frac{F_T - F_S}{F_T}$. Using Hertz theory, we can redefine the fluid load fraction in terms of the moduli, which eliminates the need to maintain constant indentation depth during measurements:

$$F' = \frac{\frac{4}{3} \cdot E_c \cdot R^{0.5} \cdot \delta^{1.5} - \frac{4}{3} \cdot E_{c0} \cdot R^{0.5} \cdot \delta^{1.5}}{\frac{4}{3} \cdot E_c \cdot R^{0.5} \cdot \delta^{1.5}} = \frac{E_c - E_{c0}}{E_c} \quad (2)$$

The effective contact modulus, E_c , includes contributions from fluid and solid stresses, while E_{c0} , a material property, is the equilibrium contact modulus of the tissue [15]. Inserting (1) into (2) and rearranging gives the following equation for the effective contact modulus of a biphasic half-space entirely in terms of controllable mechanical conditions and measurable material properties:

$$E_c = \frac{E_{c0}}{1 - \left(\frac{E_t}{E_t + E_{c0}} \cdot \frac{\dot{\delta} \cdot R}{\dot{\delta} \cdot R + E_{c0} \cdot k} \right)} \quad (3)$$

Materials and equipment

A single 12mm diameter osteochondral plug was used to demonstrate and validate the method. The sample was removed from the center of the medial femoral condyle of a mature bovine stifle. The custom microtribometer shown in Figure 1 was used to perform the indentation measurements on the cartilage. Smooth ($R_a < 80\text{nm}$) and impermeable stainless steel spherical ball bearings were used as the indenter; the diameter was either 6.35mm or 3.18mm as noted. The sample was submerged in phosphate buffered saline (PBS) during the tests. The load cell consists of a calibrated cantilevered beam ($0.95 \pm 0.0005\text{mN}/\mu\text{m}$) and a capacitive displacement sensor ($\pm 7\text{nm}$) to measure beam deflection. A $250\mu\text{m}$ piezoelectric stage with capacitive displacement feedback ($\pm 25\text{nm}$) is used to control each indentation. The sample is positioned with an X-Y translation stage and aligned relative to the Z-axis with a 2-axis tilt-stage prior to measurement.

Characterization by spherical indentation

The sample was indented at 100, 50, 10, 5, 1, 0.5, and 0.1 $\mu\text{m/s}$, either in that descending order or in a randomized order, as noted. The stage was commanded to travel either 150 μm or 50 μm into the sample as noted. The actual indentation depth and indentation rate were measured using the difference between measurements of the stage and the load cell displacements. Following the last indent in each variable-rate series, the Z-stage was held at 150 μm until equilibrium was reached to determine E_{c0} .

One practical challenge with characterizing cartilage properties, in general, is the identification of ‘full’ equilibrium. There are no universally observed standards for determining the amount of time required to observe full equilibrium, however, many investigators use a criterion involving a critical rate of change of indentation depth. In this study, equilibrium was declared when the indentation depth changed by less than 1 μm over a five minute window.

The surface was located using the position for which the first statistically significant non-zero force was detected. The probe was then pulled 3 μm above the surface to prepare for indentation. The Z-stage was then driven to the desired depth at the prescribed rate and immediately retracted at 10 $\mu\text{m/s}$ (except for the final equilibration measurement). This retraction rate helped to pull expelled fluid back into the sample. The sample was then given one minute to fully normalize between indents.

The amount by which a substrate effectively stiffens an elastic layer during contact depends on the dimensionless layer thickness, $\tau = t/a$ (t is the layer thickness), as described by Hayes *et al.* [16]. Unfortunately, the solution to this model requires expertise in numerical computation, which makes it inconvenient for general use. Stevanovic *et al.* [17] provided an approximate analytic solution to the problem based on the formulation from Chen and Engel [18]. According to their solution, the force exerted by a fictitious semi-infinite solid (F_{inf}) is a factor of f_p smaller than the force measured for a bonded layer (F_{layer}) of the same material when indented to the same depth (the substrate must be >40x stiffer than the layer for validity):

$$F_{\text{inf}} = F_{\text{layer}} \cdot f_p = F_{\text{layer}} \cdot \left(1 - 1.04 \cdot \exp \left(-1.73 \cdot \tau^{0.734} \right) \right)^3 \quad (4)$$

Each force measurement was corrected based on (4) to give the force of an equivalent semi-infinite solid (to eliminate the substrate effect).

Because cartilage and other biphasic materials can have fibrillated, bumpy or otherwise poorly defined surfaces, the surface was not defined by the location at which force was first detected. Instead, each force-displacement curve was fit to Hertz equation:

$F_t = \frac{4}{3} \cdot E_c \cdot R^{0.5} \cdot (\delta - \delta_{\text{off}})^{1.5}$, where δ_{off} represents the offset above the ‘true’ surface of the half-space. This method forces the mechanics to define the surface and eliminates surface location uncertainty. Figure 2 demonstrates how the corrected force-displacement data (unfilled) was fit to Hertz’s model (red line). Generally, the effective surface started a few

microns below the location at which forces were first detected, which is consistent with the measured magnitude of the surface roughness. The equilibrium measurement used the offset from its parent indent (the last indent in the series).

Once the contact moduli data were obtained for each indentation rate, the unknown tensile modulus, E_t , and permeability, k were obtained by fitting the data to (3). To assist other researchers in implementing this method, we have developed a template and user guide, which can be downloaded from our website at: <http://research.me.udel.edu/~dlburris/publicationsOther.html>.

Characterization by plane-ended indenter

Creep indentation with a porous-permeable plane-ended indenter tip is considered the gold standard for indentation testing to characterize biphasic properties. We used it here as a measure of validation for the present method. A 1.6mm diameter porous punch was used to indent the same 1.3mm thick cartilage sample in the same location as the Hertzian measurement. The osteochondral core was glued to the bottom of a bathing chamber which was filled with PBS, and the cartilage surface was leveled perpendicular to the indenter tip with a 2-axis tilt stage. A 50mN preload was applied to ensure complete contact at the interface and establish the zero-time reference surface. Following 5 minutes of preload the indenter was commanded to maintain a constant 200mN load [9]; the test was stopped when the rate of deformation change fell below $1\mu\text{m}$ in five minutes. The contact stress was $\sim 0.1\text{MPa}$ and the maximum deformation was $120\mu\text{m}$. For comparison, the pressures and depths during spherical indentation ranged from $0.02\text{--}0.46\text{MPa}$ and $41\text{--}121\mu\text{m}$, respectively. The creep deformation under constant loading was analyzed by a custom LBT curve-fitting program [19–21] which simultaneously determined Poisson's ratio, aggregate modulus, and permeability.

To consider the prominent tension-compression nonlinearity of the solid phase and address the large interstitial fluid pressures found in practice [7], we developed a tension-compression nonlinearly elastic (TCN) finite element model in FEBio [22]. The solid matrix of cartilage was defined as an isotropic homogeneous Neo-Hookean material reinforced by fibers in three orthogonal directions. The energy function of the fibers followed an exponential-power law, where E_t is the tensile modulus defined at zero tensile strain. The three mechanical properties, aggregate modulus, tensile modulus and permeability, were determined by curve-fitting the experimental creep response.

Results

The processed contact modulus results for spherical indentation at monotonically decreasing rates are provided in Table I. The high quality of each fit to the Hertzian contact model is reflected by the proximity of the coefficient of determination (R^2) to 1.

The effective contact modulus is plotted as a function of indentation rate in Figure 3. The effective contact modulus is a sigmoidal function of indentation rate. Below $0.1\mu\text{m/s}$, the effective contact modulus approaches the equilibrium contact modulus as shown by the logarithmic axis-scaling on the right of Figure 3. The effective contact modulus approaches

an asymptotic limit at high speeds; according to the HBT model, that limit is equal to the sum of the equilibrium contact modulus and the tensile modulus. Between these speeds, the tissue effectively stiffens with speed as it becomes more and more difficult to force fluid through the cartilage at the rate of indentation. Fitting the dataset to Eq. 3 gives $E_f=8.0\text{MPa}$, $k=0.00217\text{mm}^4/\text{Ns}$, and $R^2=0.996$. The solution to the fit is unique in the HBT model because the tensile modulus and permeability have independent effects on the tissue response [9]. To demonstrate this fact, the fitted properties have been adjusted and the resulting solutions plotted in Figure 4. Changes in permeability shift the inflection speed without affecting the asymptote (Figure 4: left). Changes in tensile modulus increase the asymptote without affecting the inflection speed (Figure 4: right). Likewise, changes in the equilibrium contact modulus shift both asymptotes by equal amounts.

Several follow-up experiments were conducted to illustrate sensitivity of the method to variations in the experimental protocol. In the first variation, the indentation rates were randomized to determine if hysteresis has significant effects; the testing conditions were otherwise constant. The order of indentation was set at: 0.5, 50, 0.1, 10, 100, 5 and $1\mu\text{m/s}$ followed by equilibration. The effects of randomization are shown in Figure 5. Monotonically descending and randomized speed conditions did not significantly affect the results of characterization: the equilibrium modulus decreased by 7%, the tensile modulus increased by 5%, while the permeability remained unchanged.

In a second variation, probe diameter was cut in half (to 3.18mm). Figure 6 (left) demonstrates that a reduction in probe size resulted in decreased equilibrium contact and tensile moduli along with increased permeability. In addition to decreasing the sampling area, the smaller probe decreased the effective sampling depth thereby making the measurement more surface-sensitive (the depth under stress is proportional to contact radius not actual penetration depth). A third variation tested the depth effect directly by limiting the indentation depth to $50\mu\text{m}$ (conditions were otherwise constant). Figure 6 (right) directly demonstrates the depth dependent properties of articular cartilage. Decreasing indentation depth by 3X caused a 57% increase in permeability, a 20% decrease in tensile modulus and a 27% decrease in the equilibrium contact modulus compared to the results from $150\mu\text{m}$ deep indentation measurements under the same conditions.

We used this method to quantitatively test McCutchen's observation that cartilage, a notoriously difficult tissue to preserve, can be preserved by drying out the tissue and rehydrating it prior to testing [1]. The sample was dried and stored in ambient lab conditions for 4 days; the sample would be extremely degraded after 4 days at ambient temperatures if not dehydrated first. It was rehydrated by submersion in PBS for several hours. The baseline experiments in Figure 3 were repeated and the results are compared in Figure 7. The permeability and tensile modulus of the preserved cartilage had increased by 18% and 9% respectively, while the equilibrium contact modulus decreased by 16%. Given the otherwise extreme nature of the storage conditions, these relatively minor changes in properties support McCutchen's suggestion that dehydration can effectively preserve the functionalities of the tissue.

Following Hertzian indentation testing, the same sample was creep-tested with a porous plane-ended indenter at the same location to determine the properties of the sample with the two gold-standard methods. The experimental results of the creep experiment, which were used by both methods, are shown in Figure 8. The fit to the LBT for plane ended indentation gives a Poisson's ratio $\nu=0.1$, aggregate modulus $H_A=0.47\text{MPa}$ and permeability $k=0.0014\text{mm}^4/\text{Ns}$ ($E=E_t=E_{c0}=0.46$ and $H_A=0.47\text{MPa}$ for a linearly elastic material with $\nu=0.1$). The fit illuminates obvious disagreement between the dataset and the model at small deformations; this is a known limitation of linear biphasic theory and occurs because linear materials are unable to support fluid pressures comparable to the elastic stress unless the layer becomes very thin relative to the contact radius; since pressure drives lubrication, this implies that linear biphasic materials cannot lubricate well. It is for this reason that LBT best-practice is to fit only the data in the upper 30% of strain measurements, which we have done here.

The TCN model better reflects the actual mechanical response of cartilage under indentation loading with $R^2>0.98$ for a fit to the whole dataset. The properties were $H_A=0.40\pm0.02\text{MPa}$, $k=0.0016\pm0.0001\text{mm}^4/\text{Ns}$, and $E_t=10.3\pm2.2\text{MPa}$; confidence intervals reflect the standard error in the estimate of the mean. The mean results from each of the three models are given in Table II for direct comparison.

Discussion

Although LBT is the most common method for characterizing biphasic materials, it is only able to represent the response of the tissue accurately when interstitial pressures are below the elastic compressive stress. The tensile modulus dominates the maximum achievable pressures and is therefore more closely related to tissue function (lubrication and load capacity) than Poisson's ratio, aggregate modulus, or even permeability [11]. The TCN model can reproduce physiological magnitudes of fluid pressure by allowing the tensile modulus to be much larger than the aggregate modulus. However, this solution requires specialized computational tools and is therefore less common as a characterization method in the literature. The HBT model has a similar capacity to predict physiological magnitudes of fluid pressure without requiring specialized computational tools, with shorter experiments and with better quantitative agreement with experimental curves (at least in this case).

The most important goal of this paper is to establish confidence in the HBT method by direct comparison to the gold standards using comparable measurements at the same location on the same sample. First, consider the tensile modulus. In this case, the result from HBT differs from TCN by less than 16%, which we consider strong agreement. Additionally, both measures agree quantitatively with published values for healthy cartilage, which range from 3.5–14 MPa [7, 23–25]. It should be noted, however, that the tensile modulus isn't as clearly defined for cartilage as it is for most engineering materials. Because the tensile stiffness comes from a collagen fiber network that aligns in the direction of loading, it tends to increase with strain. Nevertheless, the models are self-supporting, which provides confidence in their respective uses for *in-situ* tensile modulus characterization, a practice with no established standard to date.

Next, consider permeability. LBT gives the smallest permeability ($k=0.0014\text{mm}^4/\text{Ns}$), TCN gives a slightly larger permeability ($k=0.0016\text{mm}^4/\text{Ns}$), and HBT gives the largest permeability ($k=0.0026\text{mm}^4/\text{Ns}$). Each of these falls in a relatively narrow band within the wide range of values found in the literature ($0.0004\text{--}0.008\text{mm}^4/\text{Ns}$) [1, 7, 9, 12, 21, 24, 26, 27].

Permeability is not a constant even from measurements made at the same location. It depends strongly on the proteoglycan content and the predominant collagen alignment, both of which vary with depth. Consequently, the permeability varies with the predominant flow direction and the effective interaction volume. As a result, the variations in observations from these experiments are reasonable given the differing flow patterns and effective interaction volumes.

Because LBT and TCN results were based on the same experimental dataset, their differences cannot be due to the possible sources cited above. In this case, differences are likely affected by differing effective dilatations. Compressive strains consolidate internal pathways to reduce permeability while interstitial fluid pressures expand internal pathways to increase permeability [12]. The LBT model only fits the data for which fluid load support is less than ~30%; lateral tensile strains are minimized and normal compressive strains are maximized by this method. The TCN model included data for which fluid load support reached 80%; using the same dataset, TCN predicted a 17% larger permeability than LBT simply by involving more high pressure data in the fit. In contrast, the permeability from HBT was more than 60% larger than either plane-ended method. In plane-ended (LBT and TCN) indentation only ~2% of the data involved more than 50% fluid load support, while 75% of the Hertzian test (HBT) involved more than 50% fluid load support. We can test this mental model by fitting only the low-speed (low pressure) range of the HBT dataset from $0.1\text{--}1\mu\text{m/s}$; in this case, the permeability decreases to $k=1.4\times 10^{-3}\text{mm}^4/\text{Ns}$, which is in-line with that from LBT.

This analysis suggests that these differences are due primarily to significant differences in the mean effective pressure of fit datasets. Manipulation of the HBT fit to better control for the pressure range illustrates that each method supports the others. Therefore, it is not a question of which is correct or incorrect, but a question of which conditions are most relevant to the application of interest. The HBT results may be the most relevant for cartilage under physiological conditions since fluid load support is typically on the order of 90% *in-vivo* [11]. However, it is important to recognize that the reported values from HBT will always be biased above those from LBT for otherwise identical conditions.

Finally, consider comparisons of the aggregate modulus. The LBT model gives an aggregate modulus of 0.47 MPa compared to 0.40 MPa from TCN. The HBT model gives the equilibrium contact modulus, not the aggregate modulus, but when $\nu<0.1$, the difference is negligible ($<0.01\text{MPa}$). At 0.47 MPa, the HBT modulus is equal to LBT, both of which are 18% greater than that from TCN. The aggregate modulus of the TCN model is reduced to compensate for the stiffening effect of internal fibers loaded in tension (particularly near the stress concentrator). In the other two models, this effect is embedded into the reported aggregate moduli.

Repeat testing revealed that the curves and fitting results were repeatable and not particularly sensitive to the order of the indents. This is perhaps surprising considering that fluid is squeezed from the tissue during indentation, especially when rates are slow. Interestingly, we have noticed that the act of pull-off actually pulls fluid back into the tissue. The apparent adhesive force in Figure 2 is attributable to suction, not adhesion. Even more interestingly, this suction effect almost entirely restores the sample to its pre-indented level of hydration, which is why there is no noticeable hysteresis effect. This suction effect in combination with the 1 minute rest is apparently enough to virtually eliminate cartilage consolidation throughout a typical series of indentation measurements.

When probe radius decreased, the modulus decreased and the permeability increased. We believe this is attributable to actual depth-dependences. The interaction depth in a Hertzian contact increases with contact radius; decreasing probe radius or penetration depth makes the measurement more surface-sensitive. The results of this study consistently suggest that the near surface has decreased aggregate modulus and increased permeability. This is consistent with results from Chen *et al.* [28] who demonstrated that human cartilage had reduced modulus and fixed-charge density toward the surface. It is also consistent with known trends of increased water content and decreased glycosaminoglycans in the superficial region [29].

The model underlying the procedure has numerous limitations related to the over-simplified material model (a porous permeable solid with different linearly elastic compressive and tensile moduli). In reality, cartilage is heterogeneous, anisotropic, nonlinear, viscoelastic, and variably permeable. In developing this model, we incorporated only the complexities that were absolutely necessary to reproduce the essential features of the measured mechanical response. A high tensile modulus is unquestionably responsible for elevating fluid pressure in the tissue and it is important that this detail be carried over into models of its mechanical response. Variable permeability does affect the results; the reported permeability from the fit can be thought of as an effective permeability in the range of conditions of the experiments. Heterogeneity also has a significant impact on results. The superficial layer is clearly softer than the rest of the tissue and our variable depth measurements reflect that. Likewise, the measurements made here reflect a composite of the entire near-surface region within a volume whose bounds scale linearly with the contact radius.

The HBT offers unique flexibility in the spatial and depth sensitivity of the method. Under normal conditions, like those described here, the smaller contact areas result in 80–95% reduced time constants relative to the gold standard for increased throughput; time is a significant challenge for these measurements since hydrated samples degrade over time. The method can be used with high confidence based on the comparisons against the gold standards and its use requires no specialized computational tools. A fitting template written by the authors is available on their website: <http://research.me.udel.edu/~dlburris/publicationsOther.html>.

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Highlights

- Describes a new method to characterize biphasic material properties
- Method uses standard indentation techniques with a Hertzian contact
- Paper directly validates the method against the gold standard

Figure 1.
The spherical indenting tribometer used in this study.

Figure 2.

A representative force-displacement curve for the cartilage sample used in this study. The spherical indenter had a radius of 3.175mm and a nominal indentation rate of 10μm/s. The raw data (solid) is corrected for substrate effects using the analytical solution of Stevanovic *et al.* [14]. Using Hertzian mechanics the zero surface offset is determined and Hertz model is best fit (red line) to the substrate corrected force-displacement data (unfilled) to determine the effective modulus.

Figure 3.

The solid line is the biphasic model fit to the experimental data. Note that the probe had a radius of 3.175mm. Right: the same data and model fit are plotted on a logarithmically scaled X-axis.

Figure 4.

Left: the effects of permeability on the model shape. Right: the effects of tensile modulus on model shape. Note that the probe had a radius of 3.175mm.

Figure 5.

Randomized order of indentation rates are compared to the monotonically decreasing indentation rates. The solid lines are the biphasic model fit to the experimental data. Note that the probe had a radius of 3.175mm.

Figure 6.

Left: the effects of probe radius on the effective contact modulus are plotted as a function of the indentation rate (in descending order with a commanded depth of 150μm). Right: the depth dependent properties of articular cartilage can be determined using the biphasic model. The Z-stage was commanded to translate 150 and 50μm into the cartilage sample with a probe of radius=1.5875mm. The solid lines are the biphasic model fit to the experimental data.

Figure 7.

The effects of dehydration preservation (4 days) on the properties of bovine articular cartilage. The solid lines are the biphasic model fit to the experimental data. Note that the probe had a radius of 3.175mm.

Figure 8.

The porous plane ended indentation creep response of the rehydrated cartilage sample. The sample was loaded to 200mN. The linear biphasic indentation model (LBT) was fit using published analysis methods: $k=0.00135\text{mm}^4/\text{Ns}$, $H_A=0.47\text{MPa}$, $\nu=0.11$ and $R^2=0.979$ (upper 30% of strain only). Fitting the tension compression nonlinear model (TCN) to the data gives: $k=0.00158\text{mm}^4/\text{Ns}$, $H_A=0.40\text{MPa}$, $E_t=10.3\text{MPa}$ and $R^2=0.984$ (entire fit from 2 seconds). The TCN model is far better able to represent the early response where fluid pressures are large.

Table I

Hertzian model fits to the substrate corrected indentation curves from Figure 2. Note that the probe had a radius of 3.175mm.

<i>vertical stage speed ($\mu\text{m/s}$)</i>	<i>indentation rate ($\mu\text{m/s}$)</i>	<i>effective contact modulus (MPa)</i>	<i>coefficient of determination (R^2)</i>	<i>fluid load fraction</i>
100.0	60.0	7.89	0.9999	0.93
50.0	35.9	7.57	0.9998	0.93
10.0	9.5	5.31	0.9999	0.90
5.0	5.6	4.38	0.9999	0.87
1.0	1.6	2.40	0.9994	0.77
0.5	0.8	1.82	0.9998	0.69
0.1	0.2	1.02	0.9998	0.45
0.0	0	0.56	N/A	0.00

Table II

Material properties obtained for a single location of the cartilage sample from HBT, LBT, and TCN characterization methods during tests with maximum penetration depths of $\sim 100\mu\text{m}$. The aggregate and equilibrium contact moduli are equal to within 0.006 MPa when $\nu < 0.1$. The asterisk denotes the fact that the fit was limited to the data in the upper 30% of strain.

<i>Property</i>	ν	H_A/E_{c0} (MPa)	k (mm ⁴ /Ns)	E_c (MPa)	R^2
HBT	N/A	0.47	0.0026	8.7	0.994
LBT	0.1	0.47	0.0014	0.46	*0.979
TCN	N/A	0.40	0.0016	10.3	0.984