Published in final edited form as:

J Mech Behav Biomed Mater. 2011 October ; 4(7): 1540–1547. doi:10.1016/j.jmbbm.2011.02.004.

The Use of Polyacrylamide Gels for Mechanical Calibration of Cartilage – A Combined Nanoindentation and Unconfined Compression Study

Cheng Li^a, Jessica Allen^a, Tamara Alliston^{a,b}, and Lisa A. Pruitt^{a,c}

^aUCSF and UC Berkeley Joint Graduate Group in Bioengineering, University of California, Berkeley, CA, 94720, United States

^bDepartment of Orthopaedic Surgery, University of California, San Francisco, SF, CA, 94143, United States

^cDepartment of Mechanical Engineering, University of California, Berkeley, Berkeley, CA, 94720, United States

Abstract

This study investigates polyacrylamide (PA) gel as a calibration material to measure the nanomechanical compressive modulus of cartilage using nanoindentation. Both nanoindentation and unconfined compression testing were performed on PA gel and porcine rib cartilage. The equilibrium moduli measured by the two methods were discernable. Nanoindentation has the advantage of distinguishing between spatially dependent constituent properties that affect tissue mechanical function in heterogeneous and hierarchically structured tissues such as cartilage. Both sets of measurements exhibited similar positive correlation with increasing gel crosslinker concentration. The compressive modulus measurements from compression in the PA gels ranged from 300 kPa—1.4 MPa, whereas those from nanoindentation ranged from 100 kPa—1.1 MPa. Using this data, a method for relating nanoindentation measurements to conventional mechanical property measurements is presented for porcine rib cartilage. It is shown that based on this relationship, the local tissue modulus as measured from nanoindentation (1.1—1.4 MPa) was able to predict the overall global modulus of the same sample of rib cartilage (2.2 MPa), as confirmed by experimental measurements from unconfined compression. This study supports the use of nanoindentation for the local characterization of cartilage tissues and may be applied to other soft tissues and constructs.

Introduction

The complex structures and heterogeneity of cartilage presents challenges to characterizing its material properties. Commonly employed mechanical tests, such as confined and

Corresponding Author: Cheng Li, UCSF and UC Berkeley Joint Graduate Program in Bioengineering, 2121 Etcheverry Hall, University of California, Berkeley, Berkeley, CA 94720, United States, Tel.: +1 510 457 5683, +1 650 587 1513. limeow@gmail.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

unconfined compression, at best treat the tissue as a poroelastic, multi-phasic, isotropic material^{1–5}. These methods give information about the global behavior of the tissue as described by, for example, the equilibrium aggregate modulus and the time constant⁶. Further, these experiments are limited in revealing and characterizing micrometer length scale (a few μm to hundreds of μm) structure-property relationships key to cartilage function. There is a strong need for more in-depth knowledge of the *in-situ* local tissue properties in a spatially-dependent manner. This would help resolve the mechanical contributions from the spatially varied structural and constituent units to the overall behavior and function of cartilage.

Local mechanical characterization of articular cartilage exploring multi-scale structure property relationships has been limited due to a number of factors. Such factors include the paucity of mechanical tools that facilitate these measurements, the development of appropriate testing and analysis techniques, as well as the identification of materials that can serve as baseline measures for such systems. In the last decade nanoindentation has evolved as a promising tool for structure-property characterization of tissues and soft biomaterials^{7–15}. Studies have addressed a number of important factors in nanomechanical testing methodology including large deformations, surface detection, adhesion, hydration, surface roughness and time-dependent behavior^{8, 12, 13, 16–20}. Nanoindentation analysis typically relies on applying Hertz contact theory to a material volume under the probe with the assumptions of material homogeneity, isotropy, and linearly elastic or elastic-plastic material response. Application of the appropriate indenter tip size and geometry, as well as the indentation protocol can be used to target localized material volumes of interest, and isolate steady state material matrix properties. For example, a 100 μm ideal conospherical tip can effectively target a region with a contact diameter of 30–50 μm at 1–3 μm of penetration depth. This may be appropriate for probing, for example, particular cellular regions within cartilage zones, or of the extracellular matrix of cartilage. It is important to note that in indenting soft tissues like cartilage have inherent surface roughness due to natural grooves, protrusions, and pockets from protein components and cellular regions (tens of micrometers in wavelength and several micrometers in amplitude)²¹. These micro- and nano-scale roughness features deform and release pockets of fluid to provide lubrication^{22, 23} or to support other essential tissue functions. In indentation of soft tissues, these surface roughness features yield easily during initial loading (and conform to the rigid indenter with contact diameters of 40–50 μm) as fluid is pushed out and before the tissue volume can sustain significant load. Therefore the “roughness” of hydrated soft materials under indentation does not contribute significantly to the differences in contact area and load-displacement data, but rather can be considered as another aspect of tissue heterogeneity that contributes to variation in the measurement. Similar to creep and stress relaxation experiments described by Mow and Lai et al.⁶, the nanoindentation technique can be controlled to rid the fluid flux-driven time-dependent behavior and capture solid matrix properties in a solid-fluid biphasic material. However, cartilage, like most soft biomaterials, have more complex behavior than can be fully described by current material models, particularly at the micrometer and smaller length scales where conventional continuum mechanics assumptions do not necessarily apply. Moreover, the complex stress field in the cartilage under the indenter tip is not fully understood. Given these limitations associated

with applying the nanoindentation technique to soft biomaterials, the measurements are not true material properties, but rather are relative measures for making comparisons and discoveries. The measured modulus may be interpreted as an *in-situ* tissue modulus distinct from the global elastic modulus of the entire tissue structure as measured by conventional large-scale mechanical testing.

A challenge in developing a protocol for nanomechanical characterization of cartilage is the availability of a control material. An appropriate reference material is one whose physical and mechanical properties resemble that of cartilage but are controllable and predictable, and hence can be more consistently characterized. Previous investigators have utilized agarose gels to serve as a reference material for soft biomaterials and biological tissues^{16, 24, 25}. However, not only are the microstructures of agarose gels variable depending on the natural source of the agarose powder and method of gel preparation, but they are also unstable and susceptible to degradation within a few days owing to disentanglement of its physical chain network^{24, 26, 27}. An alternative material, polyacrylamide (PA) gel used commonly in biological research¹⁸ is a synthetic chemical compound that solidifies to form one large chemically crosslinked molecule that is more stable compared to agarose gel and from our experience is able to retain its mechanical integrity for well over one week. PA gels can also be tailored to span the modulus range of cartilage tissue, with elastic moduli spanning 0.2 MPa to over 1 MPa, which is within the modulus range of cartilage tissue^{6, 28, 29}. This study proposes to use PA gels as a reference material for the mechanical characterization of cartilage and other soft biomaterials. We hypothesize that there is a link between the material and its structural and compositional units and that there is a relationship relating the global elastic modulus and the nanoindentation derived tissue modulus. The goal of this pilot study is to propose a method using mechanically graded polyacrylamide gels against which nanoindentation of cartilage and other complex soft biomaterials can be calibrated.

Materials and Methods

Polyacrylamide Gels

Materials for making polyacrylamide (PA) gels were obtained from Bio-Rad (Hercules, CA). For this study, the monomer acrylamide concentration of each gel was held constant (at 20% or 30% w/v) while the Piperazine diacrylamide (PDA) crosslinker concentration was varied from 0.5% to 3% w/v. The unit w/v is measured in grams of solute per mL of solution, and is commonly used in chemistry to represent the concentration of a solution. A summary of the PA gels and the test schemes is provided in Table I. The desired acrylamide and piperazine diacrylamide volumes were added to a solution containing 0.01M Hepes Buffer (pH 8.5) and TEMED (1/2000 dilution) in deionized water. The Hepes solution was vortexed for 30 sec and degassed in a vacuum chamber for 1 hr. To initiate the polymerization reaction, 10% (w/v) ammonium persulfate solution was added to the degassed mixture at a 1/200 dilution (final conc. 0.05% (w/v)). The solution was carefully mixed with a pipette and then poured into a vertical mold that was 7 cm by 8 cm in width and height and approximately 1 mm in thicknesses. Gels were stored in the 0.01M Hepes solution overnight at 4 °C before testing. For each gel formulation, 3–5 specimens were cut

from the gel slab with a 6 mm cylindrical punch and then subjected to unconfined compression. In addition, each gel formulation was subjected to 7 or more indents using nanoindentation (described below).

Porcine Cartilage

Fresh rib cartilage was obtained from a local abattoir and immediately dissected. Cylindrical specimens of porcine cartilage ($n=4$) were obtained with biopsy punches. These specimens were 6.8 mm in diameter and 4–6 mm in thickness and were taken from porcine rib cartilage slices in the sagittal plane and subsequently stored in phosphate buffered saline (PBS) at 4°C until use. The rib cartilage disks excluded the bone. The disks were tested as soon as they were dissected; however, storage times varied from one to three days due to schedule constraints around completing unconfined and nanoindentation tests on the same specimens. Four specimens were tested in unconfined compression, and on one of these specimens, 17 indents were performed using nanoindentation. Only one specimen was tested using nanoindentation because it was decided a priori that the nanoindentation data should be collected within 24 hours of bulk testing. This takes into consideration accelerated tissue degradation *ex vivo*.

Unconfined Compression Tests

The compression loading protocols were fine-tuned for either the gel or cartilage in order to minimize testing time and to span the relevant linear stress-strain regions. The Bose Electroforce 3200 mechanical test system (Bose Corporation, Eden Prairie, MN) was used for unconfined compression. A 10 N load cell and two custom compression platens 2 cm in diameter were used to test the delicate gel samples. Each PA gel was immersed in Hepes buffer (0.01 M, pH 8.5) and loaded between the two metal platens at room temperature. Contact was established at 0.1–0.2N of preload. Each gel sample was ramp loaded at 0.02mm/s at each of the 1% strain steps (~0.01 mm in displacement) up to a total strain of 8%, and allowed to relax at each step for up to 360s. The gel samples were more delicate and prone to fracture compared to the harvested cartilage samples. Therefore, the loading protocol—pre-load, strain rate, and total strain, and time of relaxation, was optimized specifically to measure the equilibrium stresses for the gel and cartilage samples in their respective linear loading regimes. To accommodate testing of the cartilage samples, a higher 450N load cell and larger platens 5cm in diameter were used. Initial sample contact with platens was established at 0.7–0.8N of preload. Each cartilage specimen was ramp loaded at 0.008mm/s through three 5% strain steps (~0.25mm in displacement) up to a total strain of 15%, and allowed to relax at each step for up to 600s. The loading schemes and relaxation responses are illustrated in Figure 1, and the load relaxation response at each step was verified to reach a load decay rate of at least 0.03N/s. Stresses and strains at the end of the relaxation periods were captured and graphed on a stress-strain plot. The equilibrium modulus was defined as the best-fit linear slope of the stress versus strain data. The R^2 values of the curve fit ranged from 0.97–0.99.

Nanoindentation Tests

Nanoindentation was performed using a Hysitron TriboIndenter (Hysitron Inc., Minneapolis, MN) with a 100 μm conospherical diamond fluid tip. The indenter tip is connected to the transducer, which was displacement-controlled, and data is presented as load versus displacement plots for loading, hold, and unloading. For all samples, indentation started with the nanoindenter tip approaching the sample from off-contact. Sample surface was clearly evident as seen by the rapid increase in load with displacement²⁵. Typical nanoindentation load and displacement data for gel and cartilage are shown in Figure 2.

The total displacements into the sample ranged from 2000 – 3000 nm with relaxation times of at least 30 seconds at maximum displacement. The loading rates ranging from 500nm/s and 2000 nm/s was found to not affect the relaxation behaviour. Hence data from all loading curves were used to calculate the quasi-equilibrium modulus. Hertz equilibrium modulus is defined as:

$$E_H = \frac{3P_{eq}}{4\sqrt{R}h_{eq}^{\frac{3}{2}}}, \quad (1)$$

where P_{eq} is the quasi-equilibrium load, h_{eq} is the corresponding displacement, and R is the radius of curvature of the spherical indenter³⁰. The elastic modulus is related to E_H as

$$E = E_H(1 - \nu^2), \quad (2)$$

where ν is the Poisson's ratio of the specimen and assumed a value of 0.499, that of an incompressible material. The stress relaxation segment of load versus time data was fit with a power law function and the theoretical equilibrium load was determined from the curve fit. The goodness-of-fit was calculated as the R^2 value and ranged from 0.62–0.98. The load decay rate for gel at the end of the hold step was on the order of 0.1 μN , whereas that for cartilage ranged from orders of 0.1 to 1 $\mu\text{N/s}$.

Results and Discussion

Polyacrylamide gel data

The equilibrium compressive modulus for the seven gel types spanning a range of crosslinker content were measured using unconfined compression and nanoindentation. These results are plotted in Figure 3 and also summarized in Table II. The results demonstrate controllable mechanical properties within the linear range of stress-strain behavior for the PA gels with the range of crosslinker concentrations. The range of elastic modulus from unconfined compression for the gels was 0.3–1.4 MPa while that from nanoindentation was 0.1–1.1 MPa (Table II). These values are comparable to both engineered cartilage constructs^{31, 32} and native cartilage tissues^{3, 6, 28, 29}.

The equilibrium modulus values of the PA gels were found to be stable over the two-week time frame without any degradation in the modulus²⁵. The results of these modulus measurements support the premise that PA gels can be prepared with homogeneous microstructures that are reproducible^{27, 33}. Moreover, the chemically crosslinked

microstructure³³ offers a much greater consistency and stability than was found in agarose gels. These results suggest that PA gel would be an excellent control material for characterization of cartilage tissue modulus as in evaluating diseased cartilage or tissue engineering constructs.

As shown in Figure 3, the trend of increasing equilibrium modulus with greater crosslinker concentration is consistent in unconfined compression and nanoindentation data. The modulus values measured by unconfined compression are higher than those from nanoindentation tests. However at the higher crosslinker concentration, the modulus values from the two methods appear to deviate from the linear correlation, possibly due to the saturation of crosslinks within the polymer network. Also, over the range of crosslinking, there is a smaller variation in nanoindentation measurements of the equilibrium modulus than for the bulk compression tests. However this is not consistent in all PA gel formulations. This could point to the relatively more homogeneous material properties at the local level of tens of squared micrometers area due to a locally uniform microstructure, compared to the global properties of tens of squared millimeters area. However, the difference in the variation is also likely due to the variations intrinsic to the selected test method and its execution. Since the relaxation states of samples in this study are comparable based on load decay measurement at the assumed steady state, the authors do not believe that the relaxation states of the tissue are responsible for the higher mean values for unconfined compression compared to nanoindentation. The data from unconfined compression equilibrium reached a lower decay rate (0.03 N/s) following relaxation from peak loads of several Newtons in PA gels (tens of Newtons in cartilage samples) than the decay rate (0.1 μ N/s) reached by data from nanoindentation following stress relaxation from peak loads of tens of μ N in PA gels (hundreds of μ N in cartilage samples).

Based on the mean values of quasi-equilibrium modulus measured by nanoindentation and unconfined compression, a calibration curve, as shown in Figure 4, was plotted which converts the local modulus to the global modulus of the material. Fitting this data to a log function produced a quantitative relationship with a Pearson's correlation coefficient R of 0.94 for the PA gel measurements. This suggests that the local and global properties of the gels are distinguishable, with the local measurements collectively contributing to the overall tissue modulus. This also validates that the assumptions of material isotropy, homogeneity, and elasticity are satisfied in the case of PA gels for both nanoindentation and unconfined compression. This calibration curve is unique in that it can relate measures of local heterogeneities to an averaged global measure of the material as a whole.

It should be noted that for the 20:1.5 PA gel, the unconfined compression data deviated further from the curve fit than other data points. Nanoindentation did not discriminate the local compressive modulus of this gel formulation with that of the next higher crosslinker concentration. This suggests that fundamental differences in the local vs. global properties can perhaps be further clarified with using both of these complimentary measurement techniques. However, as noted above, overall trends in both unconfined and nanoindentation modulus measurements of the 20% w/v PA gel formulations indicate a plateau in equilibrium modulus at higher crosslinker concentrations. Further studies with higher

concentrations of the acrylamide gel formulations with various crosslinker concentrations will elucidate material property changes at crosslinker saturation levels.

Porcine cartilage data

The quasi-equilibrium modulus values of porcine rib cartilage were measured using the unconfined compression and nanoindentation test protocols described above. Four specimens of porcine rib cartilage were measured by unconfined compression, and one of these specimens, with a measured modulus of 2.2 MPa using unconfined compression, was further subjected to nanoindentation testing. The results of the unconfined compression and nanoindentation tests are provided in Figure 5. The average equilibrium modulus for the unconfined compression tests was 2.2 MPa (ranging from 1.8–2.6 MPa) while for the nanoindentation tests it was 2.7 MPa (ranging from 1.1–4.4 MPa).

Unlike articular cartilage, porcine rib cartilage is known to be a generally more homogeneous tissue with random collagen orientation throughout the hyaline cartilage^{34, 35}; thus it was expected that its material behaviour would be more similar to that of PA gels compared to articular cartilage. In fact, the mean equilibrium modulus values of porcine rib cartilage, 1.4 MPa and 1.1 MPa corresponding to unconfined compression and nanoindentation respectively, are on the same order of magnitude as the stiffest 30:3 (% w/v acrylamide: % w/v crosslinker) gel formulation (Table II). However, in contrast to PA gels, the variation within the nanoindentation data for cartilage was greater than variation within the unconfined compression data—a standard deviation of 1.0 vs. 0.3. This is consistent with the larger range of data on relaxation states observed under nanoindentation compared to compression. This would slightly overestimate the true equilibrium value of cartilage taken with the nanoindentation technique. The relatively large variation in the cartilage nanoindentation data however is also related to the greater heterogeneity that exists within the cartilage tissue—chondrocytes and extracellular matrix structures over tens of squared micrometers area—that is not present in the PA gels. This data also supports the notion that the nanoindentation modulus measurement can be a good indicator for evaluating local property variations within the tissue. Such spatial property information can be very useful for mapping cartilage quality for particular cellular and structural units over a given tissue area that appears homogeneous at the global tissue level.

Despite this difference, and considering the overall similarity in physical properties and in mean equilibrium modulus between the PA gels and the cartilage tissue, the measurements obtained from nanoindentation for cartilage were converted to their equivalent global value using the relationship shown in Figure 4 (developed for the PA gels). The equivalent average modulus using this conversion is 1.8 MPa and this is within 1 standard deviation of the actual values measured by unconfined compression (Figure 5). A summary of the porcine cartilage measurements is included in Table II for ease of comparison to the PA gels. These findings reveal the extent to which the PA gel calibration curve can be applied over the specified modulus property range and extrapolated to predict local and global material property relationships for rib cartilage.

Conclusions

This work demonstrates that polyacrylamide gels can serve as effective calibration materials for nanoindentation of cartilage and likely other soft hydrated biomaterials. The results of unconfined compression and nanoindentation tests indicate the modulus of the PA gels can be tailored with crosslinking to match approximate modulus values of the cartilage tissue. A method for relating nanoindentation measurements to conventional global mechanical property measurements has been suggested for both PA gels and porcine rib cartilage; the potential application of this relationship to more heterogeneous tissues with predominant preferred microstructural orientation such as articular cartilage requires further study. Nanoindentation can be used to measure the local properties of interest. In homogeneous and isotropic materials, it can be interpreted at the macroscale level to give a sense of its functionality as a whole structure. This pilot study supports the use of nanoindentation for the localized characterization of cartilage tissues and engineered cartilage constructs.

Bulk mechanical testing of healthy articular cartilage reveals that the equilibrium compressive elastic modulus is on the order of 1 MPa^{1-4} ; however, this global modulus of the tissue may not provide insight into local property changes that occur in disease progression, nor constituent contributions to the structural behavior of this complex tissue. A quantifiable measurement of spatially varying elastic modulus values at the constituent length scales can offer valuable information about the local biochemical and biomechanical transformations at work in the progression of osteoarthritis or in tissue remodeling³⁶. The knowledge gained from nanoindentation experiments of tissues and biomaterials can then be extended to efforts in disease prevention and tissue repair.

Acknowledgements

The authors would like to thank Drs. Philipp Thurner, Kevin Cheng, Kurt Koester, and Shikha Gupta for their expertise and support with testing. Funding was provided by NSF ADEPT (Applied Design and Engineering Project Teams) Fellowship.

References

1. Armstrong CG, Mow VC. Variations in the intrinsic mechanical properties of human articular cartilage with age, degeneration, and water content. *J Bone Joint Surg Am.* 1982 Jan; 64(1):88–94. [PubMed: 7054208]
2. Athanasiou KA, Agarwal A, Dzida FJ. Comparative-study of the intrinsic mechanical-properties of the human acetabular and femoral-head cartilage. *Journal of Orthopaedic Research.* 1994; 12(3): 340–349. [PubMed: 8207587]
3. Kempson GE, Freeman MA, Swanson SA. The determination of a creep modulus for articular cartilage from indentation tests of the human femoral head. *J Biomech.* 1971 Jul; 4(4):239–250. [PubMed: 5122817]
4. Sokoloff L. Elasticity of aging cartilage. *Fed Proc.* 1966 May-Jun; 25(3):1089–1095. [PubMed: 5940992]
5. Hayes WC, Mockros LF. Viscoelastic properties of human articular cartilage. *J Appl Physiol.* 1971 Oct; 31(4):562–568. [PubMed: 5111002]
6. Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic creep and stress-relaxation of articular cartilage in compression - theory and experiments. *Journal of Biomechanical Engineering-Transactions of the Asme.* 1980; 102(1):73–84.

7. Kaufman JD, Song J, Klapperich CM. Nanomechanical analysis of bone tissue engineering scaffolds. *J Biomed Mater Res A*. 2007 Jun 1; 81(3):611–623. [PubMed: 17187400]
8. Ebenstein DM, Kuo A, Rodrigo JJ, Reddi AH, Ries M, Pruitt L. A nanoindentation technique for functional evaluation of cartilage repair tissue. *J Mater Res*. 2004;273–281.
9. Franke O, Durst K, Maier V, Goken M, Birkholz T, Schneider H, Hennig F, Gelse K. Mechanical properties of hyaline and repair cartilage studied by nanoindentation. *Acta Biomater*. 2007 Nov; 3(6):873–881. [PubMed: 17586107]
10. Gupta HS, Schratte S, Tesch W, Roschger P, Berzlanovich A, Schoeberl T, Klaushofer K, Fratzl P. Two different correlations between nanoindentation modulus and mineral content in the bone-cartilage interface. *J Struct Biol*. 2005 Feb; 149(2):138–148. [PubMed: 15681230]
11. Ferguson VL, Bushby AJ, Boyde A. Nanomechanical properties and mineral concentration in articular calcified cartilage and subchondral bone. *J Anat*. 2003; 203(2):191. [PubMed: 12924819]
12. Gupta S, Carrillo F, Li C, Pruitt L, Puttlitz C. Adhesive forces significantly affect elastic modulus determination of soft polymeric materials in nanoindentation. *Materials Letters*. 2007; 61(2):448–451.
13. Li C, Pruitt LA, King KB. Nanoindentation differentiates tissue-scale functional properties of native articular cartilage. *J Biomed Mater Res A*. 2006 Sep 15; 78(4):729–738. [PubMed: 16739105]
14. Akhtar R, Schwarzer N, Sherratt MJ, Watson RE, Graham HK, Trafford AW, Mummery PM, Derby B. Nanoindentation of histological specimens: Mapping the elastic properties of soft tissues. *J Mater Res*. 2009 Mar; 24(3):638–646. [PubMed: 20396607]
15. Chaudhry B, Ashton H, Muhamed A, Yost M, Bull S, Frankel D. Nanoscale viscoelastic properties of an aligned collagen scaffold. *J Mater Sci Mater Med*. 2009 Jan; 20(1):257–263. [PubMed: 18763020]
16. Ebenstein DM, Pruitt LA. Nanoindentation of soft hydrated materials for application to vascular tissues. *J Biomed Mater Res A*. 2006; 69(2):222–232. [PubMed: 15057995]
17. Ebenstein DM, Wahl KJ. A comparison of JKR-based methods to analyze quasi-static and dynamic indentation force curves. *J Colloid Interface Sci*. 2006 Jun 15; 298(2):652–662. [PubMed: 16455101]
18. Hansma P, Yu H, Schultz D, Rodriguez A, Yurtsev EA, Orr J, Tang S, Miller J, Wallace J, Zok F, Li C, Souza R, Proctor A, Brimer D, Nogues-Solan X, Mellbovsky L, Pena MJ, Diez- Ferrer O, Mathews P, Randall C, Kuo A, Chen C, Peters M, Kohn D, Buckley J, Li X, Pruitt L, Diez-Perez A, Alliston T, Weaver V, Lotz J. The tissue diagnostic instrument. *Rev Sci Instrum*. 2009 May. 80(5):054303. [PubMed: 19485522]
19. Kesari H, Doll JC, Pruitt BL, Cai W, Lew AJ. Role of surface roughness in hysteresis during adhesive elastic contact. *Philos Mag Lett*. 2010 Dec 1; 90(12):891–902. [PubMed: 21152108]
20. Donnelly E, Baker SP, Boskey AL, van der Meulen MC. Effects of surface roughness and maximum load on the mechanical properties of cancellous bone measured by nanoindentation. *J Biomed Mater Res A*. 2006 May; 77(2):426–435. [PubMed: 16392128]
21. Mow VC, Ratcliffe A, Poole AR. Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. *Biomaterials*. 1992; 13(2):67–97. [PubMed: 1550898]
22. Walker PS, Dowson D, Longfield MD, Wright V. "Boosted lubrication" in synovial joints by fluid entrapment and enrichment. *Ann Rheum Dis*. 1968 Nov; 27(6):512–520. [PubMed: 5728097]
23. Hou, JS.; Holmes, MH.; Lai, WM.; Mow, VC. Biomechanics of diarthrodial joints. New York: Springer-Verlag; 1990. Squeeze film lubrication for articular cartilage with synovial fluid.
24. Scandiucci de Freitas P, Wirz D, Stolz M, Gopfert B, Friederich NF, Daniels AU. Pulsatile dynamic stiffness of cartilage-like materials and use of agarose gels to validate mechanical methods and models. *J Biomed Mater Res B Appl Biomater*. 2006 Aug; 78(2):347–357. [PubMed: 16470817]
25. Li, C. Local properties of articular cartilage--A combined nanoindentation and biochemical characterization approach. Berkeley: University of California; 2008.
26. Stolz M, Raiteri R, Daniels AU, VanLandingham MR, Baschong W, Aebi U. Dynamic elastic modulus of porcine articular cartilage determined at two different levels of tissue organization by

- indentation-type atomic force microscopy. *Biophys J*. 2004 May; 86(5):3269–3283. [PubMed: 15111440]
27. Normand V, Lootens DL, Amici E, Plucknett KP, Aymard P. New insight into agarose gel mechanical properties. *Biomacromolecules*. 2000 Winter;1(4):730–738. [PubMed: 11710204]
28. Kleemann RU, Krockner D, Cedraro A, Tuischer J, Duda GN. Altered cartilage mechanics and histology in knee osteoarthritis: Relation to clinical assessment (ICRS grade). *Osteoarthritis Cartilage*. 2005 Nov; 13(11):958–963. [PubMed: 16139530]
29. Kiviranta P, Lammentausta E, Toyras J, Kiviranta I, Jurvelin JS. Indentation diagnostics of cartilage degeneration. *Osteoarthritis Cartilage*. 2008 Jul; 16(7):796–804. [PubMed: 18222712]
30. Hertz H. On the contact of elastic solids. *J. Reine Angew. Math*. 1881; 92:156–171.
31. Martinez-Diaz S, Garcia-Giralt N, Lebourg M, Gomez-Tejedor JA, Vila G, Caceres E, Benito P, Pradas MM, Nogues X, Ribelles JL, Monllau JC. In vivo evaluation of 3-dimensional polycaprolactone scaffolds for cartilage repair in rabbits. *Am J Sports Med*. 2010 Mar; 38(3):509–519. [PubMed: 20093424]
32. Katakai D, Imura M, Ando W, Tateishi K, Yoshikawa H, Nakamura N, Fujie H. Compressive properties of cartilage-like tissues repaired in vivo with scaffold-free, tissue engineered constructs. *Clin Biomech (Bristol, Avon)*. 2009 Jan; 24(1):110–116.
33. Peyton SR, Putnam AJ. Extracellular matrix rigidity governs smooth muscle cell motility in a biphasic fashion. *J Cell Physiol*. 2005 Jul; 204(1):198–209. [PubMed: 15669099]
34. Mattice JM, Lau AG, Oyen ML. Spherical indentation load-relaxation of soft biological tissues. *J. Mater. Res*. 2006; 21(8)
35. Forman JL, Kent RW. Modeling costal cartilage using local material properties with consideration for gross heterogeneities. *J Biomech*. 2010 Dec 17.
36. Tang S, Souza R, Hansma P, Ries M, Alliston T, Li X. Local tissue properties of human osteoarthritic cartilage correlate with magnetic resonance T1rho relaxation times. *Journal of Orthopedic Research*. In Press.

Figure 1.

Representation of a loading scheme consisting of three sequential loading steps (top) and their corresponding load relaxation response over time (bottom) during unconfined compression. The loading schemes for PA gel and cartilage samples were each tailored to their properties and geometry. Gel sample: linear loading range=8% strain, loading step=1% strain increments, ramp rate=0.02mm/s, hold time=360s. Cartilage sample: linear loading range=15% strain, loading step=5% strain, ramp rate =0.008mm/s, hold time= 600s.

Figure 2. Representative load vs. displacement data (top) and load vs. time data (bottom) for PA gel and cartilage during nanoindentation.

Figure 3.

Comparison of equilibrium modulus values for the polyacrylamide gel types from unconfined compression and from nanoindentation. The vertical line helps to visually separate the 20% PA gel data on the left and the 30% PA gel data on the right.

Figure 4.

Mean (SD) equilibrium modulus values measured by nanoindentation vs. Mean (SD) equilibrium modulus values from unconfined compression fit to a log function. The equation for the fit is determined to be: $y=1.3119+1.125 \log(x)$, where y is the equilibrium modulus measured by unconfined compression, and x is that measured by nanoindentation.

Figure 5.

Unconfined compression and nanoindentation measurements for quasi-equilibrium modulus of porcine rib cartilage. Note the mean (SD) reported for four samples of cartilage measured with unconfined compression, and one sample (17 indents) measured with nanoindentation.

Table I

Gel Type	%w/v Acrylamide	%w/v PDA	Nanoindentation	Compression
20:0.5	20	0.5	x	x
20:1.5	20	1.5	x	x
20:2	20	2	x	x
20:2.5	20	2.5	x	n/a
30:1	30	1	x	x
30:2	30	2	x	x
30:3	30	3	x	x

Table II

	Unconfined Compression Modulus [MPa]	Nanoindentation Modulus [MPa]
Gel Sample	<i>Mean (SD), n=3</i>	<i>Mean (SD), k = 7</i>
20:0.5	0.3 (0.07)	0.1 (0.08)
20:1.5	0.5 (0.16)	0.3 (0.03)
20:2	0.8 (0.16)	0.3 (0.03)
20:2.5	N/A	0.5 (0.02)
30:1	0.5 (0.07)	0.2 (0.03)
30:2	1.2 (0.1)	0.5(0.03)
30:3	1.4(0.06)	1.1 (0.08)
Procine Rib Cartilage Sample	<i>Mean (SD), n=4</i>	<i>Mean (SD), k=17</i>
A	2.2 (0.3)	2.7 (1.0)
<i>n=number of samples of gel or cartilage, k=number of indents</i>		