The Nanoindentation of Soft Tissue: Current and Developing Approaches

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Mechanical testing of biological materials is a continuously developing field that requires the testing of hierarchical structures. This article discusses the use of nanoindentation as a valuable tool for assessing multiple length scales as well as parts of complex biological systems. The multiple issues that must be accounted for while testing biological materials are also presented. In addition, dynamic nanoindentation is introduced as a method for testing soft tissues under optimized loading frequencies encountered in everyday movements.

INTRODUCTION

Biological materials are currently subject to intensive research[1,2] since they offer a unique library of approaches for the improvement of structural and engineering materials.[3] As biological materials are implemented into a material science environment (and vice versa) a wide range of issues regarding their mechanical behavior arises.

Biological materials cannot be considered homogenous at classical length scales due to their hierarchical structure and therefore it is important to implement different testing techniques to characterize the materials properties. Similar to any highly specialized material, biomaterials' testing is subjected to many restrictions such as small quantities and/or small dimensions thus requiring the application of highly localized techniques. One of these techniques is nanoindentation (NI), which has been widely established in all fields of materials sciences.[4–6]

Biological materials can be distinguished with respect to their mechanical properties. Hard tissues such as bone have higher hardness and Young's moduli, while soft tissues such as cartilage usually are highly visco-elastic and their mechanical response is strongly dependant on loading rate and time.[6] Both types of materials present highly optimized structures, which have an ideal organization for the relevant load (such as walking and structural strengthening of the body) and environment conditions (different media from neutral to acidic), and also have the ability to adapt to changing conditions. Thus a biological material in an organism is in dynamic equilibrium, where an optimum composition is always required. However, this is not the case for artificial implants which need to be designed to mimic a biological material as closely as possible in order to be used under natural loading conditions. Since implants are usually not reabsorbed, it is important to minimize the mismatch in mechanical properties and stress shielding effects.[7,8] These would cause the tissue around the implant to lose some of its original mechanical strength. One of the aims of bioengineering is to replace the damaged tissue with repair tissue of the same quality and consistency. This requires a histological and structural analysis of the tissues as well as mechanical characterization data of both healthy and repair tissue under conditions similar to the in-vivo environment.

There are several approaches in classical materials testing which can also be applied to the characterization of biomaterials. However, all the techniques have to be modified to test small volumes under in-vitro conditions. Hydration and the hydration degree is a less important issue for hard tissues, but as some soft tissues have water contents up to 80%,[9] it is paramount that tests are carried out in the appropriate medium.[10–12] However, it has been shown that even bone, which is considered a hard tissue, can increase its hardness by 17.7–30% and the modulus by 15–50%.[10,11] This effect could also be observed in NI experiments at varying hydration degrees.[12] Therefore the test-

How would you…

…describe the overall significance of this paper?

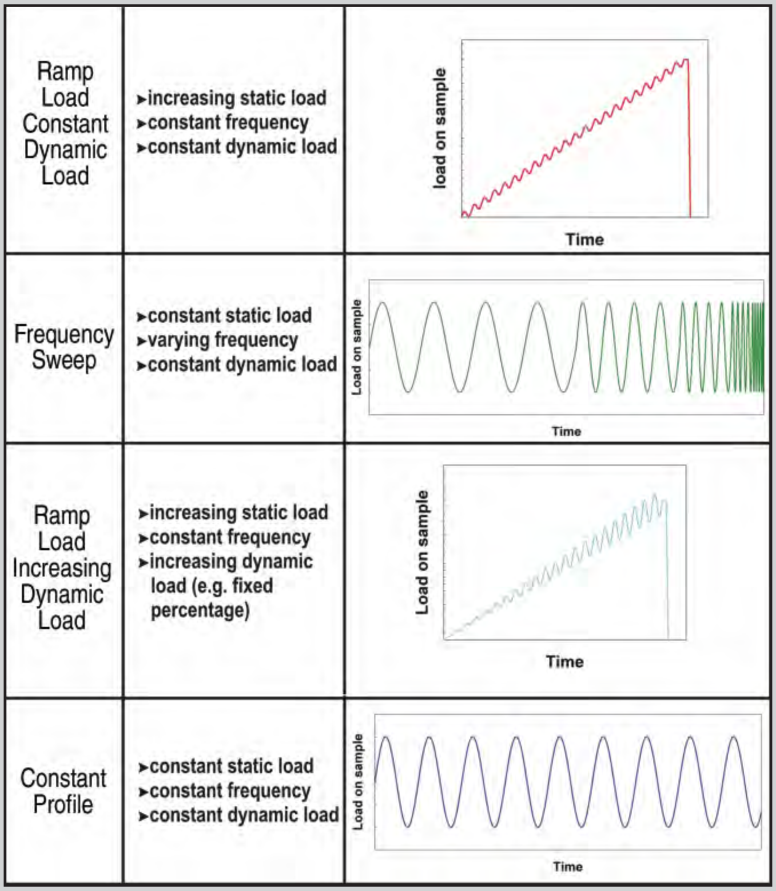
The paper gives a brief overview of the techniques currently used to test soft tissues, focusing on nanoindentation. Dynamic nanoindentation is presented as a possible method for testing soft tissues in order to account for a very strong time dependency and very low elastic moduli. Additionally, issues related to testing biological materials such as hydration and loading conditions are discussed.

…describe this work to a materials science and engineering professional with no experience in your technical specialty?

Joints are subjected to a wide range of loading conditions varying both in time and frequency (i.e., running vs. walking) in everyday life. Hence, it is important to test biological materials in their natural environment. The use of dynamic testing allows us to better understand the time-dependent material response by providing a measurement for both storage and loss moduli.

…describe this work to a layperson?

This work is focused on testing biological samples under conditions as close as possible to the natural environment. The data generated can be used to better understand joint damage mechanisms (e.g., osteoarthritis), improve implants, and as a guide for the design of biomimetic materials.



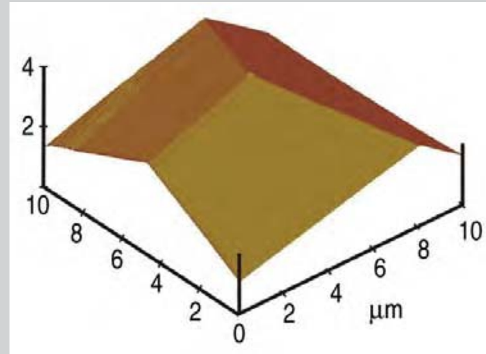


Figure 2. An AFM image of a three-sided Berkovich tip.

Figure 1. Load profiles for dynamic testing by depth-sensing indentation.

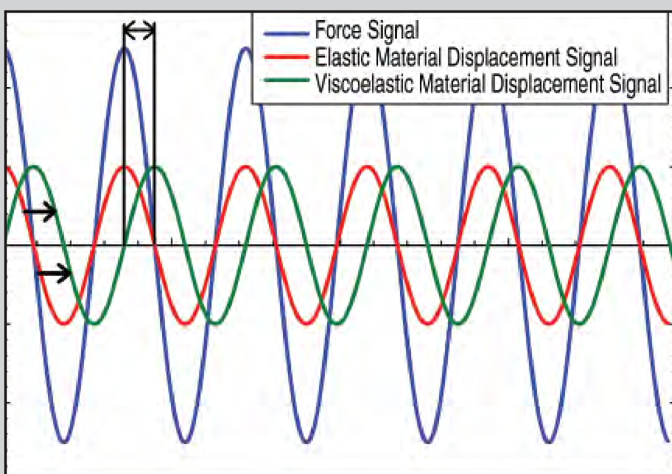


Figure 3. Materials response under dynamic loading for an elastic-plastic and a visco-elastic material.

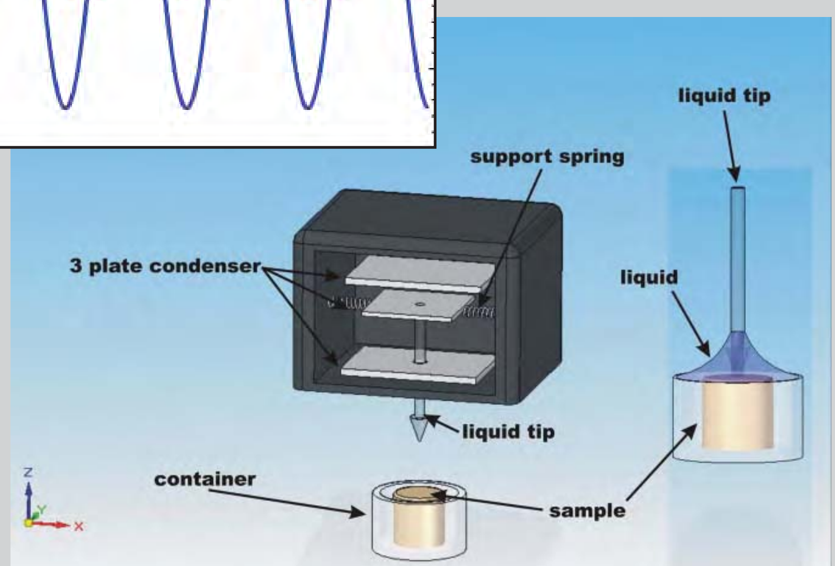


Figure 4. The setup used for in-vitro testing; inset: contact of the liquid tip with the sample and the meniscus of the test media around the shaft.

ing conditions and the sample history have to be well documented. Inappropriate storage or transport routines may invalidate the results, as tissue quickly decays. The hierarchical nature of biomaterials is essential to their function. However, this introduces a challenge in testing multiple length scales. For example, the mechanical behavior of complex macroscopic samples can be tested by standard techniques while the properties of a given sample component must be carried out at the micro- and/or nano-scale. Nanoindentation can close the gap between the length scales, especially as not all structures are readily available and can vary from subject to subject. The input from NI can be used to optimize repair tissues and to help understand the changes in mechanical properties caused by various diseases such as osteoporosis and osteoarthritis. While most testing techniques still rely on static materials properties, the influence of time-dependant behavior is a major point for the repair of soft tissues such as cartilage. Furthermore everyday loading of a joint will happen with varying frequencies. Regular walking, especially the heel strike, causes a shock wave that is passed through the body as a transient and is a possible cause for osteoarthritic degeneration.[13] A recent study by K.A. Gillepsie et al. shows that frequencies up to 400 Hz occur in a regular gait.[14] Since viscoelastic materials and their mechanical behavior are governed by the loading rate,[6] frequency, etc., testing at varying frequencies is necessary to enhance the understanding of this complex system. Figure 1 presents the different load profiles for dynamic testing of biomaterials by depth-sensing indentation.

MATERIALS AND METHODS

Nanoindentation has proven to be a valuable tool in many applications[5] such as metals, coatings, ceramics, polymers, and foams. While issues such as the indentation size effect in metals are dis-

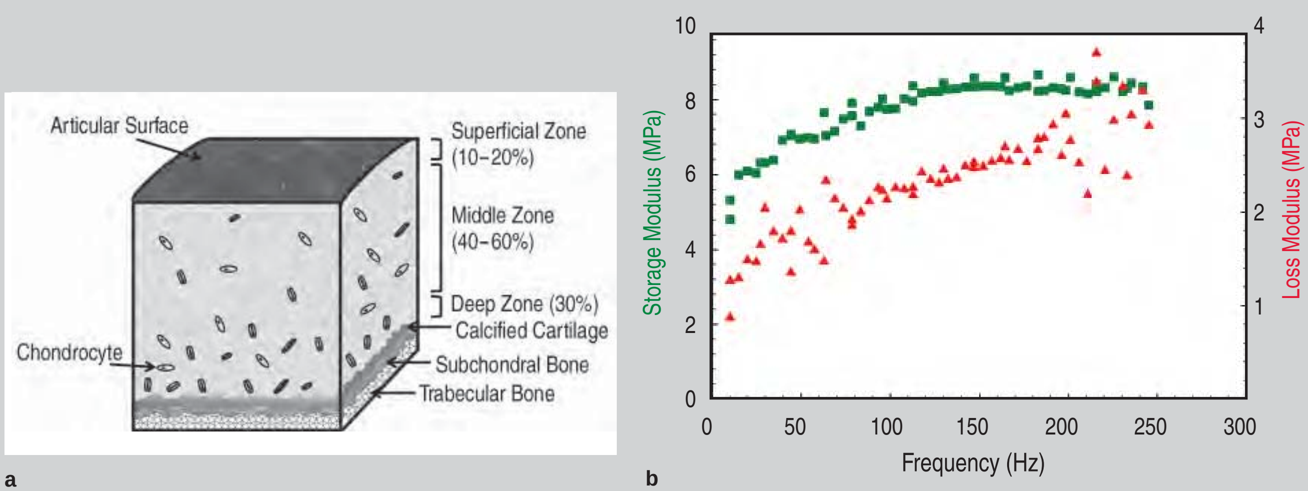


Figure 5. (a) A schematic of healthy hyaline cartilage; (b) frequency sweep in the superficial zone of a fresh hyaline cartilage sample in phosphate buffered saline. A static load of 1 mN and a dynamic load of 1 μN were used in a range from 10–250 Hz in a Hysitron Triboindenter (Hysitron Inc., Minneapolis, Minnesota). The data was analyzed using a Kelvin solid.

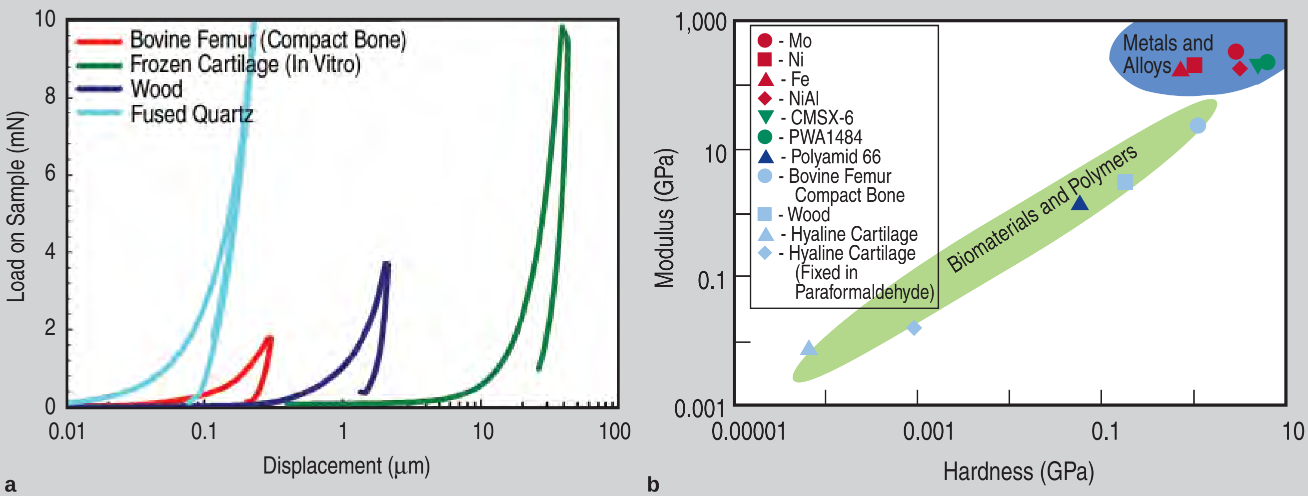


Figure 6. (a) The load–displacement curves of fused quartz and three different biomaterials (wood, bone, and cartilage). The cartilage sample was tested under in-vitro conditions, but was frozen prior to testing in PBS. The other two samples were tested in dry condition. Note the logarithmic scale on the displacement axis. The data for fused quartz was obtained with a Hysitron Triboscope. All other data is from a Nanoindenter XP (MTS Nano Instruments, Oak Ridge, Tennessee). (b) Young's modulus and hardness data for various materials; all data was obtained using a Nanoindenter XP. The shaded areas are ranges of materials that can be tested by NI with carbides having moduli close to 1,000 GPa.

cussed and widely understood,[15–17] there are new challenges regarding testing of materials with very low elastic moduli. The model currently used in most NI experiments was derived in 1992 by W. Oliver and G. Pharr,[18] who found a way to implement the idea proposed by J. Pethica, R. Hutchings, and W. Oliver to use a tip shape function from a separate calibration in a new model taking the non-linearity of the unloading curve into consideration.[19] Figure 2 shows an atomic force microscope (AFM) image of a classical three-sided Berkovich tip, which is used frequently in NI experiments. The approach by Oliver et al. differed from the ideas proposed earlier by M. Doerner and W. Nix,[20] who assumed a linear relation between load and displacement for the unloading segment.

A valuable addition to this so-called quasi-static testing is the superimposition of a sinusoidal force signal during the loading and unloading of the sample. While the paper by Oliver and Pharr already considers dynamic testing, it assumes an elastic-plastic behavior of the tested material.[18] This method, the so-called continuous stiffness method, can be used for materials without time dependence in the mechanical response. However, materials exhibiting a viscoelastic behavior will exhibit a phase shift between the load and the displacement (Figure 3). This can be used to determine the storage and the loss modulus of a polymeric material.[6,21–23]

Recently more attention has been given to the dynamic indentation and the models applied in the evaluation of the results. While the classical approach consisting of a dashpot in parallel alignment to a spring, also known as the Kelvin or Voigt model, can describe most viscoelastic materials, it lacks the ability to accommodate instantaneous elastic recovery. The simple addition of a spring in series with the described set-up can achieve a better description of those materials and be implemented into NI as proposed by W. Wright et al.[23] Since viscoelasticity is time-dependant its influence can be minimized for some materials by using very high unloading rates.[24,25] Nevertheless a hold segment seems to be sensible for most materials.[26] J. Mattice et al. also used the hold segments to determine another property of viscoelastic materials, the so-called relaxation time.[27]

Testing soft tissues by dynamic nanoindentation can account for a very strong time dependency and a very low elastic moduli on the order of several hundred kilopascals to a few megapascals. The measurement of these low-modulus materials requires an optimization of the NI equipment, which was carried out for, as an example, a Triboindenter (Hysitron Inc., Minneapolis, Minnesota used here). The testing equipment was tuned to allow for stable measurements in a wide range of frequencies by increasing the overall stiffness of the system. The use of a 1D-transducer (nanoDMA2) further enhances the high stiffness of the system. The indentation is done in three steps: the z-motor does a rough approach, while the attached piezo-scanner increases the precision for the final approach. This allows for very small displacement limits for the transducer itself (≈ 5 μm), thus making the determination of the transducer constants simple. While the spring constant and the electrostatic-force constant are determined in an air indent without an oscillary motion, the damping is determined in a frequency sweep from, for example, 10–250 Hz applying a small static and alternating load. In addition, standard tip and frame calibrations need to be carried out.

NANOINDENTATION OF SOFT BIOLOGICAL MATERIALS

As mentioned in the previous section, testing of materials with very low moduli requires different calibrations from regular quasistatic NI testing. However, the sensitivity depends mainly on the total mass of the indenter, the damping of the system, and the stiffness. S.A.S. Asif et al. propose low damping and mass in combination with a optimized stiffness.[21] Further information on indentation of soft materials can be found in References 6, 22, and 26. In order to introduce tests at in-vitro conditions, a liquid tip can be used, which allows a liquid film of up to 3 mm on the sample surface (see Figure 4). The longer shaft of the liquid tip allows for machine calibrations in the testing media and thus to account for changes in damping behavior. Testing in liquids also causes meniscus forces on the indenter, thus it is important that the indenter is fully submerged in the liquid prior to testing. A minimization of the deformation caused by the surface approach and detection is achieved by using a two-step approach. In the first step a high set-point is used to penetrate the liquid. The deformation before the actual indent is reduced by using a lower contact force in the second step. A change in set-point is a prerequisite for sensible testing and can be done with the current equipment.

Another important issue currently under intensive investigation is the adhesion of the material to the indenter.[28,29] This will cause an increase in the energy dissipated during the indentation process and as a consequence the values obtained for the loss modulus will be influenced. While adhesion is a minor influence for NI carried out on metals, it becomes increasingly important for polymers and biomaterials, especially influencing the selection of the applied load during dynamic testing. It has been shown on polymeric materials that adhesion is a big influence for very soft materials.[28] The forces required to obtain high displacements are usually very small and thus adhesion forces, which are usually negligible in hard materials,[30] can add up to a significant percentage of the overall maximum load. Adhesion will thus cause an extra phase shift, which can have significant influence on the loss modulus.

As mentioned earlier, cartilage has a wide range of frequencies where it is mechanically loaded. Furthermore the structure of cartilage, as of any other biomaterial, is highly optimized for the loading condition encountered in everyday life. Thus mechanical testing should be carried out in the relevant loading direction rather than in the cross section. Figure 5a and b shows a schematic cross section of a cartilage sample and the mechanical properties of the superficial zone in a frequency sweep from 10–250 Hz using the classical Kelvin model to evaluate the results. Further literature on the mechanical testing of cartilage can be found in References 27, 31–34.

Figure 6a shows classical load displacement curves for fused quartz and hard biomaterials (bovine femur in dry condition and wood) in comparison to a soft tissue sample. In Figure 6b the Young's moduli and hardnesses are shown for a variety of materials to show the potential of biomaterials. Since the displacement even at low loads is much higher in soft tissues than for metals, it is very important to be aware of the increasing machine influences such as electrostatic force constant and spring stiffness. Those two parameters as well as the damping coefficient are correlated to the displacement of the transducer and have to be determined in a calibration. However, as most of the movement before the indent is done by the z-stage and the piezo, they can be considered constant in the displacement range of the used system.

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References

1. M.A. Meyers et al., *Progress in Materials Science,* 53 (2008), pp. 1–206.
2. P. Fratzl and R. Weinkamer, *Progress in Materials Science,* 52 (2007), pp. 1263–1334.
3. I.A. Aksay and S. Weiner, *Current Opinion in Solid State and Materials Science,* 3 (1998), pp. 219–220.
4. D.M. Ebenstein and L.A. Pruitt, *Nano Today,* 1 (2006), pp. 26–33.
5. C.A. Schuh, *Materials Today,* 9 (2006), pp. 32–40.
6. M.R. VanLandingham, *J. Res. Natl. Inst. Stand. Technol.,* 108 (2003), pp. 249–265.
7. O.L.A. Harrysson et al., *Materials Science and Engineering: C,* 28 (2008), pp. 366–373.
8. P. Sevilla et al., *Journal of Alloys and Compounds,* 439 (2007), pp. 67–73.
9. A. Maroudas et al., "Physiochemical Properties and Functional Behavior of Normal and Osteoarthritic Cartilage," *Articular Cartilage Biochemistry,* ed. K.E. Kuettner, R. Schleyerbach, and V.C. Hascall (New York/Wiesbaden: Raven Press, 1985), pp. 311–330.
10. S. Hengsberger, A. Kulik, and P. Zysset, *Bone,* 30 (2002), pp. 178–184.
11. J.-Y. Rho and G.M. Pharr, *Journal of Materials Science: Materials in Medicine,* 10 (1999), pp. 485–488.
12. A.K. Bembey et al., *J. Mater. Res.,* 21 (2006), pp. 1962–1968.
13. L.H. Light, G.E. McLellan, and L. Klenerman, *Journal of Biomechanics,* 13 (1980), pp. 477–480.
14. K.A. Gillespie and J.P. Dickey, *Clinical Biomechanics,* 18 (2003), pp. 50–59.
15. W.D. Nix and H. Gao, *Journal of the Mechanics and Physics of Solids,* 46 (1998), pp. 411–425.
16. K. Durst, B. Backes, and M. Goken, *Scripta Materialia,* 52 (2005), pp. 1093–1097.
17. K. Durst et al., *Acta Materialia,* 54 (2006), pp. 2547–2555.
18. W.C. Oliver and G.M. Pharr, *J. Mater. Res.,* 7 (1992), pp. 1564–1583.
19. J.B. Pethica, R. Hutchings, and W.C. Oliver, *Phil. Mag. A,* (4) 48 (1983), pp. 593–606.
20. M.F. Doerner and W.D. Nix, *J. Mater. Res.,* 1 (4) (1986), pp. 601–609.
21. S.A.S. Asif, K.J. Wahl, and R.J. Colton, *Review of Scientific Instruments,* 70 (1999), pp. 2408–2413.
22. G.M. Odegard, T.S. Gates, and H.M. Herring, *Experimental Mechanics,* 45 (2005), pp. 130–136.
23. W.J. Wright, A.R. Maloney, and W.D. Nix, *International Journal of Surface Science and Engineering,* 1 (2007), pp. 274–292.
24. Y.-T. Cheng and C.-M. Cheng, *Materials Science and Engineering: A,* 409 (2005), pp. 93–99.
25. Y.-T. Cheng and C.-M. Cheng, *Applied Physics Letters,* 87 (2005), p. 111914.
26. B.J. Briscoe, L. Fiori, and E. Pelillo, *Journal of Physics D: Applied Physics,* (1998), p. 2395.
27. J.M. Mattice et al., *Journal of Materials Research,* 21 (2006), pp. 2003–2010.
28. K.J. Wahl et al., *Journal of Colloid and Interface Science,* 296 (2006), pp. 178–188.
29. D.M. Ebenstein and K.J. Wahl, *Journal of Colloid and Interface Science,* 298 (2006), pp. 652–662.
30. D. Tabor, *Journal of Colloid and Interface Science,* 58 (1977), pp. 2–13.
31. W.C. Hayes et al., *Journal of Biomechanics,* 5 (1972), pp. 541–551.
32. D.M. Ebenstein et al., *Journal of Materials Research,* 19 (2004), pp. 273–281.
33. O. Franke et al., *Acta Biomaterialia,* 3 (2007), pp. 873–881.
34. K. Gelse et al., *Arthritis & Rheumatism,* 58 (2008), pp. 475–488.

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