Nanoindentation Measurement of Articular Cartilage From an In vivo Loading Model

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Introduction: Repetitive and forceful hand tasks can give rise to joint pain and induce upper musculoskeletal diseases. Measuring the effects of loading on the mechanical properties of cartilage helps relate exposure to outcome, as well as improve assessment and intervention of disease. Recent efforts have used nanoindentation to measure the local mechanical properties of cartilage in small models such as the rabbit metacarpophalangeal joint (MCP).[1] However, there are challenges to interpreting the measured data, as standardized testing protocols and analysis methods for soft tissue nanoindentation are yet to be established. This work presents an alternative measurement method using nanoindentation, and compares it to the conventional Oliver-Pharr method (O-P)[2] for evaluating the elastic modulus of articular cartilage from an in vivo joint flexion model.

Materials and Methods: Samples from the MCP joint of skeletally mature New Zealand White rabbits were used (n=5). Samples from the MCP joint of skeletally mature New Zealand White rabbits were used (n=5). The digits of the left forepaw were flexed at 1 Hz, 2h per day for 80h. The forepaw was stored in -80 C and thawed prior to testing. The 2nd digits were dissected to test the articular surfaces. The bone ends of the specimens were embedded in poly(methyl methacrylate) (PMMA) which was cured in a fluid polymer well on a 15 mm metal atomic force microscope (AFM) disc. Tissue was immersed in phosphate buffered saline (PBS) during testing. Nanoindentation was performed by using the TriboIndenter (Hysitron, Inc. Minneapolis, MN) and a 100 μm radius of curvature conospherical diamond tip. The tip established contact with the sample at ~1-2 μN of preload. The loading profile operated in displacement feedback control and consisted of a loading and unloading rate of 400 nm/s, and a hold period of 30-45 s, which allowed creep to dissipate and the samples to achieve near equilibrium. Samples were fixed in 10% formalin, and paraffin sections were stained with Safranin O and Iron Hematoxylin following standard protocols.

To evaluate the relaxed modulus, Eeq, the Hertz contact model [3] coupled with a stress relaxation correction was used. The (pseudo) equilibrium load was defined as the load reached at the end of the hold period in the load vs. time plot. Eeq was evaluated as Eeq = 3P / 4√Rh^(3/2), where P is the (pseudo) equilibrium load, h is the corresponding displacement, and R is the radius of curvature of the spherical indenter. The Hertz elastic modulus is related to Eeq: Eh = Eeq(1- ν^2), where ν is the Poisson's ratio of the specimen. The load vs. time relaxation data was fit to a power law function and the theoretical equilibrium load was determined from the curve fit. The relaxation parameter was defined as the (pseudo) equilibrium load normalized to the theoretical equilibrium load. Only sufficiently relaxed data (relaxation parameter < 3) were included in the analysis. Six to 12 indentations were averaged per MCP. For comparison, the reduced modulus was also calculated using the conventional O-P method.[2] Briefly, the stiffness, S = dp/dh was calculated from the slope of the initial unloading data by fitting a power law function to the upper 25-95% of the load at initial unloading. The reduced modulus, Er = S√π / 2√A, where A is the contact area determined from the ideal tip area function for a spherical tip. The Young's modulus is related to Er: E = Er(1-ν^2), where ν is the Poisson's ratio of the specimen, and assuming the indenter has infinite modulus.

Results: The average relaxed modulus Eeq of the MCPs showed that three out of four flexed samples had an increase in modulus in the flexed left paw (Figure 1). Although the two analysis methods showed consistent trends between the flexed and unflexed samples (Table I), only the presently proposed method detected a significant difference in sample 3 (p<0.025). However, this difference may be the result of a combination of in vivo loading conditions and local variations in the biochemistry and microstructure, as observed in the corresponding histology slides (data not shown). This may also explain the relatively large S.D. in the data.

The average Eeq from the proposed analysis method were 24-50% less than the Er values from the O-P method. The S.D. of Eeq were also generally lower than those of Er (Table I). Power law curve fitting to the stress relaxation data showed good fits with R^2 values > 0.96. Examination of the relaxation parameters evaluated ranged from 0.82-5.32. A few curves did not reach sufficient equilibrium and were excluded in the averaged Eeq values.

Discussion: The newly proposed indentation analysis method demonstrates promising results for evaluating cartilage elastic modulus in the rabbit MCP model. This method uses the equilibrium force and displacement data and does not make assumptions about the unloading behavior in biological tissues under indentation. This method also extracts data that are more comparable in their state of relaxation, eliminating further confounding factors in the measurements. This helps to explain the decreased modulus and variation in the data. Future studies directly correlating the elastic modulus with specific indicators from tissue biochemistry and structure will increase understanding of the changes in joint mechanical properties due to loading. Moreover, confidence in the proposed testing and measurement protocol can be increased by evaluating the method in additional cartilage-like biomaterials.

References: 1. Li C et al., JBMR A 78(4):729-38 (2006) 2. Oliver WC et al., J Mat Res, 7:1564 (1992) 3. Hertz H., J.Reine Angew Math. 92:156-171 (1881)

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Table I: Comparison of Eeq and Er values and their S.D.

	L. Flexed		R. Unflexed	
	Eeq +/- SD [MPa]	Er +/- SD [MPa]	Eeq +/- SD [MPa]	Er +/- SD [MPa]
1	0.89 +/- 0.43	1.79 +/- 1.18	0.75 +/- 0.39	1.20 +/- 0.58
2	1.65 +/- 0.51	2.17 +/- 0.70	1.33 +/- 0.42	1.77 +/- 0.62
3	0.62 +/- 0.33	1.00 +/- 0.48	0.28 +/- 0.10	0.56 +/- 0.24
4	1.10 +/- 0.33	1.51 +/- 0.50	1.50 +/- 0.61	1.98 +/- 0.82
5c	1.10 +/- 0.67	1.58 +/- 0.65	0.92 +/- 0.51	1.41 +/- 0.66

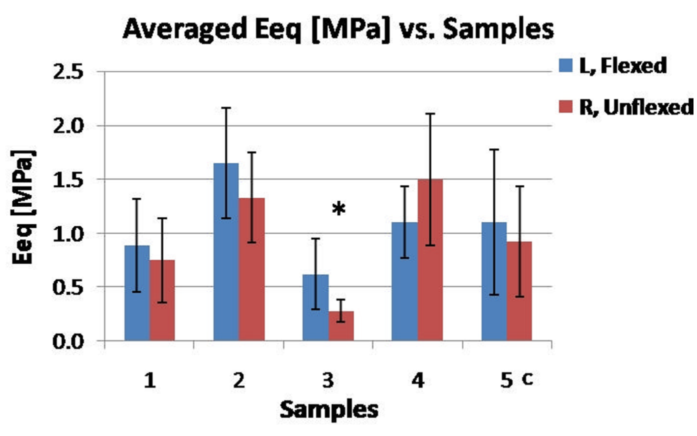

Figure 1. Averaged equilibrium modulus and S.D. for five samples of MCP. Sample 5c indicates a control sample (no flexion in either left or right sides).