ANALYSIS OF FORCE-DISPLACEMENT CURVES OF CARTILAGE AND BIOMATERIALS

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INTRODUCTION

Nano-mechanical testing equipments have made probing small volumes of sample possible, allowing measurement of local mechanical behavior of complex tissues such as articular cartilage. However, current techniques and theories developed for traditional engineering materials are insufficient for analyzing complex soft biomaterials. A few studies have shown significant adhesive effects in the load-displacement data of compliant polymers such as poly(dimethyl siloxane) (PDMS) using the Johnson-Kendall-Roberts (JKR) adhesion model.[1-3] It is suspected that indentation of hydrated tissues might have similar adhesive effects. However, no study has addressed this. In this study, an improved measurement protocol is discussed for determining the nano-mechanical properties of cartilage tissues; the issues of adhesion and surface-find are addressed in calculating reliable values of the local tissue modulus which are necessary to study the mechanical function of musculoskeletal tissues. Force-displacement curves of PDMS, agarose gel, and articular cartilage are compared.

MATERIALS AND METHODS

Sample preparations

The metacarpophalangeal (MCP) joint of a skeletally mature New Zealand White rabbit was used. The joint was dissected open. The distal end of the carpal bone was cut and embedded in poly(methyl methacrylate) PMMA, which cured in a fluid polymer well on a 15 mm metal atomic force microscope (AFM) disc. Nanoindentation was performed on the articular cartilage surface of the carpal bone. Tissue was immersed in phosphate buffered saline (PBS) to keep hydrated throughout testing. A 5% agarose gel (Apex GP, Genesee Scientific, San Diego, CA) was made and poured into a fluid polymer well on a 15 mm metal AFM disk.[4] The gel was immersed in dH_2O for indentation in fluid and. PDMS was provided by Dr. Luke Lee (BioMEMS, Univ. California, Berkeley). A 4 mm^2 sample was glued to a 2 cm metal platen with double-sided tape. The sample was placed inside a fluid polymer well and immersed in dH_2O for fluid indentation and was exposed to air for indentation in air.

Nanoindentation and analysis

Indentation experiments were performed using the TriboIndenter (Hysitron, Inc. Minneapolis, MN) with a fluid extension 100 µm radius of curvature conospherical diamond tip. In order to apply adhesion models to analyze indentation data, complete force curves capturing the absolute initial contact point between the tip and the sample, $\delta_{contact}$, and the pull-off force, P_{adh}, must be obtained.[3,5] The tip approached the surface initially from out-of-contact, and achieved detection of the sample surface at ~1-2 µN of preload. Feedback control allowed a constant rate of 100 nm/s for a total forward displacement of 1500-3000 nm. A hold period of up to 30 s was interjected between loading and unloading segments of the samples to allow creep to dissipate. To validate the appropriateness of the Oliver-Pharr method[7] for analysis of full force indentations in fluid, PDMS in fluid data were evaluated first, and the results from Oliver-Pharr analysis were compared to the direct curve-fitting method using the JKR model.[6] At least three good indents were averaged per sample.

RESULTS AND DISCUSSION

The load-displacement data as shown in Fig. 1(a-b) revealed a large amount of adhesion in PDMS, as indicated by the pull-off force, P_{adh}. In comparison, no adhesion was observed in the gel or the tissue (c). The load-displacement data of PDMS in fluid showed the characteristic jump to contact point, $\delta_{contact}$, disappear (b), possibly due to repulsive electrostatic forces effectively negating the attractive adhesive forces between the tip and the sample surface.[8] This form of the data indicates a distinct initial zero displacement, $\delta_{i,}$ defined as the initial detection of the sample surface in the Oliver-Pharr analysis.

The shape of the load-displacement curves for the gel and the cartilage is very similar as expected, revealing slightly more hysteresis than PDMS. Furthermore, from the fluid indentation data, a slight force offset of < 2 µN appeared at the end of the unloading segments. This is likely a result of capillary interactions developed during tip withdraw between the fluid and the long shaft of the fluid extension tip, even after tip had withdrawn from the sample surface. This did not seem to affect the data analysis results.

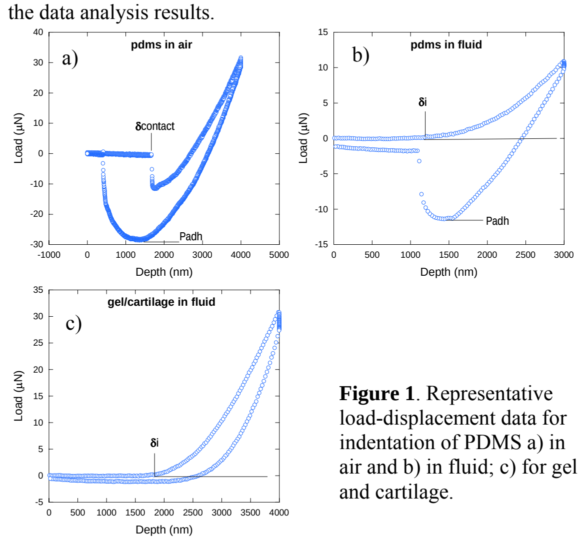

Figure 1. Representative load-displacement data for indentation of PDMS a) in air and b) in fluid; c) for gel and cartilage.

Using the JKR curve-fitting method, the reduced modulus, Er, for PDMS was 1.13 +/- 0.14 MPa for indentation in air and 1.06 +/- 0.11 for indentation in fluid, as expected to be the same. The Oliver-Pharr analysis of data from indentation in fluid also yielded similar modulus value of 1.00 +/- 0.14 MPa (Table I). The values ranged from 0.97-1.39 MPa for PDMS indented in air, and from 0.93-1.17 MPa for PDMS indented in fluid. Using the Oliver-Pharr analysis method, the values for Er ranged from 0.84-1.24 MPa. This shows that the Oliver-Pharr method can give a good approximation to the measured reduced modulus of PDMS in fluid.

With the application of the Oliver-Pharr method to cartilage, the Er for the cartilage in its close-to-physiological condition ranged from 0.17-0.76 MPa resulting in an average modulus of 0.41 +/- 0.31 MPa (Table I). This is a decrease of 81% compared to the previously measured value of 2.2 (0.8) MPa using the original open-loop indentation technique and analysis.[9]

Table I: Average reduced modulus in MPa (S.D.) for indentations in fluid

	Er (S.D.) (MPa)	Model
PDMS	1.06 (0.11)	JKR
	1.00 (0.14)	Oliver-Pharr
Gel	0.93 (0.27)	Oliver-Pharr
Cartilage	0.41 (0.31)	Oliver-Pharr

CONCLUSION

In summary, this work presents the first application of full-force displacement control indentation of soft gel and tissue in a fluid environment. This study suggests that adhesive effects are negligible for indentation of hydrated soft gel and cartilage samples, and that the Oliver-Pharr method can be used to calculate the reduced modulus of these samples. Future studies will aim to further eliminate uncertainties in tissue property measurement through examining effects of such as material hysteresis and surface roughness.

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