Application Note #1500

Characterization of the Elastic Properties of Cartilage Tissue by Indentation

Articular cartilage is a specialized connective tissue suited for the distribution of contact loads within diarthordial joints. It is a biphasic material that exhibits anisotropic and nonlinear elastic behavior. The liquid phase, primarily water, makes up 65% to 80% of the cartilage by weight. The solid phase consists of dispersed proteoglycans within an extracellular matrix (ECM) of collagen and glycoproteins. The structure contains four zones based on the arrangement of the collagen fibril network as shown in Figure 1.[1,2] Mechanical properties vary across the four zones, meaning that high-spatial-resolution is needed for characterization of the localized regions of the tissue. This application note shows how Bruker's Hysitron® BioSoft™ In-Situ Indenter was successfully used to probe local mechanical properties across the sample, and examines the applicability of various data analysis models.

The Hysitron BioSoft In-Situ Indenter is an exceptional tool for the characterization of tissue. Indentation is a popular mechanical characterization technique capable of nondestructive and in-situ measurements of biomaterials that can provide a greater understanding of a tissue's characteristics.[3] The confined volume of the indentation zone allows one to probe different areas of the tissue for local properties.
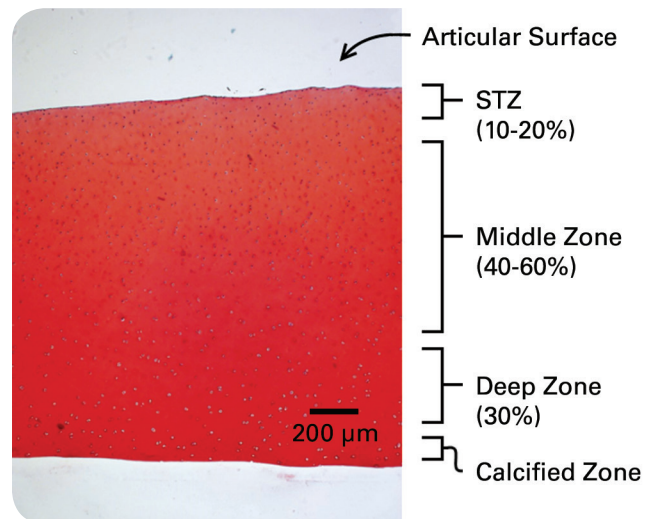

Figure 1. Microscope image of cartilage cross section. The four zones that make up the cartilage are marked, along with the thickness of the zones as a percent of total thickness. The deep zone is nearest to the bone, then come the middle zone, superficial tangential zone, and articular surface as you move further outward.
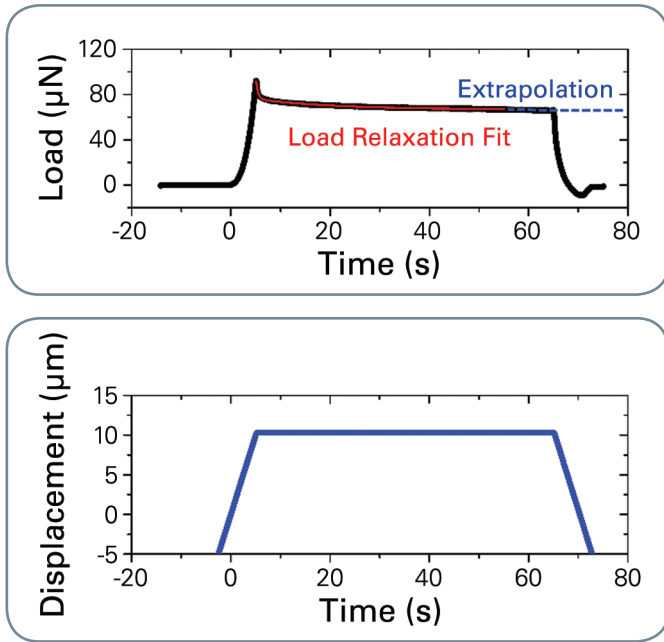
Figure 2. Representative load (top) and displacement (bottom) vs. time curves for indentation into cartilage. Initially, the load and displacement are increased up to the peak displacement, which is then held while the load relaxes. The load relaxation is fit to obtain P_∞.

Model	Modulus (kPa)	χ^2_v
Hertz	315.8 ± 14.2	31.4
Sneddon[6]	329.5 ± 15.8	35.6
Mooney-Rivlin[7,8]	125.2 ± 6.4	0.841
Fung[7]	131.4 ± 7.6	0.781

Table 1. Elastic moduli obtained by fitting various models to a depth profile into the articular surface.

Experiment

A Hysitron BioSoft indenter attached to an inverted microscope was used to indent articular cartilage submerged in phosphate-buffered saline with a 20 μm spherical probe. Load relaxation tests were performed where the peak displacement was held constant while the load was monitored. The hold period was fit using an exponential decay equation:

Equation 1:

$$P = P_\infty + P_1 * e^{-t/\tau_1} + P_2 * e^{-t/\tau_2}$$

where P_∞ is the load extrapolated to infinite time when fluid flow has ceased. At this point, hydraulic pressure is relieved and only the phase-separated response of the cellular ECM remains.[3,4]

Indentation Depth Profile

A depth profile obtained on articular surface is shown in Figure 3. Indentations were performed on the same sample location while allowing sufficient time for the cartilage to recover between tests, which resulted in good repeatability. The extrapolated loads were calculated using Equation 1. Several models were fit to the data to obtain the elastic modulus of the solid phase, as shown in Table 1. The JKR model reduced to Hertz upon fitting. The large reduced chi squared, χ^2_v, values of Hertz and Sneddon indicate poor model fits. However, both the Fung and Mooney-Rivlin hyperelastic models fit the data well. The hyperelastic models contain an additional parameter to account for non-linear elastic behavior of the tissue. The moduli given are for zero strain. A Mooney-Rivlin analysis of indentations into several locations on the surface results in a modulus of 135.2 ± 9.8 kPa, which is comparable to results found in other studies.[4,5]
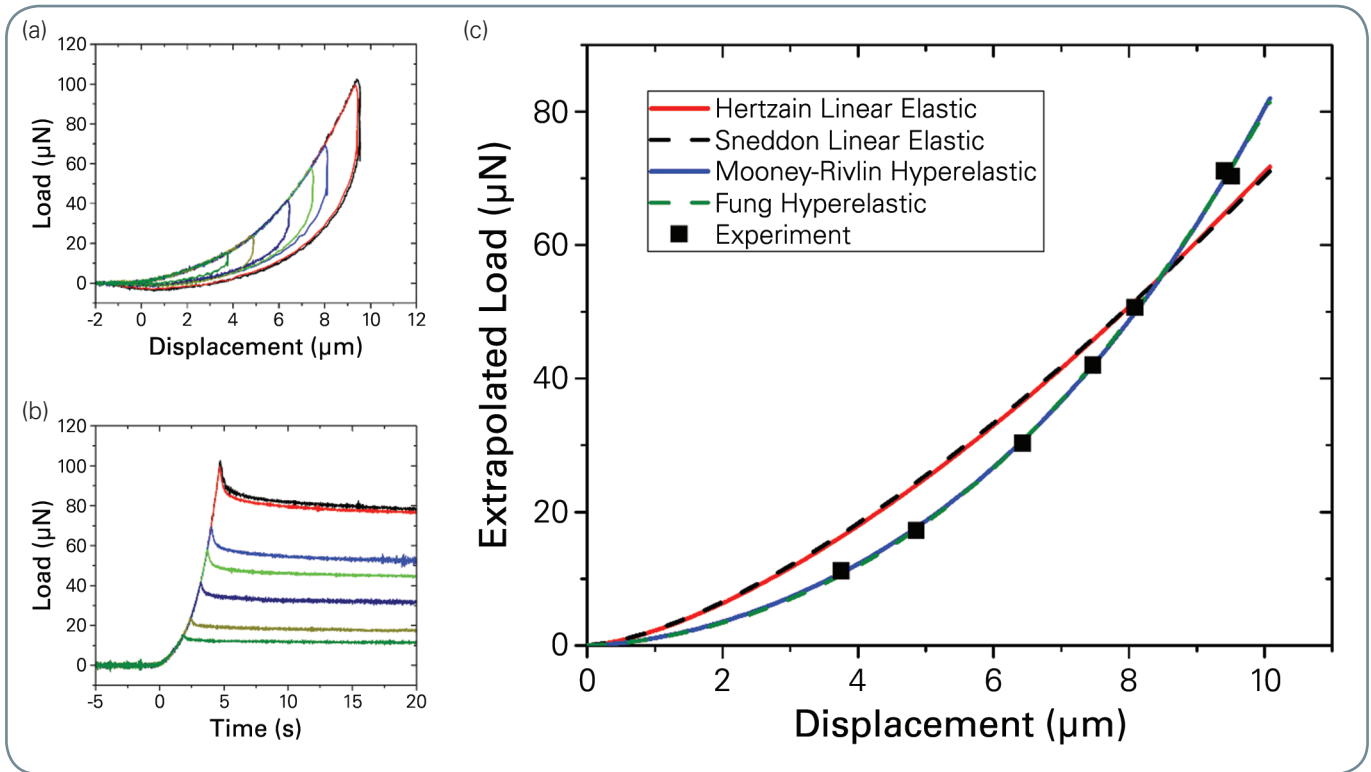
Figure 3. Depth profile into articular surface showing load vs. displacement (a) and time (b). The hold periods were extrapolated to find the load at infinite time, P_∞ which is plotted against peak displacement (c) and fit with several indentation models.

Cross-Sectional Profile

Indentation of the cross-sectional surface gives the property gradients across zones of the cartilage. Three lines of 11 indentations were performed with 100 µm spacing. Results from a Mooney-Rivlin analysis are shown in Figure 4. The modulus is observed to decrease from the deep zone to the STZ. This makes sense as the fluid content, which inversely correlates with modulus, increases near the STZ.[1] The modulus approaches that measured on the articular surface, but plateaus at a slightly higher value. This anisotropy is likely due to preferential alignment of the collagen fibrils parallel to the articular surface. The greater deviation observed near the deep zone may be attributed to an increased disparity of the material structure between indent locations.
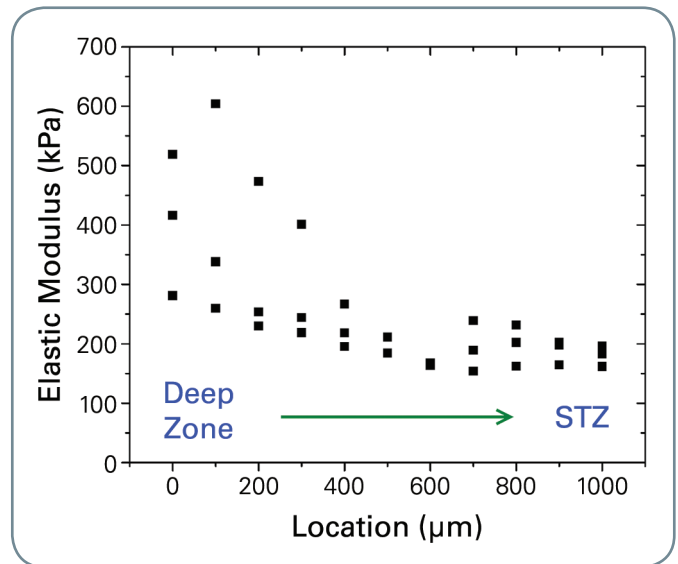

Figure 4. Elastic modulus vs relative location from indentation of cartilage cross section showing an increased modulus in the deep zone.

Conclusion

The Hysitron BioSoft is a powerful tool for testing biological tissues, including the characterization of anisotropy, homogeneity, and property gradients. For indentation depths that are small in comparison to the probe size, Hertz theory has been shown to work well. However, for large depths, where strains are greater, hyperelastic models are clearly better suited.[7]

References

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