NANOINDENTATION OF FINGER JOINT CARTILAGE IN A FLUID CELL

Li, C; ^Ebenstein, DM; ^# King, K; +^Pruitt, LA
+*Department of Mechanical Engineering, University of California at Berkeley 94720
^UCSF/UCB Bioengineering Graduate Group, University of California at San Francisco 94143

INTRODUCTION

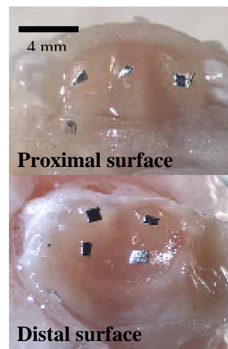
Assessing the mechanical properties of diarthrodial joint cartilage is important for the treatment of osteoarthritis and the development of functional tissue-engineered cartilage. The traditional techniques of confined and unconfined compression performed on cartilage plugs[1-3] and more recent in-situ indentation measurements[4] have shown articular cartilage mechanical properties to be site, depth, and direction-dependent. Further these techniques are limited to larger specimens with relatively smooth joint surfaces. In a preliminary study, nanoindentation performed on rabbit finger joint cartilage has been shown effective in detecting local mechanical properties.[5] Nanoindentation can target the large variety of positions and topographies found in small areas allowing direct assessment of structure-function relationships at the tissue-scale (10^{-4}-10^{-2} m) and micro-scale (10^{-7}-10^{-4} m) levels. Such information can provide critical insight into the mechanisms of cartilage function in load support and lubrication, as well as joint degeneration. This study aims to 1) validate the modified nanoindentation technique for mechanical measurements of articular cartilage in a fluid environment and 2) evaluate hydration as a critical testing parameter. In-situ compressive reduced modulus was measured for healthy rabbit metacarpophalangeal (MCP) joint cartilage immersed in saline with a specialized fluid cell tip at fine spatial resolution.

MATERIALS AND METHODS

Joint Dissection: The MCP joints from skinned digits of skeletally mature New Zealand White rabbits were frozen and thawed prior to testing. Carpal and phalange bones were cut near their proximal and distal ends, respectively, and cleaned. The outer connective tissue was removed, the joint capsule was pierced and the joint opened by cutting the local ligaments.

Sample Preparation for Nanoindentation: The long ends of the proximal and distal finger bones were embedded individually in PMMA mounted on 15 mm metal atomic force microscope discs. While the distal specimen was embedded with the bone shaft perpendicular to the metal disc, the proximal specimen was mounted at a 60° angle to metal disc to expose the corresponding cartilage surfaces. Each sample was securely placed in individual wells of a 24-well tissue culture plate. Under the dissecting microscope, small reflective markers were placed at positions of interest on the cartilage surface for identification under the nanoindentor optics (Figure 1). Samples were frequently flushed with phosphate buffered saline (PBS) to retain hydration during sample preparation and finally fully submerged in PBS for nanoindentation testing.

Figure 1: MCP sample with markers in place



Nanoindentation Protocol: All indents were performed using the Hysitron TriboIndenter (Hysitron, Inc., Minneapolis, MN) with an 100 µm radius of curvature conospherical fluid cell diamond probe tip. For the validation study, several positions of interest were identified and 2-4 indents 100 µm apart were obtained per position. For the dehydration study, two distal MCP specimens were indented at several positions over the course of 16 h at periodic time intervals. One was tested fully submerged in the fluid cell (hydrated) with evaporation over time leading to the exposure of the tissue to air. The other joint was tested in the fluid cell, but starting with the water level just below the surface of the cartilage that was to be tested (non-hydrated). A trapezoidal load function with a peak load of 200 µN and load, hold, and unload of 10 s each was applied at each indent site. Reduced modulus values (Er) were calculated from the unloading curves following the method of Oliver and Pharr.[6] The reduced modulus is related to the elastic modulus, E, by: $1/Er = (1-\nu_i^2)/E_i + (1-\nu^2)/E$, where the subscript i refers to the indenter material. Reduced modulus values are reported here to avoid presuming Poisson's ratio, ν, for rabbit cartilage. The Poisson's ratio is expected to fall in the range of 0-0.42.[1]

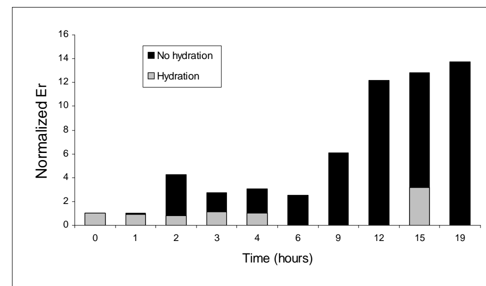
RESULTS

Reduced moduli on distal and proximal surfaces of a single digit are shown in Table 1. The results over the time course of the hydration study are summarized in Figure 2 as normalized modulus (Er_t/Er_{t0}).

Table 1: Average reduced modulus in MPa (S.D.)

Distal Positions	2.2 (0.8)	**Proximal Positions**	6.8 (2.9)
Position 1	1.4 (0.3)	Position 5	8.2 (0.6)
Position 2	2.1 (0.4)	Position 6	5.4 (3.8)
Position 3	3.3 (0.5)	Position 7	6.8 (3.3)
Position 4	2.0 (0.5)	**All Positions**	4.4 (3.1)

Figure 2: Hydration study of MCP joints (Normalized E_r as a function of hydration and time)



DISCUSSION

The reduced modulus for articular cartilage commonly reported in the literature is 1 MPa[1-3]. Using the fluid cell, the overall reduced modulus for a single digit joint cartilage was 4.4 MPa, rather than the 84 MPa reported in the previous study.[5] The proximal surface was again stiffer than the distal surface. Five out of seven positions displayed relatively consistent measurements. The two positions with large S.D. suggest local mechanical property variability at the micro-scale level, likely due to structural differences in these regions. For the hydration study, the averaged distal cartilage modulus after the first 2 h ranged from 11.9-26.1 MPa for the non-hydrated case while the values for the hydrated case only ranged from 2.6-7.6 MPa. After 15 h, the hydrated case ranged from 9-16 MPa while the non-hydrated case reached 30-85 MPa. Thus fluid hydration appears to be an important control parameter for measuring functional modulus of articular cartilage. Future work correlating structural and biochemical features with mechanical variability in the cartilage tissue will expand the use of this technique in a clinical setting.

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AFFILIATED INSTITUTIONS FOR CO-AUTHORS:

Department of Medicine, University of California at San Francisco 94143