DEPTH-DEPENDENT ORTHOTROPIC TENSILE AND COMPRESSIVE PROPERTIES OF HUMAN PATELLAR CARTILAGE

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INTRODUCTION

It is well established that articular cartilage exhibits inhomogeneity, anisotropy and nonlinearity in its mechanical response [1-4]. However, the simultaneous measurement of tensile and compressive properties along multiple loading directions and as a function of depth through the articular layer remains a major experimental challenge. Measurements of tissue inhomogeneity have been reported for the tensile [1,5] and compressive [6-10] responses of cartilage. Measurements of tissue anisotropy have been reported in tension [1,5,11] and more recently in compression by our group [12]. Some investigators have used osmotic swelling of articular cartilage to extract the tensile properties of its solid matrix [13,14]. We have recently combined and extended several of these techniques to report the anisotropic properties of bovine articular cartilage in tension and compression [15,16]. In the current study, these techniques are applied to the measurement of the anisotropic tensile and compressive equilibrium modulus of human patellar articular cartilage, as a function of depth. Measurements are performed along 3 loading directions: parallel to the local split line direction (1-direction), perpendicular to the split line (2-direction) and perpendicular to the articular surface (3-direction).

METHODS

Sample Preparation: Four human fresh frozen cadaver knees were dissected (1 female, 3 males, 39±6 years old), and full thickness patellar cartilage plugs (Ø 6 mm) were harvested from the central region of the patella. On the day of testing, all subchondral bone was removed using a sledge microtome (Model 1400; Leitz, Rockleigh, NJ) equipped with a freezing stage (Hacker Instruments, Fairfield, NJ). The split line direction, the preferred direction of collagen in the superficial zone, was determined and marked. Using a digital caliper, the thickness of the cartilage plug was then measured (2.1±0.3mm) and the tissue was sliced into three equal layers, denoted as the superficial, middle and deep layers. Cubic specimens (0.9mm×0.9mm×~0.7mm) in each layer of cartilage were prepared relative to the split-line direction [12,15]. For each layer of tissue, 12 samples were divided into 3 experimental groups to study the concentration effects along a particular loading direction. For each specimen in a group, three external bath concentrations were used (0.015M, 0.15M, or 2M NaCl).

Loading Protocol: A custom designed unconfined compression device, mounted on an inverted microscope (Olympus IX-70, Olympus, Melville, NY), was used to uniaxially load the cubic samples. After the specimen equilibrated in a bath solution for 40 minutes, the specimen was imaged. Compression was then applied in 2% increments from 0 to 20% strain *relative to the free-swelling state.* The resultant normal stress (σ_{ii}) was recorded at equilibrium, and images of the specimen's surface were acquired. The bath solution was replaced by another concentration and the loading protocol repeated. Strain analysis was performed using optimized digital image correlation [8], producing accurate axial strains (ε_{ii}). The incremental Young's moduli ($E_{Yi} = \Delta s_{ii} / \Delta e_{ii}$, i=1,3) were determined for all specimens.

Biochemical Analysis: The wet and dry weights of each cubic specimen were measured, and the samples were digested using papain (10 µl per mg; Sigma, St. Louis, MO) at 60°C overnight. The glycosaminoglycan (GAG) content was measured using the Blyscan Assay (Accurate Chemical, Westbury, NY) for sulphated GAGs with chondroitin-4-sulphate as the standard.

RESULTS

Typical stress vs. strain and modulus vs. strain responses for superficial, middle and deep layer tissue are shown in Figs. 1&2, respectively. An initial nonlinear stress-strain response was observed, indicating a strain-softening behavior, followed by a linear stress-strain or constant modulus-strain response. When the applied strain is less than the free swelling strain, the cartilage matrix is under a net tensile strain, and the E_{Yi}'s represent tensile moduli. As compression increases and the swelling strain is overcome, a smooth transition in both stress-strain and modulus-strain behavior is observed (Fig. 3), and the E_{Yi}'s represent compressive moduli. This swelling behavior was

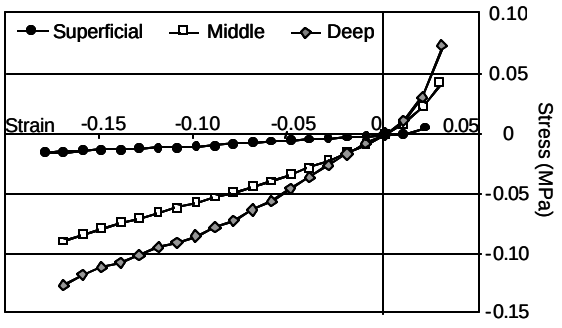

Figure 1: Stress vs. matrix strain response of cartilage loaded in 3-DIR in 0.15M NaCl

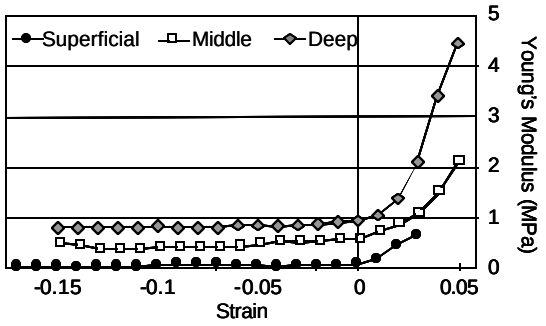

Figure 2: Modulus vs. matrix strain response of cartilage loaded in 3-DIR in 0.15M NaCl

observed in the superficial, middle and deep layers of cartilage, though the degree of swelling was non-uniform across the depth of the tissue, as indicated in Table 1 and the magnitude of tensile strain in Figs. 1,2. Superficial zone cartilage experienced the least amount of swelling, with increasing swelling observed with increasing depth. Correspondingly, the tensile modulus at the free-swelling reference state, summarized in Fig. 3 for all loading conditions, was smallest in the superficial zone and increased with increasing depth (i.e. ~ 2MPa in superficial vs. ~ 6MPa in deep layer), with the middle layer exhibiting swelling magnitudes and tensile properties more similar to that of the deep zone. The compressive moduli, measured when the applied strain overcame the swelling strain, are also summarized in Fig. 3. The superficial layer exhibited the smallest compressive modulus (~0.3MPa along 1-DIR in 0.015M) and increased with depth to ~1MPa in the deep layer. GAG content of the specimens from each layer of tissue was highest in the middle zone with the superficial layer exhibiting a significantly lower GAG content (Table 1).

DISCUSSION

This study reports the depth-dependent and strain-dependent tensile and compressive moduli of human patellar cartilage along the three directions characterizing material symmetry. With decreasing NaCl concentration, the osmotic pressure induced by proteoglycans increases, placing the tissue in a greater state of swelling. Thus, both tensile and compressive properties achieve their highest values under hypotonic conditions, and smallest values under hypertonic conditions (Fig. 3). As expected from prior studies [6-10], the compressive modulus along the 3-direction increases with depth. A new finding of this study is that this depth-dependent variation is similar along the 1- and 2-directions as well. Within the superficial layer, the tensile modulus along the 1-direction is greater than the 2- and 3-directions, however this trend varies with increasing depth. Direct measurements

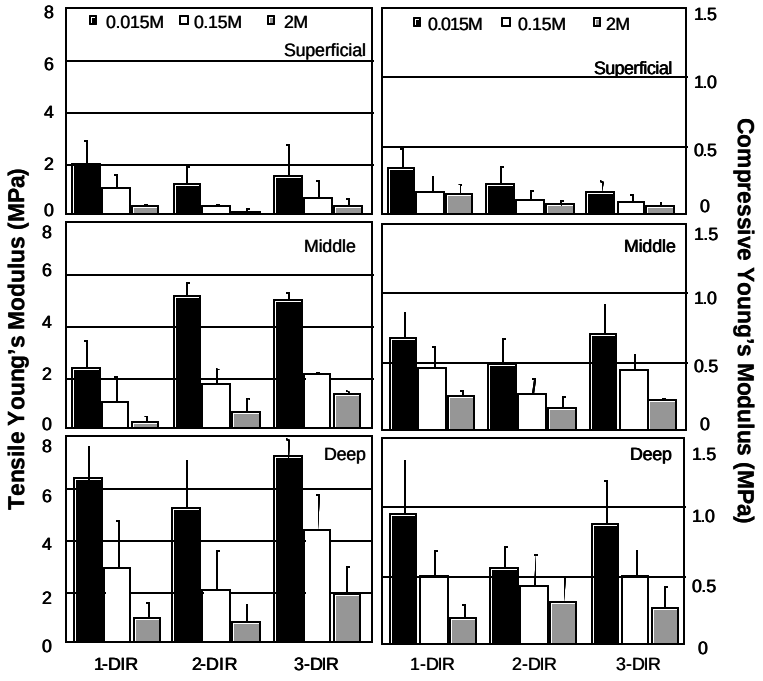

Figure 3: Summary of tensile & compressive properties

of tensile properties [1,5,11] indicate that the superficial layer should exhibit higher tensile moduli along the 1- and 2-directions than in the middle and deep layers. This trend was not observed in the current study because the maximum tensile moduli were measured under free-swelling conditions. The inhomogeneous GAG distribution however yielded smaller osmotic pressures and swelling strains in the superficial layer (Table 1). Thus the tensile moduli were not measured under identical swelling conditions in all three layers. The results of this study provide some of the most extensive measurements performed on a single full thickness sample of articular cartilage, yielding inhomogeneous, anisotropic and strain-dependent properties in human patellar tissue. These measurements can be used to develop more comprehensive constitutive models of articular cartilage.

Table 1: Swelling strain and GAG content with depth

	Superficial	Middle	Deep
Swelling Strain (%)	3.44±0.39	4.83 ± 0.17	6.53 ± 0.70
GAG content (% wet weight)	1.41 ± 0.85	5.16±2.75	3.62±1.01

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