

Depth dependent osmotic and swelling properties of cartilage

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ABSTRACT

Articular cartilage is a low-friction, load-bearing tissue located at joint surfaces. It experiences static and dynamic forces including shear, compression and tension. We investigate the relationship between structure and function by measuring the osmotic and mechanical properties in cartilage layers as a function of the distance from the articular surface. Atomic force microscopy is used to probe the mechanical properties at high spatial resolution. The mechanical measurements are complemented by osmotic swelling pressure observations made on the same samples using a novel tissue osmometer. The results show that the osmotic modulus significantly depends on the distance from the articular surface. Its value is highest in the deep zone and lowest in the middle zone.

INTRODUCTION

Cartilage is a connective tissue located at both distal and proximal ends of the bone surface. Its major biological functions include load bearing, shock absorption and lubrication [1]. The tissue's unusual and unique properties originate from the architecture and organization of its polymeric components. The cartilage matrix is mainly composed of collagen (10 - 20 %), proteoglycans (5 - 15 %), and water (70 - 80 %). The triple helical structure of the collagen fibers gives cartilage the ability to sustain tensile stress [2]. The collagen fibrils form a three-dimensional network, which contains proteoglycan (PG) assemblies. The dominant cartilage proteoglycan is aggrecan, a large, bottlebrush shaped molecule, which interacting with hyaluronan and link protein forms large aggregates (size 1-4 micrometers). The osmotic pressure exerted by the charged groups along the PG molecules leads to swelling of the PG assemblies. The swelling is constrained by the collagen network thus placing it under tension. The negatively charged aggrecan-hyaluronan complexes are highly hydrated, and thus provide compressive resistance to cartilage.

Cartilage matrix is synthesized by the chondrocytes. The volume of chondrocytes varies between 1 - 6 %. These cells ensure that cartilage functions properly by facilitating fluid exchange within the matrix, which is essential to absorb nutrients and to remove waste products [3].

Figure 1 illustrates the organization of the main macromolecular components of cartilage extracellular matrix. The aggrecan-hyaluronan complexes are enmeshed in the fibers of the collagen network.

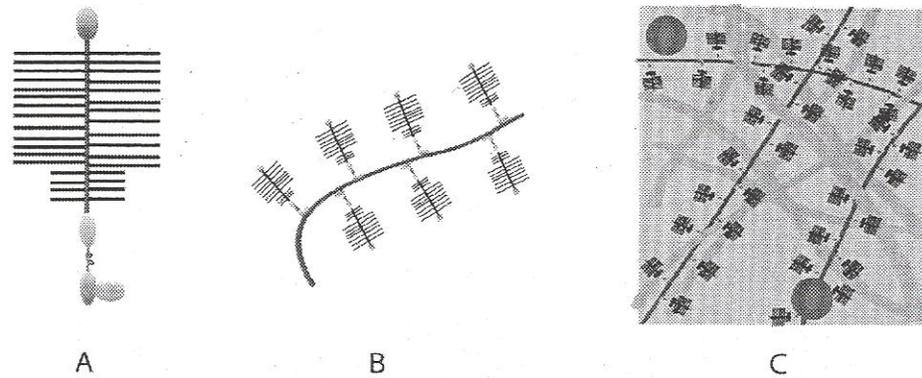


Figure 1: Hierarchy of cartilage. (A) Aggrecan bottlebrush. (B) Aggrecan-hyaluronan complex. (C) Aggrecan-hyaluronan aggregates in the collagen matrix.

The objective of the present work is to determine the osmotic properties in cartilage layers as a function of the distance from the articular surface. Such insights are important for understanding cartilage biomechanical behavior and evaluate possible mechanisms associated with cartilage development and degeneration.

EXPERIMENTAL

Sample preparation

Bovine cartilage from the superficial, middle, and deep zones were obtained from 5-6 different sites of a 2 year-old femur head. For histological and microscopic analysis tissue samples were fixed by formalin and embedded in paraffin.

Tissue micro-osmometry and atomic force microscopy (AFM) were performed on unfixed cartilage specimens.

Histological staining of cartilage samples

The histological changes of cartilage slices were investigated by light microscopy using histochemical stains. First the cartilage samples were fixed in 10% formalin for 24 hr. Then paraffin-embedded, 8-micrometer thick sections were prepared and stained with histochemical methods for haematoxylin-eosin (H & E) and alcian blue.

The histological staining was made by the American Histolabs, Inc., (Gaithersburg, MD).

AFM (Atomic Force Microscopy)

AFM imaging was and indentation measurements were made using made using a commercial AFM (Bioscope I with Nanoscope IV controller, Veeco, Santa Barbara, CA). Silicon cantilevers were used (OMCL by Olympus, Tokyo, Japan), which have a nominal spring constant of 42 N/nm, resonant frequency of 300 kHz, and nominal tip radius of 7 nm. AFM

force measurements were used to determine the stress-deformation curves. Data analysis was made using procedures reported previously [8,9]. Statistical significance was measured using the student t-test.

Osmotic swelling pressure measurements

The water uptake (hydration) of cartilage was determined by a Tissue Micro-Osmometer (TMO) developed at the Section on Tissue Biophysics and Biomimetics, NICHD, NIH [4]. It contains a gold-coated quartz crystal, which oscillates in a mechanically resonant shear mode under the influence of a high frequency AC electric field applied across the thickness of the crystal. This apparatus allows us to measure the water uptake of very small tissue specimens (< 0.1 mg). When the tissue absorbs water the resonance frequency decreases. In the linear region the mass of the absorbed water can be obtained from the frequency shift Δf (in Hz) using the Sauerbrey relation [5]

$$\Delta f = K \Delta m \quad (1)$$

where Δm is the mass change, and K is a constant, which depends on the density of the quartz crystal and the shear velocity of sound waves in the quartz crystal.

From Δm , the weight fraction w of the tissue can be determined

$$w = \frac{m_{dry}}{m_{dry} + \Delta m} \quad (2)$$

where m_{dry} is the weight of the dry sample.

All crystals used in the TMO instrument were cleaned by exposure to ultraviolet light (UV) for 5 minutes. Then the crystals were washed in a 2% SDS solution for 30 minutes, immersed in distilled water for 10 minutes, and dried with nitrogen. Finally, the crystals were again exposed to UV light for 10 minutes before being used.

Thin (< 5 μm) tissue samples were attached to the surface of the crystal and equilibrated for 2-3 hours with solutions of known water vapor pressure. The reversibility of the sorption measurements was checked by changing (decreasing and then increasing) the water vapor pressure in the surrounding environment.

Osmotic swelling pressure measurements were also made by the osmotic stress technique described elsewhere [6,7]. Cartilage samples were equilibrated with poly(vinyl pyrrolidone) solutions of known osmotic pressure. Deswelling was achieved by enclosing the specimens in a semipermeable membrane (dialysis bag). In this experiment the equilibration time was approximately 2 days.

The osmotic swelling pressure measurements and mechanical measurements were made at 25 ± 0.1 °C.

RESULTS AND DISCUSSION

Figure 2 shows the AFM images of the bottlebrush shaped aggrecan molecule (left image) and that of the collagen meshwork (right figure). In the latter, due to the lower

resolution, the individual constituents are not distinguishable. Image analysis shows that the cross-sectional diameter of the aggrecan molecule is approximately 30 - 50 nm and the length of the bottlebrush is about 600 nm. The average mesh-size of the collagen network is ≈ 300 nm.

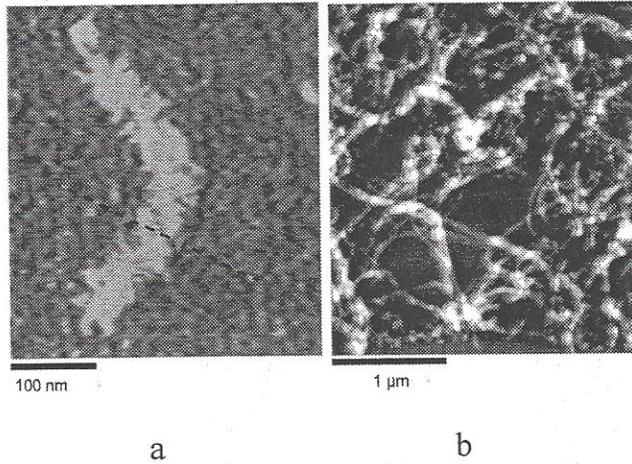


Figure 2. AFM images of aggrecan bottlebrush (a) and collagen matrix (b).

Figure 3 illustrates depth dependent histological changes of chondrocytes in the cartilage matrix. In the superficial zone, the chondrocytes are predominantly parallel to the surface, in the middle zone they are randomly distributed, while in the deep zone they exhibit a columnar arrangement.

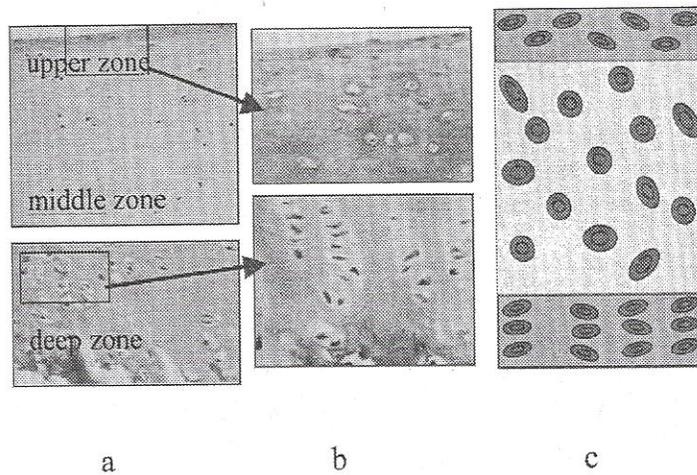


Figure 3. H & E (a) and alcian blue (b) staining of cartilage specimens. The cartoon (c) shows the schematic diagram of cartilage tissue.

Figure 4a shows typical force vs deformation curves measured by AFM nanoindentation on cartilage samples from different zones. The data indicate that the stiffness increases in the order middle zone < superficial zone < deep zone. The middle zone is softer than the deep zone

by a factor of approximately 5. The stiffness of the deep region slightly exceeds that of the superficial zone probably because the molecules are densely packed close to the bone surface.

Figure 4b shows the osmotic swelling pressure Π_{sw} of the same cartilage samples as a function of the swelling degree $1/w$. In the unloaded state $\Pi_{sw} = 0$ since the osmotic pressure of the PG assemblies is counter balanced by the elastic pressure of the collagen network [10]. The water content is greatest in the middle zone, followed by the superficial and the deep zones. In the presence of osmotic stress ($\Pi_{sw} > 0$) the swelling degree decreases. The increase of Π_{sw} is fastest in the deep zone and slowest in the middle zone. The observed trend is consistent with the results of the mechanical measurements shown in Figure 4a. It can be seen that the Π_{sw} vs $1/w$ curves crossover at $1/w \approx 4.8$ corresponding to the swelling degree of cartilage in the body.

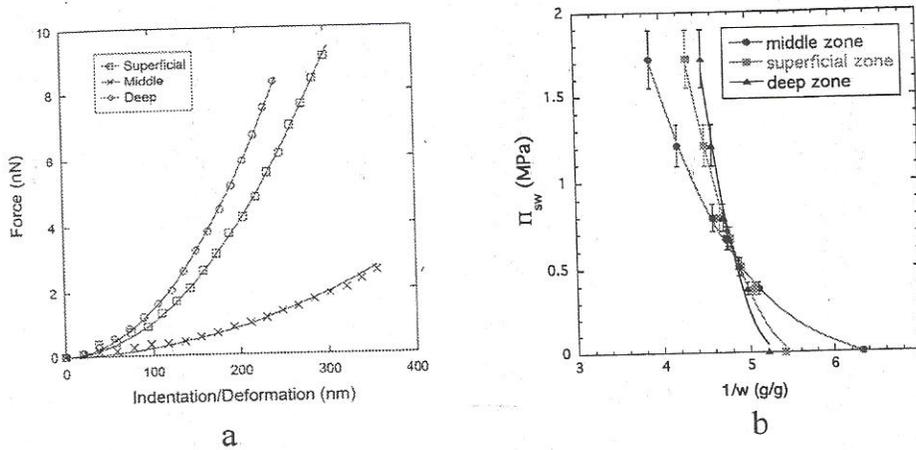


Figure 4. Typical force vs deformation (figure a) and swelling pressure vs weight fraction (figure b) dependencies measured in different cartilage layers.

The load bearing ability of the tissue is determined by the osmotic compression modulus

$$M_{os} = w \frac{\partial \Pi_{sw}}{\partial w} \quad (3)$$

An estimate from the initial slopes of the curves shown in Figure 4b yields the following values: $M_{os} = 670 \pm 60$ kPa (middle zone), $M_{os} = 980 \pm 110$ kPa (superficial zone), and $M_{os} = 1180 \pm 140$ kPa (deep zone). These values are in the range obtained for bovine cartilage samples from confined compression measurements [11].

CONCLUSIONS

The main objective of this work is to evaluate cartilage biomechanical properties as a function of depth from the articular surface using mechanical (force vs indentation) measurements in tandem with osmotic swelling pressure observations. It is found that the superficial and deep zones have a higher stiffness than the middle zone. The value of the osmotic modulus was determined for each zone. The osmotic modulus is the lowest in the

middle zone, which, therefore, determines the overall resistance of the tissue to compression forces.

The present experimental approach may provide a useful tool to quantify changes in cartilage biomechanical properties during growth and development, or with aging and degenerative joint disease. It may also help to engineer cartilaginous tissues with designed mechanical and osmotic properties.

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REFERENCES

1. Mow, V.C.; Zhu, W.; Ratcliffe, A. Structure and function of articular cartilage and meniscus, in *Basic Orthopedic Biomechanics*, eds. Mow, V.C. and Hayes, W.C. Raven Press, New York, 1991.
2. Won, C.; Sah, L.R. *Biomechanics of Articular Cartilage*, in *An Introductory Text to Bioengineering (Advanced Series in Biomechanics)*, eds. Chien, S.; Chen, P.C.Y.; Fung, Y.C. World Scientific Publishing Co. 2008.
3. Ateshian, G.A.; Hung, C.T. The natural Synovial Joint: Properties of Cartilage, *J. Engineering Tribology*, 220, 657-670 (2006).
4. Horkay, F.; Horkayne-Szakaly, I.; Basser, P.J. Measurement of the Osmotic Properties of Thin Polymer Films and Biological Tissue Samples, *Biomacromolecules* 6, 988-993 (2005).
5. Sauerbray, G.Z. Use of quartz vibrator for weighing thin films on a microbalance. *Z. Phys.* 155, 206-212 (1959).
6. Horkay, F.; Zrinyi, M.: Studies on Mechanical and Swelling Behavior of Polymer Networks Based on the Scaling Concept, 4. Extension of the Scaling Approach for Gels Swollen to Equilibrium in a Diluent of Arbitrary Activity, *Macromolecules* 15, 1306-1310 (1982).
7. Horkay, F.; Tasaki, I.; Basser, P.: Osmotic Swelling of Polyelectrolyte Hydrogels in Physiological Salt Solutions, *Biomacromolecules*, 1, 84-90 (2000).
8. Lin, D.; Horkay, F. Nanomechanics of polymer gels and biological tissues: A critical review of analytical approaches in the Hertzian regime and beyond. *Soft Matter*, 4, 669-682 (2008).
9. Chandran, P.L.; Dimitriadis, E.K.; Basser, P.J.; Horkay, F. Probing interactions between aggrecan and mica surface by the atomic force microscopy. *Journal of Polymer Science Part B: Polymer Physics* 48, 2575-2581 (2010).
10. Basser, P.J.; Schneiderman, R.; Bank, R.A.; Wachtel, E.; Maroudas, A.: Mechanical properties of the collagen network in human articular cartilage as measured by osmotic stress technique. *Arch. Biochem. Biophys.* 351, 207-219 (1998).
11. Schinagl, R.M.; Gurskis, D.; Chen, A.C.; Sah, R.L.: Depth-dependent confined compression modulus of full-thickness bovine articular cartilage. *Journal of Orthopaedic Research* 15, 499-506 (1997).