OSMOTIC SWELLING BEHAVIOR OF BOVINE CARTILAGE

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Introduction

Cartilage is a complex tissue whose extracellular matrix mainly consists of charged proteoglycan (PG) assemblies imbedded in a fibrous collagen network.[1,2] PGs attract water and repel each other due to their negative charges generating a high swelling pressure. Collagens are proteins that form the fibrillar meshwork providing cartilage with its tensile strength. Cartilage extracellular matrix is composed of 70 to 80% water, 14 to 18% collagen (mainly collagen type II), 7 to 10% PGs, and about 1 to 2% other collagens (types IX, X, and XI). Between the large collagen fibers are a meshwork of smaller collagen fibers and the PG assemblies. The PG aggregates provide the cartilage with its swelling ability and compressive resistance under external load, while the rigid collagen is necessary for shape retention.

Mechanically, cartilage exhibits nonlinear and anisotropic characteristics.[3-8] In particular, its elastic properties are influenced by the anisotropic arrangement of the collagen fibers, which are parallel to the surface in the superficial zone, randomly oriented in the middle zone, and orthogonal to the cartilage-bone interface in the deep zone.[12] The elastic modulus of articular cartilage is in the range 0.5–1.5 MPa. However, much higher values (up to 15-20 MPa) have been reported in joints during dynamic loads. The high dynamic stiffness of cartilage can be attributed to its relatively low permeability. Water cannot be easily squeezed out of the matrix but is pressurized thus supporting the high stresses. Experimental results also demonstrate anisotropy of diffusion of solute molecules. It was observed that the diffusion coefficient of macromolecules varied throughout the thickness of the tissue due to anisotropic diffusion in different zones.

Various biomechanical models have been developed to interpret the anisotropic and non-linear behavior of cartilage matrix.[13] However, an understanding of the relationship between the structure and the functional properties of the tissue remains incomplete at this time. The osmotic compression modulus that defines the load-bearing ability of the tissue can be obtained from the concentration dependence of the osmotic pressure. The aim of this study is to determine the osmotic properties of cartilage in the different zones as a function of the distance from the articular surface.

Experimental

Materials. A large number of cartilage samples from different sites of the bovine femoral head (20 to 26 months old animals) were studied. The cartilage covering the femoral head of the rear limbs was sampled. 5 mm wide and 0.5 mm thick sections were prepared representing the superficial, middle and deep zones (**Figure 1**).

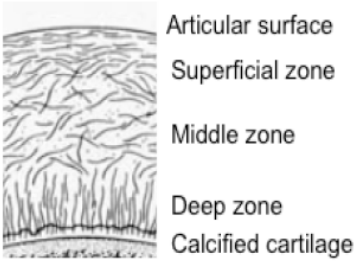

Figure 1. Schematic drawing of cartilage showing the arrangement of collagen fibers in different zones.

Osmotic Deswelling Measurements. The water content of cartilage was controlled by using the osmotic stress technique.[14-16] Tissue specimens were incubated in aqueous polymer solutions of known osmotic pressure. In this environment the cartilage loses water until its osmotic swelling pressure is in equilibrium with the surrounding solution. To set the osmotic pressure we used poly(vinyl pyrrolidone) (PVP) solutions. The polymer concentration was varied in the range from zero to 30% (w/w). The swelling pressure measurements were made in the presence of 0.1 M NaCl.

Cartilage samples were immersed in each PVP solution for 2 hr at 25 °C. In previous studies we determined that 1 hr incubation of cartilage was sufficient to attain equilibrium. However, we waited 2 hr to ensure equilibrium.

High power light microscope images were captured using a CCD video camera. The dimensions of the cartilage specimens were recorded as a function of time until equilibrium was established. The water content was determined gravimetrically by recording the weight of the samples immediately after equilibration and after drying in an oven at 80 °C for about 24 h.

The reversibility of the swelling process was checked by reswelling the deswollen cartilage specimens in 0.1 M NaCl solution.

Histology. To investigate the histological changes of the cartilage slices a light microscopic examination was performed using histochemical stains. The tissue samples were fixed in 10% formalin for 24 hr and paraffin-embedded. 8-micrometer thick sections were prepared and stained with histochemical methods for haematoxylin-eosin (H&E), alcian blue, safranin-O (the latter stains GAGs red) and Masson's trichrome method, which stains collagen blue.

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

Results and Discussion

Figure 2 shows typical images of cartilage samples captured before and after the deswelling process. It can be seen that shrinking is isotropic indicating that each sample responses uniformly to changes in the osmotic environment. At the macroscopic level the difference in the local orientation of the collagen fibers is averaged and no macroscopic anisotropy can be detected.

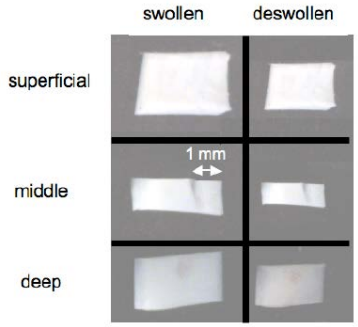

Figure 2. Images of cartilage specimens of different zones in the fully swollen state and after deswelling in PVP solution.

Histological analysis reveals important morphological differences as a function of the depth (**Figure 3**). In the upper layer the chondrocytes (cartilage cells) are smaller and oriented parallel to the articular surface. In the middle zone the chondrocytes are randomly oriented, while in the deep layer they are arranged perpendicularly to the cartilage-bone interface. These observations are consistent with the orientation of the collagen fibers as illustrated schematically in Figure 1.

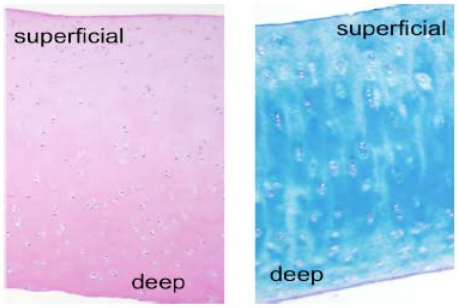

Figure 3. Histological staining of cartilage with H&E (left) and alcian blue (right). Original magnification is 40X in both cases.

The composition and morphology of the cartilage matrix modulates the

load bearing properties of the tissue. To compare the hydration properties of the different layers we equilibrated cartilage slices with polymer solutions of known osmotic pressure. Decreasing the osmotic pressure in the surrounding liquid phase results in cartilage deswelling.

The extent of tissue dehydration can be characterized by the quantity

$$Y = \frac{c - c_0}{c_0} \qquad (1)$$

where c is the concentration of the tissue sample in equilibrium with a polymer solution of arbitrary osmotic pressure, and c_0 is its initial concentration.

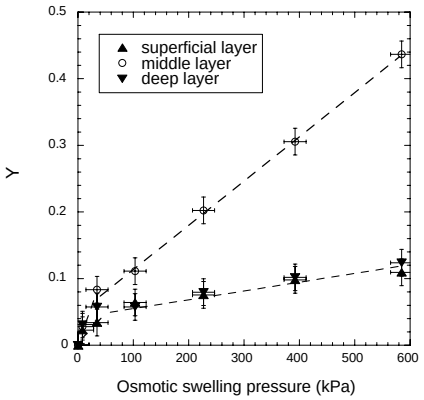

Figure 4. Cartilage dehydration as a function of the osmotic pressure.

Figure 4 shows the variation of Y as function of the osmotic pressure. It is clear from the figure that the water loss is much greater in the middle zone than in either the superficial or the deep zones. This finding suggests that collagen orientation strongly affects the dehydration behavior of cartilage. It is likely that in the superficial and deep zones the oriented collagen fibers squeeze together the highly swollen PG assemblies, thus increasing the packing density. Further dehydration of these closely packed regions requires higher osmotic stresses than that required in the middle zone where the macromolecules are randomly packed.

Conclusions

In this work we investigated the swelling properties of cartilage as a function of the depth from the articular surface. Histological analysis and osmotic deswelling measurements were made on cartilage slices from different zones. Osmotic measurements provide insight into the complex interplay between the macromolecular components of the cartilage matrix and their hydration properties. It appears that correlation exists between the orientation of collagen fibrils and the hydration behavior of the tissue. The water retention is stronger in the superficial and deep zones, where the collagen fibers are ordered, than in the middle zone where the packing density is smaller and the polymer molecules are randomly oriented.

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