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Anisotropic hydraulic permeability in compressed articular cartilage

Boris Reynaud, Thomas M. Quinn*

Cartilage Biomechanics Group, Ecole Polytechnique Fédérale de Lausanne (EPFL), AA B019, CH-1015 Lausanne, VD, Switzerland

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Abstract

The extent to which articular cartilage hydraulic permeability is anisotropic is largely unknown, despite its importance for understanding mechanisms of joint lubrication, load bearing, transport phenomena, and mechanotransduction. We developed and applied new techniques for the direct measurement of hydraulic permeability within statically compressed adult bovine cartilage explant disks, dissected such that disk axes were perpendicular to the articular surface. Applied pressure gradients were kept small to minimize flow-induced matrix compaction, and fluid outflows were measured by observation of a meniscus in a glass capillary under a microscope. Explant disk geometry under radially unconfined axial compression was measured by direct microscopic observation. Pressure, flow, and geometry data were input to a finite element model where hydraulic permeabilities in the disk axial and radial directions were determined. At less than 10% static compression, near free-swelling conditions, hydraulic permeability was nearly isotropic, with values corresponding to those of previous studies. With increasing static compression, hydraulic permeability decreased, but the radially directed permeability decreased more dramatically than the axially directed permeability such that strong anisotropy (a 10-fold difference between axial and radial directions) in the hydraulic permeability tensor was evident for static compression of 20–40%. Results correspond well with predictions of a previous microstructurally-based model for effects of tissue mechanical deformations on glycosaminoglycan architecture and cartilage hydraulic permeability. Findings inform understanding of structure-function relationships in cartilage matrix, and suggest several biomechanical roles for compression-induced anisotropic hydraulic permeability in articular cartilage.

Keywords: Cartilage; Hydraulic permeability; Anisotropic; Compression

1. Introduction

Mechanical loading of articular cartilage involves deformations and fluid flows within its extracellular matrix. Fluid flow plays important roles in load-bearing (Mow et al., 1984) and joint lubrication (McCutchen, 1962), and may influence solute transport through the avascular matrix (Maroudas, 1975) and mediate cell responses to compression (Quinn et al., 1998). Relationships between mechanical loading and matrix fluid flows are therefore central to the function and physiology of cartilage and related tissues.

Pressure-driven fluid flows in cartilage are governed by the matrix hydraulic permeability, a property of porous materials representing the proportionality between area-averaged flow velocity and fluid pressure gradient (Bear, 1972). Values of this parameter in cartilage are typically in the range of $\sim 0.1\text{–}10 \times 10^{-15}\,\mathrm{m^2/Pa\,s}$ (Mow et al., 1984; Frank and Grodzinsky, 1987; Chen et al., 2001; Jurvelin et al., 2003). Cartilage hydraulic permeability is determined primarily by the density of matrix proteoglycans (Zamparo and Comper, 1989), and varies with tissue composition and mechanical deformations (Maroudas, 1975; Mow et al., 1984; Chen et al., 2001). Direct measurement of hydraulic permeability in cartilage (by applying a pressure gradient and measuring the flow rate) is difficult because of the extremely low fluid

*Corresponding author. Tel.: +41 21 693 83 50; fax: +41 21 693 86 60.
E-mail address: thomas.quinn@epfl.ch (T.M. Quinn).

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velocities involved and because of flow-induced matrix compaction, which can deform the tissue and thereby alter the parameter being measured (Mansour and Mow, 1976; Barry and Aldis, 1990). Cartilage hydraulic permeability is therefore often measured indirectly and in combination with other matrix properties (Frank and Grodzinsky, 1987; Chen et al., 2001). As a result, direction-dependent mechanical behavior of cartilage (Jurvelin et al., 2003) is difficult to interpret because independent data for the direction-dependence of hydraulic permeability and other properties are sparse. Compression-induced anisotropic hydraulic permeability has been hypothesized to play important physiological roles in cartilage lubrication (McCutchen, 1962), load bearing (Oloyede and Broom, 1994), and mechanotransduction (Quinn et al., 1998). Anisotropic hydraulic permeability has been observed in cartilage-like materials (Higginson et al., 1976; Iatridis et al., 1998; Gu et al., 1999), indirectly suggested by consolidation experiments (Oloyede and Broom, 1994), and has been predicted to arise from anisotropic matrix structure or from microstructural alterations during tissue compression (Quinn et al., 2001), but has not yet been directly observed in articular cartilage (Mow and Guo, 2002). Preliminary efforts in this area remain inconclusive partially because they have relied upon measurements of hydraulic permeability made in different directions within different explants (Hedbys and Mishima, 1962; Iatridis et al., 1998; Gu et al., 1999; Jurvelin et al., 2003), rather than direct observation of anisotropy in individual specimens as has been achieved with other tissues (Kohles et al., 2001).

Our objectives were therefore to measure hydraulic permeability in two different directions within individual statically compressed cartilage explants. A direct method was employed wherein small pressure gradients were applied to minimize flow-induced matrix compaction, with microscope-based measurement of fluid flow rates.

2. Methods

Refrigerated humeri of 18-month old cows were obtained within 24 h of slaughter. Four millimeter diameter osteochondral cores were taken from the proximal articular surface using a biopsy drill and bone saw (Stratec, Oberdorf, Switzerland) under irrigation with phosphate buffered saline (PBS; without Ca^{2+} or Mg^{2+}). Using a microtome (Leica RM 2135) a superficial cartilage layer $\sim 100\,\mu m$ thick was removed and then a cartilage disk ~ 650–$950\,\mu m$ thick was cut consisting primarily of intermediate zone cartilage. Explant disks were stored frozen in PBS containing protease inhibitors (Complete tablets, Boehringer Mannheim) and 0.1 mg/ml sodium azide (Sigma), then defrosted for 2 h in PBS prior to experiments. Free-swelling explant dimensions were measured under a dissection microscope and used as references for applied deformations.

Explant disks were sandwiched between two precision-machined plexiglass blocks. Along the explant axis, 300 μm diameter holes were drilled through both blocks, providing conduits to PBS reservoirs (Fig. 1). Both plexiglass blocks also contained 4 mm diameter × 100 μm deep recesses to keep explants centered during mounting and compression. An O-ring around explants functioned as a gasket. Between the explant radial boundary and the O-ring, a second 300 μm diameter hole was drilled into the lower plexiglass block, providing a conduit to another PBS reservoir. Care was taken during assembly to avoid air bubbles inside the apparatus. The explant therefore represented the only significant flow resistance between the three PBS reservoirs (Fig. 2). In experiments, the reservoir above the explant was a column of PBS in flexible silicone tubing (MasterFlex) which applied a small, constant hydrostatic pressure to a 300 μm diameter region on the explant upper axial surface. Outflows through a corresponding surface on the lower axial surface (axial outflow) and through the explant radial edge (radial outflow) were directed into two glass capillaries (Figs. 1 and 2) of 340 μm inner diameter (Drummond Scientific) leading to atmospheric conditions.

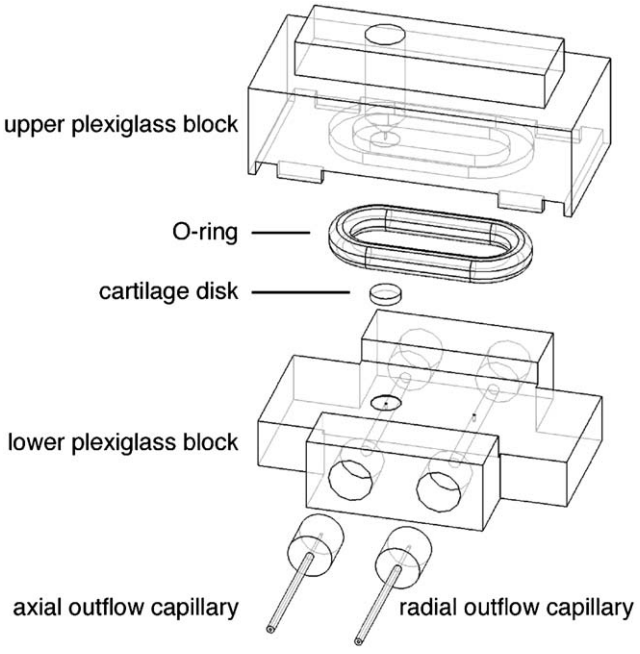

Fig. 1. Exploded view of apparatus used to measure hydraulic permeability of statically compressed cartilage explants. Cylindrical explant disks were sandwiched between precision-machined plexiglass blocks. Above and below explants, 300 μm diameter holes centered along the explant axis provided conduits for fluid flow.