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Solute transport across the articular surface of injured cartilage

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ABSTRACT

Solute transport through extracellular matrix (ECM) is important to physiology and contrast agent-based clinical imaging of articular cartilage. Mechanical injury is likely to have important effects on solute transport since it involves alteration of ECM structure. Therefore it is of interest to characterize effects of mechanical injury on solute transport in cartilage. Using cartilage explants injured by an established mechanical compression protocol, effective partition coefficients and diffusivities of solutes for transport across the articular surface were measured. A range of fluorescent solutes (fluorescein isothiocyanate, 4 and 40 kDa dextrans, insulin, and chondroitin sulfate) and an X-ray contrast agent (sodium iodide) were used. Mechanical injury was associated with a significant increase in effective diffusivity versus uninjured explants for all solutes studied. On the other hand, mechanical injury had no effects on effective partition coefficients for most solutes tested, except for 40 kDa dextran and chondroitin sulfate where small but significant changes in effective partition coefficient were observed in injured explants. Findings highlight enhanced diffusive transport across the articular surface of injured cartilage, which may have important implications for injury and repair situations. Results also support development of non-equilibrium methods for identification of focal cartilage lesions by contrast agent-based clinical imaging.

Introduction

Solute transport through the extracellular matrix (ECM)[1] of articular cartilage is an ubiquitous aspect of cartilage physiology, as well as an essential process for contrast agent-based cartilage imaging [1–3]. Articular cartilage is composed primarily of a water-rich ECM which surrounds chondrocytes (cartilage cells) and consists mainly of proteoglycans and collagen type II as structural components [4,5]. Since cartilage is avascular, chondrocytes depend on solute transport through the ECM for delivery of nutrients, growth factors, and cytokines, disposal of wastes, and deposition of newly synthesized matrix molecules [1,6]. Contrast agent-based clinical imaging of articular cartilage such as X-ray computed tomography (CT) and magnetic resonance (MR) imaging also relies upon transport of contrast agents through cartilage ECM, mainly through diffusion [7–9].

Mechanical injury is likely to have important effects on solute transport through cartilage, and particularly across the articular surface. Cartilage injury is associated with: (i) changes in ECM composition and structure, such as collagen network rupture, diminished glycosaminoglycan (GAG) content, and increased water content, (ii) chondrocyte death; and (iii) articular fissures associated with tissue mechanical failure [10–14]. Previous studies have established that GAG and water content in cartilage correlate strongly with solute transport parameters [15–19]. However, the effects of injury-associated articular fissures on solute transport are still largely unknown. One previous study reported that the diffusion flux of ioxaglate through the articular surface was significantly higher in mechanically injured cartilage [20]. This suggests that transport of a wide range of solutes may be affected by mechanical injury, with important implications for cartilage physiology and contrast agent-based clinical imaging.

Non-invasive techniques for contrast agent-based clinical imaging have been developed to evaluate cartilage biochemical composition and integrity, such as delayed gadolinium-enhanced MR imaging of cartilage (dGEMRIC) [21–23] and CT-based methods [2,19,24]. These techniques assume that contrast agent reaches a steady-state distribution within cartilage ECM, which poses a serious drawback in clinical application due to the long equilibration periods required and the difficulty (if not impossibility) of achieving a true steady-state distribution *in vivo* [25,26]. A previous study reported that it is possible to detect cartilage injury shortly after the injection of contrast agent by measuring its diffusion instead of its equilibrium distribution, thereby avoiding long waiting times for equilibration [20]. Therefore, study of both equilibrium and non-equilibrium transport in injured cartilage may provide useful information for development of contrast agent-based imaging of articular cartilage.

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[1] *Abbreviations used:* ECM, extracellular matrix; CT, computed tomography; MR, magnetic resonance; PBS, phosphate buffered saline.

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In this study, we hypothesize that cartilage mechanical injury can have important effects on solute transport across the articular surface since injury can alter the composition and structure of cartilage ECM and increase the area available for transport by introduction of surface fissures. The effective partition coefficients and diffusivities of various solutes were measured for transport across the articular surface of cartilage explants injured by an established protocol. Experiments included solutes with a wide range of molecular weights (from 0.4 kDa fluorophores to 40 kDa dextrans), a growth factor-like protein (insulin), an ECM macromolecule (chondroitin sulfate), and a CT contrast agent (sodium iodide).

Methods

Osteochondral explants

Adult (18-month old; skeletally mature) bovine femurs (each joint from a different animal) were obtained fresh from a local slaughterhouse. Cylindrical osteochondral explants of 5 mm diameter were obtained from the femoral head using a power drill and stored frozen at −20 °C in phosphate buffered saline (PBS) containing 0.1 mg/ml sodium azide and protease inhibitors (Sigma–Aldrich). Frozen osteochondral explants were thawed at room temperature prior to experiments. Just prior to the application of injurious mechanical compression (below), the cartilage on osteochondral explants was trimmed to 2.5 mm diameter using a biopsy punch (Miltex). Explants were assigned randomly into 3 groups (uncompressed controls, injured, and sliced positive controls) for use in partition coefficient, diffusivity, biochemical and mechanical characterization experiments (below).

Mechanical injury

Osteochondral explants were subjected to radially unconfined axial compression within a precision mechanical loading apparatus consisting of a load cell (Model 31, Honeywell) and displacement actuator (LTA-HL, Newport) mounted in an aluminum and stainless steel frame. Explants were compressed between a steel loading post on the articular surface, and a steel and plexiglass support chamber. The displacement actuator raised the support chamber to push the explant against the loading post which was attached to a load cell. The device was interfaced to a PC using instrumentation software (LabVIEW) for continuous recording of stress and strain. Loading conditions were determined from previous studies for the induction of relatively high strain rate impact-like damage [12]. Injurious loading consisted of a single compressive ramp at strain rate 0.7 s^{-1} and peak stress 14 MPa. The high strain rate loading from this protocol was reported to cause surface fissures in approximately 90% of cases, tissue swelling, and GAG loss [12,27,28].

Injured explants were kept in PBS at room temperature for 16 h before proceeding with experiments. The 16-h waiting period after injury was designed to allow the explant plenty of time to achieve its free swelling state after compression, but also to begin assessment of changes of solute transport at a relatively early stage following injury.

Sliced positive controls for injury-induced fissures

The injurious loading protocol tended to introduce fissures in the cartilage articular surface with a depth of roughly 200 μm (Fig. 1B). To investigate the effects of this increase in surface area due to fissures on effective transport parameters, positive controls were generated by slicing the articular surface of uncompressed explants. Slices involved a vertical 200 μm deep cut across a diameter of the articular surface using a vibrating blade microtome (VT1200S, Leica) (Fig. 1C and F). Sliced explants were kept in PBS at room temperature for 16 h before proceeding with experiments.

Solutes

Fluorescein isothiocyanate (FITC; 0.4 kDa) and FITC-dextrans (4 and 40 kDa) and sodium iodide were from Sigma–Aldrich. FITC-insulin (5.8 kDa) and FITC-chondroitin sulfate (20 kDa) were conjugated by established protocols [29]. FITC, insulin from bovine pancreas, and chondroitin sulfate sodium salt from shark cartilage were from Sigma–Aldrich. Before experiments, FITC-conjugated solutes were filtered using PD-10 columns (GE Healthcare Bio-Sciences) to remove trace amounts of free FITC molecules. Solutes were selected to have a wide range of molecular weights and similarities to macromolecules which have important functional roles in cartilage. FITC and sodium iodide are small, rigid molecules; dextrans and chondroitin sulphate are linear polysaccharides; and insulin is a globular protein. Chondroitin sulphate is one of the major components of cartilage ECM, insulin is structurally similar to insulin-like growth factors and sodium iodide is a commonly used CT contrast agent for cartilage imaging.

Partitioning measurement

For each solute, 18 explants obtained from 3 to 4 joints were randomly distributed into 3 groups (uncompressed control, injured and sliced) for partitioning measurements. All explants in partitioning experiments were carved to have identical dimensions in order to minimize the confounding effects of solute attachment to explant surfaces [30]. Cartilage explants (2.5 mm in diameter) were sectioned to 600 μm thickness with a microtome (VT 1200, Leica Microsystems) with the articular surface intact, and allowed to equilibrate for 16 h in an absorption bath of FITC, FITC-conjugated solute or sodium iodide (15 μM FITC, 25 μM FITC-dextran 4 kDa, 4 μM FITC-dextran 40 kDa, 85 μM FITC-insulin, 70 μM FITC-chondroitin sulfate, 67 μM sodium iodide). The next day, the explant was blotted lightly using a tissue to get rid of excess bath solution on the surface, moved into a desorption bath (150 μL of PBS), and again left to equilibrate overnight to obtain the final desorption bath concentration. Both absorption and desorption were conducted at 22 °C.

For fluorescent solutes, the equilibrium absorption and desorption bath concentrations were measured directly from fluorescence with a plate reader (LB940, Berthold Mitras) while for sodium iodide a colorimetric iodide assay was used [31]. Pilot studies were conducted to establish linear calibrations between fluorescence intensities and solute concentrations for all fluorescent solutes; and between absorbance (at 610 nm) and iodide concentrations for sodium iodide. After desorption, explants were lyophilized (Labconco FreeZone 2.5). Wet and dry weights of cartilage explants were measured using an analytical balance (AL204, Mettler Toledo). Explant water contents were the difference between wet and dry weights.

The solute effective partition coefficient K was defined as the ratio of solute concentration within the cartilage fluid to that within surrounding fluid. K was determined from conservation of solute in the desorption bath [32]:

$$K = \frac{c_d V_d}{V_f (c_a - c_d)} \tag{1}$$

where c_a and c_d represent the equilibrium adsorption and desorption bath solute concentrations, V_d is desorption bath volume and V_f is explant fluid volume. K was termed an "effective" partition coefficient because the measurement reflected several factors