Video Article

An Experimental and Finite Element Protocol to Investigate the Transport of Neutral and Charged Solutes across Articular Cartilage

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Abstract

Osteoarthritis (OA) is a debilitating disease that is associated with degeneration of articular cartilage and subchondral bone. Degeneration of articular cartilage impairs its load-bearing function substantially as it experiences tremendous chemical degradation, *i.e.* proteoglycan loss and collagen fibril disruption. One promising way to investigate chemical damage mechanisms during OA is to expose the cartilage specimens to an external solute and monitor the diffusion of the molecules. The degree of cartilage damage (*i.e.* concentration and configuration of essential macromolecules) is associated with collisional energy loss of external solutes while moving across articular cartilage creates different diffusion characteristics compared to healthy cartilage. In this study, we introduce a protocol, which consists of several steps and is based on previously developed experimental micro-Computed Tomography (micro-CT) and finite element modeling. The transport of charged and uncharged iodinated molecules is first recorded using micro-CT, which is followed by applying biphasic-solute and multiphasic finite element models to obtain diffusion coefficients and fixed charge densities across cartilage zones.

Video Link

The video component of this article can be found at https://www.jove.com/video/54984/

Introduction

Molecular transport plays a vital role in the homeostasis of articulating joints, delivery of therapeutics to articular cartilage and contrast-enhanced cartilage imaging [1,2,3]. Factors such as cartilage integration and intactness, solute charge and size as well as osmolality and concentration of bath in contact with cartilage may influence the transport rate [4,5,6]. The transport of solutes, either neutral or charged, can be different between articular cartilage zones, because each zone consists of different concentrations and orientations of major extracellular matrix molecules, namely proteoglycans (PGs) and collagen type II [1,7,8,9,10,11]. More importantly, the transport of charged solutes can be highly dependent on the concentration of proteoglycans comprising negative fixed charges within the extracellular matrix which increases across articular cartilage [8,9]. Those parameters particularly fixed charge density (FCD), orientation of collagen fibrils and water content variation across cartilage may undergo alterations as osteoarthritis (OA) progresses, thereby signifying the importance of studying diffusion across cartilage.

In the current study, a protocol based on previously established experimental and computational studies [6,8,9] is proposed to accurately investigate diffusion under various boundary conditions using neutral and charged solutes in a finite-bath model of diffusion. The proposed methods are composed of micro-Computed Tomography imaging (micro-CT) of a system including cartilage and a finite-bath supported by advanced biphasic-solute and multiphasic finite element models. These models enable obtaining the diffusion coefficients of neutral and charged molecules as well as FCDs across various zones of articular cartilage. Using these models, one can gain better understanding of the behavior of the diffusing neutral and charged molecules that could be used to investigate the interactions between cartilage and overlaying finite-bath.

Protocol

NOTE: The protocol presented here is adopted from the experimental and computational procedures of recent research papers [6,8,9]. The protocol is illustrated in **Figure 1**.

The cadaveric materials were collected with permission from veterinary faculty of Utrecht University.

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1. Sample and Bath Preparation

1. Drill out cylindrical osteochondral plugs (diameter of 8.5 mm) from cadaveric equine femoral condyles using custom-made drill bit (**Figure 1**) while spraying cool Phosphate-buffered Saline (PBS) to prevent overheating and subsequent cartilage damage.
2. Heat-shrink the osteochondral plugs with a plastic shrinking sleeve to minimize the lateral diffusion of the overlaying bath.
    1. Mount the osteochondral plug with cartilage at the top initially inside the plastic shrinking sleeve and follow by blowing hot air to it. Add wet cotton pieces on the surface of cartilage to prevent heat-related damage.
3. Prepare finite-baths of charged (*e.g.,* 650 µL, 420 mM, ioxaglate, Molecular Weight (MW) = 1,269 Da, charge = -1) and neutral (650 µL, 420 mM, iodixanol, MW = 1,550 Da) solutes separately.
4. Load the prepared finite-baths on the surface of cartilage using a syringe and place a cork plug on the wrapped sample to prevent evaporation during experiments at RT (**Figure 2A**). To study the neutral solute transport, place the iodixanol bath and to study the negatively charged solute transport place ioxaglate bath onto the cartilage surface.

2. Imaging and Image Processing

1. Place the wrapped samples isolated with cork plug on a custom-made holder attached to the motorized stage of a micro-CT. Place the sample so that cartilage surface covered with contrast agent solution is facing upward.
2. Scan using the micro-CT (voxel size of 40 × 40 × 40 μm^3, scan time of 2 min , tube voltage of 90 kV and tube current of 180 µA) a field of view consisting of cartilage, subchondral plate, and finite-bath in several time points until equilibrium state (48 h) is reached (**Figure 2A**). The equilibrium state is achieved when concentration values do not change over time.
3. Register the 3D images in different time points based on the initial image to facilitate positioning of the Region of Interest (ROI) using manufacturer's software (*e.g.,* Analyze).
4. Convert the 3D reconstructed micro-CT images into 2D Tagged Image File Format (TIFF) stack before processing them using manufacturer's software.
5. Globally segment (ImageJ>Adjust>Threshold) the cartilage from the subchondral bone and overlying bath in the software.
6. Obtain the average grey value of cartilage at different time points (ImageJ>Analyze>Measure) using the generated cartilage mask in the previous step.
7. Given the initial bath concentration and initial contrast agent concentration in the cartilage, use a linear calibration curve to convert the average grey values to the actual concentration of solutes. Previous data support the fact that grey values maintain a linear relationship with the concentration of contrast agents.
8. Plot the solute concentrations versus experimental time points.

3. Computational Modeling

NOTE: The diffusion in this problem is assumed to take place in 1D (along the z-axis), which complies with the experimental boundary condition. Therefore, the geometry could be arbitrarily created.

1. Build finite-bath based cartilage multi-zone models: 1) cartilage consisting of superficial zone (20% of the total cartilage thickness), middle zone (50% of the total cartilage thickness) and deep zone (30% of the total cartilage thickness) [12] and 2) finite-bath in FEBio [13,14] (**Figure 2B**).
2. Assign the mechanical and physical properties of different zones of cartilage and bath in FEBio. Young's modulus (10 MPa) was assumed to be high enough to resist the osmotic pressure exerted by the overlying bath and therefore protect the cartilage from excessive deformations.
    1. Use a hydraulic permeability of 10^{-3} mm^4/Ns and Poisson's ratio of 0. Use actual solute diffusion coefficient of the bath in the simulations [8,9].
3. Generate mesh (8-node trilinear hexahedral elements) and refine it near the boundaries (**Figure 2B**) [8,9].
4. Biphasic-solute model
    1. Apply initial solute concentration in the bath and effective pressure corresponding to it. Look at the description of effective pressure in [9,15].
    2. Run the model in transient mode to obtain solute concentration versus time curves according to the prescribed diffusion coefficients in different cartilage zones.
5. Multiphasic model
    NOTE: The electric fluctuation between bath and tissue can be circumvented by adding two monovalent counter-ions to both the bath and the tissue.
    1. For steady-state models: use the same effective fluid pressures and concentrations in cartilage and overlying bath while increasing FCD to its desired value.
    2. For transient models: create a well-stirred finite-bath by keeping the solute diffusion coefficient in the bath sufficiently high. Inject the solute from the bath-air interface into the bath to reach its desired concentration value.
    3. Transient: remove the prescribed solute concentration boundary condition in the previous step and revert the diffusion coefficient of the finite-bath to its actual diffusion coefficient.
    4. Run the model to obtain solute concentration-time curves based on applied FCDs and diffusion coefficients in different cartilage zones.
6. FEBio-MATLAB interface
    1. Develop a MATLAB code to automatically perform simulation in FEBio and plot concentration-time curves (FEBio-MATLAB interface) [8,9].
    2. Change diffusion coefficients and FCDs in cartilage zones using FEBio-MATLAB interface. Run models in FEBio and extract solute concentration-time curves [8,9].

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3. Compare the obtained solute concentration-time curves with the experimental data and obtain sets of diffusion coefficients and FCDs in different cartilage zones based on minimum root mean square error (RMSE) [8,9].

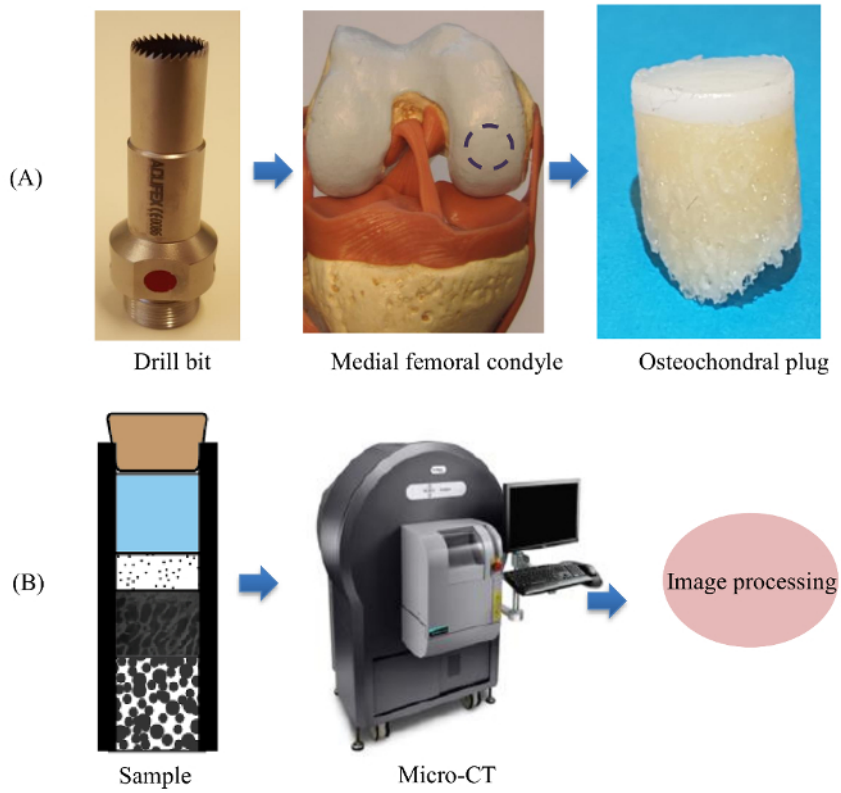

Figure 1: Experimental Setup. A) Sample extraction procedure using a custom-made drill bit. **B**) Micro-CT imaging procedure to monitor diffusion process. Please click here to view a larger version of this figure.

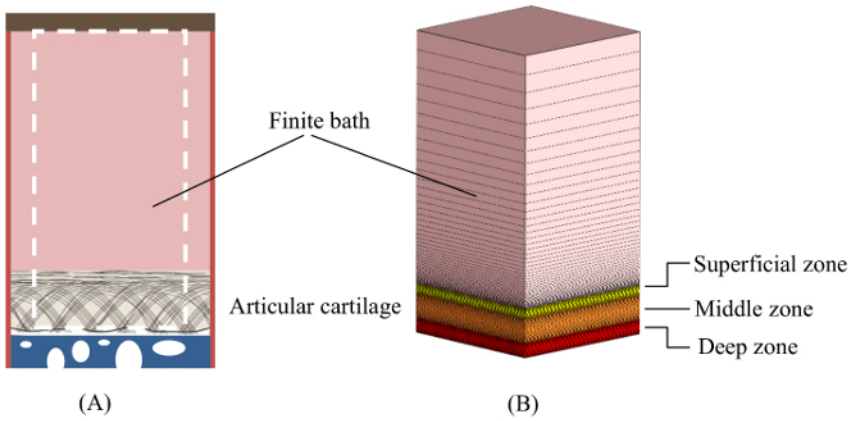

Figure 2: Schematic. A) Experimental design. **B**) Multi-zone computational model consisting of the finite bath, superficial, middle and deep zones of cartilage and associated mesh. Please click here to view a larger version of this figure.

Representative Results

The representative results provided here are adopted from previous research papers [6,8,9,16].

In OA, articular cartilage undergoes significant changes most importantly GAG loss, and collagen fibril damage [17,18,19]. Those changes may affect the diffusive behavior of solutes through articular cartilage [20,21]. We studied axial diffusion of two iodinated contrast agents, *i.e.* iodixanol (charge = 0) and ioxaglate (charge = -1), in cadaveric equine osteochondral plugs using micro-CT. To quantify the diffusion process of a neutral solute (iodixanol), a biphasic-solute model and a charged solute (ioxaglate) multiphasic model were developed in FEBio that considered the zonal structure of cartilage. The biphasic-solute and multiphasic models could predict the diffusion of iodixanol and ioxaglate across articular cartilage (**Figure 3**). These models enabled obtaining diffusion coefficient of iodixanol (biphasic-solute) and diffusion coefficient as well as FCD (ioxaglate) in different cartilage zones [8,9].

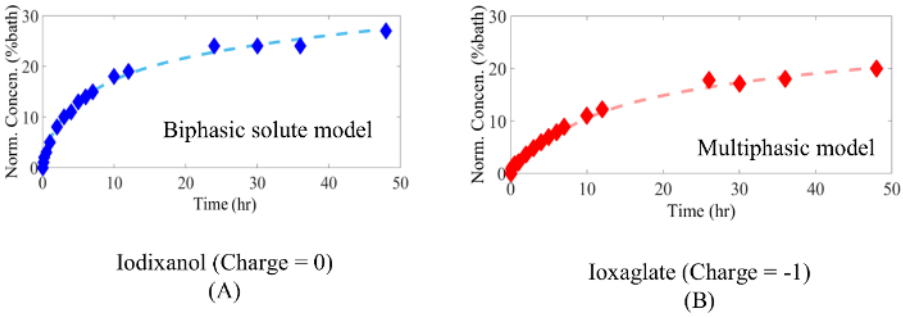

Figure 3: Computationally Curve-fitted Data. A) Multi-zone biphasic-solute (dashed) versus experimental data and **B**) multiphasic models fits (dashed) versus experimental data (symbol) [8,9]. Please click here to view a larger version of this figure.

Discussion

We presented an experimental protocol combined with a finite element modeling procedure to study the diffusion of neutral and charged solutes across articular cartilage. According to our recent studies, the proposed models could accurately describe the transport of both neutral (biphasic-solute) and negatively charged (multiphasic) solutes across different zones of articular cartilage [8,9]. It is widely believed that articular cartilage becomes functionally limited through loss of its vital components such as negatively charged GAG macromolecules as well as collagen fibrils during OA progression [21,22,23,24]. Using the technique proposed in this study, one can potentially examine the healthiness of articular cartilage. The transport of neutral solute may be augmented in OA primarily because of lower interactions between the solute as well as GAGs and collagens. On the other hand, the transport of negatively charged solutes could help acquire information with respect to the concentration of fixed charges of proteoglycans, thereby giving some indication of the level of OA progression.

The biphasic-solute and multiphasic models that were developed based on the emergence of finite-bath concept according to the previous studies could serve as platforms by which accurate estimation of zonal properties of articular cartilage could be provided. Limitations associated with large baths of contrast agents, namely possible beam-hardening artifacts, and assigning a single diffusion coefficient to articular cartilage [7,20,25,26,27,28,29,30] drove the motivation to develop the current study. In the future OA research, our developed models might potentially find applications for early OA diagnosis.

There are some critical steps required both in experiments and computational simulations. To preserve cartilage integrity during experiments, one would require adding sufficient amounts of protease inhibitors to prevent subsequent enzymatic activities. In case of using one sample for more than one experiment, wash-out time for the penetrated solutes after equilibration was approximately 48 h. The wash-out efficiency needs to be checked using micro-CT. When using our set up employing equine cartilage, the minimum volume of the overlying bath equivalent to an infinite bath was calculated to be five times higher than cartilage volume. Moreover, to perform finite element modeling of diffusion, it is critical to apply actual solute diffusion coefficient in the bath since deviation from that could affect the outcomes.

In the underlying finite element models, solute size effect was not implemented and therefore could not be studied. Our proposed finite-bath model offers some advantages, namely understanding diffusion process in the articulating joints more appropriately, and reducing beam hardening artefacts. Our proposed technique combining experiments and computational models enables scrutinizing the diffusion attributes of cartilage when positively charged contrast agents are applied. The knowledge of actual solute diffusion coefficient in the bath seems to be crucial as that might significantly affect the accuracy of the obtained diffusion coefficients across articular cartilage. This would require either obtaining the diffusion coefficient of bath experimentally or readily using literature values.

In conclusion, we proposed a general protocol consisting of experiments and computations to investigate the transport of charged and uncharged solutes across articular cartilage. Using the protocol, one can successfully obtain diffusion coefficients and fixed charged densities in different cartilage layers.

Disclosures

The authors have nothing to disclose.

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