Comparison of convective and diffusive flows in articular cartilage

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BME 4010
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INTRODUCTION

Nutrient transport in an avascular environment is one of the major barriers to successful transplant of natural and engineered tissues. Without vascularization, transport occurs mainly by diffusion. Here we will examine the mechanisms of nutrient transport in cartilage, a tissue with naturally low vascularity which still manages to maintain metabolic activity. Articular cartilage is important in the function of joints, in particular, in preventing loads from causing mechanical trauma to the surrounding bone. Chondrocytes, the biosynthetic cells of the cartilage, require nutrients in order to maintain the extracellular matrix that gives the tissue its functionality [1]. In articular cartilage, physical loading provides a mechanism for creating convective nutrient transport and allows nutrient flow to occur more quickly than in other avascular diffusion-limited systems [2]. Cartilage thus represents an important model of effective nutrient distribution in diffusion-limited tissues.

Previous research has used applied electric fields to model the physical loading that causes convective fluid flow of various solutes in cartilage. Convective flow is driven by the electroosmotic and electrophoretic forces. Electrophoresis occurs when a charged particle migrates through a neutral background due to an applied current and electroosmosis is the bulk flow of liquid over a charged surface under the applied current [3]. Charged solutes thus experience both electroosmotic and electrophoretic forces, and neutral solutes only experience electroosmotic forces. Garcia et. al [2] found that both charged and neutral solutes showed changes in flux under an applied field, indicating that electroosmotic flow is very important in creating convection.

The objective of this experiment is to determine the relative importance and timescales of convective and diffusive transport in hyaline cartilage. This is accomplished by measuring transport of fluorescein, a negatively charged fluorescent protein, through an Ussing chamber. In the first portion of the experiment, diffusion is measured via spectroscopy. We then induce an electric potential to simulate the convective flow induced *in vivo* by physical loading. Fluxes under diffusive and convective conditions are compared to determine the relative importance of the two transport methods. Fluorescein data is also compared to known flux and diffusivity data of fluorescein di B-D galactoside, a larger and uncharged compound, to determine solute effects on transport.

MATERIALS AND METHODS

Diffusive Flow

The experimental setup consisted of a section of bovine articular hyaline cartilage (thickness 1.95mm) placed in an Ussing chamber as shown in figure 1. A peristaltic pump (Masterflex) controlled flow of fluorescein and PBS through the Ussing device and its upstream and downstream compartments, ensuring all were well mixed. The upstream chamber, a 15mL conical tube, contained 12 mL of the 0.5mg/mL fluorescein. The downstream chamber, a cuvette, contained 7.5mL PBS. Electrodes were also set up in the Ussing chamber in preparation for convective flow experiments. The downstream chamber was placed in-line with the spectrophotometer and absorption was measured using Ocean Optic Spectrophotometry software with an integration time of 3 seconds. Absorbances were read at 487nm and 600nm. The values read at 600nm were considered

background fluctuations and subtracted from values read at 487nm, the fluorescein wavelength. All data analysis was done using this $A_{600\text{-}400}$ value.

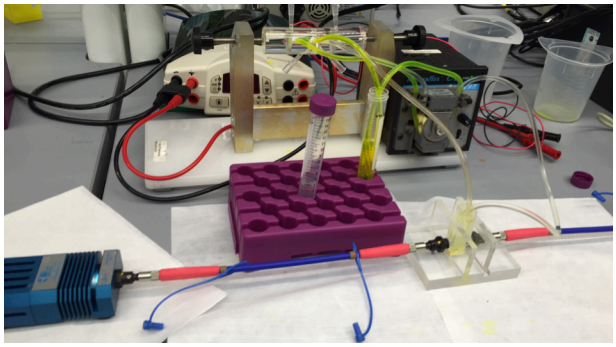

Figure 1. Experimental setup

Convective Flow

An electric potential was induced over the Ussing device to examine convective flow. Electrodes were connected initially such that the upstream solution contacted the positive terminal and the downstream contacted the negative terminal. Current was set to a constant 5mA. After 15 minutes, the electrodes were reversed to change the current direction. Absorbance was measured in the same manner as described for diffusive flow.

Calibration

A calibration curve for absorbance was created using 7 dilutions (1, ½, ¼, 1/8, 1/16, 1/32, 0) of a .025 mg/mL fluorescein solution. Absorbance was measured in the same manner as described for diffusive and convective flow and converted to concentration using Beer's law, which describes a linear relationship between absorbance and concentration $A = \varepsilon bc$ where A is absorbance, c is concentration, ε is the molar absorptivity and b is the path length.

Analysis

Flux for each condition was calculated as

$$F = \frac{\Delta C_s^d}{\Delta t}\frac{V^d}{A}$$

where ΔC_s^d represented the change in downstream concentration of solution, V^d represents the downstream compartment volume, and A represents the cross-sectional area of exposed cartilage. $C_s^d V^d$ was plotted against time, and $\frac{\Delta C_s^d}{\Delta t}V^d$ was taken as the slope of the linear regression of this plot. For the case of convective transport after switching the electrodes, the regression was taken after current reached a steady state as shown in figure 3.

For the cases of diffusive transport without exposure to current, diffusivity was calculated as

$$D = \frac{F}{\Phi\frac{C_s^u}{\delta}}$$

where F represents the flux calculated as above, Φ is the porosity which was taken to be .75, C_s^u is the upstream solute concentration, and δ is the thickness of the cartilage. This equation for diffusivity is derived from Fick's law using the assumption that the Fick's law term $\frac{\partial C_s}{\partial x}$ can be approximated as $\frac{C_s^u}{\delta}$ given that the downstream concentration of solute is much greater than the upstream solute concentration. All calculations assumed a molecular weight of 376 g/mol for fluorescein and 656 g/mol for fluorescein di B-D galactoside. Cartilage thickness for the fluorescein experiments was measured to be 1.95mm, downstream volume was 7.5mL, upstream concentration was 0.5mg/mL, and the cross-sectional cartilage area was .1133cm^2. For the fluorescein di B-D galactoside experiments, cartilage thickness was 0.5mm, downstream volume was 8mL, upstream concentration was 1.25mg/mL, and the cross-sectional cartilage area was also .1133cm^2.

RESULTS

Linear regression on the calibration curve gave R^2 values of .955 and .996 respectively as show in figure 2.

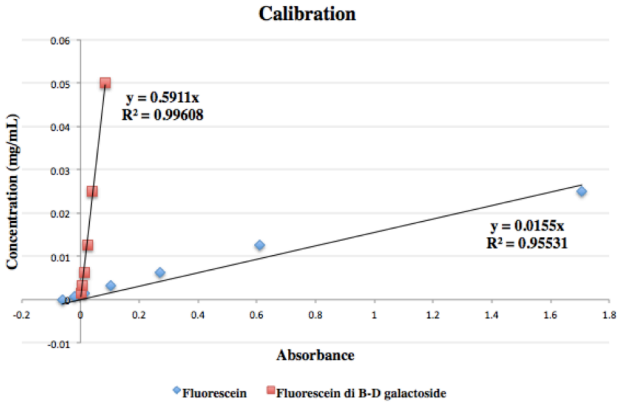

Figure 2. Calibration curves for fluorescein and fluorescein di B-D galactoside.

For fluorescein experiments, diffusive flux was measured from t=0s to t=2045s. Forward convective flux was measured from t=2045s to t=2947s. The current was switched at t=2947s. After allowing time for equalization, reverse convective flux was measured from t=3133s to t=3808s. The same method was applied to analysis of fluorescein di B-D galactoside, for which diffusive flux was measured from t=0 to 1142s, forward convective from t=1142 to 1904s, and reverse convective from t=2046 to 2707s. These results are shown in table 1 along with the calculated diffusivities for each compound. Figure 3 shows concentration times downstream volume versus time for each compound.

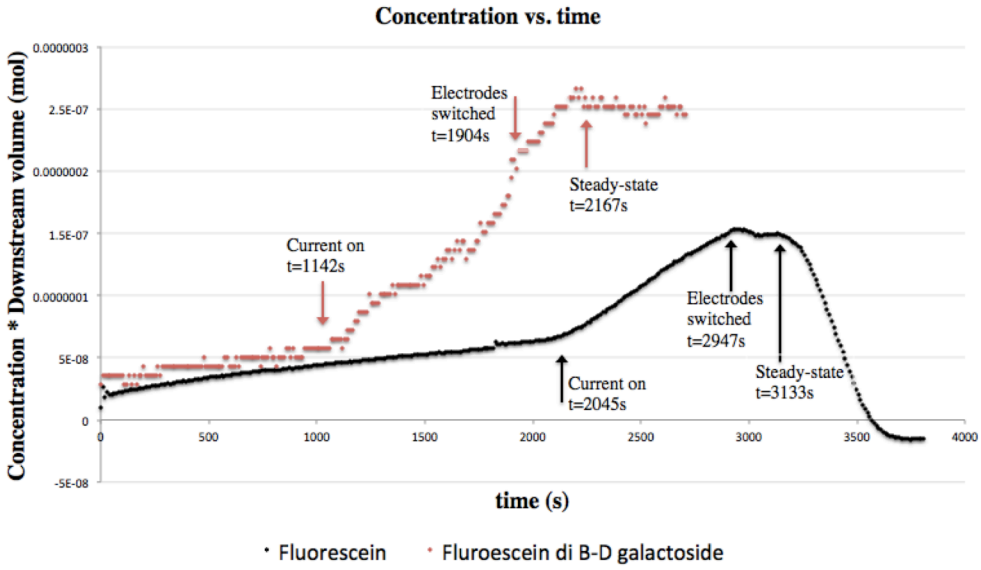

Figure 3. Concentration of solutes was determined by converting spectrophotometric data with Beer's law. The slope of this curve represents the flux times the cross sectional cartilage area (which was the same for all solutes). Arrows show the times at which current was applied. Flux changes at these points are easily observed as changes in slope since cross-sectional area is constant.

	Fluorescein	Fluorescein di B-D galactoside
Diffusive flux (mol/cm^{2}s)	1.79594E-10	2.2495E-10
Forward convective flux (mol/cm^{2}s)	9.62832E-10	1.12606E-09
Reverse convective flux (mol/cm^{2}s)	-2.70802E-09	-2.00446E-10
Diffusivity (cm^2/s)	3.51141E-05	7.87023E-06

Table 1. Fluxes and diffusivities for fluorescein and fluorescein B-D galactoside show increased flux in convective conditions.

DISCUSSION

Both fluorescein and fluorescein di B-D galactoside showed obvious flux changes when subjected to changes in applied electric field. Each showed an increase in forward flux with the initial applied current. Given that this indicates flow from the positive to negative terminal, this result is indicative of a dominant electroosmotic force. An electrophoretic force would have caused motion of the negatively charged fluorescein in the opposite direction. This corresponds with the results of Garcia et al.[1], who observed the same direction of flux for other negatively charged solutes. When the current was reversed, flux direction did as well, indicating the success of the model. *In vivo*, this reverse flux would correspond to flux caused by expansion of the cartilage after load is relieved.

For both fluorescein and fluorescein di B-D galactoside, the flux increased almost exactly five-fold when the initial current was applied, which also corresponds to the findings of 2.4 to 10 fold increases found by Garcia et al. With such a large increase, it is easy to see how convection would be much more important than diffusion in nutrient transport in cartilage.

Fluxes differed in each condition between fluorescein and fluorescein di B-D galactoside, with fluorescein having a generally lower flux except in the case of the reversed field. Generally one would expect that the fluorescein, which has a higher diffusivity, would have a higher flux. Our result is likely due to the electrophoretic effect, which would have increased flux in direction opposite to the current for only the charged species, and to the fact that the upstream concentration of fluorescein di B-D galactoside was much higher than that of fluorescein. The greater flux of fluorescein in the reverse direction can also be attributed to the much lower upstream solute concentration than in the fluorescein di B-D galactoside system.

The diffusivity of fluorescein is greater than that of fluorescein di B-D galactoside, in line with the fact that diffusivity generally increases with molecular weight. Fluorescein di B-D galactoside's diffusivity of $7.9*10^{-6}$ cm^2/s is comparable to the diffusivity of raffinose, a neutral solute of molecular weight 594 Da, which was measured by Garcia et al to be $2.9*10^{-6}$ cm^2/s. Diffusivity of fluorescein, 3.5*10-5cm2/s, was less comparable to the similarly charged and weighted thymidine (242 Da) which Garcia et al measured at $.32*10^{-5}$ cm^2/s [1]. Though the molecular weight difference could have played a part in the difference in diffusivities, the use of a much thicker cartilage slice (1.95mm) than was used in either the Garcia or fluorescein di B-D galactoside experiments (both .5mm) may also have contributed to this error.

The use of applied current as a model makes it difficult to translate the magnitudes of naturally occurring mechanical forces to the desired applied current. A more accurate model would observe flux due to actual mechanical compression and expansion of the chambers. Further work could mitigate this limitation with use of mechanical loading by syringe pump or other methods. However, this experiment was still able to show that convection plays a significant role in solute transport through cartilage and that this effect is due to bulk electroosmotic fluid flow more than electrophoretic flow. It could be argued that convection by mechanical loading is the

evolutionary alternative to convection through vascularization. Though this solution may not be applicable to other tissues with higher oxygen needs than cartilage, it would be interesting to see if inducing mechanical loads could make a difference in the success of poorly vascularized tissue grafts.

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