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Short communication

Diffusion of antibiotics in intervertebral disc

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ABSTRACT

Delivering charged antibiotics to the intervertebral disc is challenging because of the avascular, negatively charged extracellular matrix (ECM) of the tissue. The purpose of this study was to measure the apparent diffusion coefficient of two clinically relevant, charged antibiotics, vancomycin (positively charged) and oxacillin (negatively charged) in IVD. A one-dimensional steady state diffusion experiment was employed to measure the apparent diffusion coefficient of the two antibiotics in bovine coccygeal annulus fibrosus (AF) tissue. The averaged apparent diffusion coefficient for vancomycin under 20% compressive strain was $7.94 \pm 2.00 \times 10^{-12}$ m^2/s (n = 10), while that of oxacillin was $2.26 \pm 0.68 \times 10^{-10}$ m^2/s (n = 10). A student's *t*-test showed that the diffusivity of vancomycin was significantly lower than that of oxacillin. This finding may be attributed to two factors: solute size and possible binding effects. Vancomycin is approximately 3 times larger in molecular weight than oxacillin, meaning that steric hindrance likely plays a role in the slower transport. Reversible binding between positive vancomycin and the negative ECM could also slow down the rate of diffusion. Therefore, more investigation is necessary to determine the specific relationship between net charge on antibiotic and diffusion coefficients in IVD. This study provides essential quantitative information regarding the transport rates of antibiotics in the IVD, which is critical in using computational modeling to design effective strategies to treat disc infection.

1. Introduction

Intervertebral disc (IVD) infection is a dangerous, devastating, and debilitating condition with growing incidence related to the aging population, an increase in spinal surgeries, hematogenous spread, particularly in immunosuppressed patients (e.g., HIV, cancer, rheumatic disease patients), and the rise of intravenous (IV) drug abuse. Currently, disc infections are difficult and costly to treat, often requiring weeks of inpatient care and IV administered drugs. Successful treatment requires antibiotic levels in the IVD above the minimally inhibitory concentrations (MIC). However, the avascular nature of the IVD, coupled with the charged nature of the disc extracellular matrix (ECM), make delivery of antibiotic drugs, which are often charged molecules, to the tissue problematic in practice.

A number of experimental studies have indicated that electrical charge plays a significant role in the penetration of antibiotics into the IVD (Conaughty et al., 2006; Gibson et al., 1987; Riley et al., 1994; Scuderi et al., 1993; Tai et al., 2002; Thomas et al., 1995). Overall, these studies either directly suggested or indicated that positively charged antibiotics have easier access to or higher uptake in the discs than negatively charged ones. We have developed a computational model of the human IVD able to predict the kinetics of antibiotic penetration into the IVD (Zhu et al., 2016). Our numerical prediction was in agreement with these empirical findings, with positively charged drugs having higher concentrations and uptakes than their negatively charged counterparts. However, this computational analysis relied on estimates for antibiotic diffusion coefficients, since quantitative values are not available in the literature. To more accurately predict drug penetration and concentration profiles in the disc, more information is needed on the value of the diffusion coefficient and net charge of relevant drugs in IVD tissues.

To our knowledge, no previous study has quantified the diffusivity of antibiotics in the IVD. Such information is necessary in order to employ computational modeling to design effective strategies to treat disc infection while maintaining drug levels above MIC. To this end, we will measure the apparent diffusion coefficient of antibiotics in IVD tissue. The diffusion coefficient of two clinically relevant antibiotics, vancomycin (positively charged)

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and oxacillin (negatively charged), in bovine coccygeal annulus fibrosus (AF) tissues were investigated in this study.

2. Materials and methods

2.1. Specimen preparation

AF specimens were prepared from the bovine coccygeal IVD. Using a corneal trephine, 6 mm cylindrical punches were cored in the axial direction from the AF section of the IVD (see Fig. 1). Using a sledge microtome with freezing stage, the cylindrical specimens were cut to a final height of 1 mm, as measured using a custom, current-sensing micrometer. A total of 20 specimens were prepared, for a sample size of n = 10 for each molecule investigated. All samples were prepared from the middle AF region of the IVD. Several specimens were prepared from each disc and then divided between the two antibiotics groups.

2.2. Diffusion measurement

The apparent diffusion coefficients of vancomycin and oxacillin were measured using a one-dimensional quasi-steady state experiment, similar to our earlier studies (Jackson et al., 2012; Jackson et al., 2008; Kleinhans et al., 2015; Yuan et al., 2009). A custom diffusion chamber was used, consisting of two acrylic solution chamber halves, separated by a specimen holder in the middle (see Fig. 2). The specimen was held in place with two rigid porous plates (hydrophilic polyethylene, 50–90 μm pore size, Small Parts, Inc., Miami Lakes, FL) to inhibit swelling, and sealed with an o-ring. The compressive strain was controlled by changing of a spacer placed between the two chamber halves. For all experiments, the compressive strain was controlled at 20%.

At the start of the experiment, an initial one hour incubation period was used to facilitate equilibration of the antibiotic in the tissue specimen. During this time, phosphate buffered saline (PBS) solution was filled in the downstream chamber, while a PBS solution containing antibiotic was filled in the upstream chamber. For oxacillin, a concentration of 100 mM oxacillin in PBS was used upstream, while, for vancomycin, a concentration of 10 mM vancomycin in PBS was used. These concentrations were based on preliminary studies for protocol optimization. A magnetic stir rod was placed in each chamber and the diffusion apparatus was placed on a magnetic stir plate at room temperature (~23 °C).

Following the one hour incubation, the downstream chamber was emptied and rinsed with fresh PBS solution. At the start of the experiment, 500 μL of fresh PBS solution as filled in the downstream chamber; the upstream chamber still contained a solution containing antibiotics as described above. The upstream and downstream chambers were both continuously stirred with a magnetic stir bar and stir plate. At 30 min intervals, the contents of the downstream chamber were collected for concentration measurements. The downstream chamber was once again rinsed with PBS, and the experiment was repeated for 10 consecutive intervals of 30 min each (for a total of 300 min). Preliminary studies showed that this was adequate time to reach steady state in the tissue. Representative experimental curves for both antibiotic molecules are shown in Fig. 3.

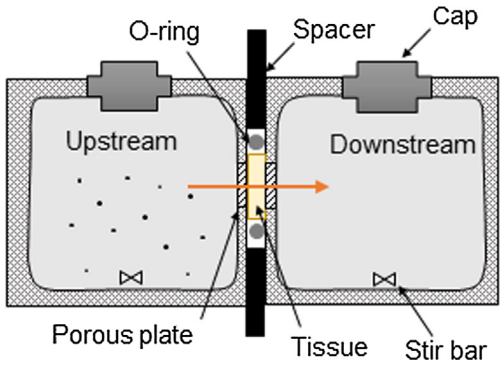

Fig. 2. Schematic of custom diffusion chamber used in 1-D steady state diffusion experiment to calculate the apparent diffusivities of antibiotics in bovine AF. The specimen is sealed between two porous plates and on the radial edge by an o-ring. The level of compression for the specimen is controlled by the size of the metal spacer between the two chamber halves; all specimens were measured at 20% compressive strain. Solute moves from the upstream chamber, through the tissue, to the downstream chamber during the experiment.

Following sample collection, the concentration of antibiotic in the samples from the downstream chamber, as well as the final concentration of antibiotic in the upstream chamber, was determined using the Folin-Ciocalteau reagent colorimetric assay with calibration curve. The absorbance was measured at 750 nm using a BioTek plate reader and Gen5 software.

The apparent diffusion coefficient, D_{app}, of antibiotic in AF tissue was determined based on the average antibiotic concentration at steady state (i.e., average concentration of last three measurements) using the following relationship:

$$D_{app} = \frac{V_{down} C_{down} h}{(C_{up} - C_{down}) A t}$$

where V_{down} is the volume of the downstream chamber, C_{up} and C_{down} are the upstream and downstream antibiotic concentrations,

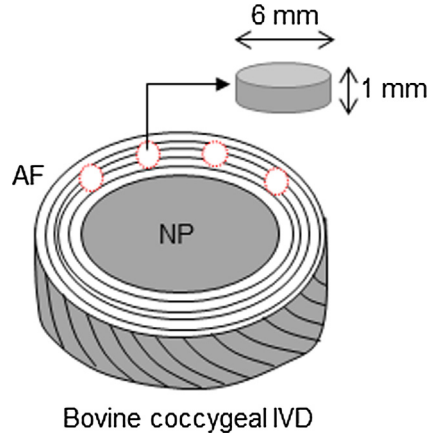

Fig. 1. Schematic showing location, orientation, and size of specimens collected from the AF region of bovine coccygeal IVDs.

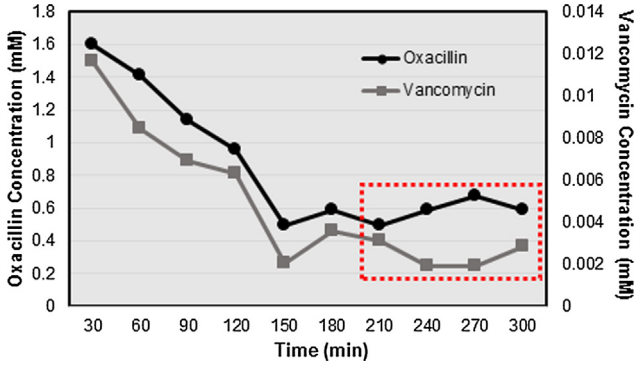

Fig. 3. Representative experimental curves for oxacillin and vancomycin samples. The experiment was carried out for 300 min, with concentration measurements taken every 30 min. The concentration at equilibrium was taken from the averaged concentrations in the final 120 min of the experiment (see box outline).

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