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Molecular transport in collagenous tissues measured by gel electrophoresis

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ABSTRACT

Molecular transport in tissues is important for drug delivery, nutrient supply, waste removal, cell signaling, and detecting tissue degeneration. Therefore, the objective of this study was to investigate gel electrophoresis as a simple method to measure molecular transport in collagenous tissues. The electrophoretic mobility of charged molecules in tissue samples was measured from relative differences in the velocity of a cationic dye passing through an agarose gel in the absence and presence of a tissue section embedded within the gel. Differences in electrophoretic mobility were measured for the transport of a molecule through different tissues and tissue anisotropy, or the transport of different sized molecules through the same tissue. Tissue samples included tendon and fibrocartilage from the proximal (tensile) and distal (compressive) regions of the bovine flexor tendon, respectively, and bovine articular cartilage. The measured electrophoretic mobility was greatest in the compressive region of the tendon (fibrocartilage), followed by the tensile region of tendon, and lowest in articular cartilage, reflecting differences in the composition and organization of the tissues. The anisotropy of tendon was measured by greater electrophoretic mobility parallel compared with perpendicular to the predominate collagen fiber orientation. Electrophoretic mobility also decreased with increased molecular size, as expected. Therefore, the results of this study suggest that gel electrophoresis may be a useful method to measure differences in molecular transport within various tissues, including the effects of tissue type, tissue anisotropy, and molecular size.

1. Introduction

Molecular transport in collagenous tissues is important for drug delivery (Bajpayee et al., 2014; Prausnitz and Noonan, 1998), contrast agent administration (Bansal et al., 2011; Kulmala et al., 2010), detecting tissue degeneration via changes in tissue architecture or composition (e.g., proteoglycan content in cartilage) (Bansal et al., 2011; Torzilli et al., 1997), cellular nutrient supply and waste removal (Knothe Tate et al., 1998; Maroudas et al., 1988), and cellular signaling (Knothe Tate et al., 2000; Swartz and Fleury, 2007). General mechanisms for molecular transport in tissues include mass transport due to a pressure gradient (i.e., fluid flow), concentration gradient (i.e., diffusion), or electric potential gradient (i.e., electrophoresis). Molecular transport within collagenous tissues is primarily impeded by cell networks (e.g., endothelium) (Mehta and Malik, 2006) and the macromolecular organization and composition of the extracellular matrix (Swartz and Fleury, 2007).

Forced fluid flow, or mass transport due to a pressure gradient, has been used to measure the permeability of collagenous tissues and tissue scaffolds (Pennella et al., 2013). A pressure gradient is induced across a tissue sample using either gravity feeding (O'Brien et al., 2007) or a piston/pump (Maroudas, 1968; Naumann et al., 1999). Darcy's law is used to calculate permeability from the flow rate, fluid pressure, and sample dimensions (Mow et al., 1984; Pennella et al., 2013). Challenges and limitations of this approach include a priori knowledge of the fluid properties (e.g., viscosity), non-uniform deformation of the specimen due to the fluid drag force, the application of high pressures, and sealing/clamping specimens (Maroudas, 1968; Mow et al., 1984; Pennella et al., 2013).

Molecular diffusion within collagenous tissues has been investigated by measuring the time-dependent transport of a solute or dye, often from a bath, in specimens due to a concentration gradient. Molecular concentrations within the tissue can be measured from cross-sectional profiles or sequential sectioning using colorimetric intensity (Brower et al., 1962; Kantor and Schubert, 1957), fluorescence (Decker et al., 2013; Knothe Tate et al., 1998; Lee et al., 2011), scintillation counting (Torzilli et al., 1997; 1998), X-ray

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attenuation in computed tomography (Arbari et al., 2015; Bansal et al., 2011; Kulmala et al., 2010; Palmer et al., 2006), or magnetic resonance imaging (Bacic et al., 1997; Kulmala et al., 2010). Site-specific molecular diffusion and relative anisotropy have also been measured using fluorescence recovery after photobleaching (FRAP) and related techniques (Leddy and Guilak, 2003; Travascio et al., 2009). A limitation of these methods is that the diffusion of large molecules (e.g., >500 Da) can exceed the available time window for in vivo imaging prior to washout (removal) of the contrast agent (Burstein et al., 2001; Tiderius et al., 2003). Moreover, long incubation times for diffusion in excised tissue specimens can be prohibitive and possibly lead to erroneous measurements due to tissue degradation (Kantor and Schubert, 1957; Maroudas, 1970; 1976). Finally, the use of a bath requires a large volume of potentially costly solute or dye molecules and can lead to build-up at the tissue interface (Arbari et al., 2015).

Electrophoresis can be used as an alternative and/or complementary method for measuring molecular transport in collagenous tissues to overcome limitations of the above methods. For example, the independent effects of fluid flow, diffusion, and electrophoresis on molecular transport in cartilage were previously investigated using an apparatus with electrodes added to a diffusion chamber (Minerva Garcia et al., 1996). Gel electrophoresis is commonly used to separate proteins of different size and charge for a variety of biochemical assays (Haas et al., 1994; Pluen et al., 1999; Rill et al., 2002). Thus, the necessary apparatus is readily available in most labs and relatively inexpensive. However, gel electrophoresis has not been previously used to measure molecular transport in collagenous tissues.

Therefore, the objective of this study was to investigate gel electrophoresis as a simple method to measure molecular transport in collagenous tissues. The electrophoretic mobility of charged molecules in tissue samples was measured from relative differences in the velocity of a cationic dye passing through an agarose gel in the absence and the presence of a tissue section embedded within the gel. Differences in electrophoretic mobility were measured for the transport of a molecule through different tissues and tissue anisotropy, or the transport of different sized molecules through the same tissue.

2. Materials and methods

2.1. Preparation of buffer and dye solutions

Tris/borate/ethylenediaminetetraacetic acid (TBE) buffer (5X) was prepared in deionized (DI) water with 54 g Trizma base ($\geq$99.9%, Sigma-Aldrich, St. Louis, MO), 27.5 g boric acid ($\geq$99.5%, Sigma-Aldrich), and 20 mL of 0.5 M ethylenediaminetetraacetic acid (EDTA). The EDTA solution was prepared by adding disodium EDTA·$2H_2O$ (99.0–101.0%, Sigma-Aldrich) to DI water under stirring and adjusting the pH to 8.0 using NaOH (Amresco, Solon, OH). As-prepared buffer solutions were passed through a 0.22 μm vacuum filter (Corning®, Sigma-Aldrich) to delay the formation of precipitates. Two cationic dyes were investigated: pyronin Y (302.8 g/mol, Sigma-Aldrich) and safranin O (350.8 g/mol, Amresco). Dye solutions were prepared by adding 5 mg of dye and 3 g sucrose (certified ACS, Fisher Scientific, Waltham, MA) to 7 mL of 1X TBE buffer.

2.2. Tissue samples and preparation

Fresh-frozen bovine flexor tendons were thawed and cut into 2.0 ± 0.2 mm thick sections either parallel or perpendicular to the collagen fibers within the proximal and distal regions which are known to experience distinct tensile and compressive forces, respectively, and exhibit corresponding histological characteristics resembling tendon and fibrocartilage, respectively (Benjamin and Ralphs, 1998; Koob and Vogel, 1987; Vogel and Evanko, 1987). Fresh-frozen bovine articular cartilage from the patella was also thawed and cut into 2.0 ± 0.2 mm thick sections parallel to the patellar surface. The planar area of all tendon and cartilage tissue sections was at least 1 cm^2. All tissue samples were stored in 1X TBE buffer for 12–24 h before embedding in agarose gels.

2.3. Gel electrophoresis

In order to accommodate tissue samples, a custom gel electrophoresis chamber was constructed using a $\sim12\times24$ cm^2 tray with a 0.07 mm diameter stainless steel wire cathode and replaceable anodes connected to an electrical power supply (BioRad PowerPac HC, Hercules, CA). The replaceable anodes were constructed out of plastic tabs (3×7 cm^2) with 0.07 mm diameter stainless steel wire wrapped twice around the bottom and extending to the top. Anodes were preemptively replaced after 45 min of use to prevent failure by corrosion.

The gel was prepared by adding 1 g agarose (molecular biology grade, Thermo Scientific, Rockford, IL) to 100 mL TBE buffer (1X). The solution was heated in a microwave oven for $\sim$2.5 min to dissolve the agarose, removed, and cooled for 5 min. The agarose solution was poured into a tray (8.95×10.55 cm^2 base) and the bottom of the comb was placed $\sim$3 mm above the base of the tray. Tendon and cartilage tissue samples were inserted into the warm liquid gel, directly against the comb. The middle lane of each gel was left open as a control lane. The agarose was solidified for 1 h at room temperature ($\sim$20°C). The solidified agarose gel was placed in the electrophoresis chamber which was filled with 1X TBE buffer to a depth of approximately 2 mm above the gel ($\sim$350 mL TBE). Dye solutions were pipetted in 10 μL aliquots into each well.

Each gel was run between 2 and 3 h at 80 V. After each run, the gel was removed from the tray, placed on a glass slide, and photographed at a resolution of $\sim$100 pixels/cm. The intensity profile of the dye in each lane was measured using ImageJ (v10.2, National Institutes of Health) and fit to a Gaussian distribution (Fig. 1) to measure the mean distance traveled by the dye. The pooled mean coefficient of determination (R^2) for all samples was 0.865. The rate of dye migration through the tissue sample, v (cm/s), was derived (Appendix A) as,

$$v=\frac{w}{t\left(1-\frac{d_i-w}{d_o}\right)} \quad (1)$$

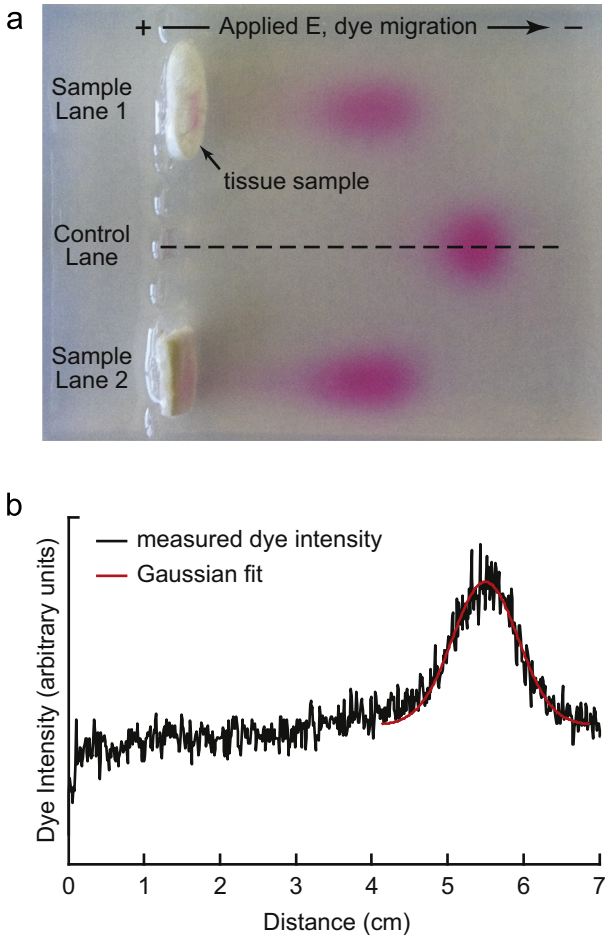

Fig. 1. Electrophoretic mobility in response to an applied electric field (E) was measured in an (a) agarose gel by the delayed migration of a cationic dye through embedded tissue samples (sample lanes) compared to the gel alone (control lane) as quantified by (b) the mean distance of migration from a Gaussian fit of the line intensity profile of each lane.

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