Oxygen Diffusion across Extracellular Matrix Derived Scaffolds

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Statement of Purpose: Biologic scaffolds derived from decellularized mammalian extracellular matrix (ECM) have been used in preclinical and clinical applications for the reconstruction of a variety of tissue structures. Although the remodeling process begins almost immediately upon surgical placement of the ECM scaffold, blood vessel development is more gradual. Cells that infiltrate the scaffold rely on diffusion for the exchange of gases, nutrients, and waste products. Diffusion can be affected by the scaffold's composition and ultrastructure. The purpose of the present study was to compare oxygen diffusion across 3 different ECM scaffold materials using Fick's first law of diffusion. The ECM scaffolds tested were porcine small intestinal submucosa (SIS), urinary bladder matrix (UBM), and urinary bladder submucosa (UBS).

Methods: The preparations of SIS, UBM, and UBS have been previously described[1]. SIS consists of the basilar portion of the tunica mucosa, the muscularis mucosa and the tunica submucosa of the small intestine; UBM consists of the basement membrane and tunica propria of the urinary bladder; and UBS consists of the tunica submucosa of the urinary bladder. Following preparation and disinfection of each ECM scaffold material, they were frozen, lyophilized, and cut into 7 x 3 cm sheets. Samples were terminally sterilized by ethylene oxide exposure with a dosage of 750 mg/hr for 16 hours.

The diffusion cell (Crown Glass, NJ) consisted of two-50 mL glass chambers separated by the ECM scaffold. Oxygen diffusion occurred between the two chambers with a circular area of 2.75 cm^2. Each chamber was filled with 50 mL of sterile filtered, degassed phosphate-buffered saline (PBS). The donor chamber was saturated with ambient air while the receiver chamber was purged of dissolved oxygen by saturation with nitrogen gas. The donor chamber's oxygen concentration was kept constant by a continuous supply of air. Both chambers were maintained at 37°C by an external, thermostatically controlled water bath.

The oxygen concentration was measured with a WTW OxiMeter 340i probe (WTW, Germany). The probe was sealed inside the receiver chamber where it recorded the oxygen concentration at ten-minute intervals for three hundred minutes. For SIS and UBM, the luminal or abluminal surfaces faced the donor chamber in separate tests. At the end of each test, the scaffold's thickness was measured using a digital micrometer.

Fick's first law of diffusion was used while assuming: linear solute concentration across the scaffold; same concentration of solute at the solute/sample inter-phase and within the chamber, and steady state conditions.

Using Fick's laws and these assumptions, the following equations were used[3]:

$$\ln\left(\frac{C_D - C_R}{C_D - C_{Ro}}\right)\left(\frac{1}{-\beta}\right) = D' t \qquad (1)$$

$$\beta = \frac{A}{z}\left(\frac{1}{V_R}\right) \qquad (2)$$

where D' is the effective diffusivity coefficient, t is time, A is the area through which diffusion occurs, z is the thickness of the test membrane, V_R is the volume of the receiver cell, C_D is the concentration of dissolved oxygen in the donor cell, C_R is the concentration of dissolved oxygen in the receiver cell at time t, and C_{Ro} is the concentration of dissolved oxygen in the receiver cell at t=0.

Results/Discussion: The results are summarized in figure 1. SIS showed a trend of side-dependent diffusivity values with a greater diffusivity of oxygen from the abluminal to luminal direction with p=0.054. This directionality trend was consistent with previous studies on the water permeability index of SIS scaffolds[2]. UBM showed no side-dependent diffusivity values, and low oxygen diffusivity values. UBS had higher diffusivity values than UBM and similar diffusivity values when compared to SIS (abluminal). The presence of a basement membrane in the UBM devices may be responsible for the low diffusivity values when compared to SIS and UBS.

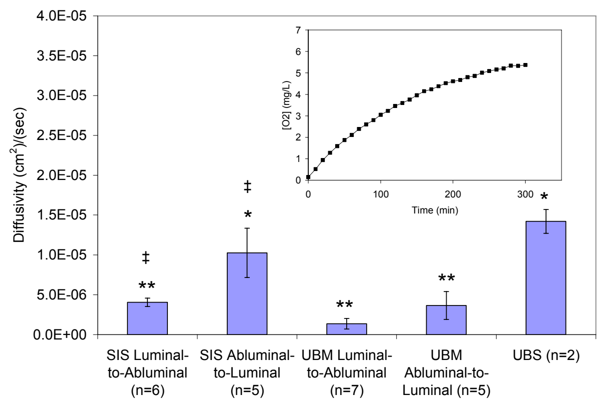

Figure 1. Diffusivity of SIS, UBM, and UBS. Data represents mean ± SEM. Inset shows a typical oxygen versus time plot. *,**, and ‡ statistically similar values (p> 0.05) based on Bonferroni's post hoc test.

Conclusions: ECM scaffolds have distinctive oxygen diffusivity properties and the presence of a basement membrane may affect oxygen transfer across an ECM scaffold.

References: 1. Freytes DO. Biomaterials 2004; 25(12):2353-61; 2. Ferrand BK. J Biomed Mater Res 1993; 27(12):1235-41; 3. Cussler EL. Diffusion 2nd Ed. 1997.