A novel method of diffusivity constant measurement in collagen hydrogels

T.L. Estus
M.C. Shaw
Physics & Bioengineering Dept., California Lutheran University, 60 W. Olsen Rd. #3750, Thousand Oaks, CA

Statement of Purpose: Controlled diffusion of small and large signaling molecules represents a crucial part of the design of successful tissue-engineered constructs. However, rapid and quantitative *in-vitro* characterization of large-molecule diffusion rates in 3D, cell-populated constructs is not straightforward. This study describes the development and critical validation of a novel method for measuring the rate of diffusion of large molecules in three-dimensional scaffolds, *in vitro*, via quantitation of their diffusivity constants. Specifically, transient diffusion experiments were performed with optically translucent Type-1 collagen or gelatin constructs and a variety of opaque dye molecules to obtain optical images of concentration gradients at regular time intervals. The experiments were performed by delivering 15.5 µL of dye solution to a cylindrical cavity introduced into the center of each construct. Image analysis revealed temporally and radially varying concentration gradients that yielded results that agreed well with those separately measured for the diffusion of glucose in sclera tissue, when analyzed in accordance with Fick's Second Law,

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad \text{(Eq. 1)}$$

where D is the diffusion coefficient, C is concentration, t is time and x is position.

Methods: Four wells of a 24 well cell culture plate were filled with 500 µL of either: (i) a 10 mL collagen solution made up of 8.0 mL PureCol (3 mg/mL), 1.0 mL 10X MEM, and 1.0 mL NaCl, or, (ii) commercially available gelatin. The cell culture plate containing collagen was then placed in an 37˚C incubator in air until the collagen had fully polymerized, whereas the gelatin specimens were gelled at room temperature. After the specimens had polymerized, each well was covered with a template lid and secured to the lab bench. A puncturing device created by attaching a 5.0 cm length of a cylindrical plastic tube to the end of a 10 mL *Normject* syringe, was then used to create a cylindrical cavity with a 15.5 µL volume and a 0.15 cm radius in the center of each collagen or gelatin construct. Each 15.5 µL cavity was then filled with an equal amount of black dye solution. The wells were then photographed using a Nikon Digital Camera attached to a Meiji light microscope over a logarithmic time scale (5, 10, 20, 50, and 100 minutes). The digital images were then analyzed using *ImageJ* to provide grey value intensity vs. radial position plots at each time interval. The Gaussian error function solution to Eq. 1 was then fit to these data to obtain the diffusion coefficients:

$$\frac{C - C_2}{C_1 - C_2} = erf\left(\frac{x}{2\sqrt{Dt}}\right) \qquad \text{(Eq. 2)}$$

Results:

Well	Diffusivity Constant (cm^2/s)	Standard Deviation
A5	5.9509E-05	8.23506E-05
B5	1.55618E-05	1.23142E-05
C5	5.48425E-06	2.69036E-06
D5	6.292E-06	3.4848E-06
Average	2.17E-05	2.56096E-05

Table 1. Diffusivity constants for collagen with standard deviation.

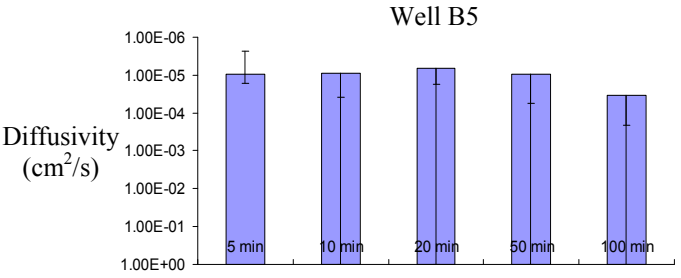

Fig 1. Diffusivity Constants for well B5.

Conclusions: Being one of the most important and recurrent processes within organisms, diffusion needs to be modeled in order to successfully predict how scaffolds will acclimate *in vivo* and to re-create *in vivo* environments *ex vivo*. The current method is suitable to not only measure diffusivity coefficients within collagen scaffolds, but any scaffold made of a hydrogel or similar substance. Here, an average diffusion coefficient, D, for dye molecules within collagen was found to be approximately $2\times10^{-5}\ cm^2/s$ with no statistically significant difference between the specimens. This value compares well with the separately reported value for the diffusivity of glucose within scleral tissue, D_{gs}, found to be, $D_{gs}=3.45\times10^{-6}\ cm^2/s$,[1]. The high standard deviation in the results was remedied with a T-test showing no statistically significant difference between the diffusivity coefficients of the individual collagen wells. There was also found to be a statistically significant difference between the diffusivity coefficients of the same well at different times, which is to be expected due to the finite quantity of dye. Future and current studies focus on the effect that fibroblasts seeded within the collagen scaffolds have upon the diffusivity coefficients.

References: [1] Bashkakov, Alexy N. et al., *Estimation of glucose diffusion coefficient in scleral tissue, 2000.*