Modeling Diffusion across Basement Membrane Hydrogels

Zdravka Cankova[1], Shiri Uriel[1], Georgia Papavasiliou[1], Eric M. Brey[1,2]
Illinois Institute of Technology[1], Hines V.A. Hospital[2]

Introduction: Basement membranes (BMs) are sheet-like extracellular matrices that separate many cells from the surrounding tissue stroma. Understanding diffusion through BMs provides insight into the nature of macromolecule transport in physiologic and pathologic processes and can also be used to assist in designing biomaterials with parameters characteristic of natural membranes. The goal of our research is to examine models for hindered diffusion in fibrous matrices and to determine their applicability to diffusion in natural BMs.

Methods: Two hydrogels were used for the study of transport*:* 1) BM isolated from dermis tissue using a technique developed in our lab[1], and 2) Matrigel (BD Biosciences), a BM extracted from a spontaneously occurring mouse sarcoma. The dermal BM extractions were induced to form gels by two methods: 1) lowering the pH or 2) raising the temperature to 37°C for 1 h. Matrigel hydrogels were prepared through incubation at 37°C for 1 h. Fiber matrix models predict the effective diffusion coefficient (D_{eff}) of a protein based on physical properties of the hydrogels. The following properties were quantified for application to the models: fiber (r_f) and pore (r_p) radii, fiber volume fraction (φ) and partition coefficient (Φ). Measurements of fiber (d_f) and pore (d_p) diameters were determined from SEM images. The φ values were calculated as the ratio of swelled gel volume to dry volume. Partition coefficients were determined by soaking the gels in a solution of fluorescently labeled protein for 24 h and quantifying the ratio of fluorescence inside the gel to that in solution. Φ values in salt solutions of different concentrations were also measured to account for changes based on electrostatic repulsion and/or binding of proteins to the BM gels. Experimental D_{eff} values are being measured using a diffusion chamber under quasi-steady state conditions.

Results/Discussion:

Characterization of Hydrogel Physical Properties: Debate exists in the literature as to the exact mechanism of BM assembly *in vivo*: temperature effects above a concentration threshold or local increase in pH at the cell surface. The physical properties, and hence diffusive properties, of the hydrogels vary depending on the mechanism of assembly (Table 1).

Table 1. Hydrogel Physical Properties

Hydrogel	d_f (nm)	d_p (nm)	φ
Dermal BM – temp	36±12	602±407	0.094±0.014
Dermal BM – pH	83±25	475±253	0.022±0.007
Matrigel	69±35[2]	105±70[2]	0.017±0.001

Φ for the dermal gel of the pH formulation was determined to be 0.723±0.104, which is lower than the predicted value of 0.976 according to the Ogston equation[3]. Preliminary results indicate that Φ for Matrigel decreases with increasing salt concentration, which may be explained as a reduction in protein binding in the gels.

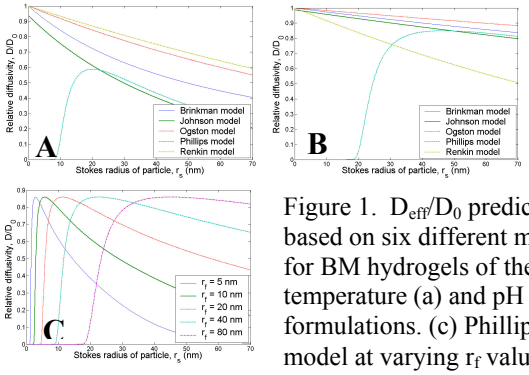

Figure 1. D_{eff}/D_0 predictions based on six different models for BM hydrogels of the temperature (a) and pH (b) formulations. (c) Phillips model at varying r_f values.

Diffusion models: Several models have been developed to describe hindered diffusion of macromolecules in fiber matrices by predicting the ratio of the diffusion coefficient in the gel (D_{eff}) and in infinitely dilute solution (D_0) as a function of the properties of the diffusing species and physical properties of the structure. The physical properties determined above were applied to six different models for the two dermal hydrogel formulations are shown in Fig. 1 (Matrigel not shown). The Phillips model failed to produce an accurate curve at small and medium solute size with the r_f values for all three hydrogels. This model has been suggested to be the most accurate for synthetic equivalents of BM but these results indicate that this is only valid for small r_f (Fig. 1c), much smaller than most extracellular matrices. While the Renkin model predicted reasonable results for dermal BM, it failed at Matrigel pore sizes.

Conclusions: The physical properties of BM hydrogels were quantified and combined with hindered diffusion models to provide predictions of diffusion of particles in BM hydrogels. This provides insight into biological transport properties and could be applied in the design of biomaterials with transport characteristics closely resembling those of natural membranes. Current work focuses on collecting experimental data to assess the accuracy of the model predictions and their applicability to natural BM hydrogels.

References:

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Acknowledgments:

This work was supported by funding from the Veteran's Administration.