Multiplexed protein quantification with barcoded hydrogel microparticles

*David C. Appleyard, Stephen C. Chapin, and Patrick S. Doyle**

Supporting Information

Introduction

To promote maximum capture and detection within the microparticle gel the porosity must be sufficient to allow diffusion of target proteins, reporter antibody, and the reporter fluorophore. A simple system was used to estimate the diffusivity of a fluorescently labeled 150 kDa IgG, allowing for direct comparison of various hydrogel compositions. Individual cylindrical poly (ethylene glycol), PEG, posts were polymerized in a microfluidic channel that provided rapid buffer exchange yet prevented elution of microparticles. Time lapse imaging of fluorescently labeled IgG penetration into the cylinder was obtained and used in combination with a simplified model to estimate diffusivity of the system.

Materials and Methods

Polymer Precursors. 15% (v/v) poly(ethylene glycol) diacrylate (PEG-DA) monomer mixture (15% PEG-DA (MW 700 g/mol), 40% PEG (MW 200 g/mol), 5% Darocur 1173, 40% 3X Tris-EDTA pH 8.0 (TE)). **20%** (v/v) poly(ethylene glycol) diacrylate (PEG-DA) monomer mixture (20% PEG-DA (MW 700 g/mol), 40% PEG (MW 200 g/mol), 5% Darocur 1173, 35% 3X Tris-EDTA pH 8.0 (TE)). **25%** (v/v) poly(ethylene glycol) diacrylate (PEG-DA) monomer mixture (25% PEG-DA (MW 700 g/mol), 35% PEG (MW 200 g/mol), 5% Darocur 1173, 35% 3X Tris-EDTA pH 8.0 (TE)).

Microfluidic Device. A chamber for diffusion analysis is detailed in Figure 1. The PDMS device has a 57μm high channel which is fed by an open reservoir allowing for monomer, buffer, and fluorescent probe exchange. All flow control is handled by a hand-operated syringe attached to the end of the channel opposite the reservoir by a short (30cm) tygon tube and pipette tips. A set of PDMS columns fence off the syringe outlet to prevent accidental loss of a polymerized particle during exchange.

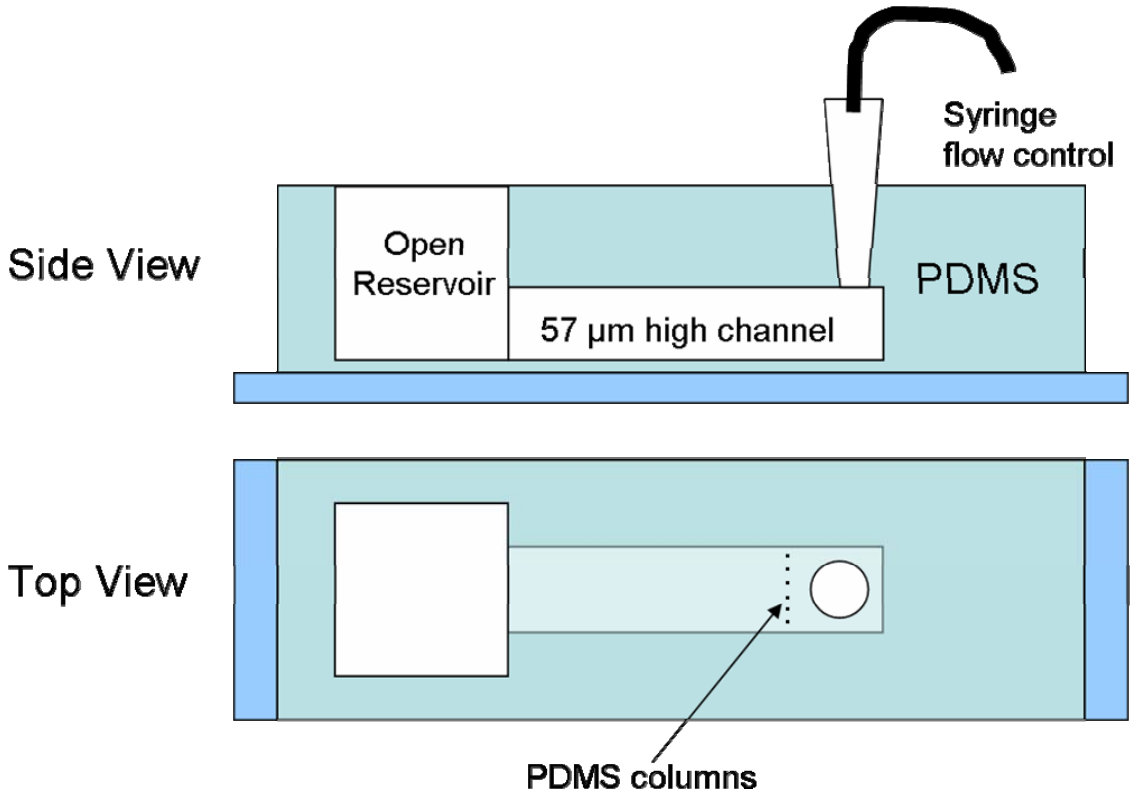

Figure 1. Cartoon PDMS device (light blue) adhered to a coverglass (dark blue) for performing diffusion analysis.

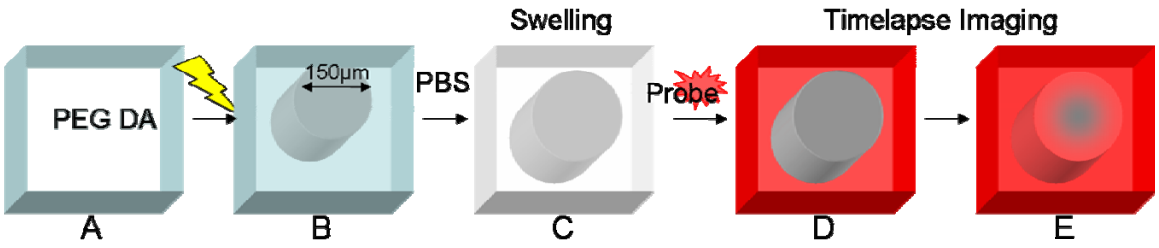

Figure 2. Schematic depicting a top down view of the method for synthesizing the circular plug particle and measuring diffusion. A) PEG diacrylate (PEG DA) precursor is loaded into the channel. B) UV exposure causes synthesis of a 150 µm diameter cylindrical post. C) PEG precursor is exchanged for 80 µl PBS and the plug particle is washed and swells to lock itself in position. D) Probe molecule is introduced into the channel and begins to penetrate the particle. E) Time lapse imaging is used to monitor penetration.

The monomer precursor being evaluated was loaded on to the channel (Figure 2A). Polymerization occurred using a single 100 ms exposure (Figure 2B). To ensure complete removal of unpolymerized monomer, 80 µl of PBS was exchanged through the device (Figure 2C). Swelling occurred in the PBS buffer after exchange and was essential in eliminating probe diffusion from the space above or below the cylindrical plugs; it also served to wedge the plug particle in the channel so it would not flow out during buffer exchanges.

Time lapse imaging was initiated just prior to a buffer exchange of 40 µl of 100 µM Cy3 labeled anti-goat IgG (Sigma Aldrich C2821) in PBST (Figure 2D). Imaging software (Andor SOLIS) coordinated a 0.05 s camera exposure (Andor Clara) with fluorescent excitation from a UV light source (Lumen 200) using a triggered shutter system (Uniblitz) to minimizing photo bleaching. Sequential images were recorded every

1s (Figure 2E). Time lapse image stacks were analyzed in MATLAB (Mathworks). The center of the circle was found for each image, and then radially averaged to provide the fluorophore penetration as a function of distance from the center. The 30 μm just outside the circle were also averaged to provide the bulk fluorescence for normalization. By cycling through the stack, both positional and temporal data was collected. To simplify fitting, only the probe concentration at the center of the circle as a function of time was used in estimation of diffusivity.

Mathematical model of diffusion. By constraining diffusion to the radial dimension through axial contact of the cylinder to the lower coverglass and upper PDMS channel a much simpler model of diffusion can be used. Axisymmetric diffusion in cylindrical coordinates for our system assuming no z-dependence has the form of equation 1:

$$\frac{\partial C}{\partial t} = \frac{D}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) \qquad \text{Eq. 1}$$

Where C is the concentration at radial position r. D is the diffusivity. We non-dimensionalize this equation by introducing dimensionless time, τ, dimensionless position, η, and dimensionless concentration, θ, as defined:

$$\tau = \frac{t}{R_0^2/D}$$
$$\eta = \frac{r}{R_0} \qquad \text{Eq. 2}$$
$$\theta = \frac{C}{C_0}$$

Where C_0 is the bulk concentration and R_0 the cylinder radius. The system is subject to the boundary conditions:

$$\theta(\eta=1,\tau)=1$$
$$\left.\frac{\partial\theta}{\partial\eta}\right|_{\eta=0}=0 \qquad \text{Eq. 3}$$
$$\theta_{ss}(\eta)=K$$

Equation 1 is a PDE which can be solved using methods defined in Deen, realizing that Eq 1 is in the form of a Sturm-Liouville with a von Neumann and Dirichlet boundary condition.[1] Resulting in a solution of:

$$\theta(\eta,\tau)=K-2\sum_{n=1}^{\infty}\left[\frac{1}{\lambda_n}\frac{J_0(\lambda_n\eta)}{J_1(\lambda_n)}e^{-\lambda_n^2\tau}\right] \qquad \text{Eq. 4}$$

Where K is a partition coefficient, J refers to the Bessel function of order 0 or order 1, λ is the solution to the characteristic equation ($J_0(\lambda_n)=0$).

Results.

Representative penetration curves for the IgG along with fit lines for the four different PEG DA concentrations are displayed in Figure 3. As the PEG DA decreases the polymer matrix becomes more porous and has an increasing void volume allowing for faster penetration of molecules and a larger partition coefficient. This is reflected in an increasing diffusivity and partition coefficient with decreasing PEG DA concentration (Table 1).

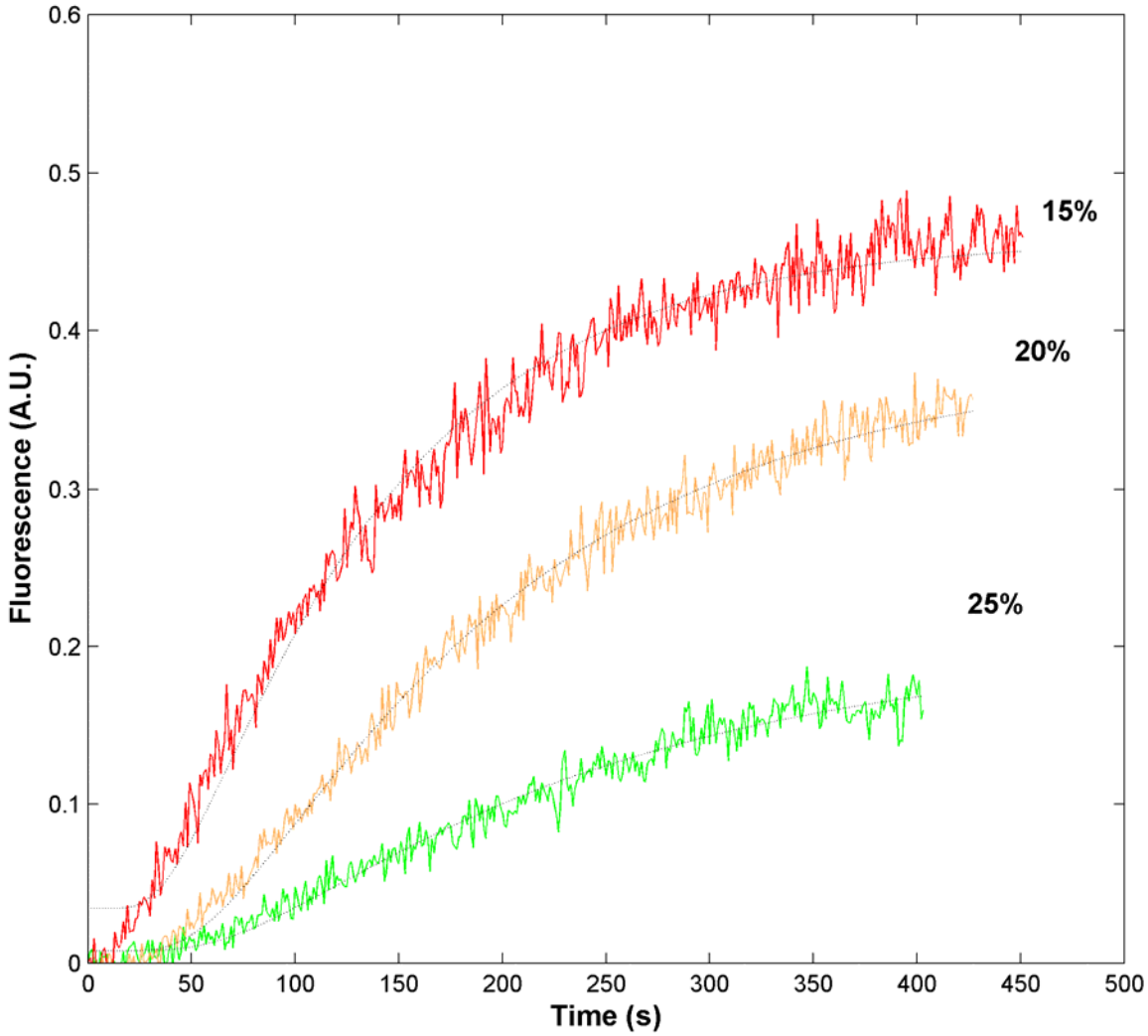

Figure 3. Representative penetration curves showing the fluorescence intensity at the center of a 150 μm diameter cylindrical plug as a function of time after the introduction of a fluorescent IgG probe. Percentage labels on curves refer to the PEG DA composition of the plug. Dashed lines reflect fit to Equation 4.

	D ($\mu m^2/s$)	t_p (s)	K
15%	86.2±2.0	65.3±1.5	0.40±0.03
20%	56.6±0.4	99.5±0.6	0.39±0.02
25%	45.5±4.2	123.8±10.4	0.19±0.01

Table 1. Extracted fitting parameters for diffusivity, D, penetration time, t_p, and partition coefficient, K, as a function of PEG DA concentration in the monomer solution from two measurements. Penetration time is calculated as $t_p=R_0^2/D$.

References:

(1) Deen, W. M. *Analysis of transport phenomena*; Oxford University Press: New York, 1998.