Inhibition of Protein Diffusion in 3-D Hydrogel Arrays for Tissue Engineering

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Statement of Purpose: The inability to simultaneously explore the influence of many distinct factors on new tissue formation is a key limitation in current tissue engineering approaches. To address this limitation, we have engineered 3-dimensional hydrogel arrays with independent soluble environments, designed to ultimately enable controlled 3-D signaling environments to study tissue development.

Methods: PEG hydrogels containing an array of 16 wells (1mm diameter, 1.25mm depth) were generated by adding PEGDA solutions with Irgacure 2959 (BASF, Ludwigshafen ,Germany) photoinitiator into the Teflon mold followed by UV crosslinking. Interpenetrating networks (IPN's) were created by swelling these arrays of wells, which we term "backgrounds", in increasing concentrations of PEGDA 575. Solutions of fluorescently labeled protein, 8k PEGDA, and 0.05%w/v I2959 photoinitiator were prepared and added into the wells of the interpenetrated background arrays. The already crosslinked background and uncrosslinked wells were then exposed to UV light to form the finished arrays. Diffusion of proteins from localized array spots was then measured in PBS. Images were taken using a fluorescent microscope. The fluorescence intensity in the wells was measured by defining a region of interest around the perimeter of the well using SimplePCI software (Compex, Sewickley, PA). The fluorescence intensity between the wells was determined by measuring the intensity of pixels on a line between wells using ImageJ software (freeware, Bethesda, MD).

Results/Discussion: The Teflon mold allows for the formation of hydrogel-based arrays (Figure 1).

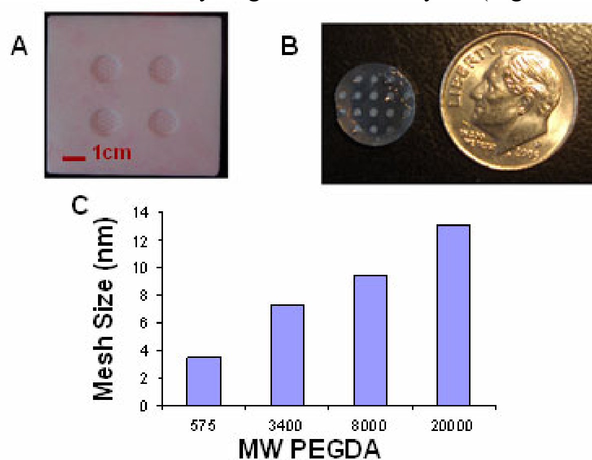

Figure 1. A. Teflon mold containing 1mm diameter posts. B. PEGDA array with spots containing plastic microspheres (added for contrast) C. The average mesh sizes were calculated from hydrogel equilibrium swelling ratios using Flory-Rehner theory.

Arrays with IPN's comprised of PEGDA 575 modulated, but did not completely inhibit, the diffusion of Lysozyme from the wells (Figure 2).

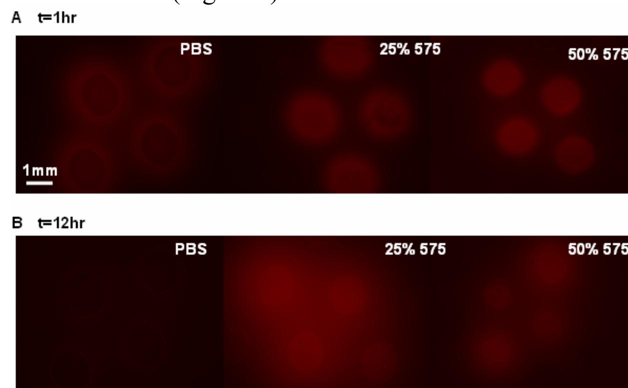

Figure 2. Lysozyme in hydrogel arrays after (A) 1 and (B) 12 hours of diffusion. Diffusion is hindered by the PEGDA 575 IPN's.

Next, to characterize the diffusion of a protein with a hydrodynamic radius about the as the calculated mesh size of the IPN, BSA was loaded into the wells and allowed to diffuse. This protein remained in the wells, resulting in the creation of environments with a distinct soluble signal (Figure 3).

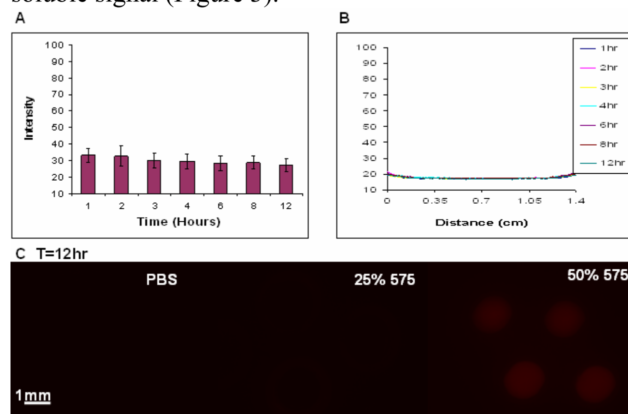

Figure 3. A. BSA is retained in the array that was interpenetrated with a 50% PEGDA 575 solution. B. No protein gradient developed between the wells suggesting that BSA was not diffusing from the wells into the background. C. After 1 hour the protein had diffused out of the backgrounds interpenetrated with PBS and 25% 575 but remained in the arrays interpenetrated with 50% 575 solution.

Conclusions: In this study hydrogel-based arrays were engineered and protein diffusion was inhibited to create independent soluble environments.