Perfusable Cell Laden Microchannel Networks in Poly(ethylene glycol) Hydrogels

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Statement of Purpose: Microscale fabrication technologies have been utilized to study fundamental biological phenomena, mimic the extracellular microenvironment and develop capillary like structures in natural hydrogels. Fabrication of perfusable, cell laden, bioactive hydrogel scaffolds holds the potential to improve diffusion limited mass transport and facilitate spatially controlled tissue formation. In this work we report a multilayer softlithographic method in which polydimethysiloxane (PDMS) and poly(ethylene glycol) diacrylate (PEGDA) are serially molded to form a perfusable PEGDA microchannel network contained within a PDMS housing. In addition, we demonstrate the ability to modulate and quantify the spatial temporal mass transport properties of solutes from the perfused channel throughout the hydrogel bulk. Finally, we demonstrate the ability to sustain cellular co-cultures within the hydrogel bulk and channel microarchitecture under physiologically relevant perfusion conditions.

Methods: *Hydrogel Microchannel Fabrication*

Perfusable PEG microchannels were fabricated using a multilayer replica molding strategy. First, PDMS was molded to a patterned photoresist master (SU-8 2100 Microchem) in order to fabricate an encasing housing with perfusion access ports. Next, the PDMS housing was overlaid with a second photoresist master (SU-8 2050 Microchem) that provides the channel microarchitecture and geometry. PEGDA prepolymer solutions were then injected into the housing around the photoresist master and exposed with a UV lamp for 3 min to form a crosslinked hydrogel matrix encased within a PDMS housing. The PDMS/PEG microchannel device was then peeled from the photoresist master and conformally sealed to cover glass for all other studies.

Mass Transport Studies

PDMS/PEG microchannels devices were fabricated as stated above with 6 kDa PEGDA at variable concentration (10% and 5% (w/v)) solutions in order to analyze the effect on hydrogel diffusivity. Toluidine blue solutions were perfused at 6 ml/hr through a 100 um channel and diffusion from the channel through the bulk was imaged every min. for 400 min. by a CCD camera mounted to a stereoscope. ImageJ was used to generate spatial temporal intensity profiles, and diffusion coefficients were obtained by fitting intensity data to 2nd order Fickian diffusion functions using Igor Pro.

Spatially Distinct Co-Cultures

NIH 3T3 fibroblasts were labeled with Cytotracker Red (Invitrogen), suspended in a 10% (w/v) 6 kDa PEGDA (3E5 cells/ml) with 14 mg/ml acryloyl-PEG-RGDS and replica molded to yield cells encapsulated within the hydrogel bulk. Human umbilical vein endothelial cells (HUVEC) were labeled with Cytotracker Green (Invitrogen), seeded within the microchannel lumen (3E5 cells/ml), and imaged at 48 hr to observe cell-cell and cell-channel interactions.

Perfused Co-Cultures

A 4:1 co-culture of HUVEC:10T ½ cells were labeled with Cytotracker Green and Red respectively, suspended within a proteolytically degradable PEG derivative (30E6 cells/ml) with 14 mg/ml acryloyl-PEG-RGDS and replica molded to yield a 250 um channel. The co-culture was perfused at 600 ul/hr for 72 hr with endothelial growth media. Time-lapse confocal imaging of the co-culture was performed in order to determine cell-cell and cell-channel interactions.

Results: *Hydrogel Microchannel Fabrication Outputs*

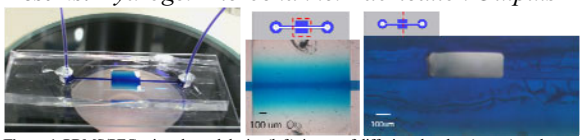
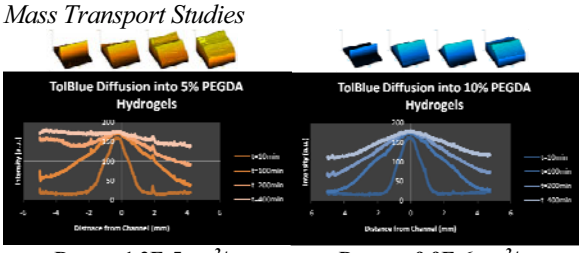


Figure 1. PDMS/PEG microchannel device (left), image of diffusion chamber (center), and cross section of microchannel (right)

Mass Transport Studies



$D_{5\%gel}$=1.2E-5 cm^2/s $D_{10\%gel}$=9.9E-6 cm^2/s

Figure 2. Spatial temporal diffusion plots and diffusivity fit values for 0.01% toluidine blue in 5% (left) and 10% (right) 6 kDa PEGDA hydrogels

Spatially Distinct Co-Cultures

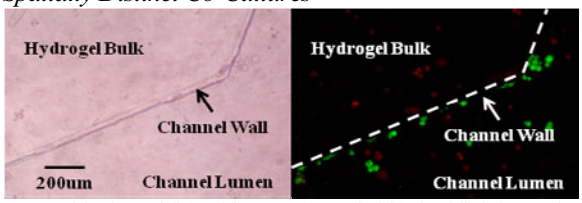


Figure 3. Light micrograph (left) and fluorescent micrograph (right) of spatially distinct co-cultures (Green=HUVEC and Red=3T3)

Perfused Co-Cultures

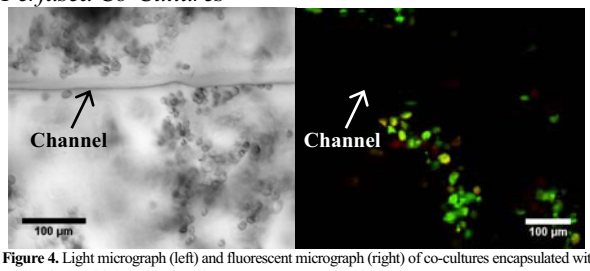


Figure 4. Light micrograph (left) and fluorescent micrograph (right) of co-cultures encapsulated within a perfused microfabricated hydrogel (Green=HUVEC and Red=10T ½)

Conclusions: In this work we demonstrate the ability to fabricate perfusable microchannel networks in PEGDA hydrogels. It was also observed that we can modulate and quantify the diffusivity of the hydrogel by varying polymer concentration. Finally, we have demonstrated that the fabrication output allows incorporation of multiple cell types in spatially distinct locations as well as the ability to culture cell under continuous perfusion conditions. Future research activities will include evaluating the affect of temporal flow regimes, microchannel geometry, and co-culture content on the ability to generate perfusable microvascular structures *in-vitro*.