Photolithographically and Softlithographically Microfabricated Poly(ethylene glycol) Hydrogels

Michael Cuchiara,[1] Jordan Miller,[1] Theodore Chen,[1] Alicia Allen,[1] Raul Villarreal[1] and Jennifer L. West[1]
[1]Department of Bioengineering, Rice University, Houston, Texas

Statement of Purpose: Fabrication of microfluidic, multicellular, bioactive hydrogel scaffolds holds the potential to improve diffusion limited mass transport and develop tissue like microarchitectures ex vivo. In this work poly(dimethysiloxane) (PDMS) and poly(ethylene glycol) diacrylate (PEGDA) are serially molded to form a perfusable PEGDA microfluidic network in a PDMS housing. Combination with photolithography has shown the ability to spatially pattern multiple cell types within the microfluidic hydrogel, with control over mass transport characteristics and with the ability for cells to form organized structures.

Methods: *Softlithographic Microchannel Fabrication*

Perfusable PEGDA microchannels were fabricated using multilayer replica molding. PDMS was molded to a patterned photoresist master to fabricate a housing with perfusion access ports. Next, the PDMS housing was overlaid with a second photoresist master for the microchannel architecture and geometry. PEGDA was then injected into the housing around the photoresist master and exposed to UV light to form a hydrogel encased within a PDMS housing. The PDMS/PEGDA microchannel device was then peeled from the photoresist master and conformally sealed to cover glass.

Photolithographic Hydrogel Structure Fabrication

In combination with the multilayer softlithographically fabricated constructs, photolithography was applied in order to photo-pattern cell laden PEGDA microstructures with independent cell types in spatially distinct locations. PEGDA solutions containing either endothelial cells (HUVEC) or neural progenitor cells (MHP 36) were exposed through different photomasks to fabricate MHP 36 pillars embedded within a perfused microfluidic HUVEC hydrogel matrix.

Mass Transport Studies

Acellular PDMS/PEG microfluidic devices were fabricated as stated above with 6kDa PEGDA and perfused with variable molecular weight solutes (Toluidine blue 305 Da, 3 kDa and 10 kDa dextran) at 600 ul/hr through a 250 um channel. Diffusion from the channel through the bulk was imaged as a function of perfusion time using a CCD camera attached to a dissecting microscope.

Spatial Temporal Cell Viability

NIH 3T3 fibroblasts were encapsulated (11E6 cells/mL) within a 6kDa PEGDA microfluidic hydrogel. The system was perfused through a 250 um channel with DMEM supplemented with 10% FBS at 600 uL/hr. Spatio-temporal cell viability was assayed (Live/Dead stain; Invitrogen) as a function of distance from the perfused microchannel (0-1500 um), culture time (0-72 hr), and static vs. perfused systems.

Perfused Tubulogenic Co-Cultures

A 4:1 co-culture of HUVEC:10T ½ cells (pericyte precursors) were labeled with Cytotracker Green and Red respectively, encapsulated within a MMP degradable PEGDA derivative (30E6 cells/ml) with 2 mM acryloyl-PEG-RGDS and replica molded to yield a 250 um channel. The co-culture was perfused at 600 ul/hr for 96 hr with endothelial growth (EGM-2, Lonza). Tubulogenic activity and interactions of the co-culture with the perfused channel were determined by perfusion of the channel with fluorescent BSA and imaged using confocal microscopy.

Results: *Hydrogel Microchannel Fabrication Outputs*

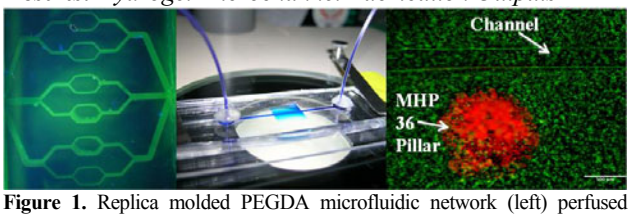

Figure 1. Replica molded PEGDA microfluidic network (left) perfused PDMS/PEGDA microfluidic device (center) and multilithographically fabricated hydrogel construct (Green=HUVEC, Red=MHP 36).

Spatial Temporal Mass Transport and Cell Viability Studies

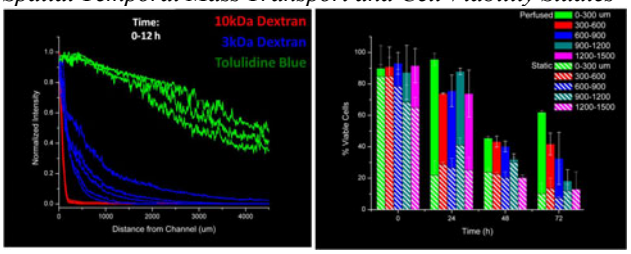

Figure 2. Spatial temporal diffusion plots for toluidine blue, 3 kDa & 10 kDa Dextran in 10% 6 kDa PEGDA (left). Spatial temporal cell viability of perfused (solid bar) and static (hashed bar) cultures (right).

Perfused Tubulogenic Co-Cultures

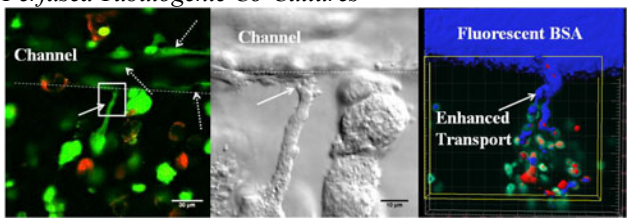

Figure 3. Cytotracker labeled co-cultures interacting with the perfused channel wall (left), phase image with increased magnification of HUVEC interaction with perfused channel wall (center) and perfusion of system with fluorescently labeled BSA shows enhanced transport at sites of tubulogenic activity.

Conclusions: We demonstrate the ability to fabricate perfusable microchannel networks and photo-pattern independent cell types within perfused PEGDA hydrogels. Enhanced mass transport within the perfused system provides spatio-temporal control over soluble - cell signaling interactions and maximizes cell viability. The ability to sustain tubulogenic co-cultures within the microfluidic hydrogel provides a tool to prevascularize scaffolds in vitro. Furthermore, enhanced mass transport observations within regions of high tubulogenic activity suggest potential anastomosis of tubules with the microchannel. Future research activities include evaluation of tubule structure interaction with perfused channels and fabrication of complex biomimetic multicellular tissue structures in vitro.

Acknowledgments: Funding support provided by NIH Biotechnology Training Grant and the NIH Quantum Grant.