Microvascular Network Formation and Integration within Perfused Microfluidic Poly(ethylene glycol) Hydrogels

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Statement of Purpose: Despite significant progress, clinically viable regenerative medicine based therapeutics are restricted to thin, avascular, and metabolically diffuse systems. Primarily this limitation is due to the lack of a perfused 3D vascular network to support both the metabolic needs of the construct during *in vitro* tissue development and as a pre-vascularized conduit to facilitate rapid vascular integration post implantation. Herein we report the integration of a proteolytically degradable microfluidic poly(ethylene glycol) (PEG) hydrogel with a tubulogenic co-culture of human umbilical vein endothelial cells (HUVECs) and smooth muscle cell precursors (10T ½ cells). We demonstrate that the device can be used to optimize vessel growth and network formation as well as perfuse a *de nuevo* pre-vascularized tissue bed from fabricated microchannels. This work has direct implications in the development of perfusable pre-vascularized tissues *in vitro* for cell culture and regenerative medicine applications.

Methods: *Microfluidic Hydrogel Device Fabrication*
PDMS-PEG microchannels were fabricated using soft lithographic followed by photolithographic methods. PDMS was first molded to a patterned photoresist master to fabricate a housing with perfusion access ports. Next, the PDMS housing was overlaid with a transparency photomask which controls the hydrogel structure geometry within the housing. A 4:1 co-culture of HUVECs:10T ½ cells were suspended within a proteolytically degradable photocrosslinkable PEG derivative ($30x10^6$ cells/ml) with 2 mM acryloyl-PEG-RGDS. The cell-PEG solution injected into the housing and exposed to UV light through the photomask to form a hydrogel structure encased within a PDMS housing.

Microvascular Network Formation
The cell laden PDMS-PEG device was sealed to cover glass and perfused at 600 µl/h with endothelial growth media (EGM-2, Lonza) at one interface of the hydrogel structure and PBS at the other to establish a gradient of nutrients, wastes, and growth factors. (Fig 1) Cultures were sacrificed at 0, 48, & 96 h, immunostained with anti-PECAM, anti-SM-αactin, and DAPI to label HUVECs, 10T ½ cells, and all nuclei respectively. Vessel structures and tubule morphology were analyzed as a function of distance from the media channel and culture time.

Perfused Microvasculature Transport and Integration
After 96 hr of culture, the microchannel was perfused with fluorescent dextran (MW = $2x10^6$, Invitrogen) to real-time image the transport of dextran throughout the construct and evaluate the spatial localization of the dextran within vessel structures.

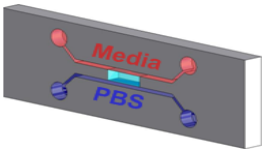

Figure 1. Softlithographic and photolithographically fabricated cell laden PDMS-PEG microfluidic device. PDMS housing (gray), EGM-2 channel (red), buffer channel (blue) & cell laden PEG hydrogel structure (cyan).

Results: *Spatiotemporal Microvascular Network Formation*

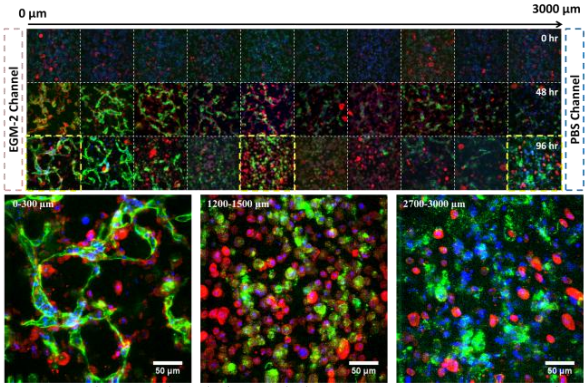

Figure 2. Microvascular tubule morphology (PECAM = green, SM-αactin = red, DAPI = blue) as a function of culture time and distance from media microchannel. (Top) Total tubule length and number increased from 0-96 h at regions less than 600 µm from the media microchannel. (Bottom) Expanded images (yellow outline) of optimal networks (left) and necrotic regions (center & right).

Perfused Microvascular Transport and Integration

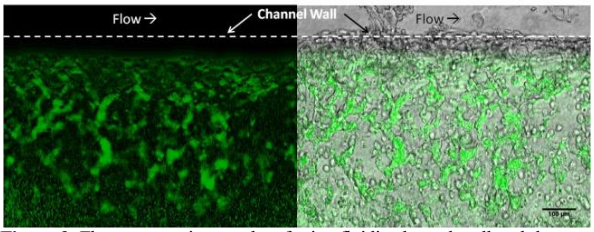

Figure 3. Fluorescent micrographs of microfluidic channel wall and dextran (green) found within microvascular structures immediately post perfusion (left). Phase-fluorescent micrograph overlay showing co-localization of dextran within vessel structures and rapid convective like transport up to 600 µm from microchannel wall (right).

Conclusions: We demonstrate the integration of a proteolytically degradable microfluidic PEG hydrogel with a tubulogenic co-culture of HUVECs and 10T ½ cells. The tubulogenic co-culture was shown to form robust, functional 3D microvascular networks as indicated by the presence of capillary lumens, positive expression of the endothelial marker PECAM, and network stabilization by pericytes positive for SM-αactin. Furthermore, we observed significant changes in capillary network properties as a function of distance from the perfused microfluidic channel (Fig 2). Total tubule number was shown to be significantly greater in the nutrient rich regions nearest the perfused microchannel (0-600 µm) at 48 and 96 h of culture. Finally, perfusion of the microfluidic network with fluorescent dextran demonstrated rapid convective like transport and co-localization of the dextran signal within tubule structures (Fig 3) which is thought to indicate a shift in tissue bed transport regimes from diffusion through a matrix to convective anastomotic transport through vessel lumens.

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