3D Microfluidic Tissue Model with Perfused Human Capillaries

Monica L. Moya[1], Yu-Hsiang Hsu[1], Christopher C.W. Hughes[1], Abraham P. Lee[1] and Steven C. George[1]
1. University of California, Irvine

Statement of Purpose: Engineering tissue constructs requires the ability to stimulate extensive vascularization to prevent tissue necrosis. To better understand this process and to design effective strategies, 3D *in vitro* biomaterial-based scaffolds are often utilized to mimic the *in vivo* microenvironment. While there has been progress in the development of these 3D *in vitro* systems, no model contains a perfused bed of human capillaries—a fundamental feature of essentially all tissue. The goal of this work is to merge microfabrication techniques with 3D cell culture to create a high throughput platform of 3D microtissues (~1mm^3) with perfused human capillaries that allow for the structural observations of cell-microenvironmental interactions. Integrating the response of cells, the stroma and the circulation in a dynamic 3D setting will create an environment conducive to the exploration of many fundamental processes and lead to design of better tissue constructs. Additionally this novel high throughput platform of perfused vascularized microtissues can be used for drug delivery and chemical toxicity screening.

Methods: Using standard polydimethlsiloxane micro-molding, a microfluidic device consisting of 2 microfluidic channels on either side of a central micro-chamber was created. For the study of angiogenesis-like processes, endothelial progenitor cell-derived endothelial cells (ECs) lined the fluidic channels (1x10^{6}cells/ml) that connected via communication pores to the central tissue chamber consisting of fibroblast (2x10^{6}cells/ml) seeded in a fibrin matrix (10mg/ml). In the tissue chamber, cells were seeded as either a dense cluster of cells (600 cells/pellet or as a cell suspension. To stimulate vasculogenic-like processes, in some devices ECs were distributed in the tissue chamber with the fibroblast as co-culture pellets (Fig 1A) or randomly dispersed throughout the channel (Fig 1B). A pressure gradient (2mm H_2O) across the tissue channel was applied once obvious network formation was identified. Flow in formed capillaries was assessed by adding 1 μm diameter polystyrene fluorescent beads into the fluid channels and tracking their movement in the formed capillaries across the tissue channel. To visualize cell interactions, cells were either transduced with a fluorescent protein or stained with CD31 markers (EC marker) and DAPI (nuclei stain).

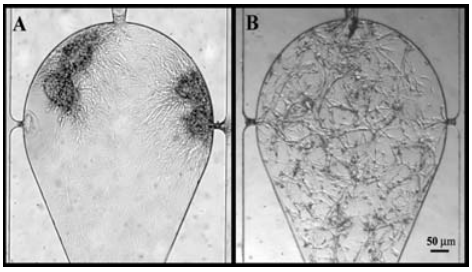

Figure 1. Images of devices with different seeding strategies at day 2.

Results: Cells in the device were cultured and remained viable through 40 days. Within a week of culture, vessel-like structures were visible within the device. (Fig2) Fluorescent microscopy allowed for visualization of lumen within the tubules. (Fig 3)

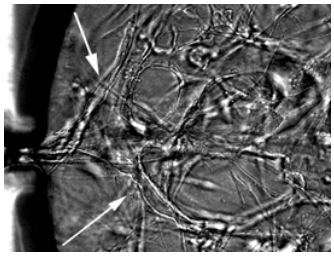

Figure 2. Brightfield image taken at day 4 depicts tubule-like structures (white arrows) forming in tissue chamber seeded with co-culture of ECs and fibroblast.

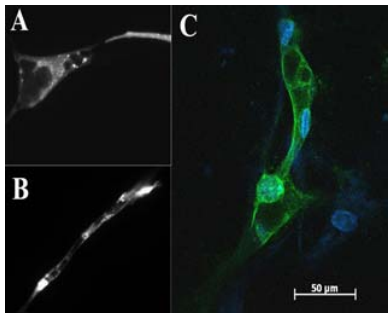

Fig 3. Fluorescent microscopy of transduced ECs (A & B) and ECs stained for C31 (green) and DAPI (blue) confirm the presence of lumen within the formed tubules

In an earlier iteration of the design, when cells were co-cultured and distributed in the central chamber, early perfusion of a partially formed vessel network was confirmed at 3 weeks. Tracking the microbeads revealed flow speeds of around 55.1μm/s ± 27.

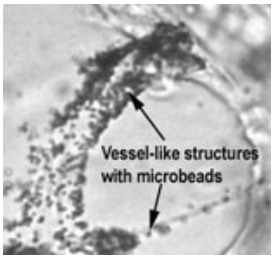

Figure 4. Microbeads (1μm, black spots) flowed from arteriole-like fluidic channel to the venule-like channel illuminated the path of partially perfused capillaries in the tissue channel.

Conclusions: This novel microfluidic system has been demonstrated to allow for the visualization of the interplay between ECs and the stroma during angiogenic and vasculogenic processes. Vessel-structures formed in the co-culture condition were shown to form functional capillaries as evidenced by the presence of lumens and the early perfusion of formed capillary network. Current work is focused on optimizing design variable and parameters as well as characterizing the vessel network formed under all the three different seeding conditions.