A Novel Engineered Niche to Explore the Perivascular Association of Adult Stem Cells and the Vasculogenic Potential of Embryonic Stem Cells

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Statement of Purpose: Numerous pathologies are characterized by poor blood vessel growth and therefore inadequate nutrient and oxygen delivery. Therapeutic angiogenesis seeks to enhance vessel growth into ischemic tissues by delivering combinations of pro-angiogenic factors with precise spatial and temporal resolution to recruit host vasculature. An alternative approach is to deliver an appropriate cell type that can provide a more physiologic mixture of pro-angiogenic cues to accelerate the recruitment of host vessels in a paracrine fashion, or that can differentiate into a functional vasculature directly. It is possible that both embryonic and adult progenitors may act in either or both of these manners to stimulate new vessel formation.

Consistent with the first possibility, we have utilized a three-dimensional (3D) fibrin-based *in vitro* cell culture model to demonstrate that pre-committed (mesenchymal stem cells, MSCs) or more highly committed (fibroblasts) mesenchymal cells stimulate capillary formation from human umbilical vein endothelial cells (HUVECs). Interestingly, while both cells types occupy perivascular niches within this system, they promote capillary morphogenesis via distinct mechanisms involving differential dependence on proteases that degrade the extracellular matrix (ECM). With respect to the second possibility, we are also interested in better understanding the factors that govern the direct differentiation of embryonic stem cells (ESCs) into vascular progenitors.

To facilitate our efforts to understand the significance of the perivascular association of most adult stem cells and to investigate how combinations of soluble and insoluble morphogens, and cues from other cell types, are integrated to coordinately govern the endothelial differentiation of ESCs, the goal of this study was to transfer our existing 3D fibrin-based model system into a novel microfluidic device, and demonstrate the ability of this device to support the formation of capillary blood vessels. Furthermore, this versatile multichannel device was explicitly designed to allow multiple discrete constructs of three-dimensional cell-laden hydrogels to be easily patterned, and to permit the superposition of soluble morphogen gradients.

Methods: Microfluidic devices were generated using standard PDMS-based soft lithography and rapid prototyping methods. Resulting PDMS devices were permanently bonded to a glass slide following treatment with an air-plasma, forming enclosed microfluidic channels. To create 3D constructs within these channels, cells suspended in fibrinogen were mixed with thrombin, immediately pipetted into one of the reservoirs in the gel chamber section (Fig. 1A), and allowed to clot for 30 min. at 37°C. Media was then added to the inlet reservoirs and gently suctioned through the main channels and the entire device placed in a standard cell culture incubator. HUVECs and stromal cells (either MSCs or fibroblasts) were seeded within fibrin gels (2.5 mg/ml) in discrete channels, separated by a third channel containing only fibrin (Fig. 1B). Over time, both HUVECs and stromal cells invade the interstitial matrix to occupy the middle channel. Experiments were carried out in microfluidic devices for up to 14 days, with capillary morphogenesis assessed qualitatively via phase contrast and confocal microscopy.

Results: Our findings to date validate the use of this novel 3D engineered niche as a model system in which to study capillary morphogenesis. Phase contrast microscopy revealed that HUVECs cultured in the presence of stromal cells within these devices form a network structure similar to a primitive capillary plexus (Fig. 1B). Confocal microscopy confirmed that the HUVECs formed capillary-like structures with well-defined lumens in the presence of stromal cells in fibrin gels constructed within the microfluidic device (Fig. 1C). Immunofluorescent staining of α-SMA confirmed an intimate perivascular association of these stromal cells (either fibroblasts or MSCs) with the endothelial cells involved in capillary-like structures (Fig. 1D).

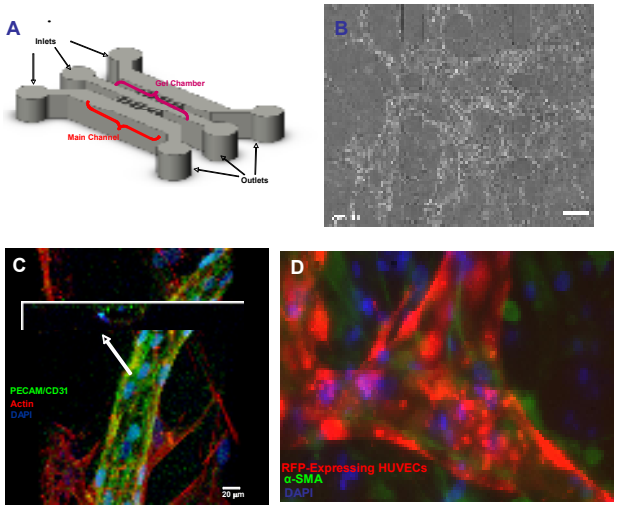

Figure 1

Conclusions: These studies demonstrate the potential of this engineered stem cell niche as a multipurpose model system in which to study capillary morphogenesis. Having shown that adult stem cells introduced into this system recapitulate their endogenous perivascular niche, our ongoing work is focused on dissecting the chemical and physical properties that define *in vivo* niches. We also intend to use this system to systematically investigate how ECM biophysical properties can be used in concert with soluble morphogen gradients and cues from other cell types to govern the endothelial differentiation of ESCs in a controllable fashion.