A Three-Dimensional Micro-Environment for Characterizing Endothelial Cell Sprouting

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Statement of Purpose: Angiogenesis is the process of forming new blood vessels that originate from pre-existing vessels. During angiogenesis, endothelial cells (ECs) migrate away from existing vessels, organize into multi-cellular capillary-like structures called sprouts, and are stabilized by additional cell types to form new blood vessels. *In vivo*, monolayers of ECs lining the inside of conduit vessels experience the 2-D environment of the luminal surface; however, when they migrate into the surrounding ECM during angiogenesis, they are exposed to the 3-D environment of the surrounding basement membrane. Cell morphologies and functions are known to be different in 2-D versus 3-D environments. *In vitro* methods can be helpful in mimicking each of these environments in a controllable and reductionist manner. We have developed a microfluidic platform (Fig. 1) capable of resembling 2-D and 3-D tissue environments. We previously studied 2-D EC chemotaxis in response to vascular endothelial growth factor (VEGF) gradients in this device.[1] Recent studies have shown the critical role of matrix stiffness in mediating EC response to VEGF.[2] Here we report on the simultaneous role of matrix stiffness and VEGF gradients in a 3-D environment designed to mimic early angiogenesis.

Methods: Soft lithography was used to fabricate the microfluidic mold. Human dermal microvascular endothelial cells (HDMVEC) were cultured on microcarrier dextran beads (d~170 mm). The HDMVEC-coated beads were mixed with a collagen-fibronectin gel pre-cursor and injected into the microfluidic device cell culture chamber. Medium (with and without VEGF) was injected into the source and sink channels, respectively, to form a stable VEGF concentration gradient across the cell culture chamber. Oscillatory rheometry was used to characterize gel viscoelasticity.

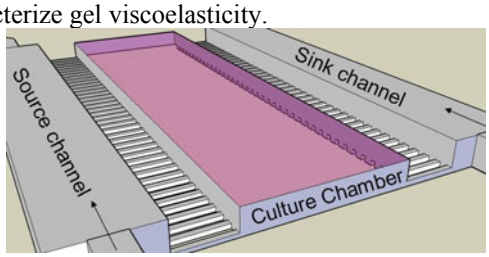
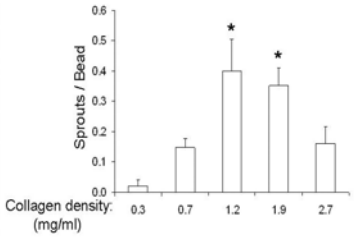


Figure 1. Microfluidic device schematic.

Results: Sprout formation of HDMVEC was analyzed within collagen matrices of varying density (0.3 - 2.7 mg/mL, corresponding to shear moduli from 8 – 800 Pa) subjected to stable gradients of soluble VEGF. These experiments revealed that endothelial sprouting into multi-cellular, capillary-like structures is optimized within intermediate collagen gel densities, (G'~100-200 Pa). At lower matrix densities, cells were more likely to lose their coordinated motion and migrate as individual cells through the matrix; while at higher matrix densities, the cells formed broad cell clusters that rarely elongated into a sprout (Fig. 2). Sprout thickness and length directly correlated with matrix rigidity, with thicker and shorter sprouts present in gels with the highest shear moduli.

Fig.2. The frequency of sprout formation within collagen gels of varying density. EC sprouting is maximized within two intermediate densities (* < 0.05).



Sprout navigation within the two intermediate collagen densities (marked * in Fig.2) was characterized in response to three VEGF profiles including steeper and shallower VEGF gradients with identical absolute VEGF concentrations (0-50 ng/ml). To quantify navigation; sprout initiation point, sprout initial angle, and sprout final angle (i.e., sprout turning) were recorded. Intriguingly, these analyses revealed that EC sprouts alter their sensitivity to VEGF depending on the matrix density, suggesting a complex interplay between biochemical and biomechanical factors. Steeper VEGF gradients and higher VEGF absolute concentrations are required to induce directional sprouting within stiffer matrices. In more compliant gels, EC sprouts that originally misaligned were able to turn and properly reorient parallel to the VEGF gradient; however, this turning phenomenon was only rarely observed in stiffer matrices. Sprouts formed within stiffer matrices also had higher linear cell densities (i.e., more individual cells per length of sprout); therefore, we hypothesize that sprout turning within stiffer gels may be more difficult due to the required large-scale collective movement of many cells.

Conclusions: We believe these are the first *in vitro* observations of EC sprout guidance and turning. Together, these results demonstrate that matrix stiffness is a mediating factor in guiding the orientation of ECs during sprouting. Corroborating earlier studies, we demonstrate that ECs response to VEGF is mediated by matrix rigidity. Because angiogenesis is critical in wound healing, tumor development, and ischemia treatment; having a deep understanding of the key regulators of this process may lead to the development of new pro- and anti-angiogenic strategies.

References:

1. Shamloo A, et al. Lab Chip. 2008, 8:1292-1299.
2. Mammoto A. et al. Nature. 2009: 457:1103-1157.