Hydrogel Arrays to Screen Angiogenesis Inhibitors and Vascular Disrupting Compounds

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Statement of Purpose: The large quantity of putative anti-angiogenesis compounds and the need to regulate angiogenesis in tissue development and pathogenesis calls for a robust, biologically-relevant screening assay to identify agents that disrupt vascular function. Many of these compounds have been characterized as putative vascular disrupting compounds (pVDCs) using assays of human vasculature in a dish. However, current assay systems to identify and characterize pVDCs typically measure two-dimensional (2D) endothelial tubule formation or endothelial cell proliferation as a measure of vascular disruption. These assays are often performed using ECM-mimicking materials such as collagen or Matrigel, which is composed of hundreds of unique proteins[1] and exhibits lot-to-lot variability that may reduce reproducibility. Recently, we have observed endothelial tubulogenesis and sprouting in assay platforms comprising well-defined, synthetic hydrogels. Here we report *de novo* tubule network formation and EC sprouting in enhanced throughput, multivariate hydrogel arrays. We hypothesized that these arrays could be used to identify and characterize inhibitors of vascular function.

Methods: Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs)[2] were encapsulated in a synthetic hydrogel composed of poly(ethylene glycol) (PEG) and tethered cell attachment and cell-degradable crosslinking peptides. Multi-arm PEG was derivatized with norbornene groups as previously described[3], and hydrogels were crosslinked with pendant cell attachment motifs (*e.g.* CRGDS) and protease-degradable crosslinking groups (GenScript). Hydrogel arrays were generated by encapsulating iPSC-ECs (Cellular Dynamics International, Inc.) in a bioactive hydrogel droplet, followed by a second encapsulation in a bioactive hydrogel post. iPSC-ECs were cultured in growth factor-containing medium (CDI) for six days and subsequently stained with Calcein-AM and Ethidium homodimer-1. Sprouting arrays were imaged using epifluorescence microscopy or confocal laser scanning microscopy (Nikon), and iPSC-ECs that had invaded into the surrounding hydrogel post were quantified using NIS Elements.

Results: iPSC-ECs exhibited sprouting behavior that was correlated with the concentration of cell-adhesion peptides and cell-degradable crosslinks within the surrounding hydrogel. Confocal laser scanning microscopy revealed primitive *de novo* tubule networks within the interior cell-dense hydrogel, and tubule network presence correlated with the presence of growth factors (GFs). iPSC-ECs that had sprouted from the cell-dense hydrogel exhibited a CD31+ phenotype and morphology that was distinct when compared to an human dermal fibroblasts, an invasive control cell type. Importantly, inhibitors of vascular endothelial growth factor (VEGF), receptor tyroskine kinases, mammalian target of rapamycin (mTOR), and matrix metalloproteinase 2 and 9 (MMP-2,9) each potently reduced iPSC-EC sprouting while not affecting iPSC-EC viability. Furthermore, VEGF inhibition reduced iPSC-EC sprouting dependent on the properties of the surround hydrogel, which suggests that hydrogel properties may impact the cellular response to inhibition. Results demonstrated the ability to screen pharmacological inhibitors in biomimetic hydrogel arrays.

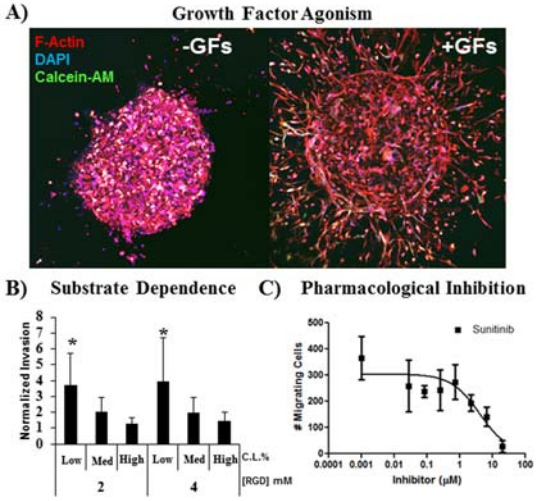

Figure 1. Biomimetic hydrogel arrays to screen iPSC-EC sprouting. A) Confocal images of iPSC-ECs encapsulated and cultured without (-GFs) and with growth factors (+GFs). B) Quantification of iPSC-EC sprouting within hydrogels containing low, medium, and high concentrations of MMP-degradable crosslinks and low and high concentration of CRGDS. C) iPSC-EC sprouting in the presence of GFs and various concentrations of Sunitinib malate, a receptor tyrosine kinase inhibitor.

Conclusions: Biomimetic hydrogel arrays identified substrate dependence and growth factor dependence of iPSC-EC sprouting. iPSC-ECs exhibited sprouting that was dependent on mTOR and VEGF receptor signaling along with MMP-2 and MMP-9 activity, as expected. Encapsulated iPSC-ECs exhibited primitive *de novo* tubule network formation within cell-dense hydrogels, which suggests that the assay described here is capable of simultaneously identifying conditions that impact tubule network formation and endothelial sprouting. Specifically, the screening approach is capable of simultaneously quantitating single-cell invasion, sprout length, survival, and tubule network formation and thus may efficiently distinguish the particular influence of pharmacological angiogenesis inhibitors and pVDCs on multiple EC functions.

References:

1.C. S. Hughes, L. M. Postovit, and G. a Lajoie, *Proteomics*, 2010, **10**, 1886–90.

2.D. G. Belair, J. a Whisler, J. Valdez, J. Velazquez, J. a Molenda, V. Vickerman, R. Lewis, C. Daigh, T. D. Hansen, D. a Mann, J. a Thomson, L. G. Griffith, R. D. Kamm, M. P. Schwartz, and W. L. Murphy, *Stem Cell Rev.*, 2014.

3.D. G. Belair and W. L. Murphy, *Acta Biomater.*, 2013, **9**, 8823–8831.