**High Throughput Screening Format Identifies Synthetic Mimics of Matrigel for Tubulogenesis Screening**

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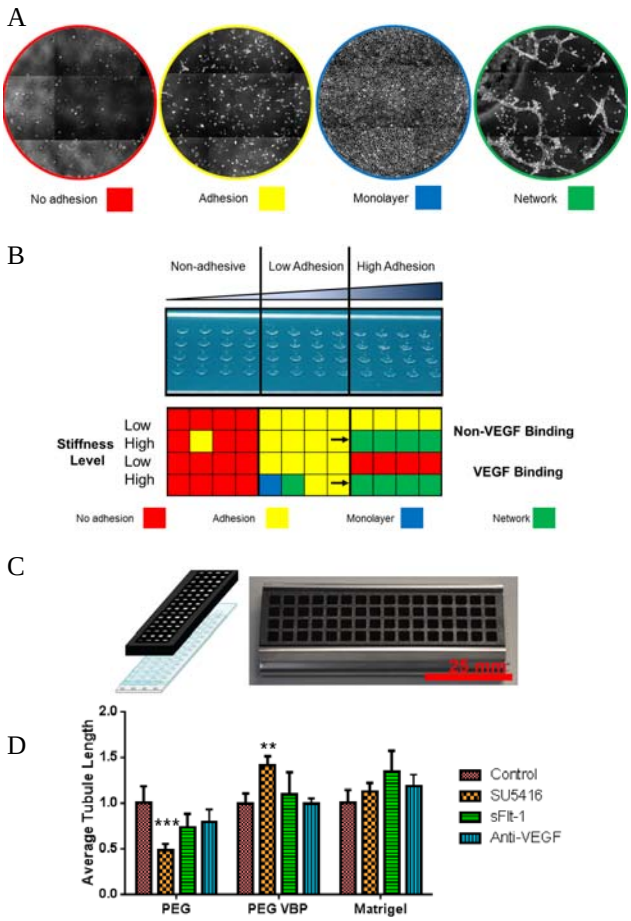
**Statement of Purpose:** Matrigel™ is a commonly used material for testing the effects of chemical compounds on endothelial cell tubulogenesis (1). While it is a simple material to utilize for in-vitro tubulogenesis assays, it is poorly characterized and composed of >1800 individual proteins, leading to a number of limitations including high batch to batch variation and presence of confounding growth factors (2). We hypothesize that the undefined components of Matrigel™ and its inherent variability contribute to decreased sensitivity to tubulogenesis inhibitors, leading to the potential identification of false negatives/positives in the screened compounds. Here, we used a high-throughput screening assay to determine chemically defined synthetic hydrogel formulations that permit Human Umbilical Vein Endothelial Cell (HUVEC) tubule formation to occur similar to Matrigel™.

**Methods:** Hydrogel precursor solutions contained varied concentrations of cell adhesion peptide, 20 kDa, 8-arm poly(ethylene glycol) (PEG), matrix metalloproteinase-labile crosslinking peptide (xlinker) and a matrix-linked Vascular Endothelial Growth Factor (VEGF)-binding peptide (VBP). Hydrogel precursor solutions were patterned onto silane-coated glass slides using gold slides with hydrophilic and hydrophobic regions as a stencil. The hydrogels were cross-linked using the photoinitiated thiol-ene reaction between thiol groups on the crosslinking peptides and norbornene groups on the PEG molecules (3). Endothelial cells were seeded on top of the hydrogel spots at a density of 8.5 x $10^4$ cells/$cm^2$ and cultured in EGM2-supplemented endothelial growth medium (Lonza) for 24 hours before imaging and fixation. To test the effects of known and experimental tubulogenesis inhibitors the hydrogel spots were physically separated using Grace Bio-Labs Proplate® slide modules and each spot was individually seeded with HUVECs and treated with chosen inhibitors. Tubulogenesis was quantified using Nikon Elements image analysis software in terms of total tubule length as well as average tubule length normalized to total cell area on the spots. Results were compared to that of Matrigel™ which were evaluated in an Ibidi™ angiogenesis plate. Statistical significance was determined using two-tailed Student's T-test with equal variance.

**Results:** The hydrogel screening format identified two synthetic formulations that mimicked Matrigel's ability to induce endothelial cell tubulogenesis. One of the formulations was designed to sequester VEGF and the other did not sequester VEGF. Comparison of the optimal PEG conditions to Matrigel™ showed increased sensitivity to vascular inhibitors in the synthetic formulations particularly to SU5416 (*p<0.001* and *p<0.01* respectively).

**Conclusions:** Our screening format identified synthetic hydrogels that are amenable to screening bioactive chemical compounds via tubulogenesis quantification. Initial screening of known vascular inhibitors showed increased sensitivity in optimized synthetic hydrogel formulations when compared to Matrigel™. Future studies will highlight the impact of substrate-mediated VEGF sequestration on the effectiveness of vascular inhibitors. Further optimization of synthetic hydrogels will result in a formulation that will be suitable for use in a scaled-up chemical compound screen that will quantify the effects of 1060 chemicals of the ToxCast Library on HUVEC tubulogenesis.




**A.** Scoring system for tubule networks on synthetic hydrogel spots. **B**. Screening synthetic hydrogels to identify conditions that permit tubule network formation (arrowheads). **C.** Grace Bio-Labs Proplate® slide modules are superimposed on hydrogel arrays to separate chemical compounds during tubulogenesis assays. **D.** Quantifying effects of vascular inhibitors on synthetic hydrogels and Matrigel. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$ compared to control.

**References:**

1. Auerbach R. Clin Chem. 2003;49: 32-40.
2. Hughs CS. Proteomics. 2010;10: 1886-1890.
3. Fairbanks B. Adv Mater. 2009;21: 5005-5010.