Validation of a screening platform for vascular toxicities utilizing synthetic substrates

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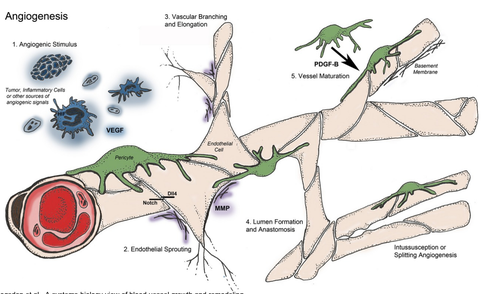
 # 3053

Abstract

Angiogenesis is an essential developmental process that remains critical throughout the lifespan in response to conditions requiring increased oxygen supply, including physiological repair and exercise. The process is complex and tightly regulated through localized delivery of angiogenic factors, matrix remodeling, cellular migration and vessel maturation. The angiogenic process is also a critical component of several disease pathologies including cancer, retinopathies, critical limb ischemia, macular degeneration and vascular malformations. For several decades, a primary *in vitro* assay to assess for vascular disruptors has relied on animal-derived biomaterials such as the Engelbreth-Holm-Swarm mouse sarcoma-derived products marketed as Matrigel®, Geltrex® and Cultrex®. This material is complex, exhibits lot-to-lot variability and is challenging in an HTS workflow due to its temperature sensitivity. In collaboration with scientists at the University of Wisconsin[1], we have developed a synthetic vascular hydrogel that enables high throughput screening (HTS) for vascular disruptors utilizing human umbilical vein endothelial cells (HUVEC) or iPSC-derived endothelial cells[2]. The hydrogel is optimized to promote a VEGF-dependent tubulogenic response which requires matrix remodeling and cellular migration. The assay detects appropriate inhibitors of these processes including Axitinib (AG013736) (IC_{50}, 300 nM), Sunitinib (SU11248) (IC_{50}, 6.3 µM), Nocodazole (IC_{50}, 23 nM) and Primostat HCl (AG3340 hydrochloride**)** (IC_{50}, < 1 µM). The assay can be run in a 96 or 384 well plate format with Z' values of >0.5 and is insensitive to Suramin (Bayer 205), a compound which disrupts Matrigel® and is a broadly referenced false positive in the Matrigel-based assay. Overall, the hydrogel platform is flexible for use in standard cell culture workflows and is suitable for co-culture and 3D organoid applications, facilitating their use for toxicity or efficacy screening applications.

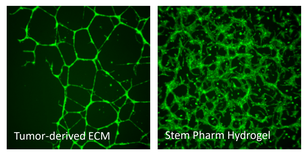
Angiogenesis



Source: Logsdon et al. A systems biology view of blood vessel growth and remodeling J. Cell Mol. Med. 2014 Aug; 18(8): 1491–1508. PMC4190897 (3) Open Access through Creative Commons

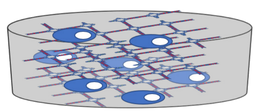
Models to explore the biology of angiogenesis are important to understand vascular diseases, toxicology and oncology

In Vitro Models of Angiogenesis, 2.5D

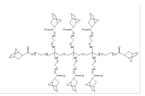


In Vitro models of Angiogenesis often use an ECM-derived from a murine tumor model, first described in 1983 and first available commercially in 1990, now marketed as Matrigel®, Geltrex® and Cultrex®. This material is complex, with 1850 unique protein identified in preparations of the material[4], exhibits lot-to-lot variability and is temperature sensitive, making higher throughput applications challenging.

Design of Synthetic Substrates



No animal-derived components
Reducing complexity
Control: crosslinking chemistry
adhesion receptor engagement
matrix degradability (or not)
Design for purpose
Optically clear
Amenable to automation



Multi-arm PEG-NB

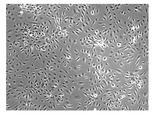
+ SH - Crosslinker - SH
Adhesion peptides -SH
+ Photoinitiation

Vascular Tubulogenesis assay

Assay Workflow

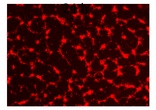
Day 1
Thaw hydrogel, add to assay plate
Photopolymerize and swell hydrogel
Label Cells (optional)

HUVEC on TCP

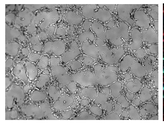


Day 2
Equilibrate hydrogels
Prepare Assay reagents
Plate Assay

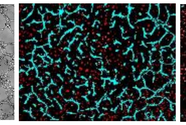
Labeled cells on hydrogel,



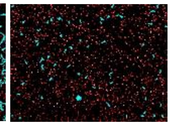
HUVEC on hydrogel, 24 h

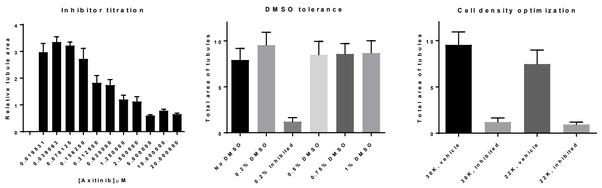


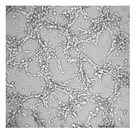
ImageJ-masked tubules
Vehicle control



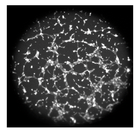
ImageJ-masked tubules
Inhibitor control







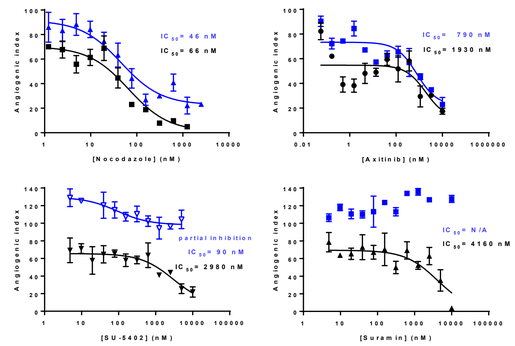
iCell Endothelial cells plated onto SP-105



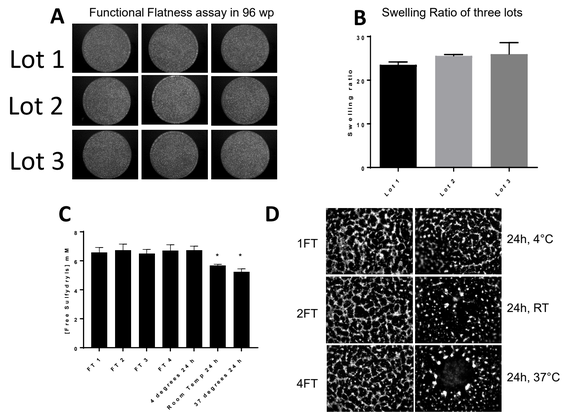
HUVEC assay in 384 wp format

We have optimized a hydrogel formulation (SP-105) that enables screening for vascular disruptors with both Human Umbilical Vein Endothelial Cells (HUVEC) and iPSC-derived Endothelial Cells (bottom left image, Cell ECs, Cellular Dynamics International). Data above, generated 24 h after plating, utilizes HUVEC cells: top left, phase-contrast image of inter-connected HUVEC cells plated in the presence of 0.2% DMSO (vehicle control), middle (vehicle only) and right (20 µM Sunitinib), CellMask™ Orange (Thermo Fisher Scientific) Plasma Membrane-stained HUVEC cells. Images were analyzed for tubule area with ImageJ software with tubules masked as blue objects. Graphs represent Axitinib titration, DMSO tolerance and cell density optimization. HUVEC experiments were performed in Ibidi® µ-Plate Angiogenesis 96 well plates. Published Z' values for the assays are 0.66 for HUVEC in 96 well plate[1] and 0.65 for HUVEC in 384 well plate[2]. Example image from the entire area of a 384 well plate (Greiner, low profile plates) demonstrating the utility of the assay for high through-put screening applications. Validated by Iwata et al., 2017.

Comparison of EHS-derived Matrix and SP105—Dose response curves



Hydrogel Stability and Reproducibility



Characterization of lot to lot reproducibility and synthetic hydrogel stability A key advantage of the synthetic hydrogel over Matrigel in this application is the ability to control lot to lot variability and its improved liquid handling capabilities. (A) For this imaging based assay, it is important that the hydrogel surface is flat, free of a meniscus or bulging effect. To demonstrate this, we have developed a fluorescence assay utilizing fluorescent beads (Tetraspeck, Thermo). Gels are polymerized and allowed to swell. Beads are placed in solution and allowed to settle on the surface, allowing detection of any surface defects. (B) Analysis of swelling ratios (swelled to dry weight ratios) of three lots of the hydrogel formulation. (C) Free thiol content in hydrogel precursor solutions was evaluated across multiple freeze-thaw cycles and following storage in multiple conditions (n = 3). *, $p < 0.05$ using a one-way ANOVA followed by post-hoc Dunnett's test compared to 1 freeze-thaw cycle. Data represents means and error bars represent standard deviations. (D) Functionality of hydrogels in the endothelial network formation assay was tested after precursor solutions were subjected to multiple freeze/thaw conditions or storage at various temperatures. Multiple freeze/thaw cycles resulted in minimal impact on the assay, while storage above freezing temperatures had a greater impact.

Assay Advantages

- Increased sensitivity over Matrigel® for many vascular disruptors[1]
- Improved material handling properties over Matrigel® (automation friendly, better reproducibility in 384 well format)[2]
- More phenotypic complexity (thicker nodes and small protrusions invading into the matrix vs. the cords observed on Matrigel)
- Lot-to-lot consistency
- Clear optical properties for imaging
- Works in 384-well format
- Formulation have been identified which support 3D tubule formation and vascularization of neural organoids [5,6]

References

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