Engineering self-assembling vascular constructs for a membrane-free microphysiological system

Kevin Conrad[1,2]; Ethan Byrne, PhD [2]; Mark Mondrinos, PhD [2]

1- LSUHSC School of Medicine; 2- Department of Biomedical Engineering, Tulane University.

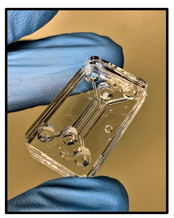
Introduction

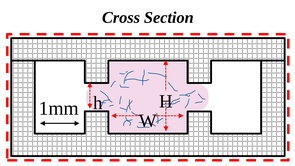
From atherosclerosis to cancer, vascular biology mediates the pathophysiology of nearly every disease; and understanding the role that vasculature plays in the initiation, propagation, and conclusion of disease is critical for fully appreciating the course of disease. Furthermore, human endothelial cells are a highly desired target for pharmaceutical intervention. Despite its importance, most current pathophysiological models do not adequately consider the critical role that vasculature plays in disease.

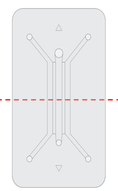
In this work, we characterize self assembling vascular networks in a vascularized triple negative breast cancer model. Human umbilical vein endothelial cells (HUVEC) and human lung fibroblasts (HLF) are seeded in a collagen-fibrin hydrogel, injected into the device, and allowed to assemble into a perfusable vascular network. To optimize our model, we characterize (1) the kinetics of vasculogenesis, (2) the morphology of our vascular networks and their dependence on hydrogel composition, and (3) permeability as a metric for vascular function. Characterizing our model in this way, will allow us to refine experimental design to yield vascular networks which are more representative of human biology.

While our device may be used in a wide variety of tissue culture applications, we currently use it in for a triple negative breast cancer (TNBC) model. TNBC tumors are propagated in a mouse model before being processed into injectable explant and incorporated into cell culture.

Device Design







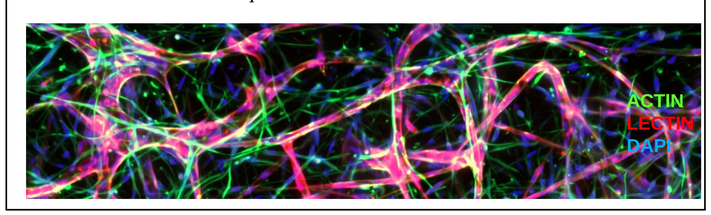
Max Dimensions:
2x1.5x12mm

General Procedure for Vascular Device Seeding:

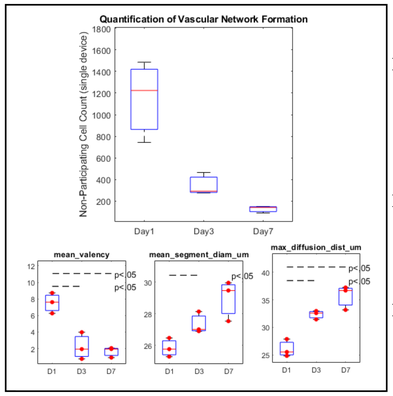
1. Add 4M HUVEC, 2M HLF/mL to hydrogel (3 mg/ML Collagen, 5 mg/mL fibrinogen
2. Add thrombin and quickly mix
3. Inject hydrogel into device and allow for fibrin formation + collagen crosslinking
4. On Day 2, add 10M HUVEC/mL to side channels, allow to adhere for 2 hours, and replace with liquid media
5. Place on rocker for a minimum of 6 days to ensure HUVEC lumen formation
   [alternate methods to induce fluid flow may be used]

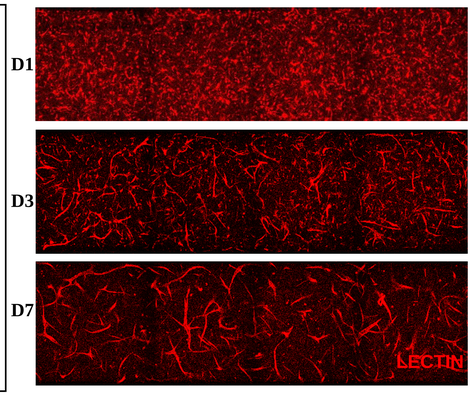
Why bother with a device?...

1. Control of fluid flow
2. Maintain 3D environment
3. Fluid/liquid ratio



Vasculogenesis Kinetics

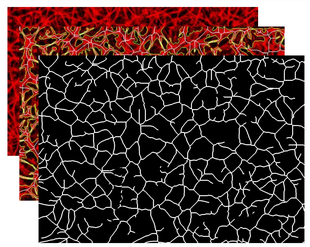




Morphometric Analysis and Optimization

Image processing

1. Neural network denoising
2. Contrast adjustment – adaptive histogram equalization
3. Smoothing and filtering
4. Removal of unnetworked cells
5. Segmentation



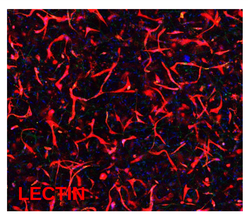
Hydrogel Optimization

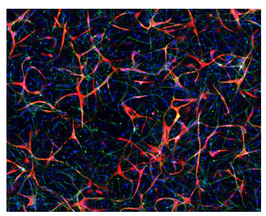
Collagen: 1.75, 3.25, 4.5 mg/mL
[5mg/mL fibrin]

Fibrin: 2, 5, 8 mg/mL
[3 mg/mL collagen]

	Col I Conc	Fibrin
Vessel Area	- -	NL
Vessel Length	- -	NL
Branchpoint count	- - -	NL
Tortuosity	-	
Segment Diameter	- -	- - -
Max diffusion distance	++	- -

- - - : p < . 01
- - : p < .05
NL: non-linear relationship



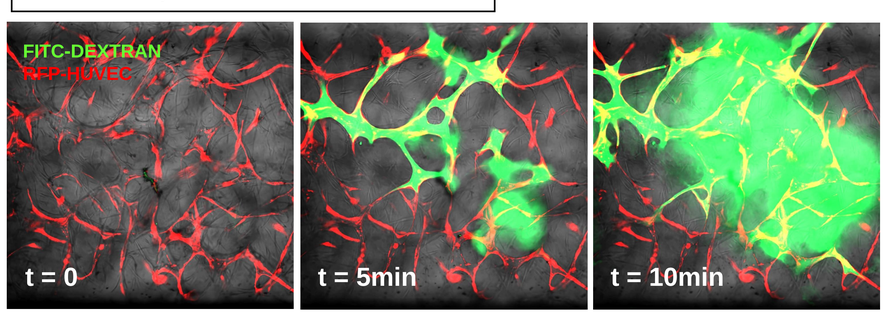


Vessel Permeability

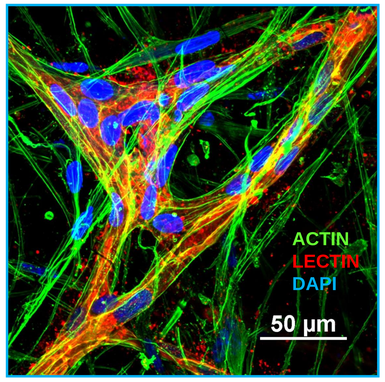
Minimum requirements for reliable lumen formation:

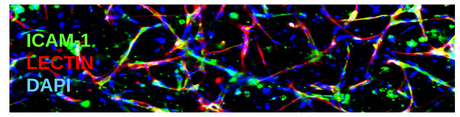
- At Least 4M HUVEC/mL seed density
- Interstitial fluid flow
- HUVEC side channel seeding to create patent vessels
- At least 6 days in culture

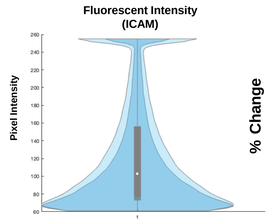
$$P = \frac{1}{I_w} * \frac{dI/dt}{I_j}$$

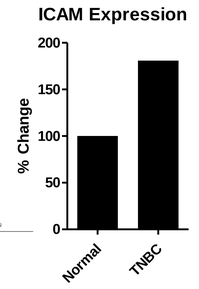


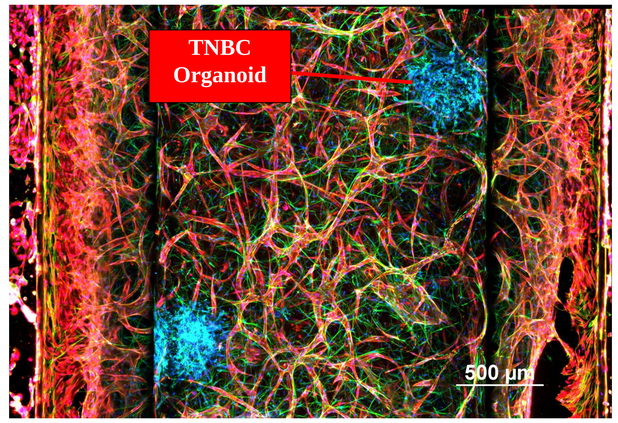
Application to TNBC

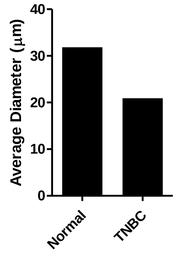












Conclusions & Future Research

In this work, we were able to morphologically characterize self assembling vascular constructs in a 3 dimensional milliscale device. Key findings include:

- Morphological analysis of vascular networks shows increase in vessel size and overall decrease in network complexity over a 7-day maturation period
- Hydrogel composition significantly affects vascular network morphology
- Established minimum requirements for vessel lumen formation
- When co-cultured with TNBC extracts, vascular networks have higher expression of inflammatory markers (ICAM-1) and smaller vessel diameter

Future work will further characterize vascular networks with and without TNBC extracts. We plan to examine:

- Additional factors impacting network morphology
- Effect of TNBC and inflammatory assays on network permeability
- Incorporation of immune cell lines to capture transmigration in response to inflammation

TULANE UNIVERSITY
SCHOOL *of* SCIENCE
& ENGINEERING