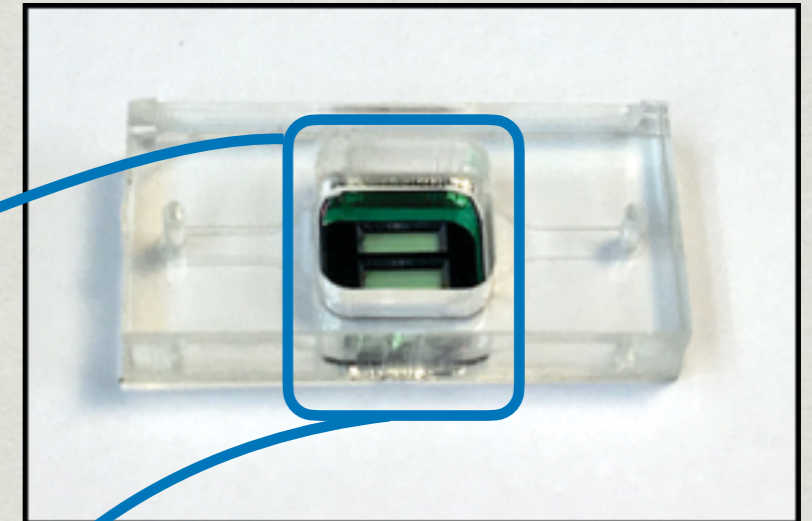
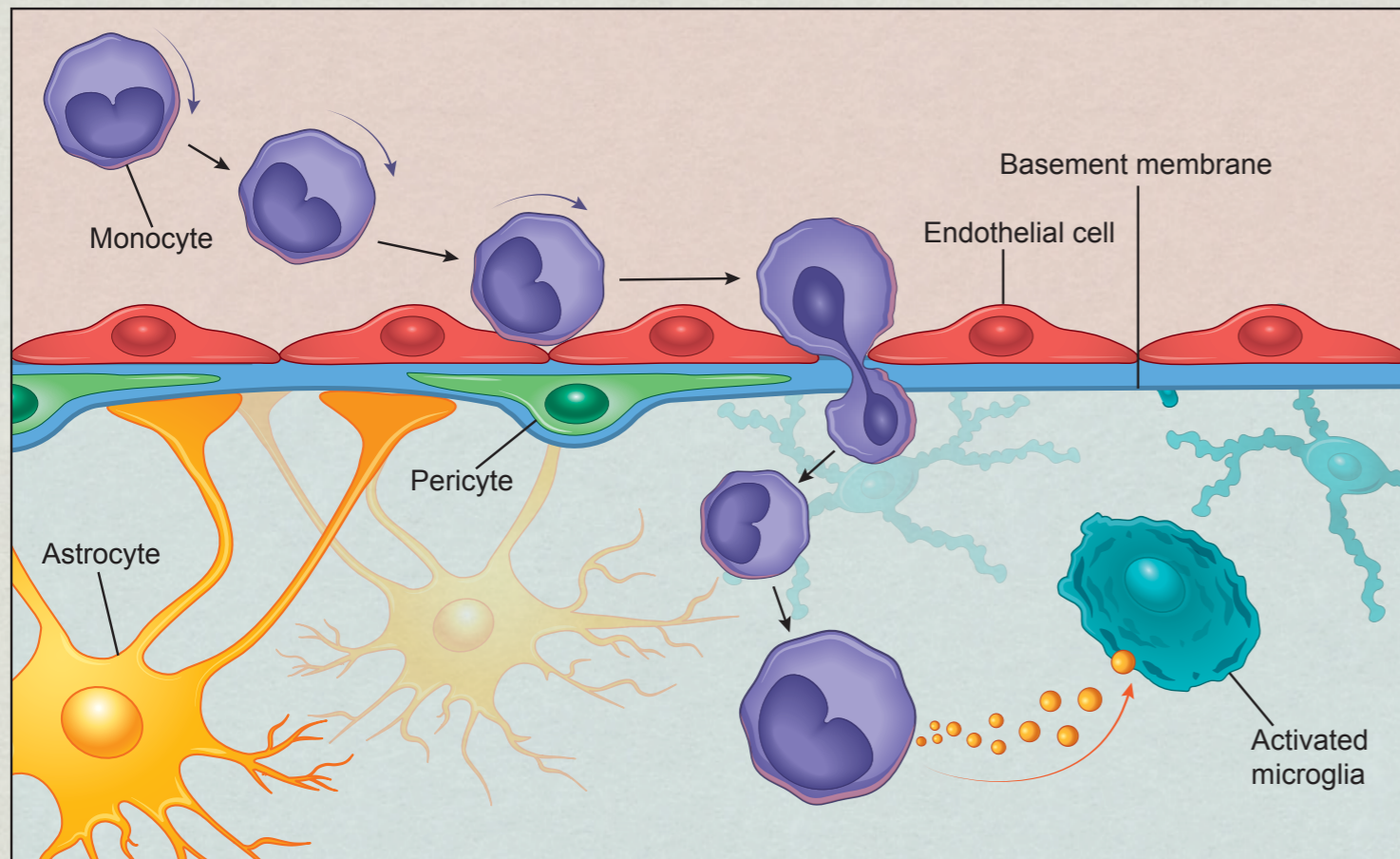


The μ SiM-hNVU – a Human BBB Platform for the Study of Brain Injury Mechanisms in Sepsis

R61: 6 Month Progress Report



Trans-Agency Blood-Brain Interface Program



R61 HL154249-01



RIT



R61 Goals

1. Develop an easy-to-distribute, easy-to-use, and reliable μ SiM platform that supports:
 - Key tasks & manipulations: cell culture, staining, flow/ leukocyte introduction, cytokine introduction.
 - Key measurements: live and fixed cell imaging, permeability, cytokine levels, glycocalyx and basement membrane status.
2. Establish and validate an iPSC-derived *h*NVU (BMECs, astrocytes, pericytes) on the μ SiM platform.
3. Reconstitute monocyte infiltration in response to septic stimuli.

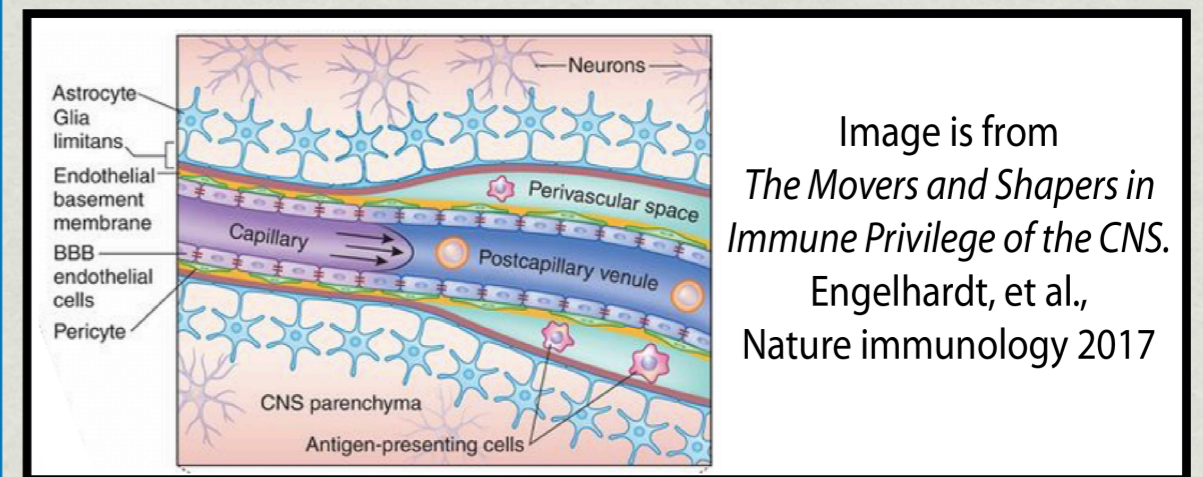
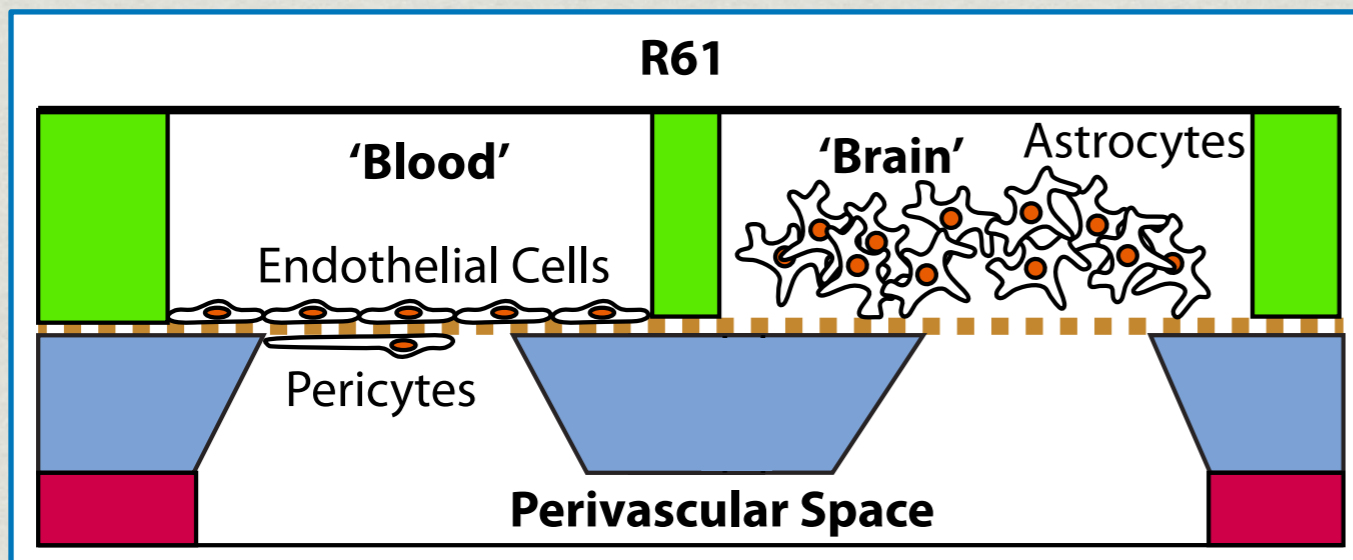


Image is from
*The Movers and Shapers in
Immune Privilege of the CNS.*
Engelhardt, et al.,
Nature immunology 2017

R61 Team



James McGrath, PhD
Richard Waugh, PhD
Jonathan Flax, MD

Trainees

Molly McCloskey (μ SiM, BBB models, Permeability)

Chloe Chen (glycocalyx, ELISA)

Samantha Romanick, PhD (EC iPSCs)

Dan Ahmad (Transmigration)

Staff

Bill Houlihan, PhD (microscopist, 'Data Blog', AFM)

Julie Kuebel (glycocalyx, AFM)

Collaborator

Angela Glading, PhD

Feyone La (Student in Glading group)

Influence of Shear Stress on Cerebral Cavernous Malformations (CCMs - KRIT1)



Benjamin Singer, MD, PhD
Katsuo Kurabayashi, PhD

RIT

Tom Gaborski, PhD
Vinay Abhyankar, PhD
Steven Day, PhD

Trainees

Louis Widom (Basement membrane imaging)

Mehran Mansouri (Flow module)

Britta Engelhardt, PhD

Trainees

Pelin Kasap (μ SiM adoption)

Sasha Soldati (Leukocyte Trafficking)

Hideaki Nishihara (iPSC EECM-BMECs)

Chiara Stüdle (COVID-19)

Collaborator Anuska Andjelkovic-Zochowski, MD, PhD

Role of BBB Gap junction proteins in stroke and cerebral amyloid antipathy (CAA)

Trainees

Muyu Situ (Student in Andjelkovic group)

Howard Su (Digital ELISA)

Detailed Gantt Chart

R61

Successful Development of the Modular μ SiM

- M1A** Successful assembly and use by non-engineering laboratories
- M1B** Successful development and application of a flow module
- M1C** Successful development and application of a TEER module
- M1D** Successful development of methods for small molecule permeability

Successful Development of the μ SiM-hNVU

- M2A** Supports co-culture of all components of the NVU
- M2B** Supports a validated BBB
- M2C** Demonstrate responsiveness to septic stimuli

R33

Advancement of the μ SiM-hNVU for brain injury studies

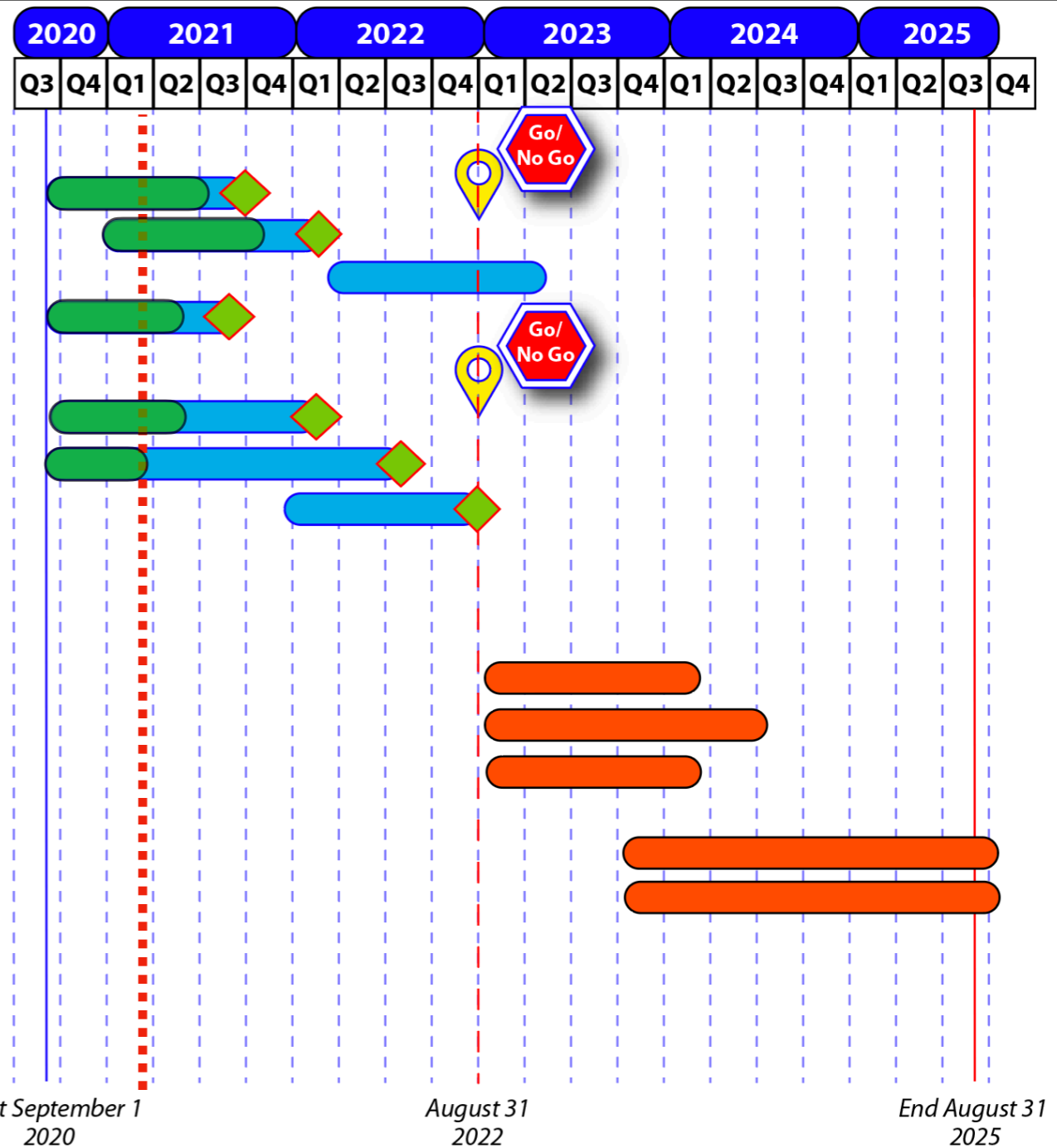
- M3A** Supports co-culture of all components derived from ApoE4 iPSCs
- M3B** Supports a validated ApoE4 BBB
- M3C** Development/Inclusion of microglia reporter cells

Application of the μ SiM-hNVU to study brain injury mechanisms

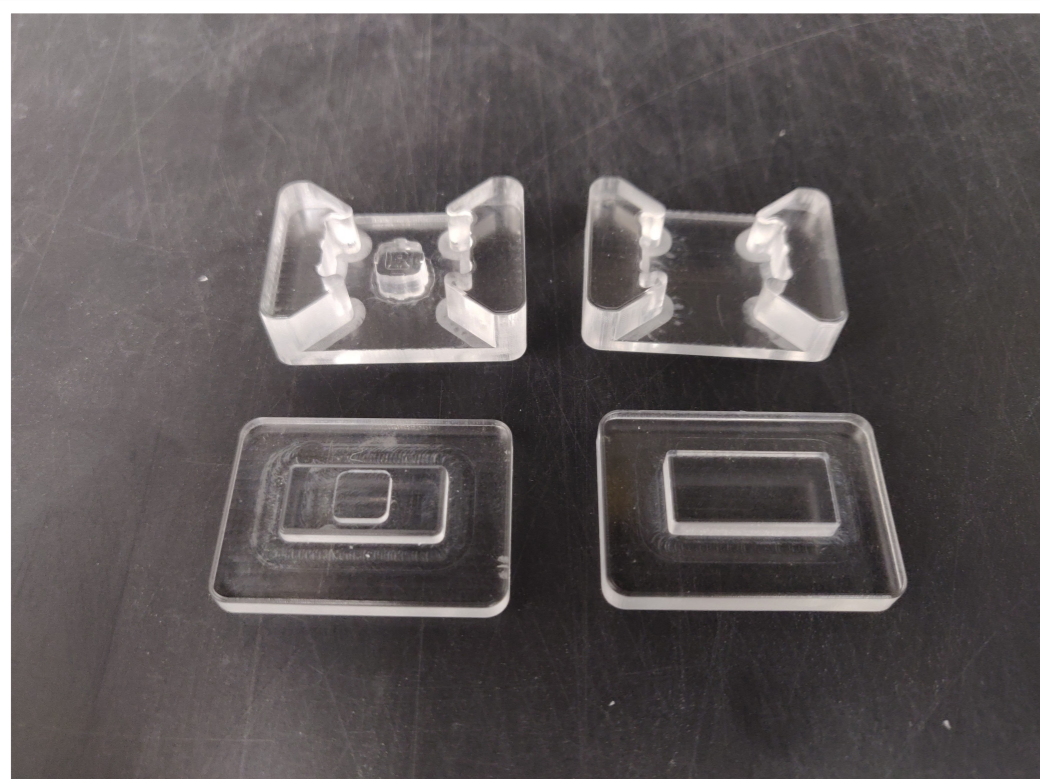
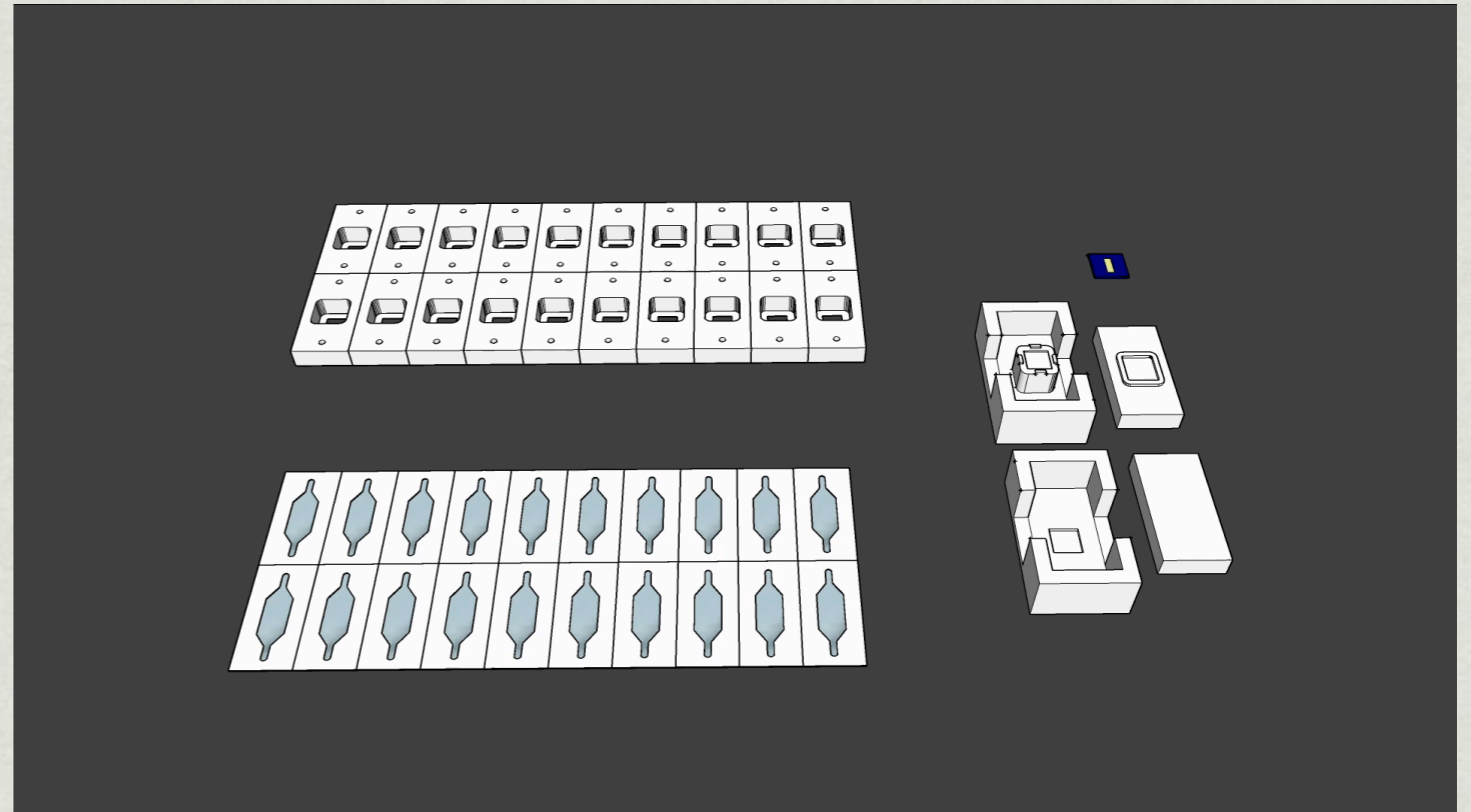
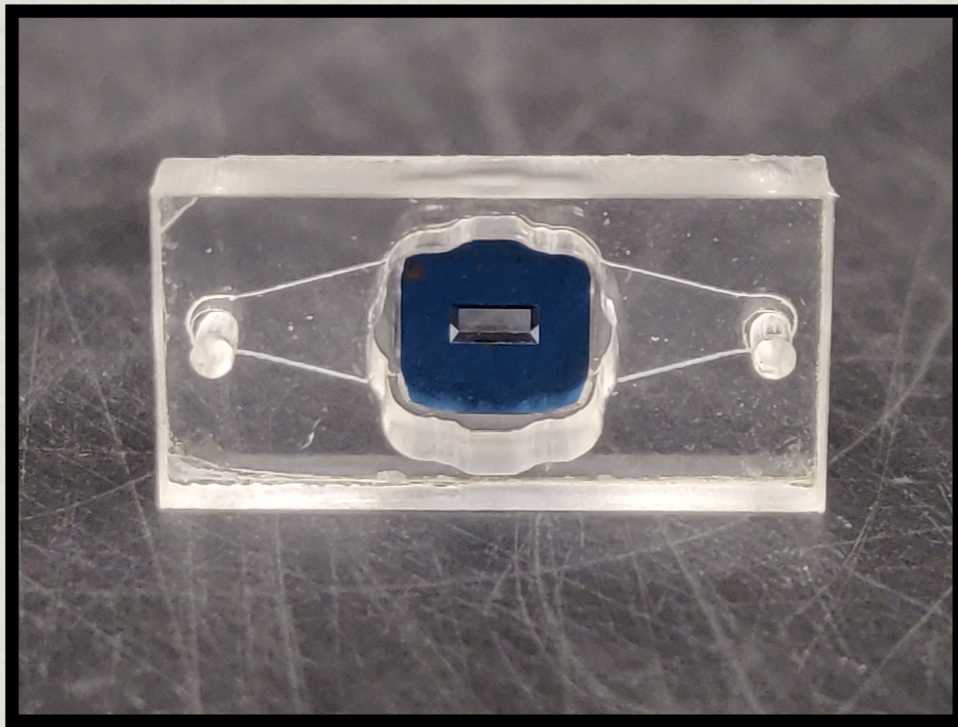
- M4A** Examine pre-activated monocytes invading/activating microglia
- M4B** Evaluate the role of circulating factors on brain injury

 Progress

 Essential Milestone  Go/No Go Decision Point



Distribution - Devices



- Distributed more than 500 device components to 7 (non-McGrath) laboratories
- Includes 3 non-engineering labs
- Includes 3 different types of membranes
 1. Nanoporous
 2. Microporous glass
 3. Dual scale (micro/nano)
- Successful assembly in all labs
- Successful cell culture in all labs
- Stocked supplies for 500 more devices at UR

Distribution - Methods & Data

Home > Data Blog

HBVP (pericyte) and hCMEC/D3 (endothelial cell) μ SiM Coculture Optimization

Posted on February 20, 2021 by Molly McCloskey — No Comments ↓

Introduction Breakdown of the blood-brain barrier (BBB) is one of the earliest signs of sepsis and is linked with long-term cognitive impairment. We are developing a coculture model of the human blood-brain barrier (μ SiM-hBBB) to elucidate mechanisms of BBB breakdown ...

[Read more >](#)

Posted in [NRG Data, R61](#)

[Edit](#)

iPSC IMR90-4 ApoE Genotyping

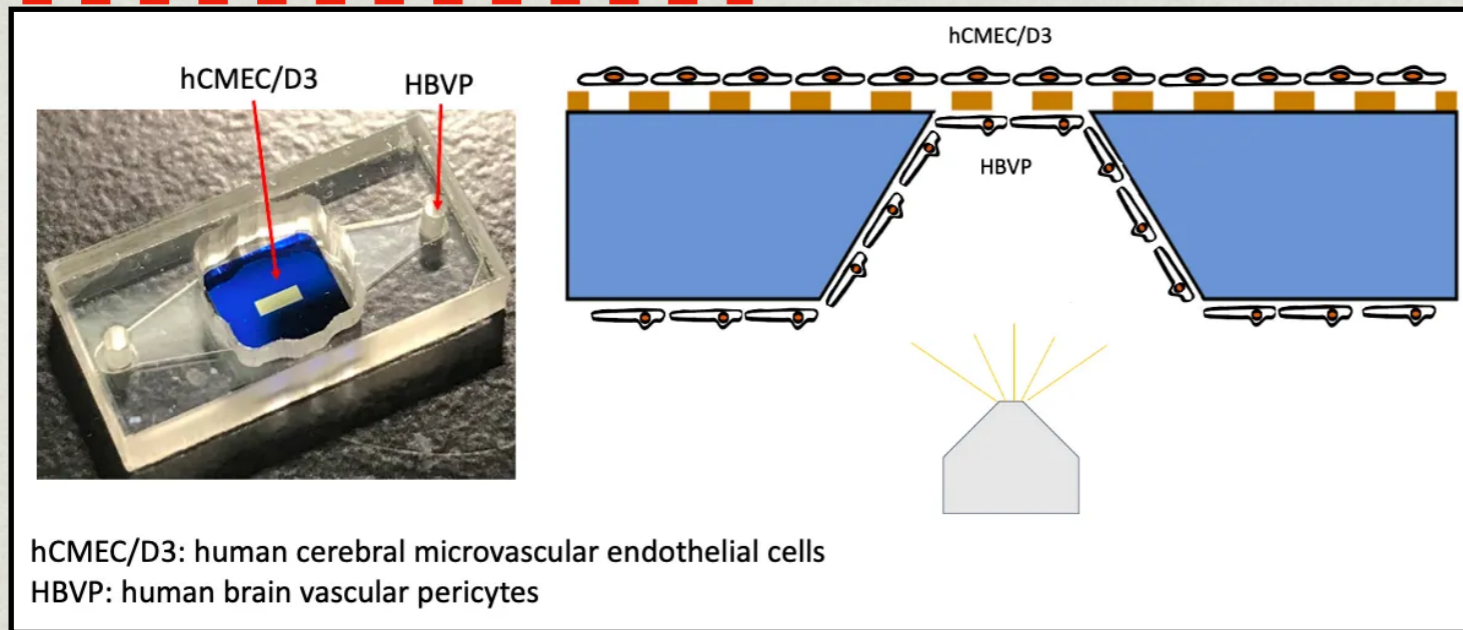
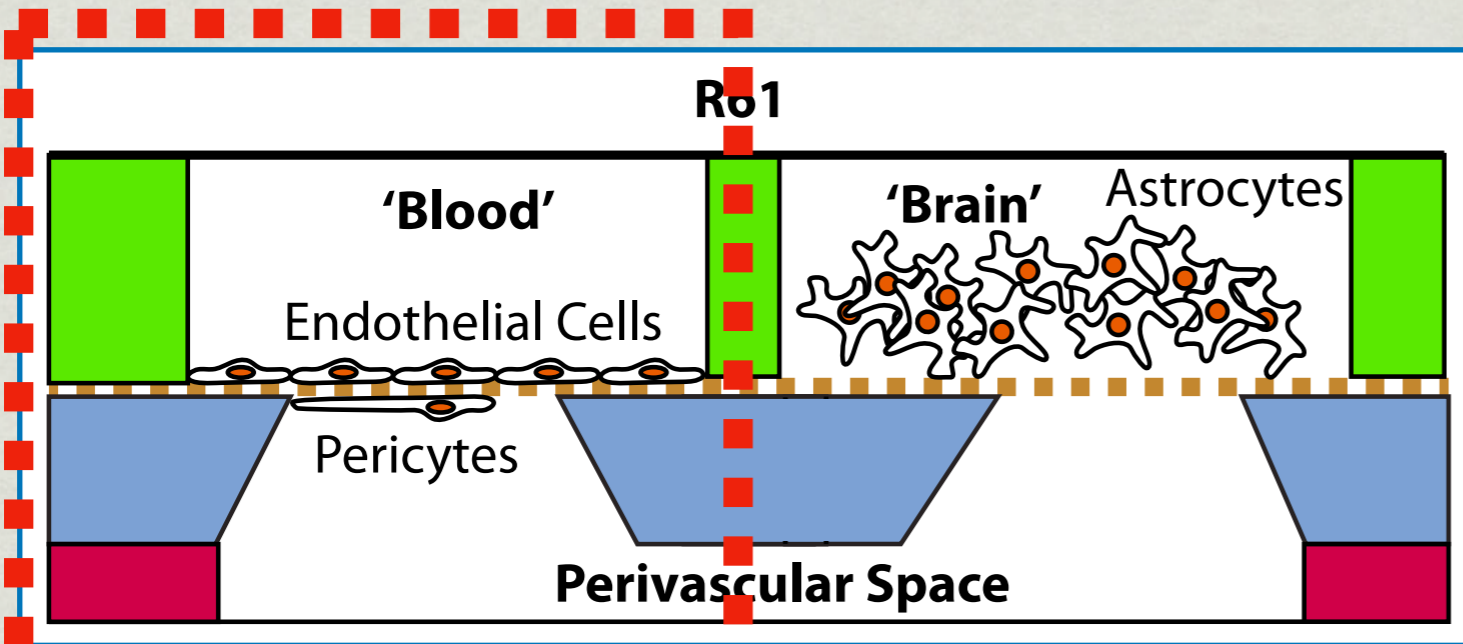
Posted on February 20, 2021 by Molly McCloskey — No Comments ↓

Introduction The ApoE4 allele of the apolipoprotein E (ApoE) gene is the greatest known genetic risk factor for Alzheimer's disease. Further it has been linked to increased susceptibility and duration of infection. On the other hand, the ApoE3 allele has ...

[Read more >](#)

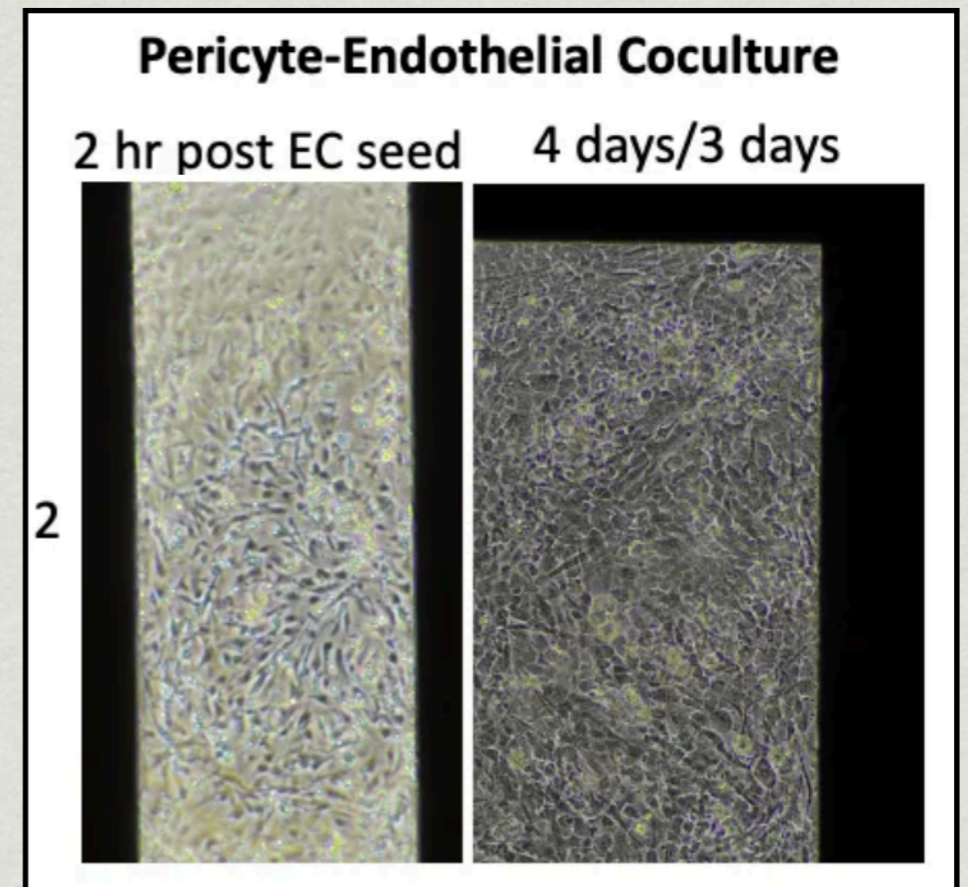
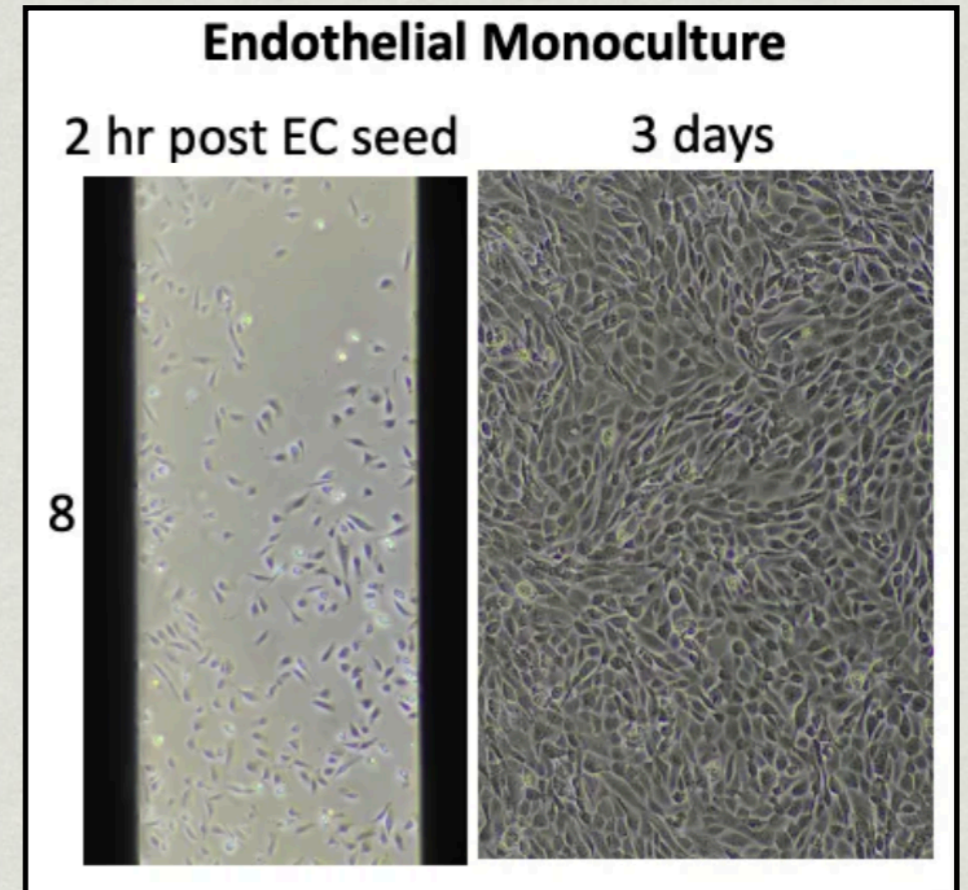
- Monthly 'small group' meetings: UR/RIT; UR/Bern; UR/Michigan
- Monthly consortium-wide 'large group' meetings with blog-based presentation and discussion
- Special meetings between teams as needed
- Community based protocol development through NRG blog - pages

BBB Development

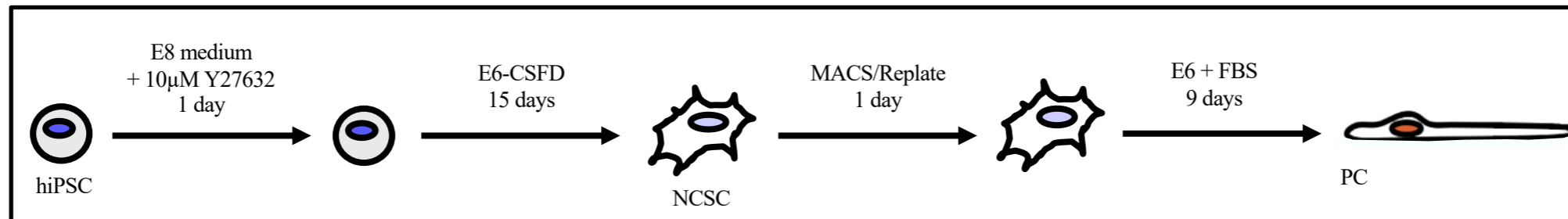


Two approaches:

- hCMECs/HBVP (commercially available primary human pericytes)
- iPSC-derived Pericytes and ECs



iPSC Pericytes

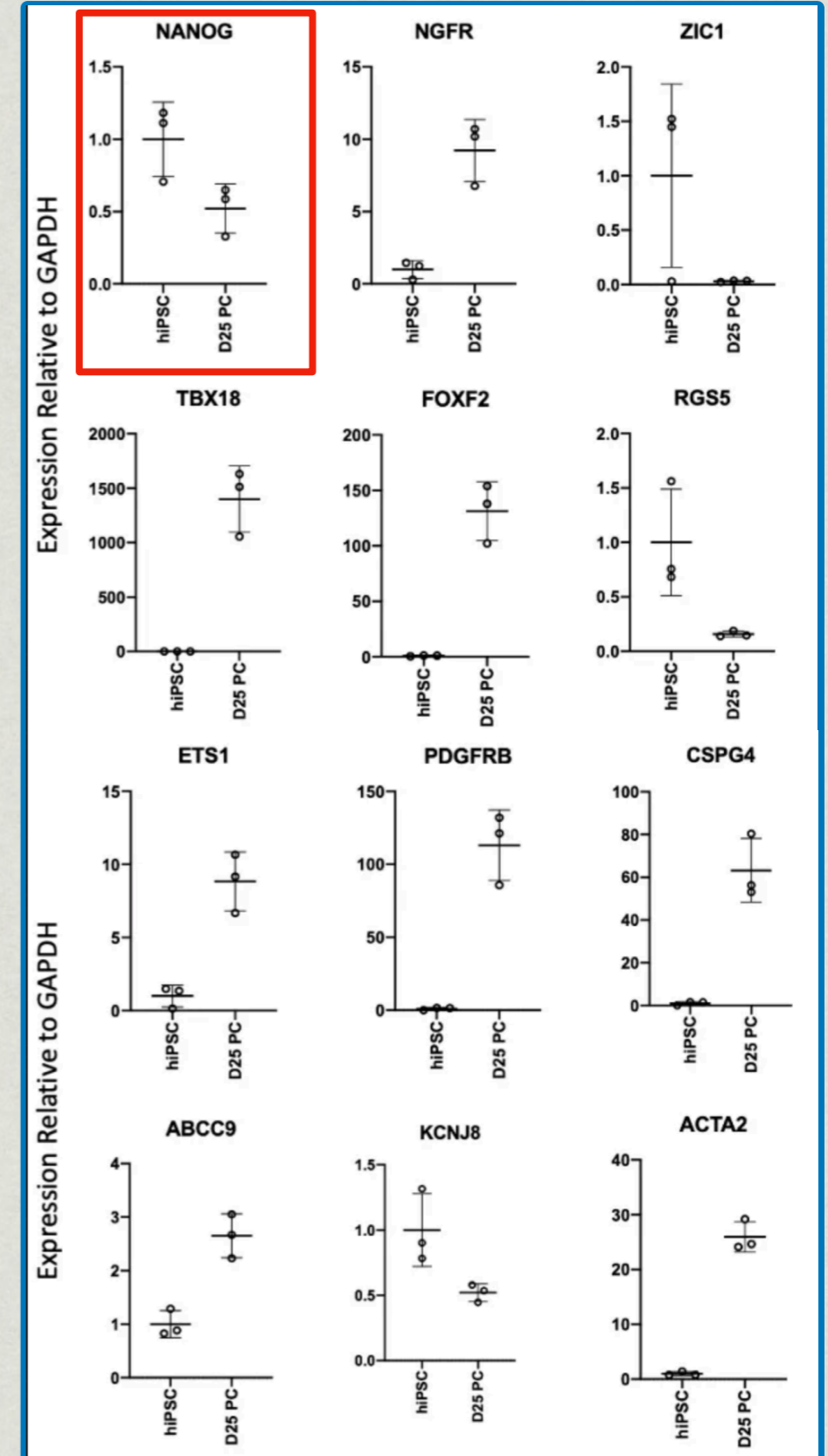
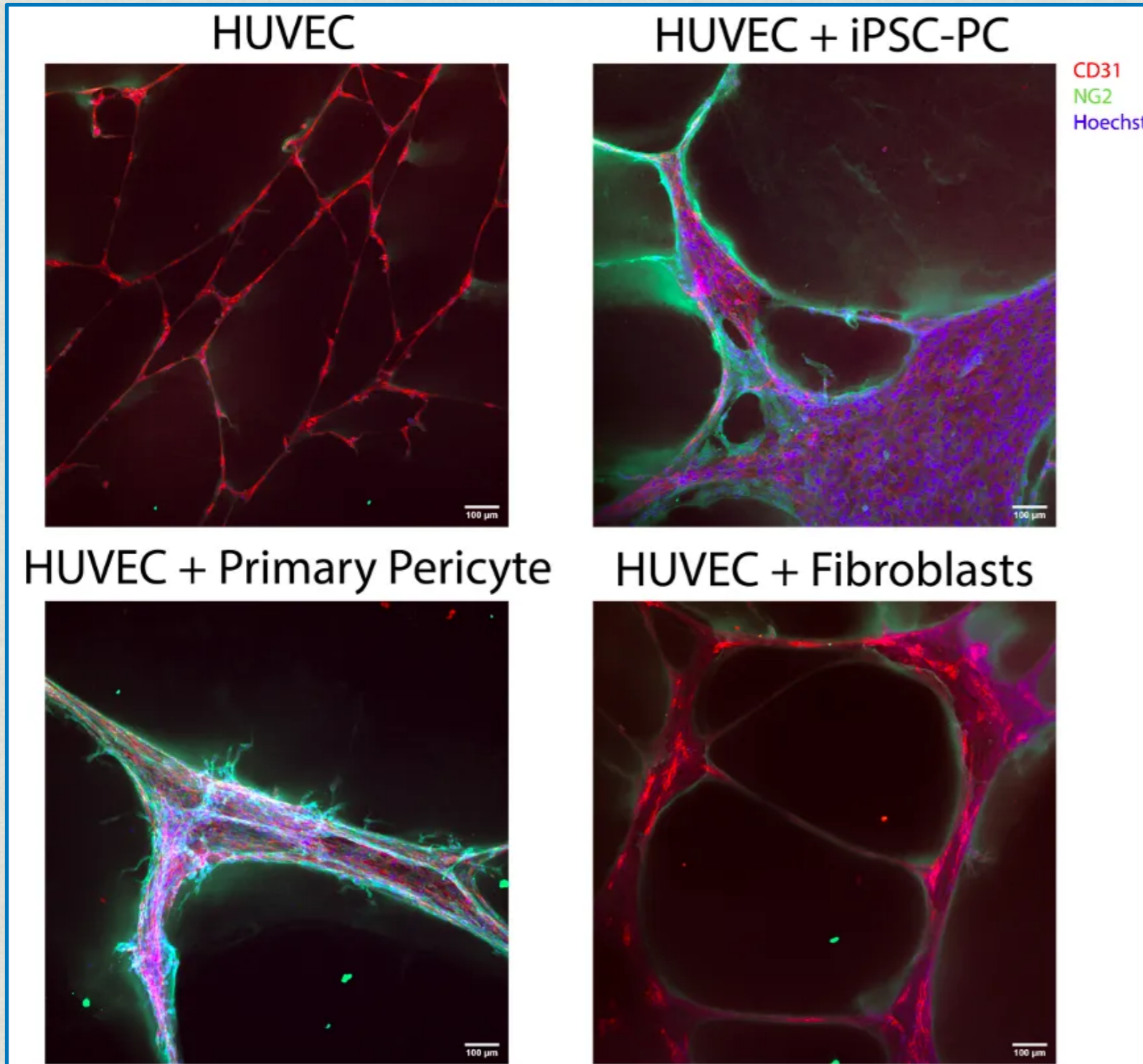


Steps for Differentiation	Medium
hiPSC Maintenance	E8 medium (DMEM/F12 basal medium supplemented with 64 mg/liter l-ascorbic acid-2-phosphate magnesium, 14 µg/liter sodium selenium, 100 µg/liter FGF2, 19.4 mg/liter insulin, 543 mg/liter NaHCO ₃ , 10.7 mg/liter transferrin, and 2 µg/liter TGFβ1)
NCSC Differentiation	E8 + 10 µM Y27632
MACS/Replate	E6-CSFD medium (DMEM/F12 basal medium supplemented with 64 mg/liter l-ascorbic acid-2-phosphate magnesium, 14 µg/liter sodium selenium, 19.4 mg/liter insulin, 543 mg/liter NaHCO ₃ , 10.7 mg/liter transferrin, 22.5 mg/liter heparin sodium salt , 1 µM CHIR99021 , 10 µM SB431542 , 10 µg/liter FGF2, and 1 µM dorsomorphin)
PC Differentiation	E6 medium supplemented with 10% FBS

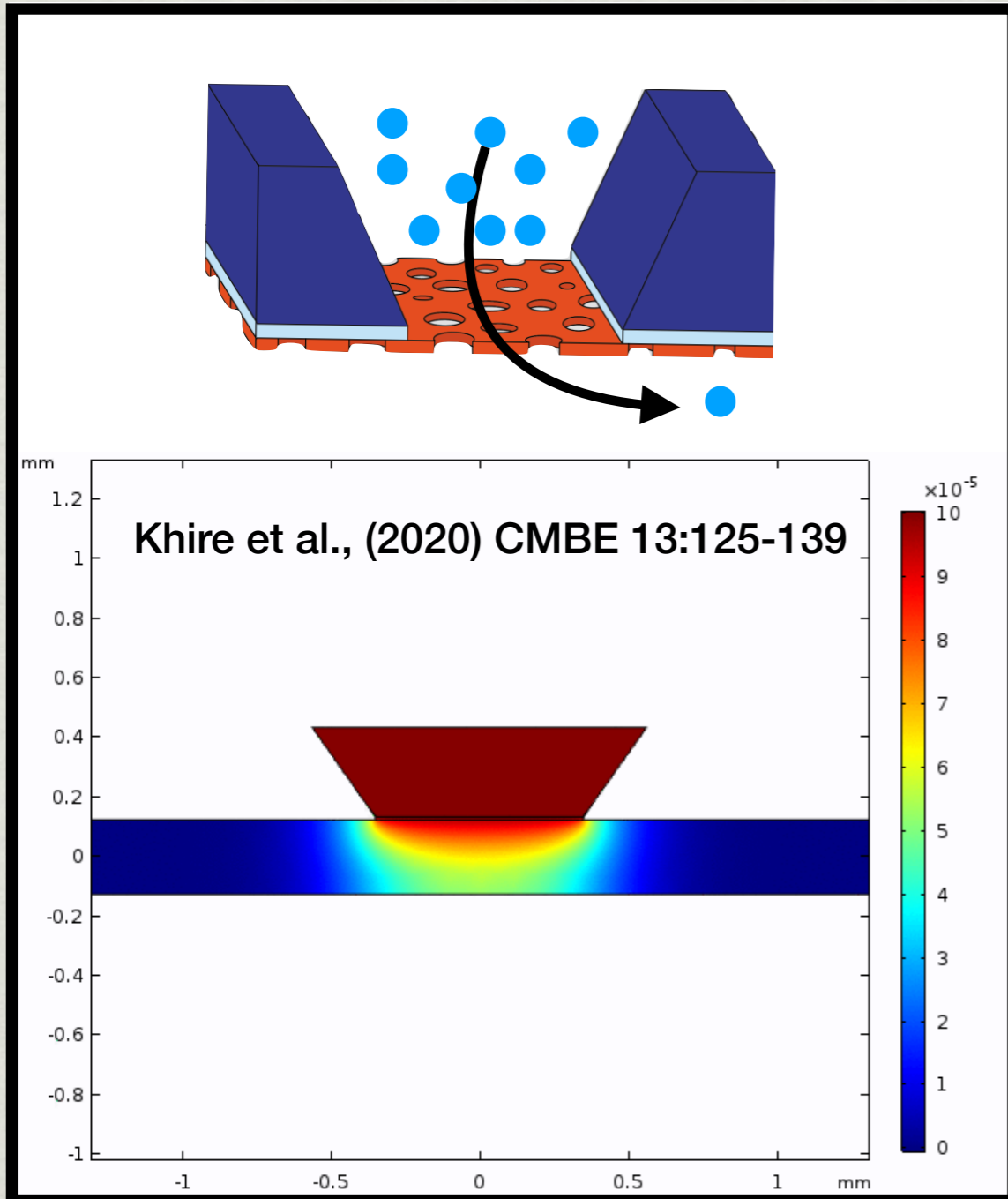
Shusta et al., Neuroscience 2019

- Commercial iPSC clone: IMR90-4
- Genotyped (by us) to be APOE3 homozygous (healthy NVU)
- Two rounds of pericyte development from IMR90-4

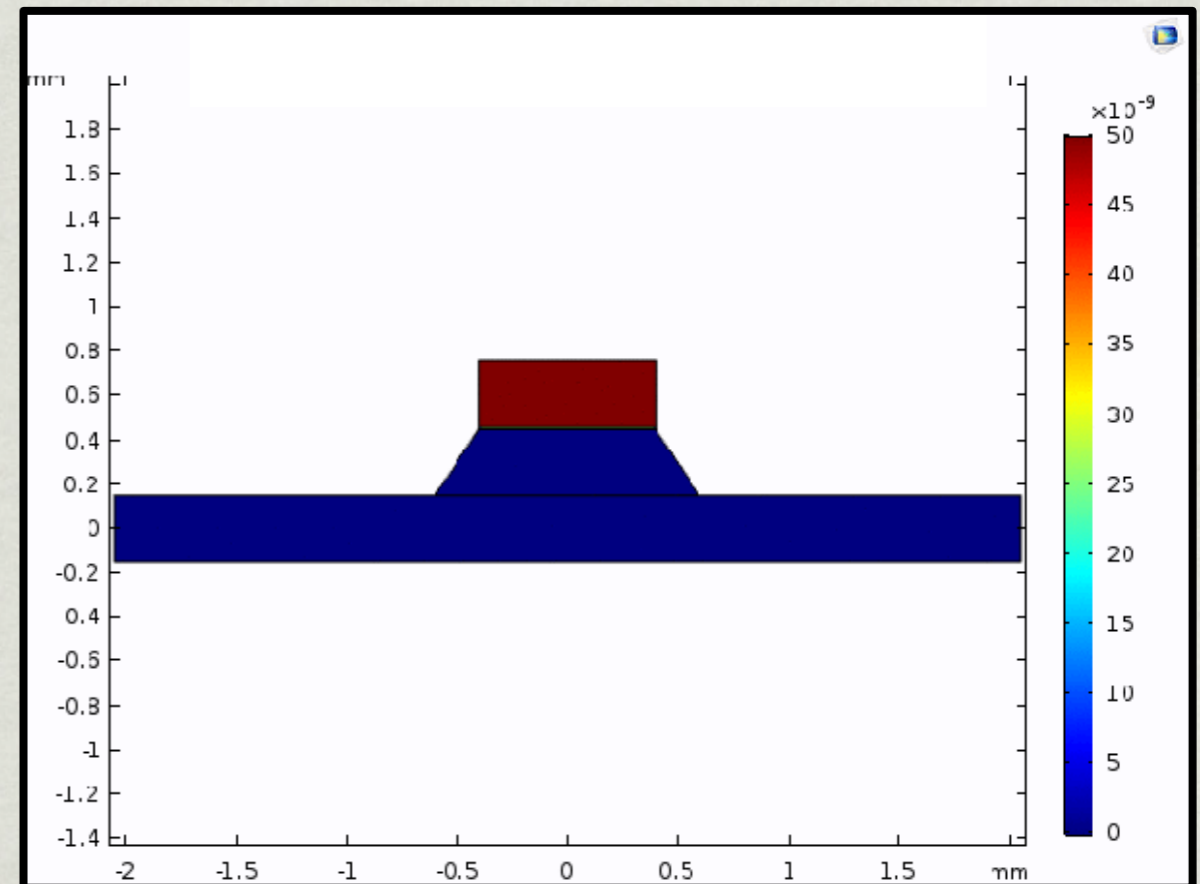
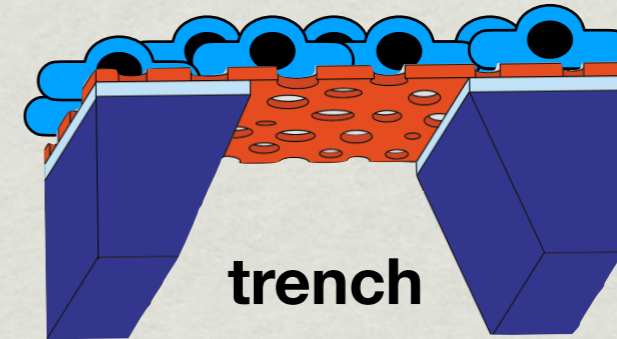
iPSC Pericytes



Permeability Measurements

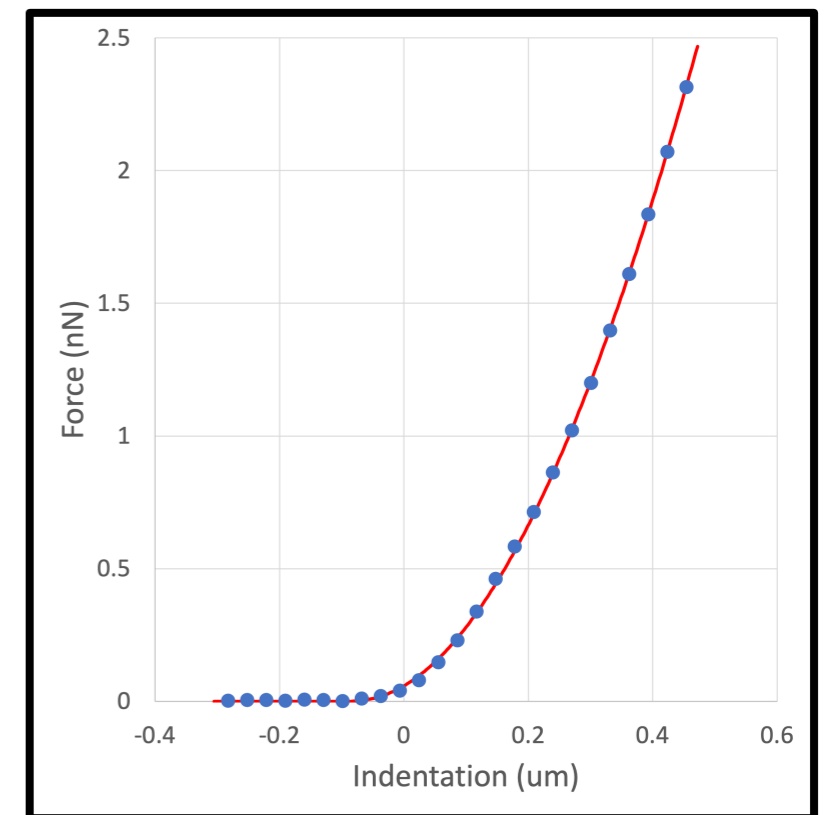
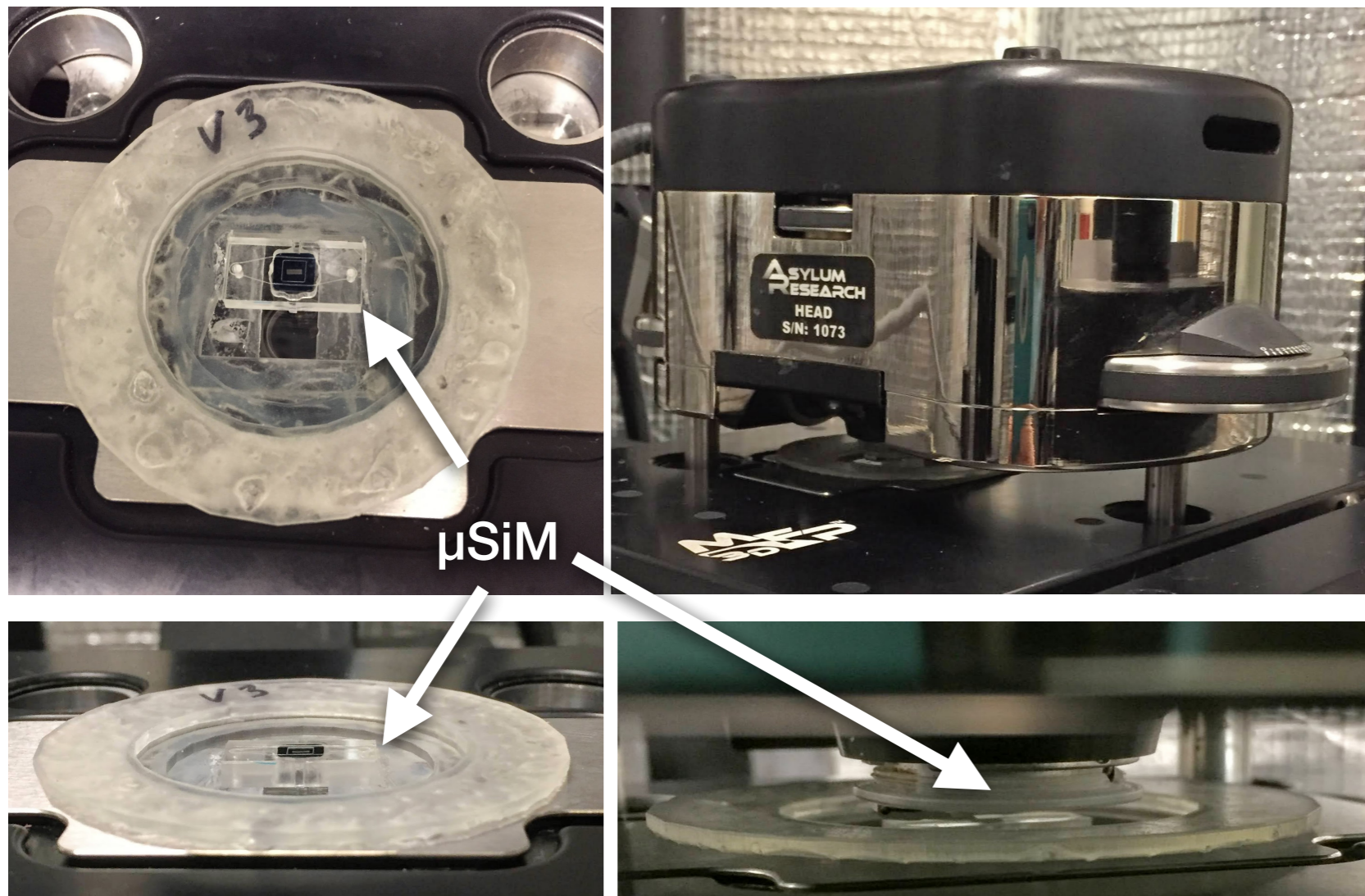


R61: flat side EC culture



- Developed confocal measurement of diffusion in trench 100 μm below membrane.
- Validated for small molecule diffusion without cells
- Next up: Application to monoculture and co-cultures
- Follow up: sampling based approach (end-point assay)

Glycocalyx Studies - AFM Measurements on ECs



$E_{cell} = 6.28 \text{ kPa}$
 $E_{gc} = 26.5 \text{ Pa}$
 $Th = 90.1 \text{ nm}$

- Also developing an ELISA-based approach for monitoring glycocalyx shedding

RIT progress update

Tom Gaborski, PhD
 Vinay Abhyankar, PhD
 Steven Day, PhD

Louis Widom (basement mebrane)
Mehran Mansouri (flow module)

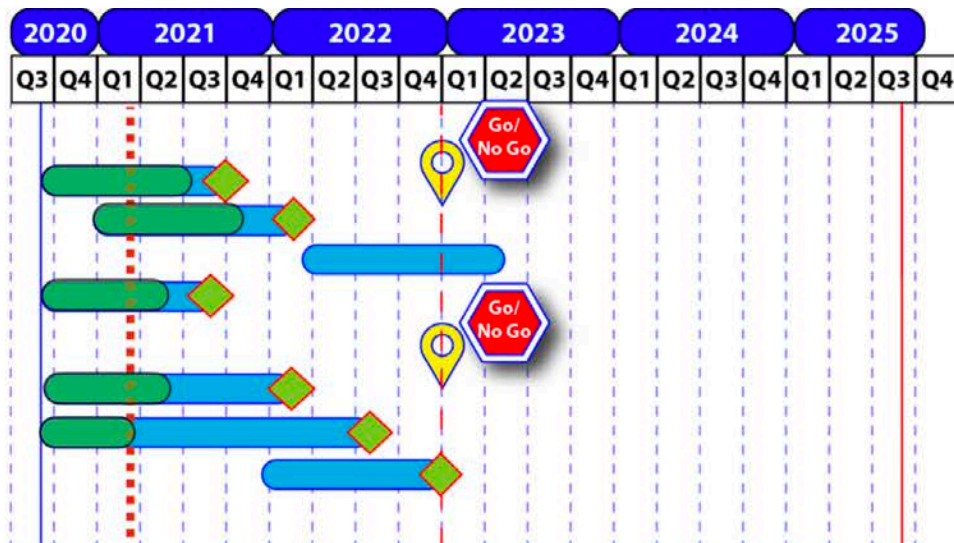
Detailed Gantt Chart

Successful Development of the Modular μ SiM

- M1A Successful assembly and use by non-engineering laboratories
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- M1C Successful development and application of a TEER module
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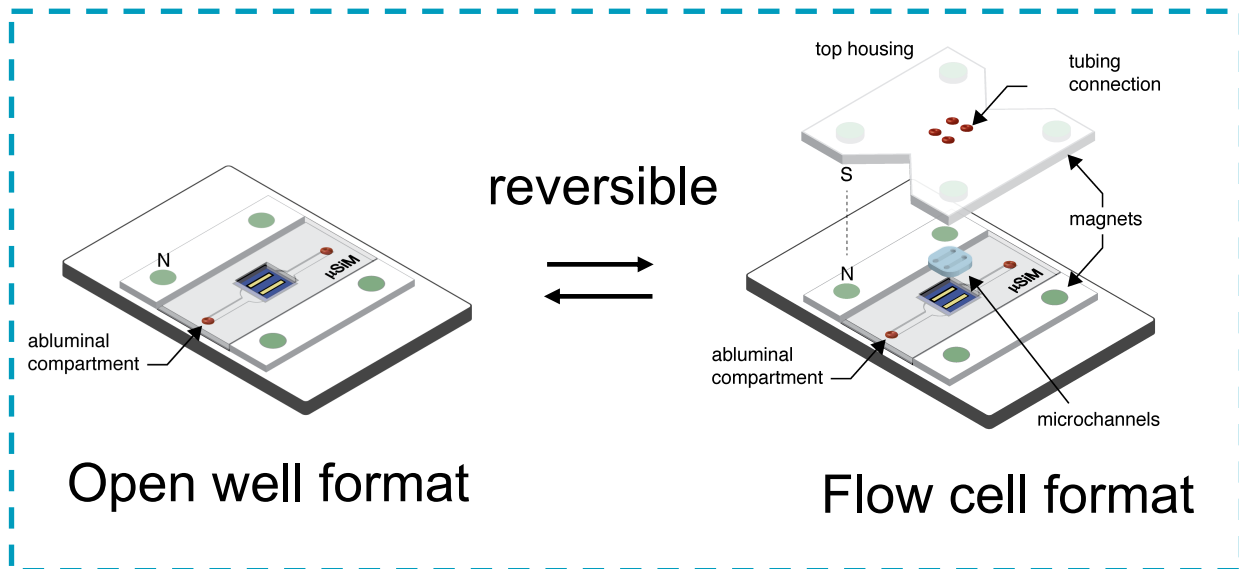
Successful Development of the μ SiM-hNVU

- M2A Supports co-culture of all components of the NVU
- M2B Supports a validated BBB
- M2C Demonstrate responsiveness to septic stimuli



Modular μ SiM

M1B: Successful development and application of a flow module

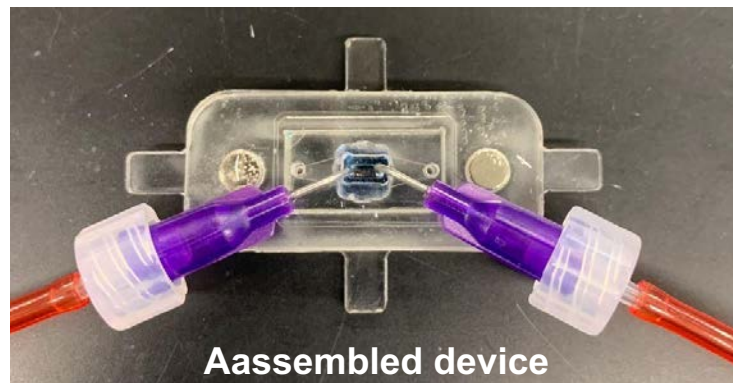
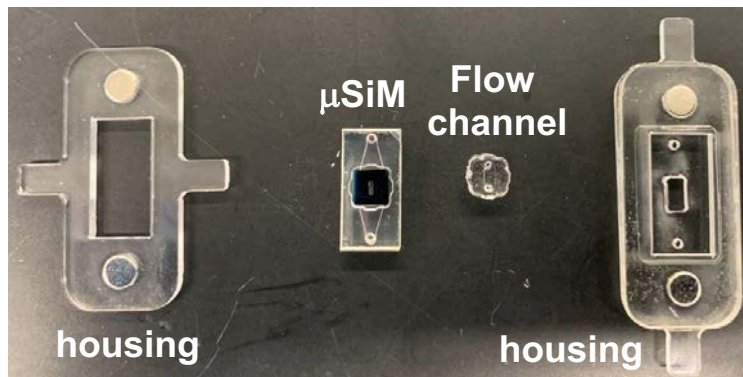
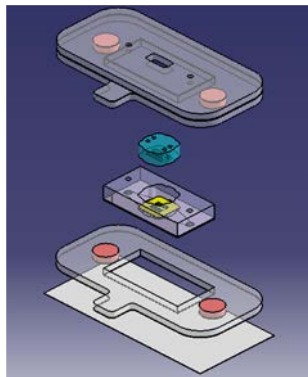


UR
Bern
Michigan

Prototyping and flow characterization

Modular μ SiM prototyping

Magnetic assembly



Progress toward milestone

- Working prototype of modular platform with reversible switching from open well to flow cell format

Modular μ SiM: flow validation

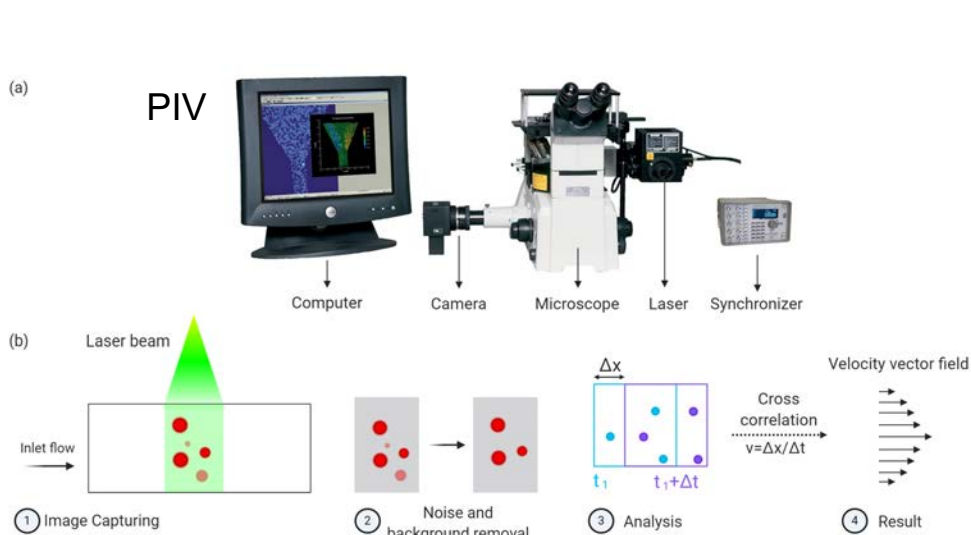
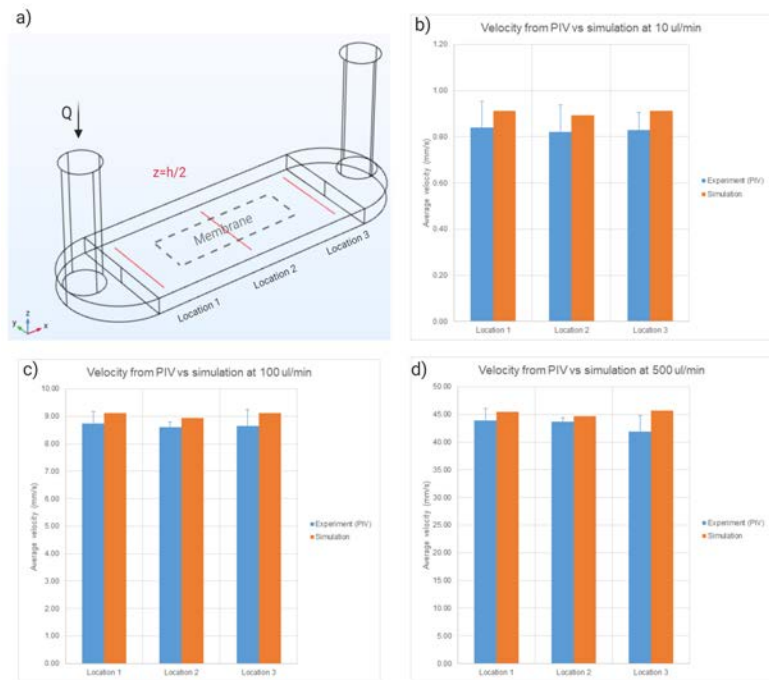


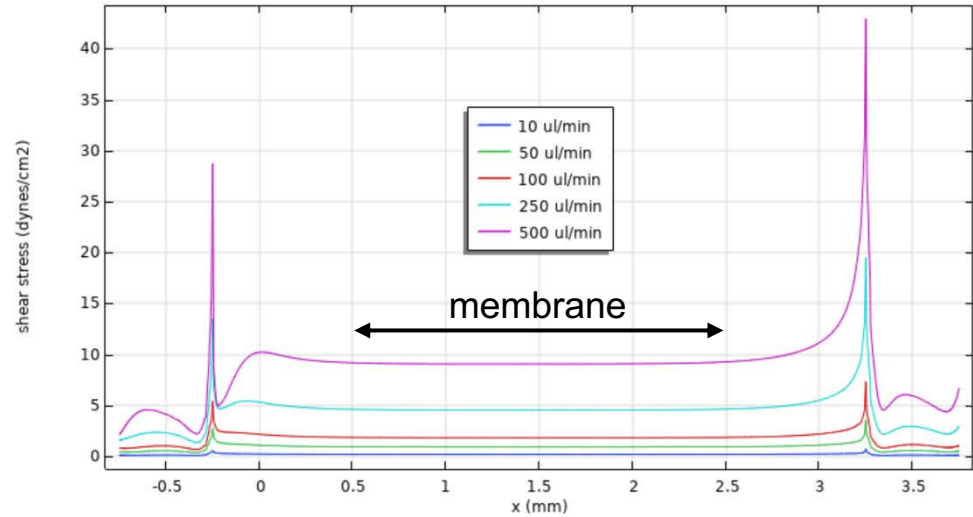
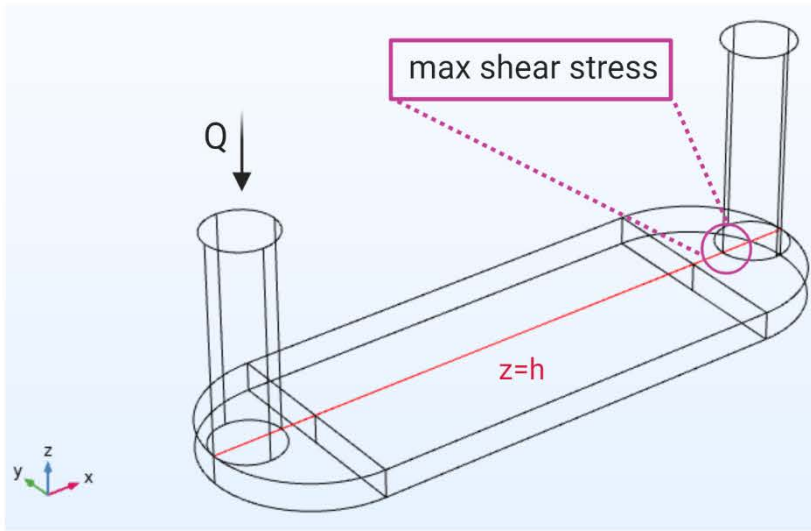
Figure 1. Schematic of (a) particle image velocimetry (PIV) setup (b) PIV analysis concept



Progress toward milestone

- Experimental characterization of flow (PIV) matches COMSOL simulation from $Q = 10 - 500 \mu\text{L min}^{-1}$

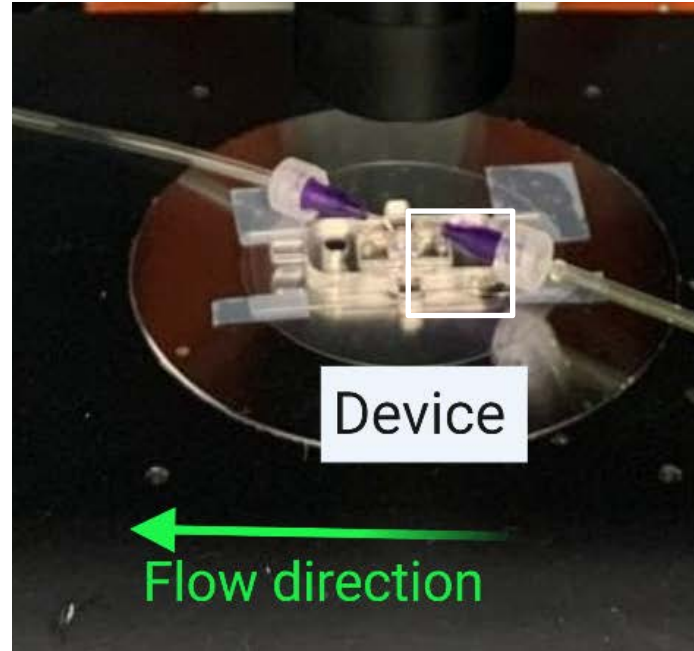
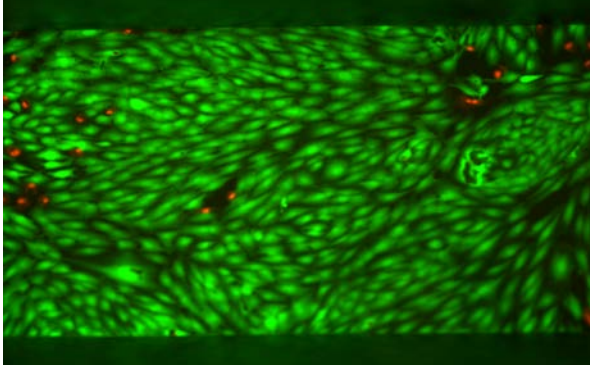
Modular μ SiM: fluid shear stress distribution



Progress toward milestone

- Identified flow conditions to satisfy requirement of $\tau < 40 \text{ dyne cm}^{-2}$ to prevent shear activation with $\tau = 1.5 \text{ dyne cm}^{-2}$ at membrane

Modular μ SiM: endothelial cell culture (24 hours)



Progress toward milestone

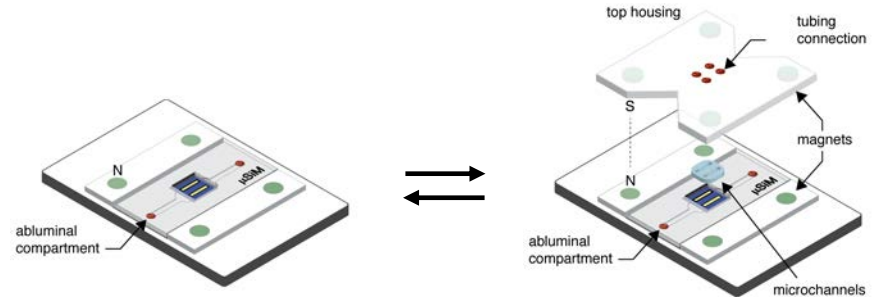
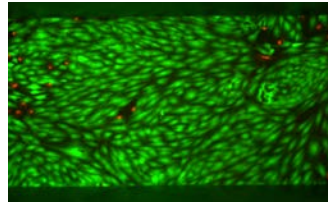
- Leak-proof endothelial culture for 24 hours with $> 90\%$ viability

Modular μ SiM: ongoing work

- Optimization of flow condition to introduce neutrophils to platform
- Confirm leak-proof operation for longer term cultures
- Local beta testing of μ SiM prototypes to ensure ease of use and functionality



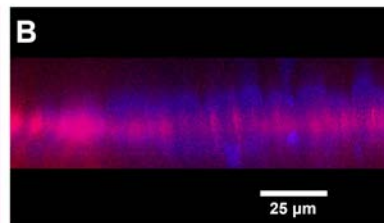
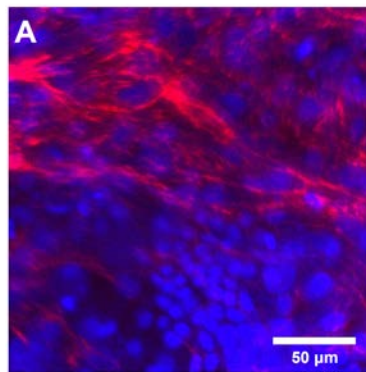
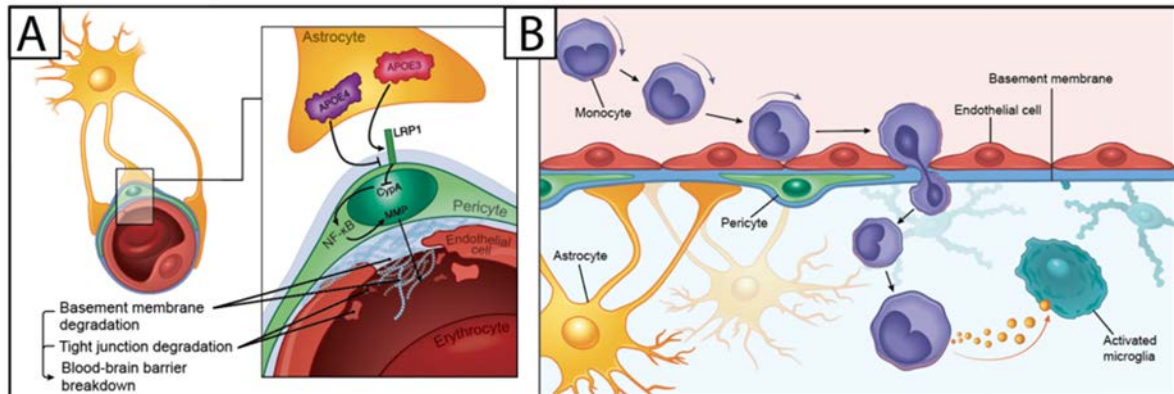
Mehran Mansouri



Basement Membrane (BM) Formation

Collagen IV deposition was assessed in:

- Human Brain Vascular Pericyte (HBVP) monocultures
- Human Cerebral Microvascular Endothelial Cell (hCMEC/D3) monocultures
- Co-cultures of both cell types

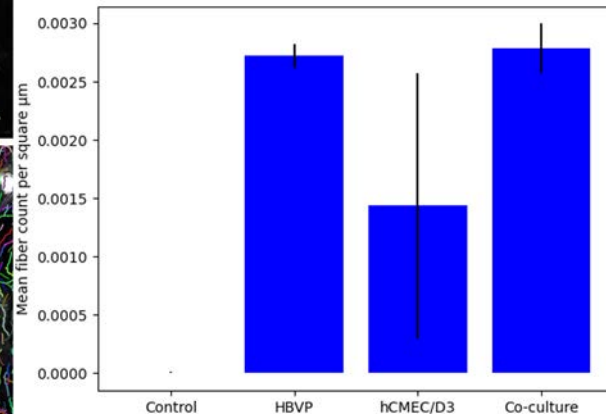
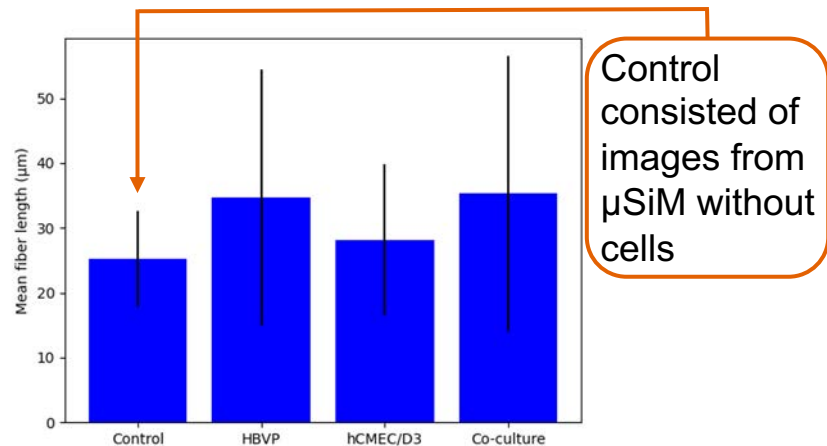
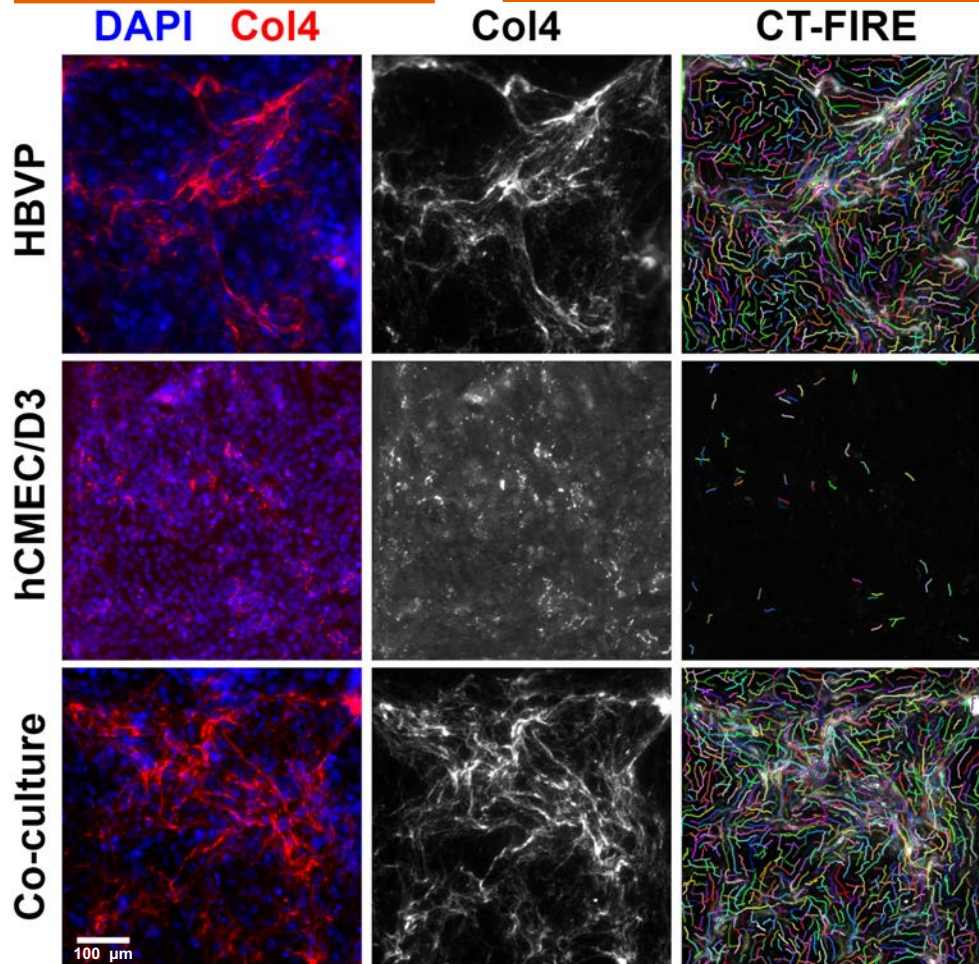


DAPI Col4

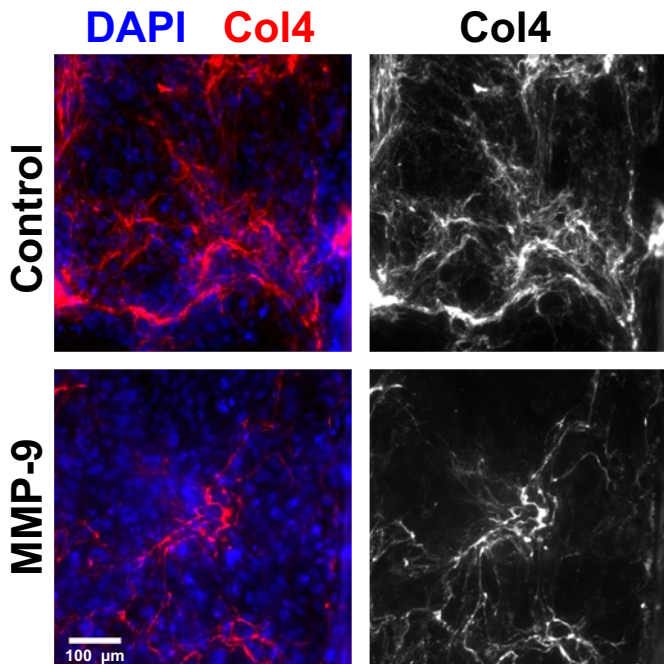
Confocal microscopy revealed collagen IV localized between the two cell layers in co-culture



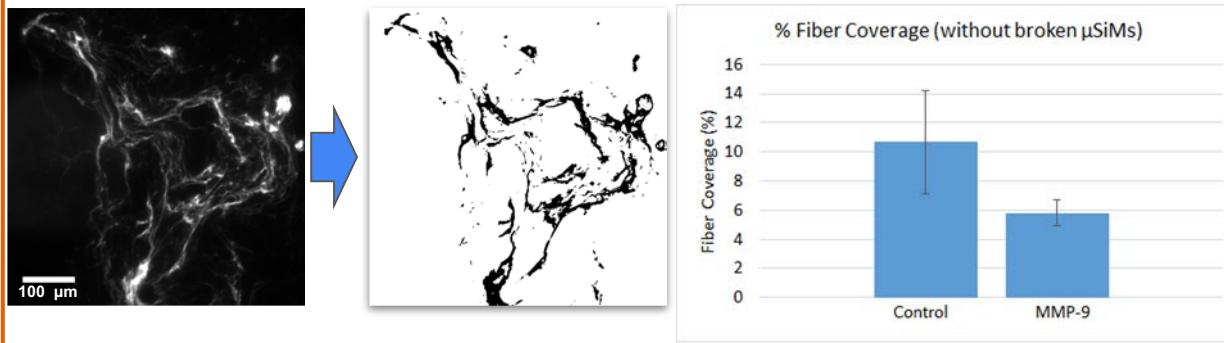
Louis Widom



MMP-9 degrades collagen IV, and is a known product of pathways initiated by pro-inflammatory signals (Rosenberg et al. 1995)



Binarization was used to determine the percentage of collagen IV fiber coverage following MMP-9 treatment



Next steps:

- Increase MMP-9 treatment replicates
- Investigate endothelial cell and pericyte motility in response to MMP-9 treatment
- Examine responses to treatment with $\text{TNF-}\alpha$



The **B**ern human **B**rain **B**arriers Team

u^b

**UNIVERSITÄT
BERN**

Sasha Soldati



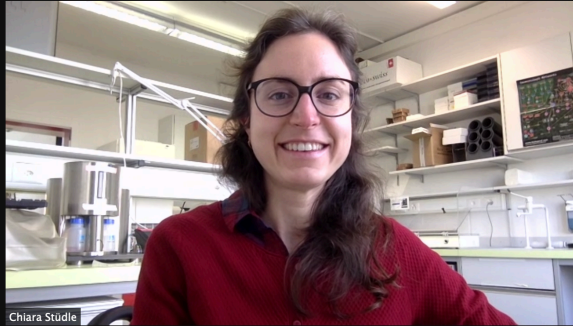
sashasoldati

Britta Engelhardt



Engelhardt, Britta (TK)

Chiara Stüdle



Chiara Stüdle

Hideaki Nishihara



hideaki.nishihara@tki.unibe.ch

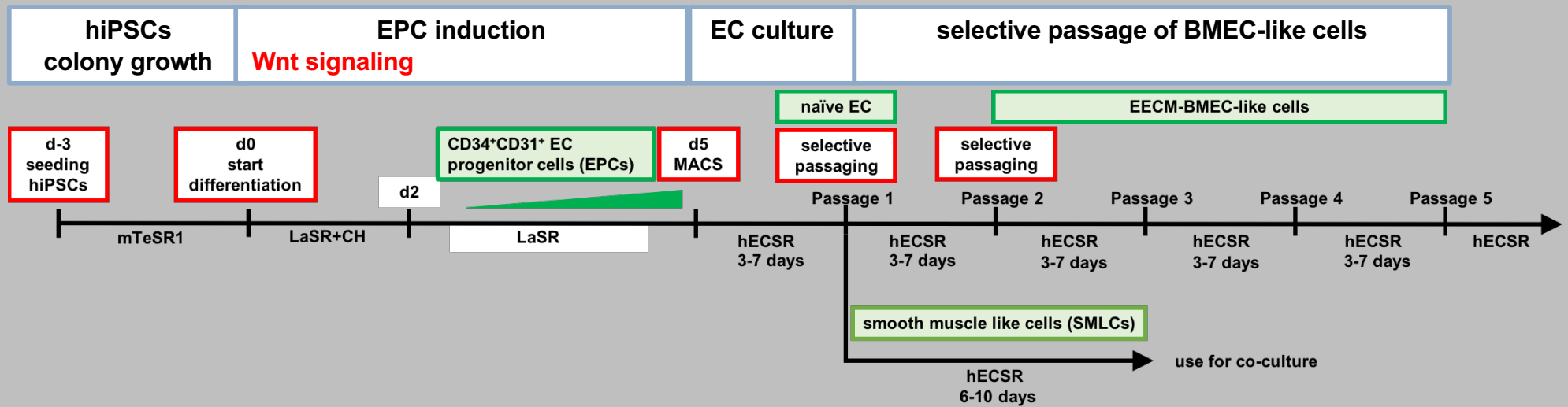
Pelin Kasab



Pelin Kasab

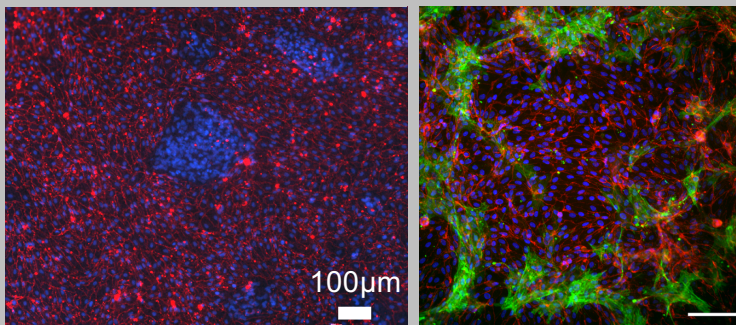
Advancing human induced pluripotent stem cell-derived blood-brain barrier models for studying immune cell interactions

Extended endothelial cell culture method (EECM)



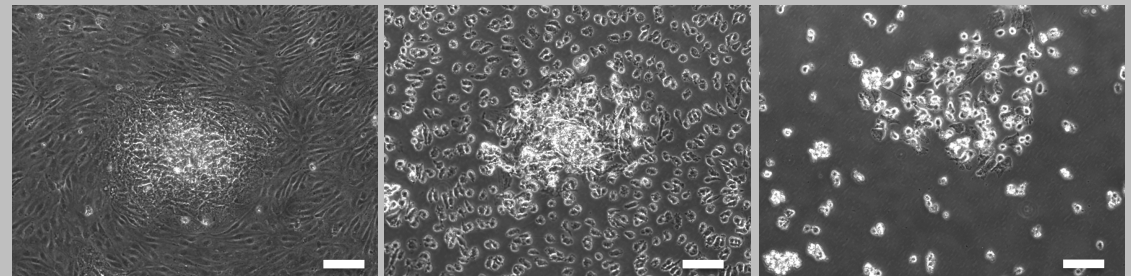
naïve ECs **VE-cadherin**

PECAM-1 **SMA**



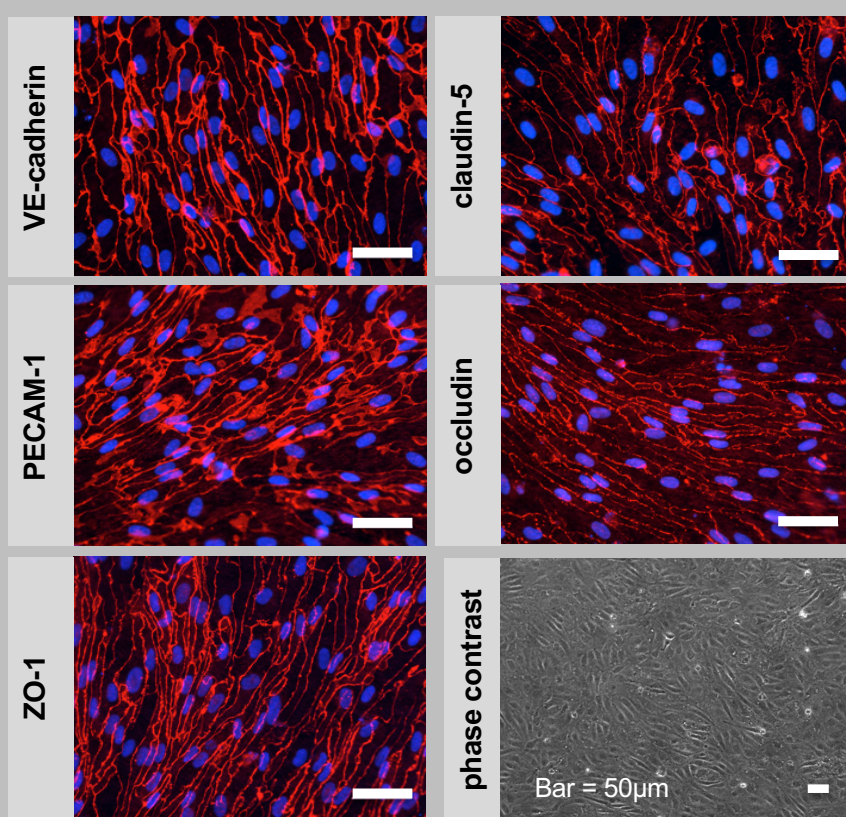
Accutase incubation

After tapping plate

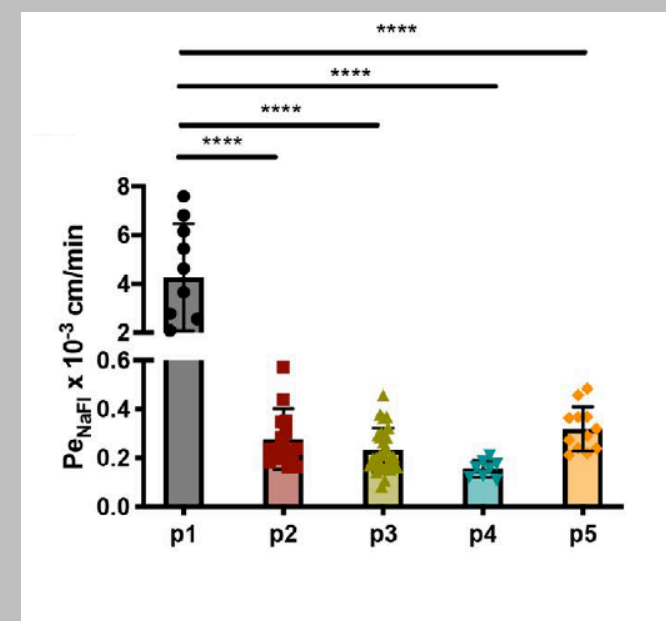
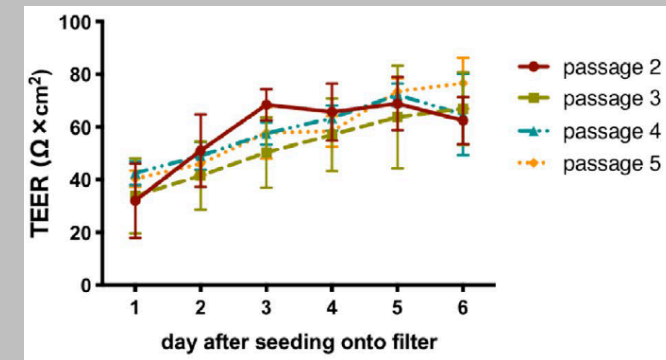


EECM-BMEC-like cells develop mature tight junctions and good barrier properties

Mature junctions

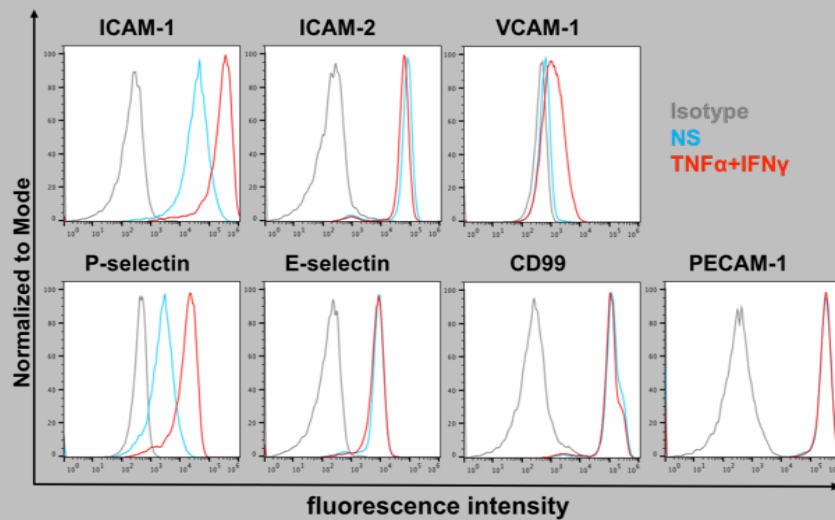


Barrier characteristics

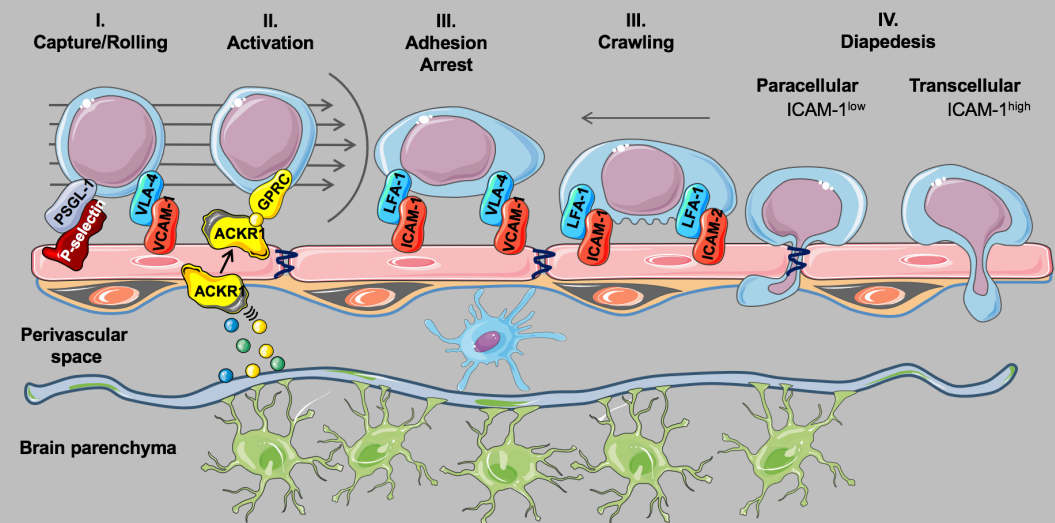


EECM-BMEC-like cells express adhesion molecules allowing for immune cell interactions

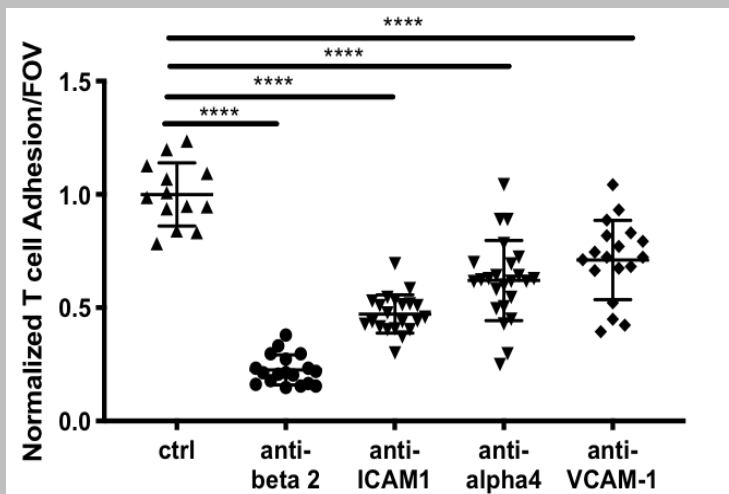
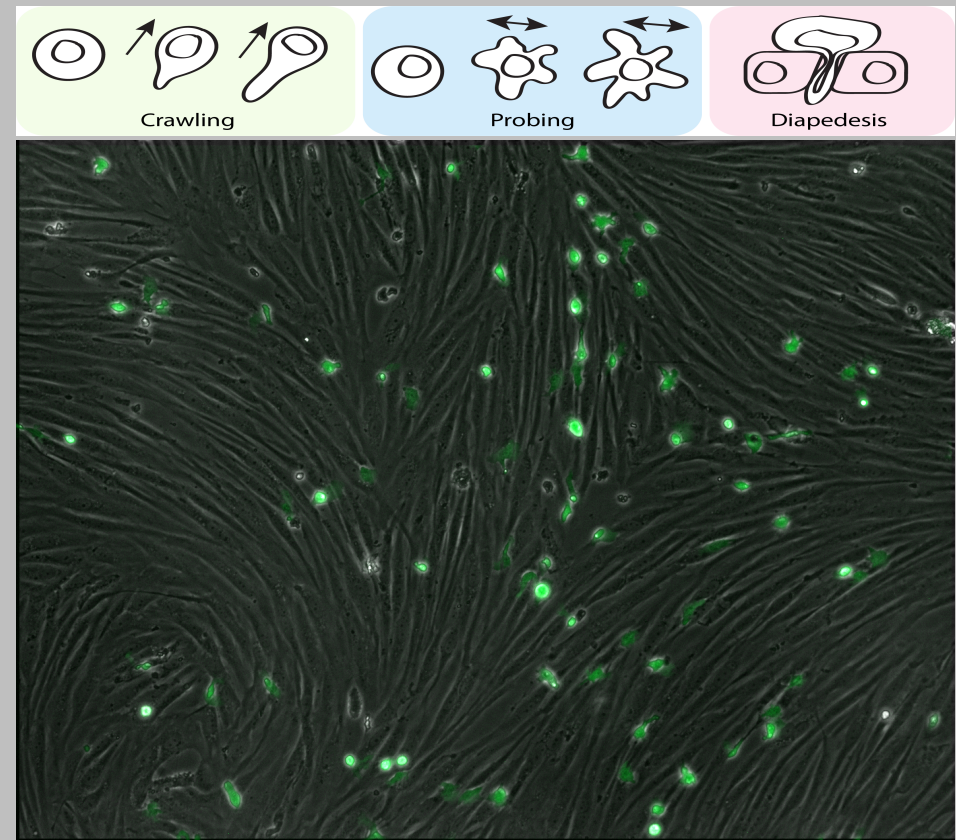
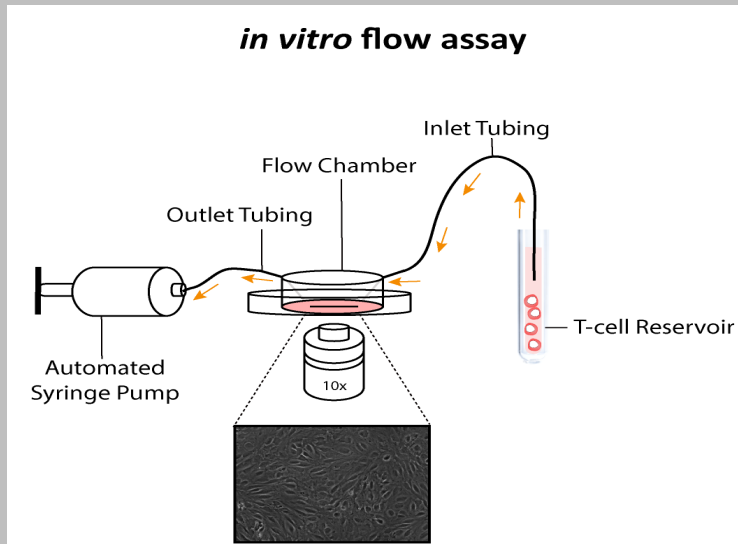
Mature immune phenotype



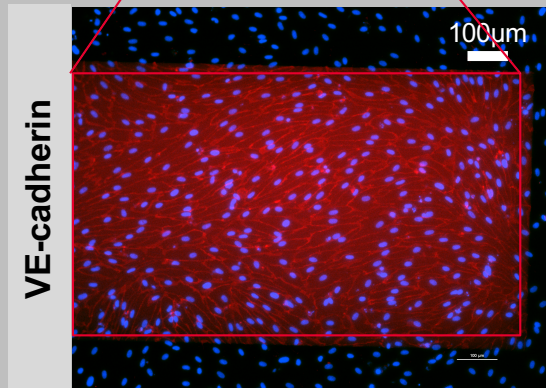
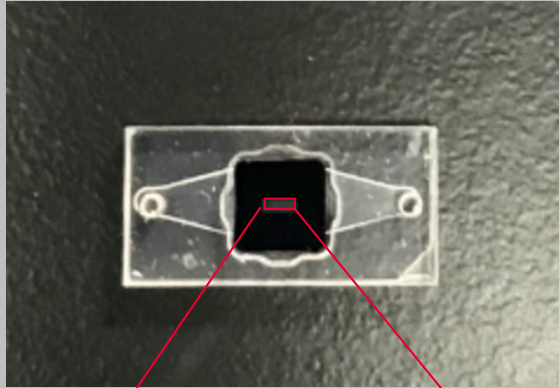
Multi-step T cell extravasation across the BBB



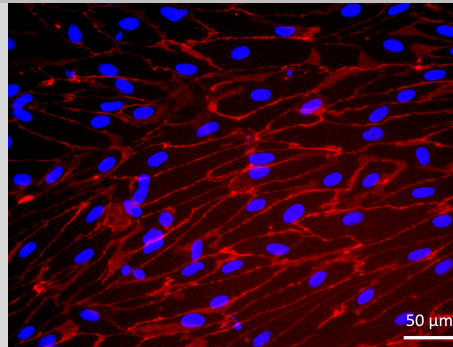
Multi-step T-cell extravasation across EECM-BMEC-like cells under physiological flow



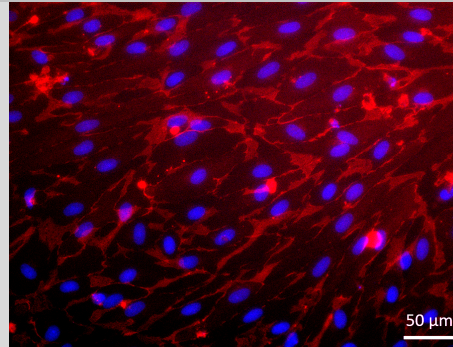
EECM-BMEC-like cells grow on nanomembranes and form mature tight junctions



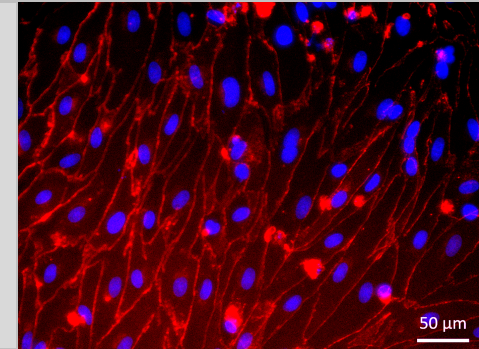
VE-cadherin



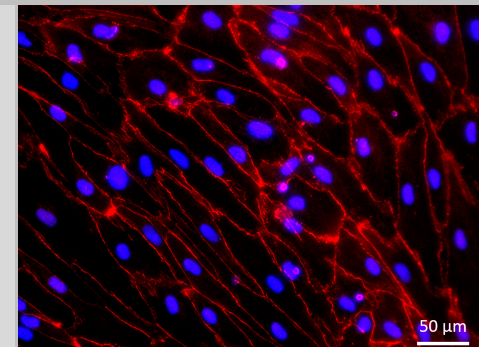
PECAM-1



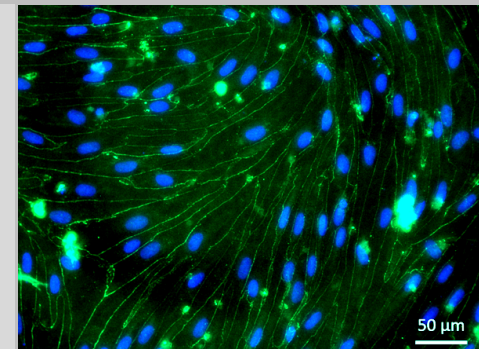
claudin-5



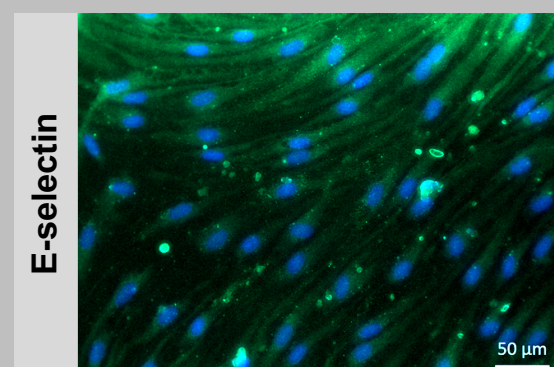
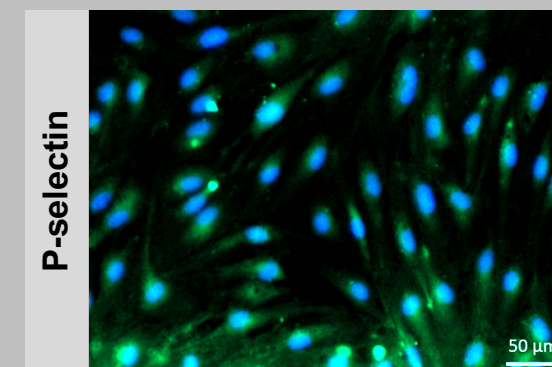
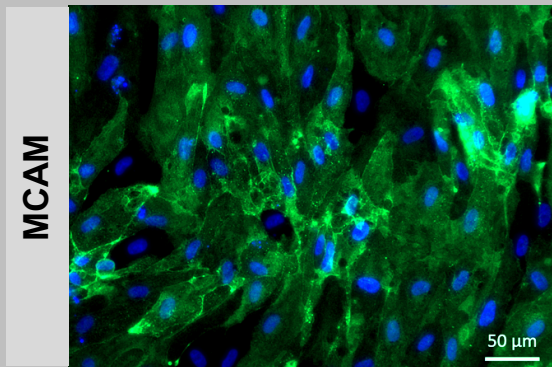
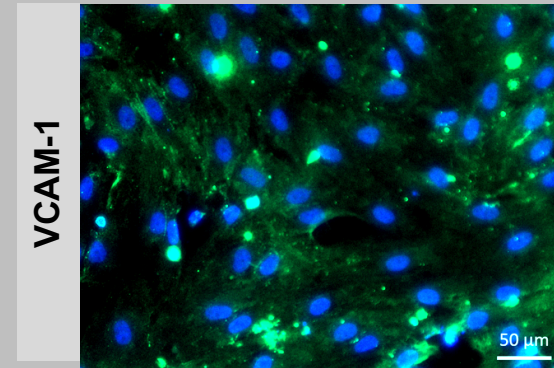
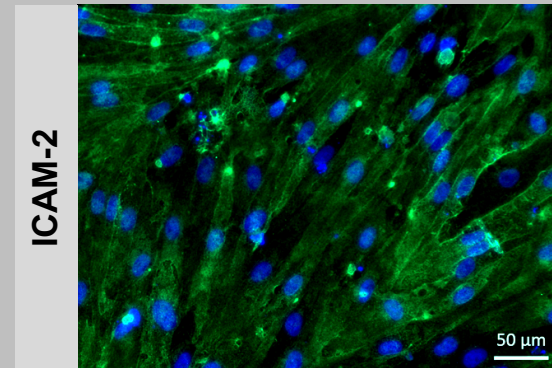
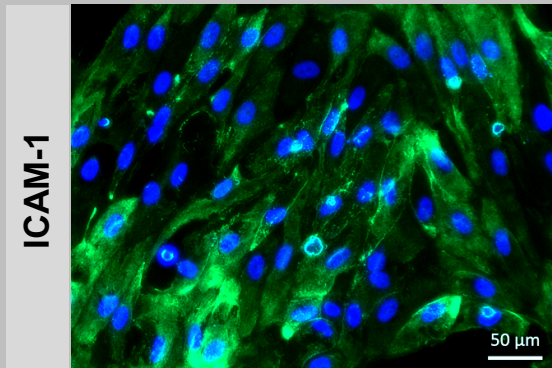
ZO-1



occludin

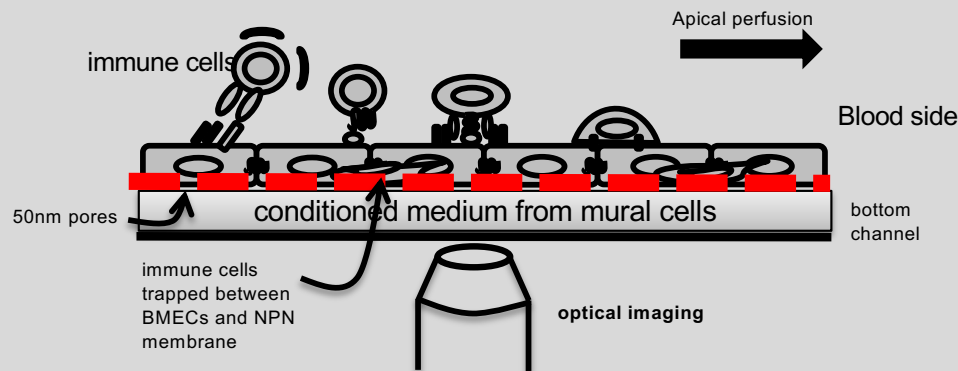


EECM-BMEC-like cells grow on nanomembranes and express cell adhesion molecules

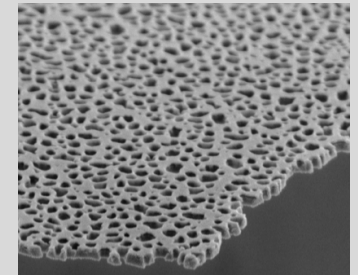


Develop a μ SiM-hiBMEC platform to study immune cell extravasation across the BBB under physiological flow

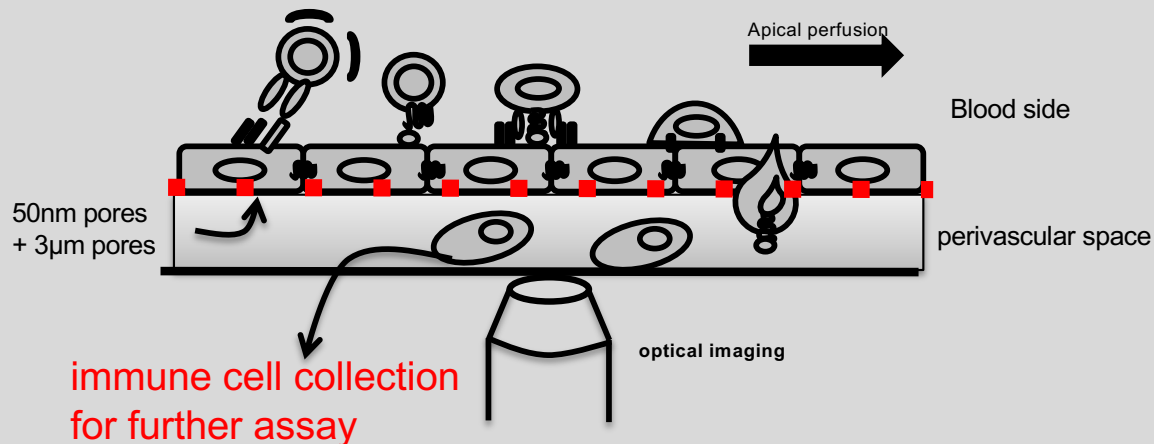
Step 1. Adapt hiPSCs derived BMECs to μ SiM device



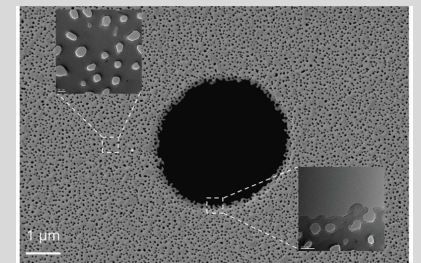
NPN membrane



Step 2. Adapt hiPSC derived BMECs to μ SiM- dual-scale membrane devices

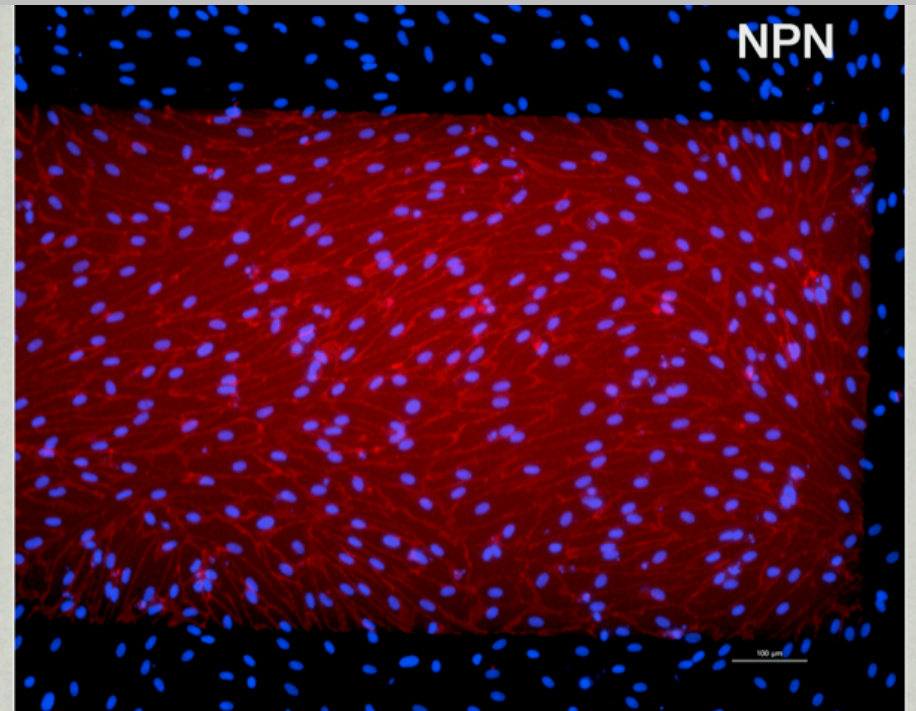
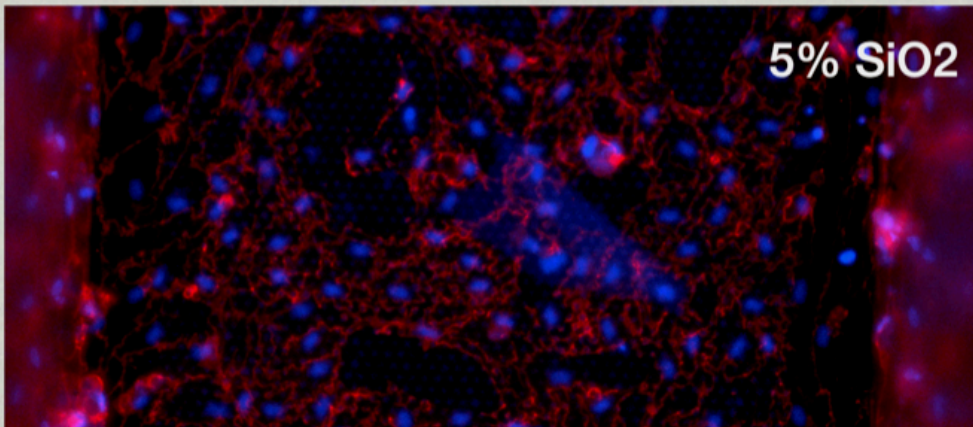
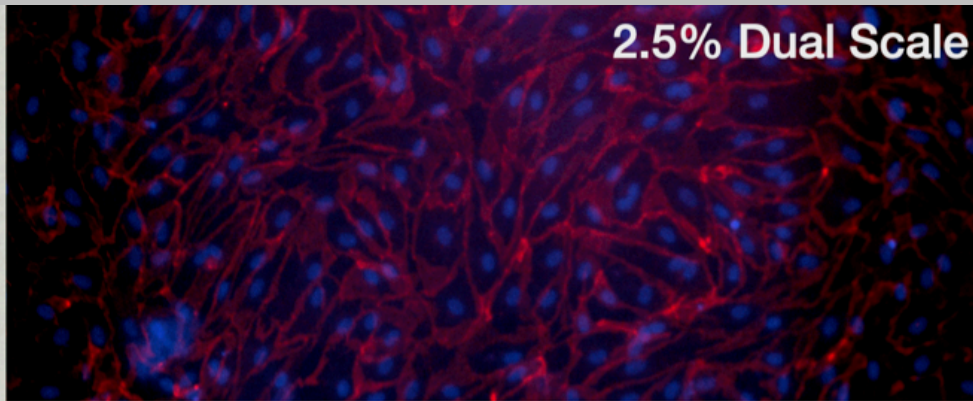


dual-scale membrane nano and micro pores



Salminen et al. *Small*, 2019

Optimizing EECM-BMEC-like cell growth on dual scale nanomembranes



University of Michigan Team



Benjamin Singer, MD, PhD

Katsuo Kurabayashi, PhD

Anuska Anjelkovic-
Zochowska, MD, PhD



Michigan Goals

R61

External testing site for μ SiM using primary cells

Digital ELISA platform for measuring cytokines in ~ 2 μ l of supernatant

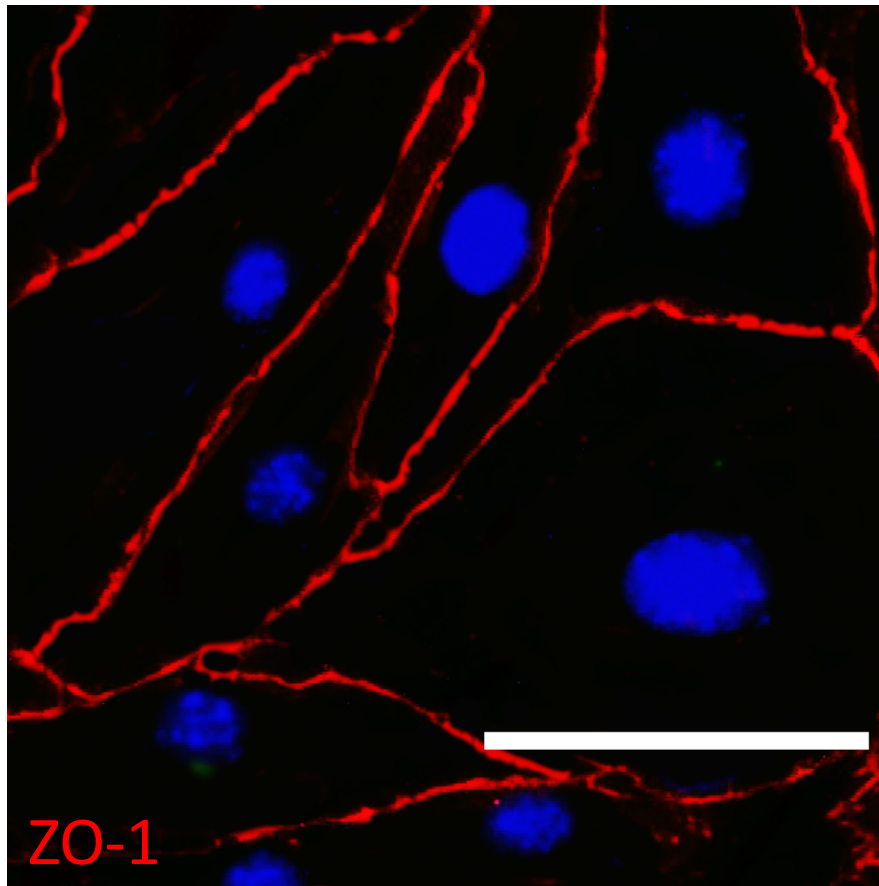
R33

BBB injury by circulating factors from patients with sepsis or noninfectious critical illness, focusing on DAMPs.

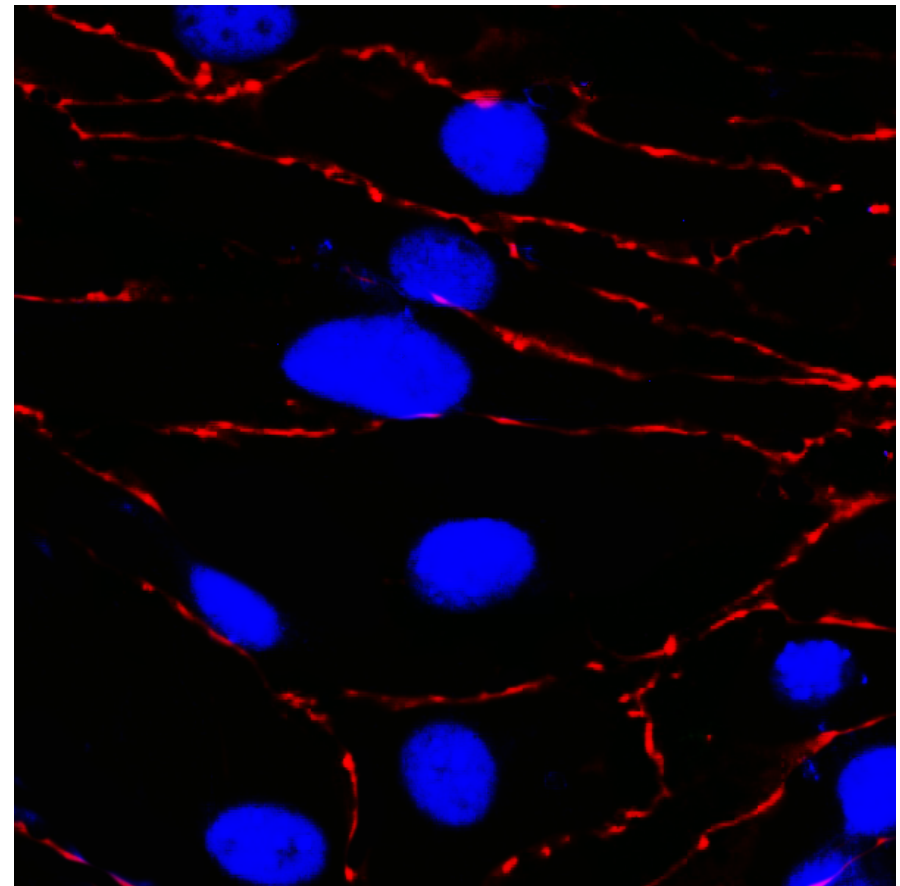


Culture of Murine Brain Endothelial Cells

control



LPS 100ng/mL x 24 hrs



Scale bar 50 μ m

Anuska Anjelkovic Zochowska
Muyu Situ



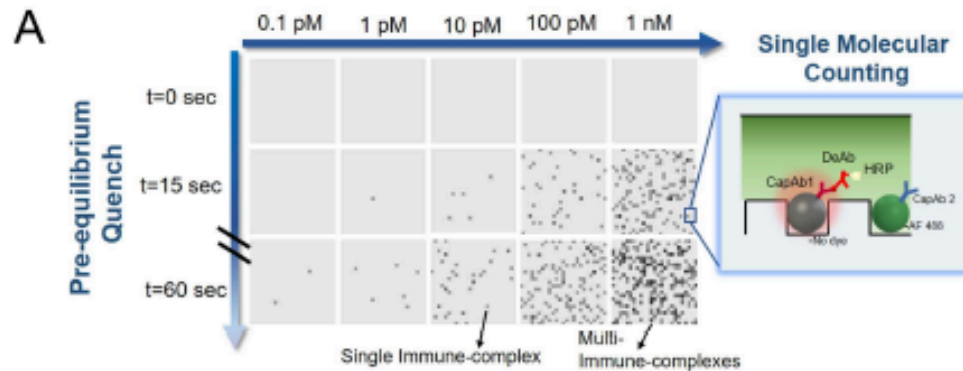
Measurement of secreted proteins

Goals:

- Small volume: repeated measurements with minimal perturbation of the culture milieu.
- High sensitivity
- High reproducibility



Measurement of secreted proteins

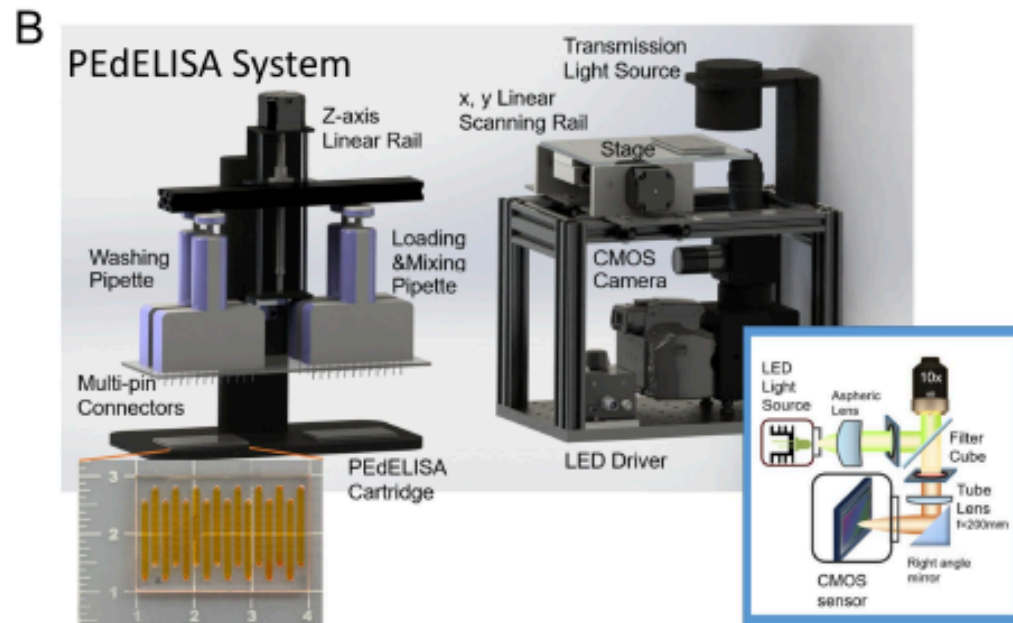


Digital ELISA platform

- Linear detection over a wide dynamic range

-based on commercially available ELISA antibody pairs

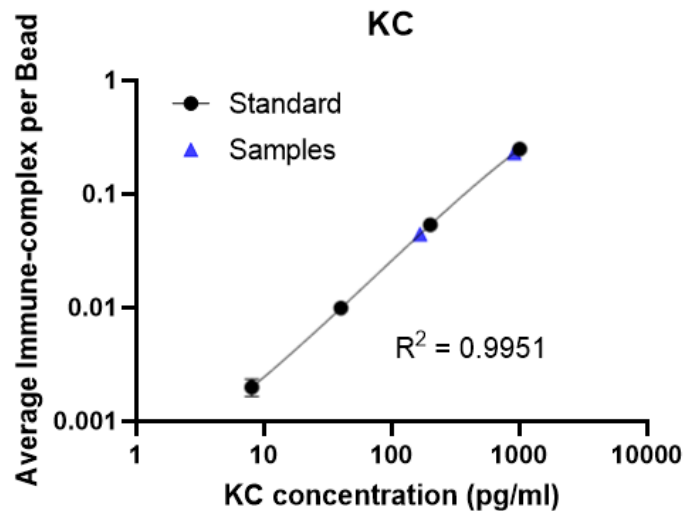
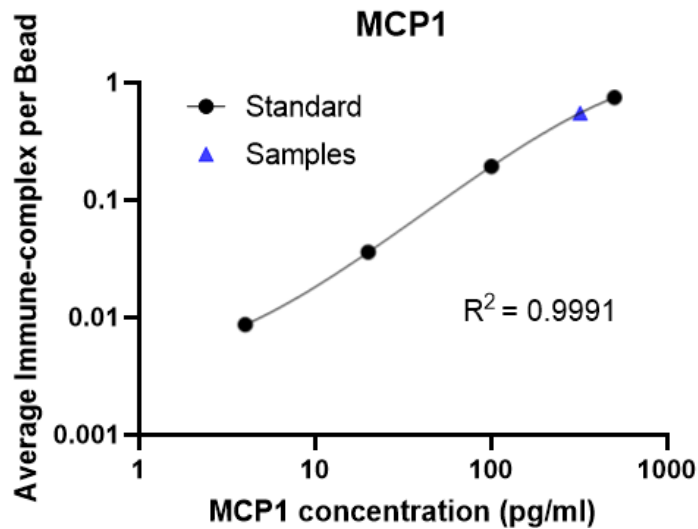
-low cost



Song et al, *Blood*, 2020



Measurement of secreted proteins



Immediate next steps

- Serial measurement of chemokine secretion from stimulated primary endothelial cells
- 2 μ l sample volume from lower chamber outlet

Katsuo Kurabayashi
Howard Su

