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Enhanced-throughput platforms to model neural vasculature on synthetic hydrogels

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WISCONSIN INSTITUTES FOR MEDICAL RESEARCH

UW COLLEGE OF ENGINEERING

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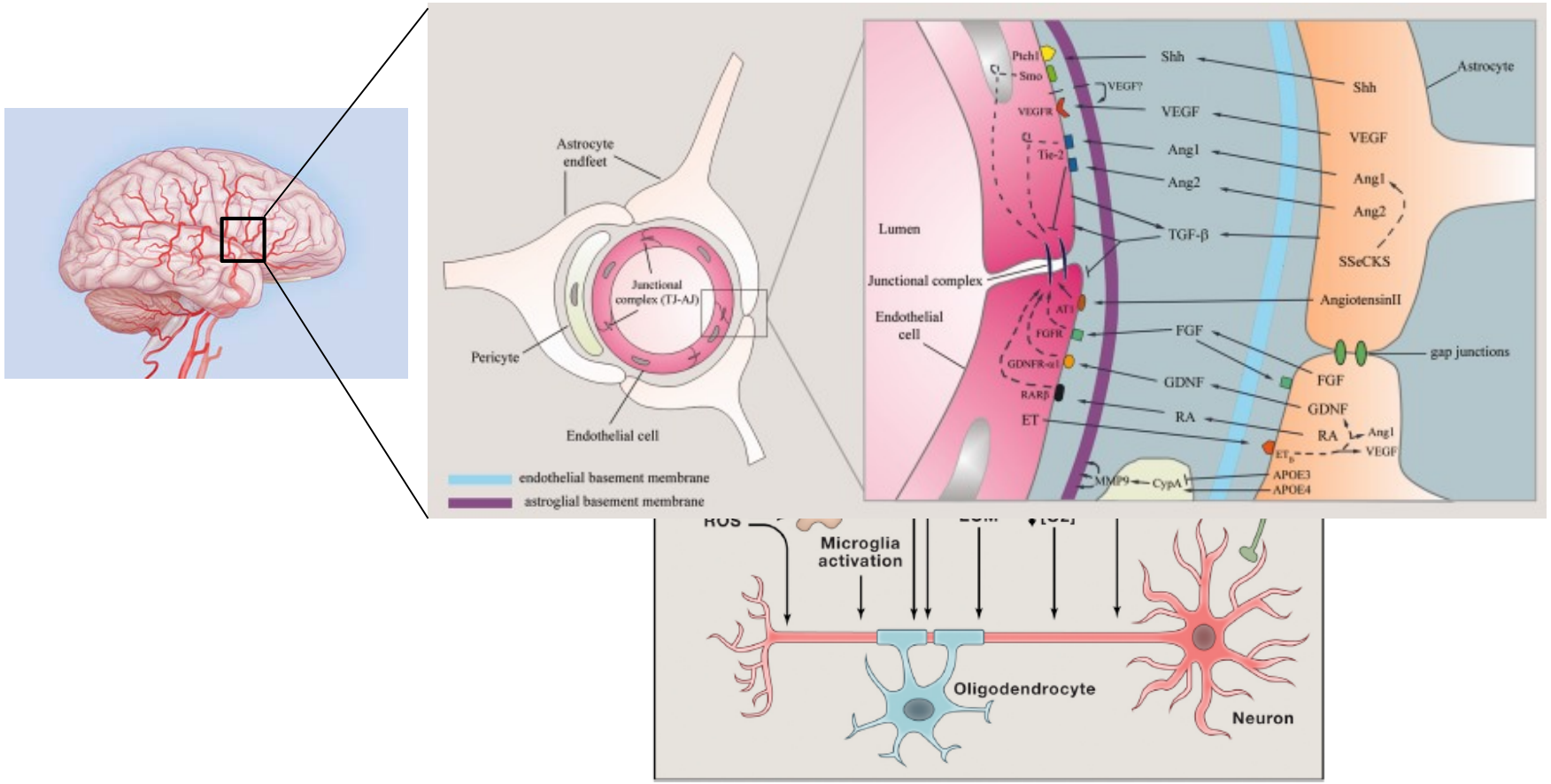
Neurovasculature and the Blood Brain Barrier

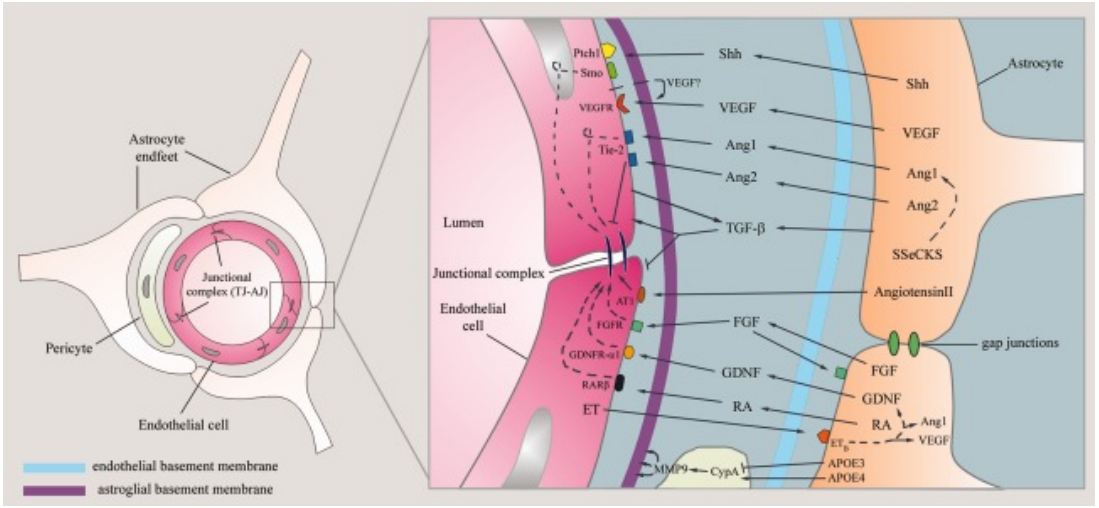


Neurodegeneration

Failure and disruption of microvasculature can lead to damage to the central nervous system

Alvarez et al, Glia 2013

The Neurovascular Unit

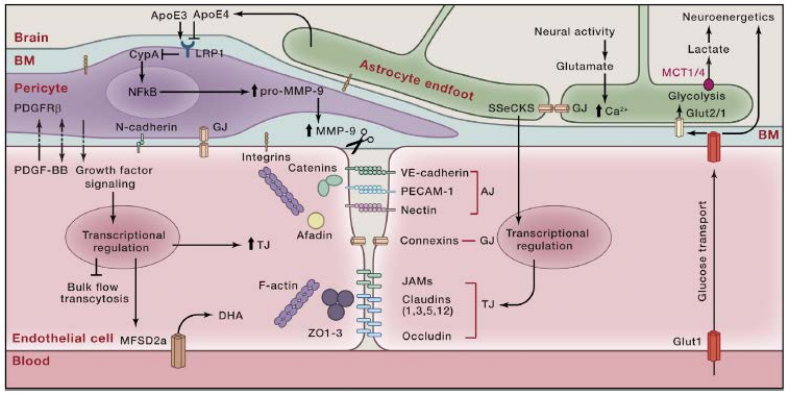


Objective:

- Generate in-vitro neurovascular unit models to detect chemicals that potentially compromise neurovascular tissue

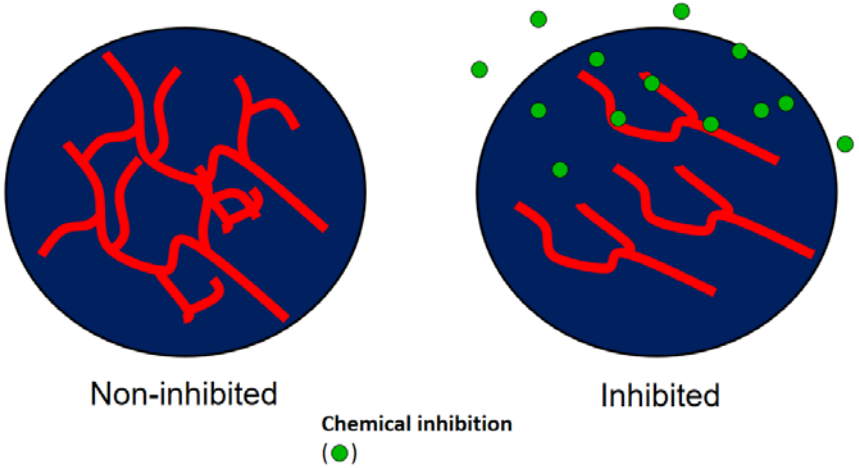
Alvarez et al, Glia 2013

Neurovascular Unit Model



Endothelial Cells	Pericytes	Astrocytes
IPSC-ECs (CDI)	Human Brain-derived Pericytes (ScienCell)	IPSC-ACs (CDI)

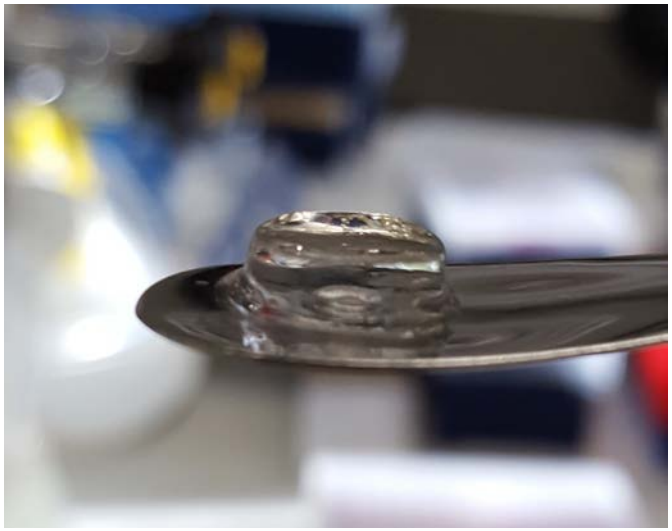
Step 1: Create environments to induce physiological cell behaviors



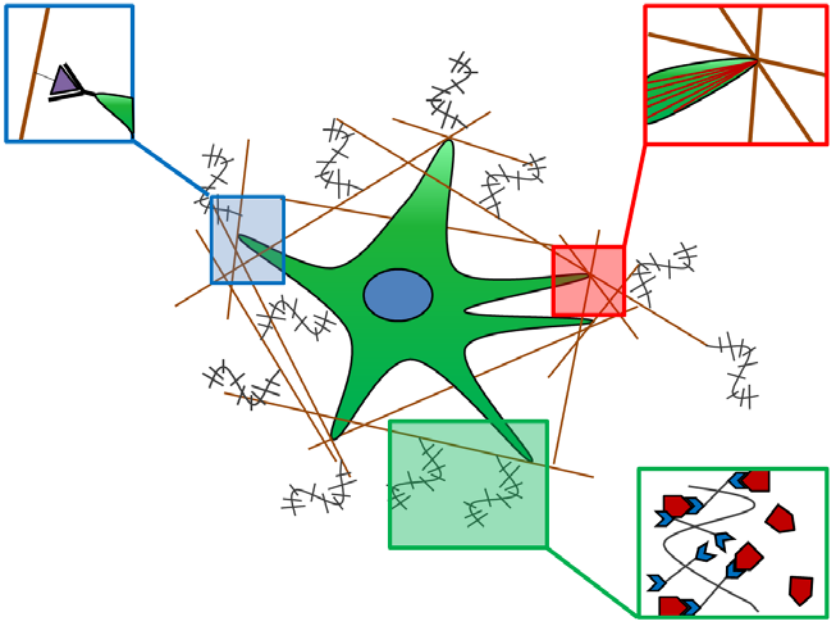
Step 2: Treat tissue models with drugs and toxins to gain insight on their effects on neurovascular networks

Zhao et al, Cell 2015

Chemically-defined hydrogels



?

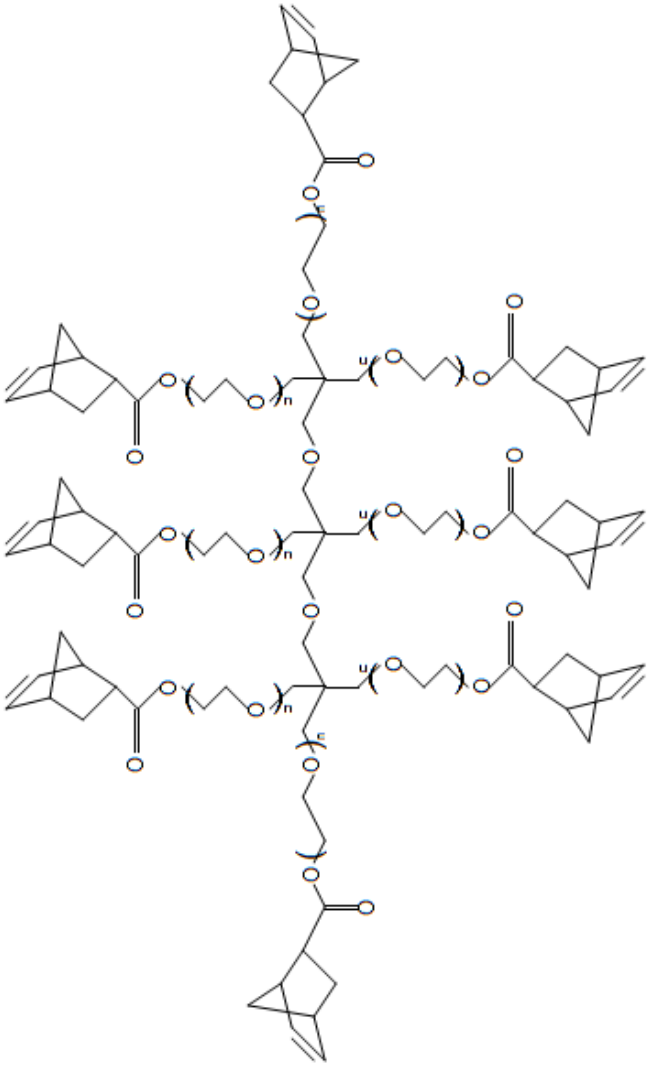


Chemically-defined hydrogels that **recapitulate specific ECM parameters can modulate cell behavior**

Lei *et al.* 2011, *Biomaterials*, 32, 39-47.

Griffith and Swartz 2006, *Nature Reviews: Molecular Cell Biology*. 7, 211-224.

Poly(ethylene glycol) hydrogel modifications

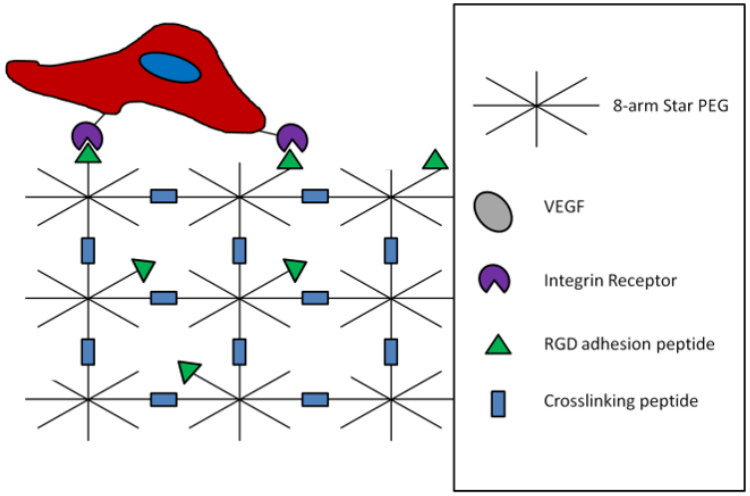


+ **C**RGDS / RGDF$_d$**C**

Cell-adhesion

+ K**C**GGPQGIWGQG**C**K

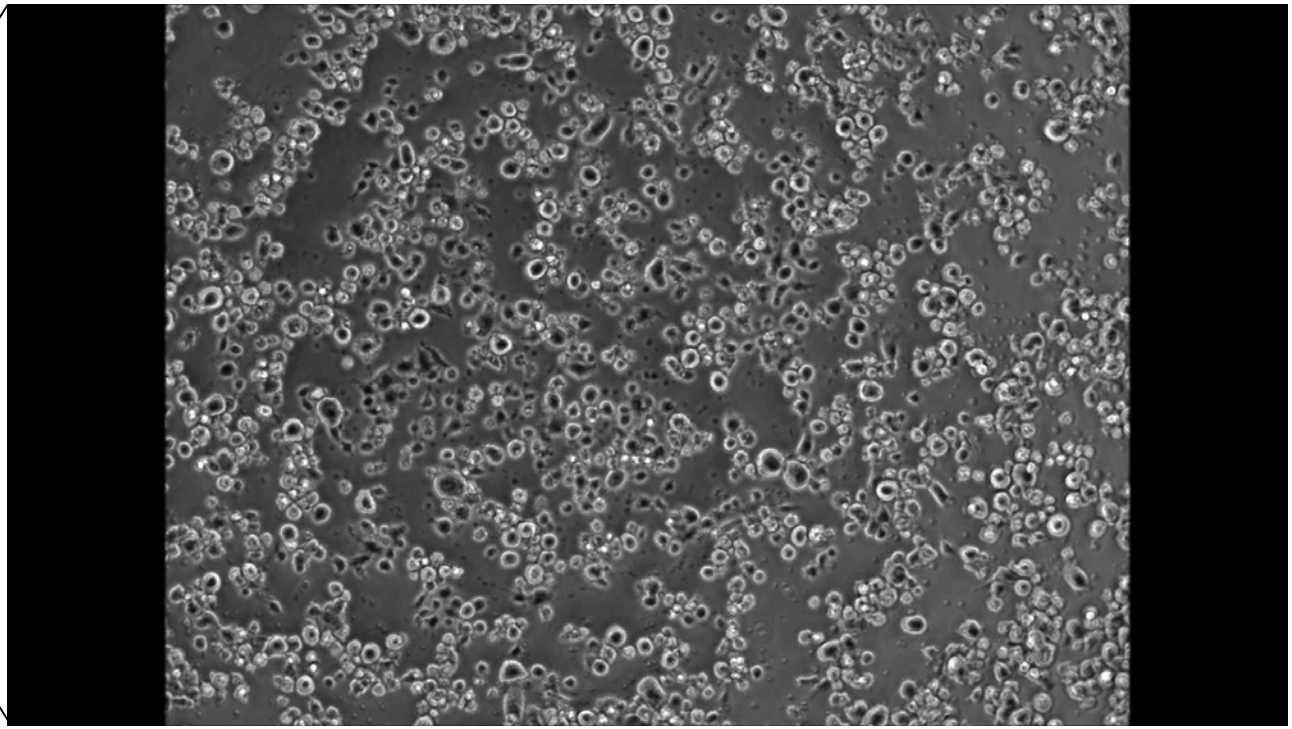
Crosslinking-stiffness



Ack. David B, Nick I, Mike T.

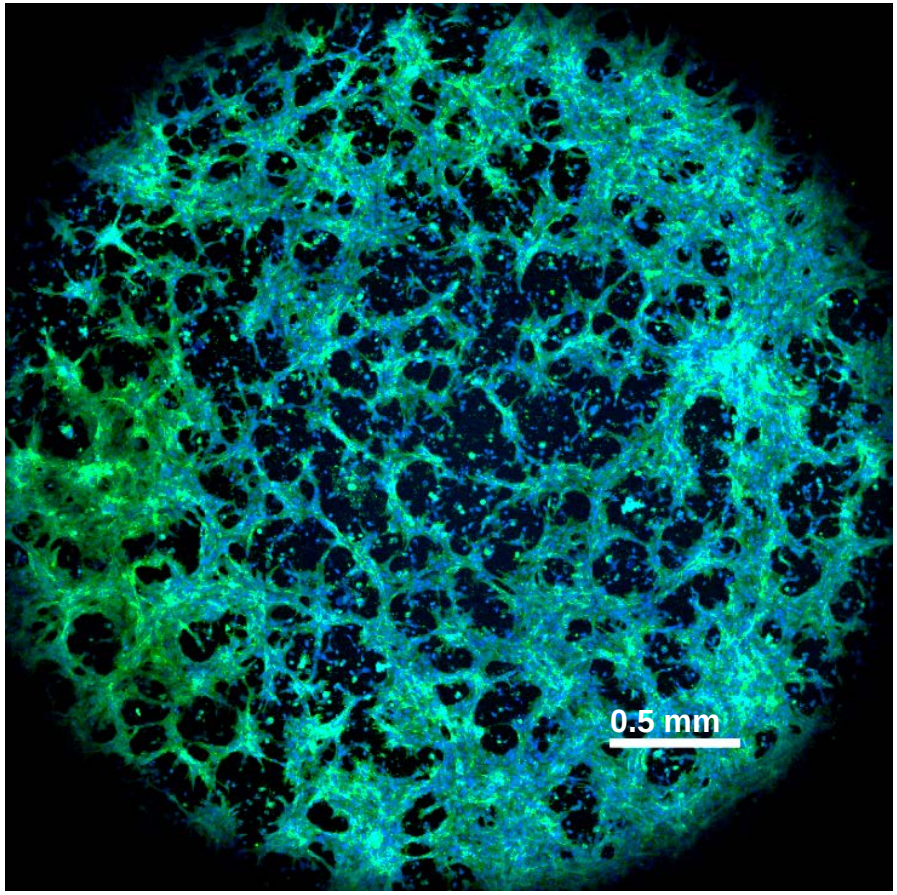
iPSC-ECs form interconnected networks on synthetic hydrogels

Synthetic

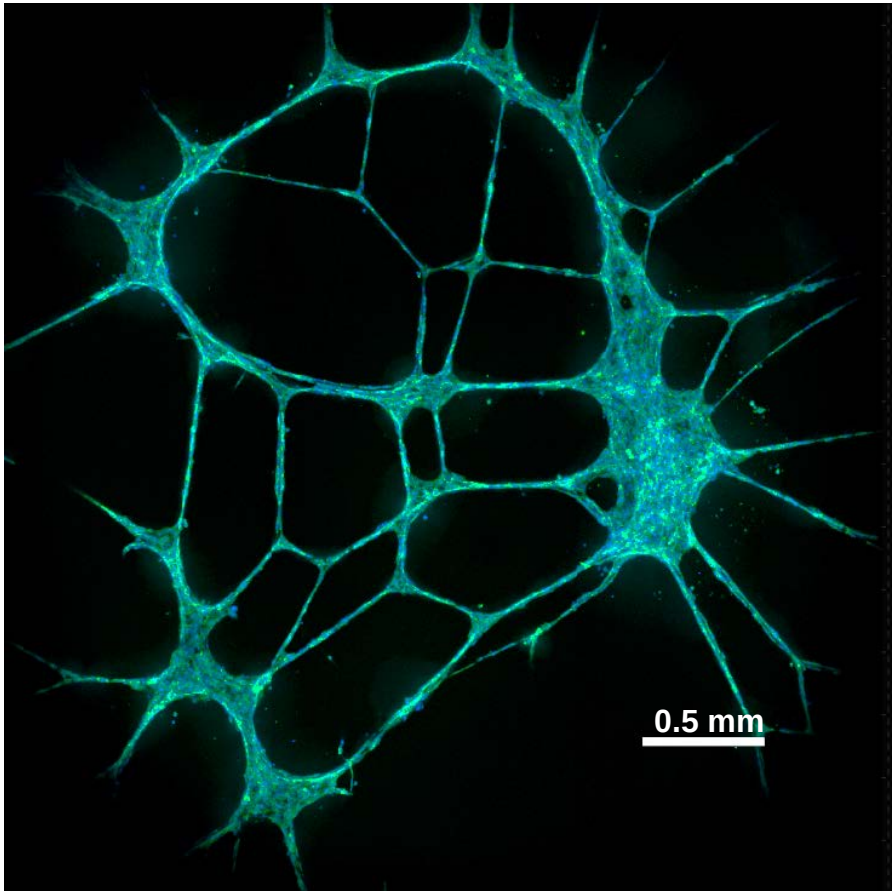


iPSC-ECs form interconnected networks on synthetic hydrogels and Matrigel

Synthetic



Matrigel

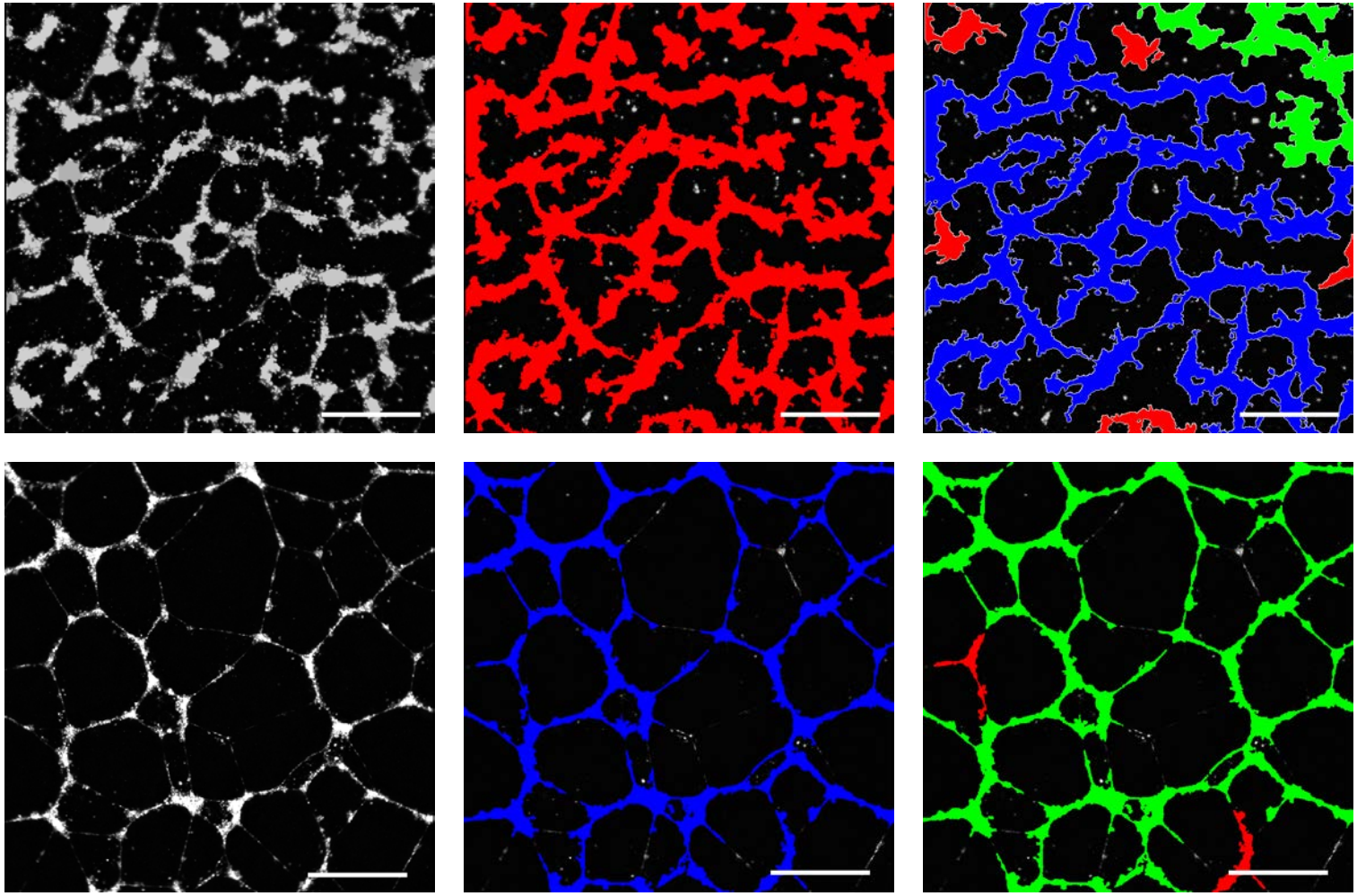


Green: CD31 **Blue:** DAPI

Network area measurements

PEG

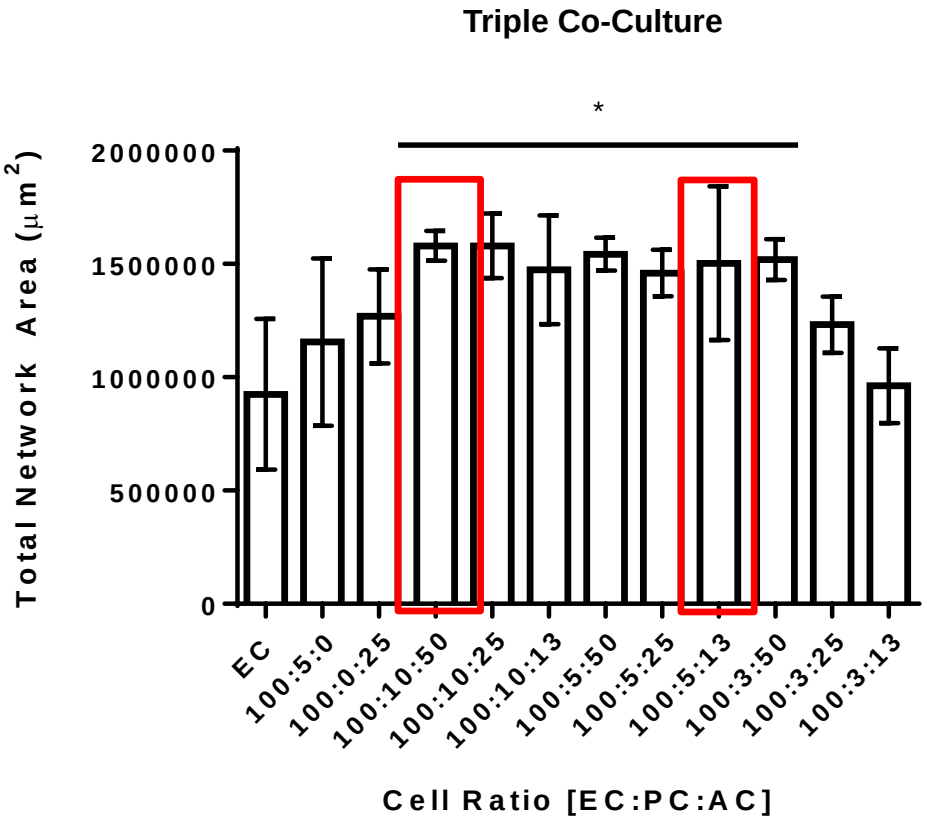
Matrigel



Contrasted Image | Thresholded | Measured Objects

Scale Bar: 0.5 mm

Total Endothelial Network Area – Pericyte and Astrocyte co-cultures



100 : 5 : 13
EC : PC : AC

Triple-Low

100 : 10 : 50
EC : PC : AC

Triple-High

Endothelial Network Area in the presence of VEGF inhibitor Sunitinib Malate

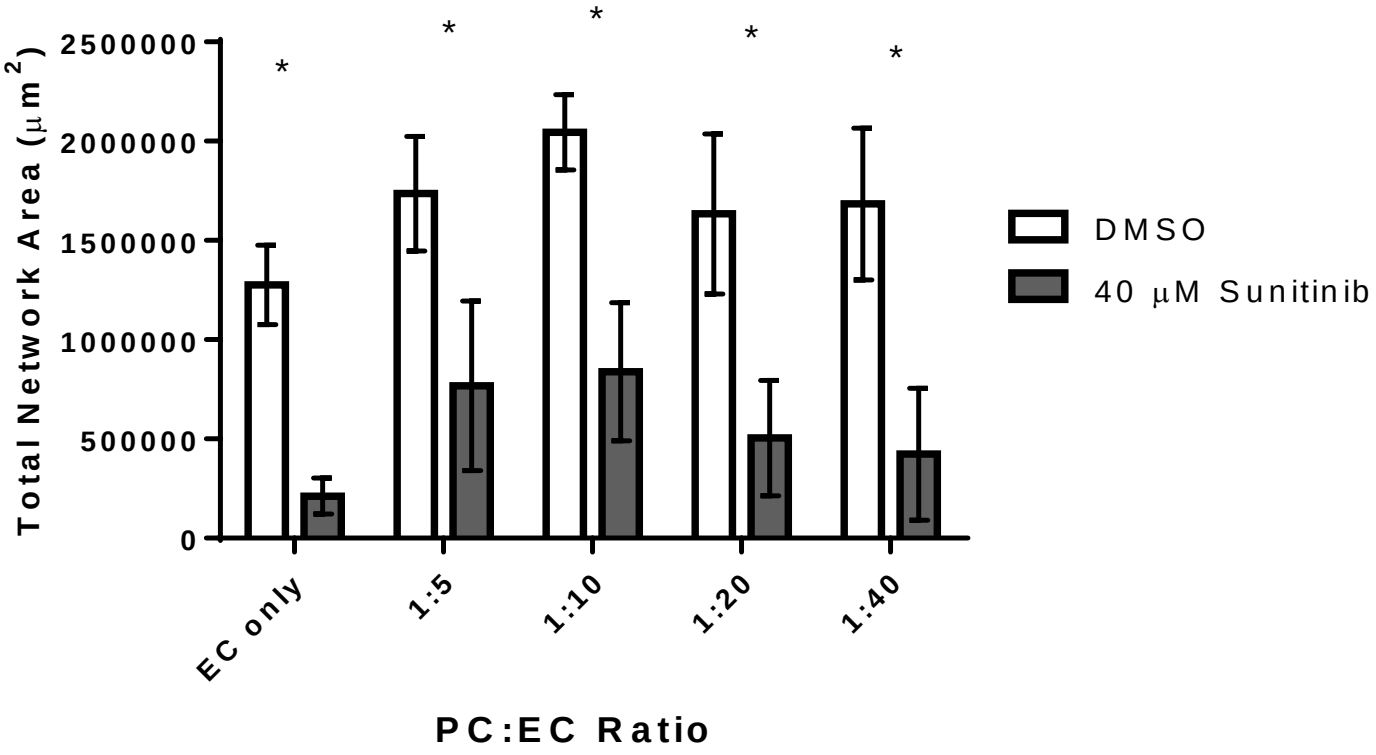
	Sunitinib
Co-culture	**Disruption (+/-)**
EC only	**?**
EC-Pericyte	**?**
EC-Astrocyte	**?**
Triple – Low	**?**
Triple – High	**?**

Astrocyte and Pericytes

Identify which cell types are affected by toxic exposure

Identify which cell types protect endothelial networks from disruption

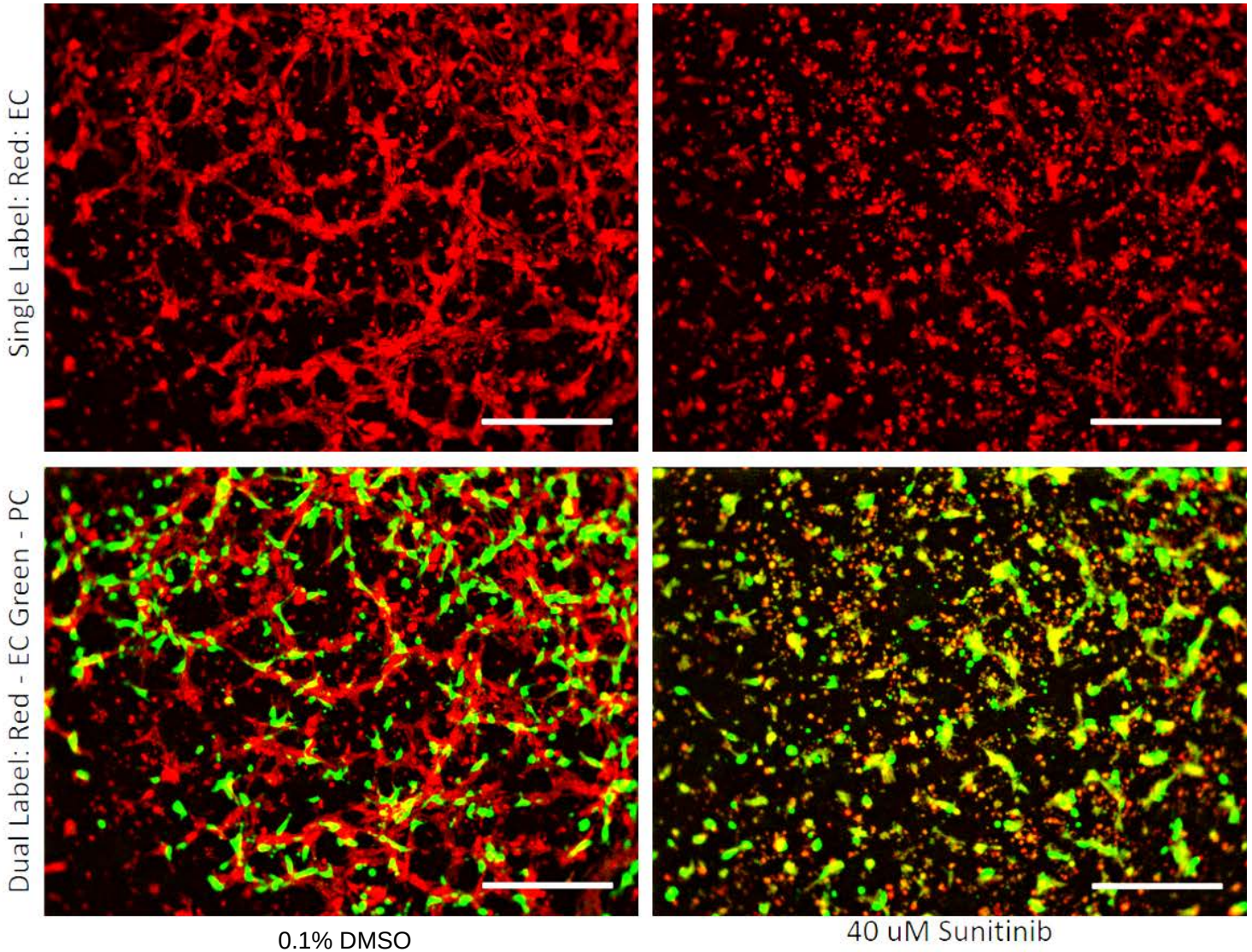
Pericytes fail to prevent Sunitinib-mediated endothelial network disruption



	Sunitinib
Co-culture	**Disruption (+/-)**
EC only	+
EC-Pericyte	+
EC-Astrocyte	
Triple – Low	
Triple – High	

Pericytes fail to prevent Sunitinib-mediated endothelial network disruption

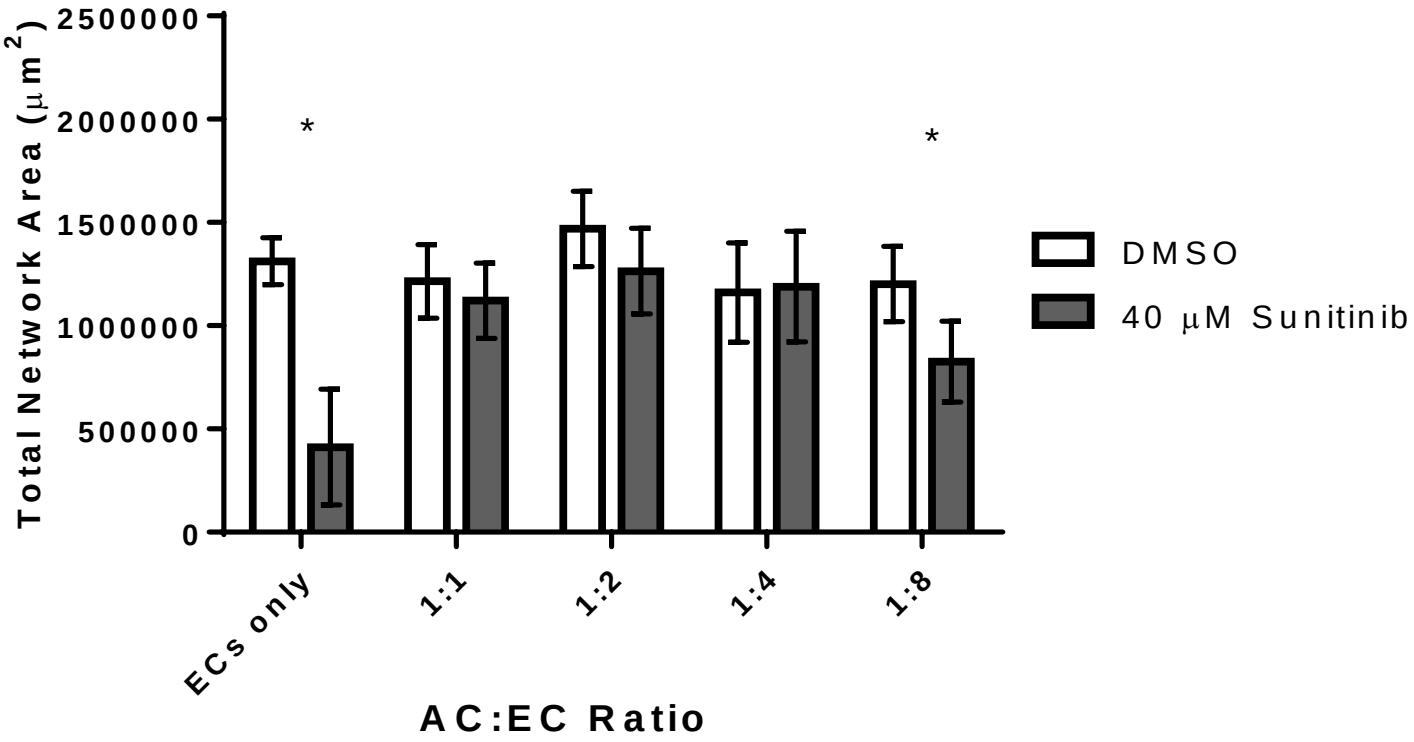
1 PC : 20 EC



Scale Bar: 0.5 mm

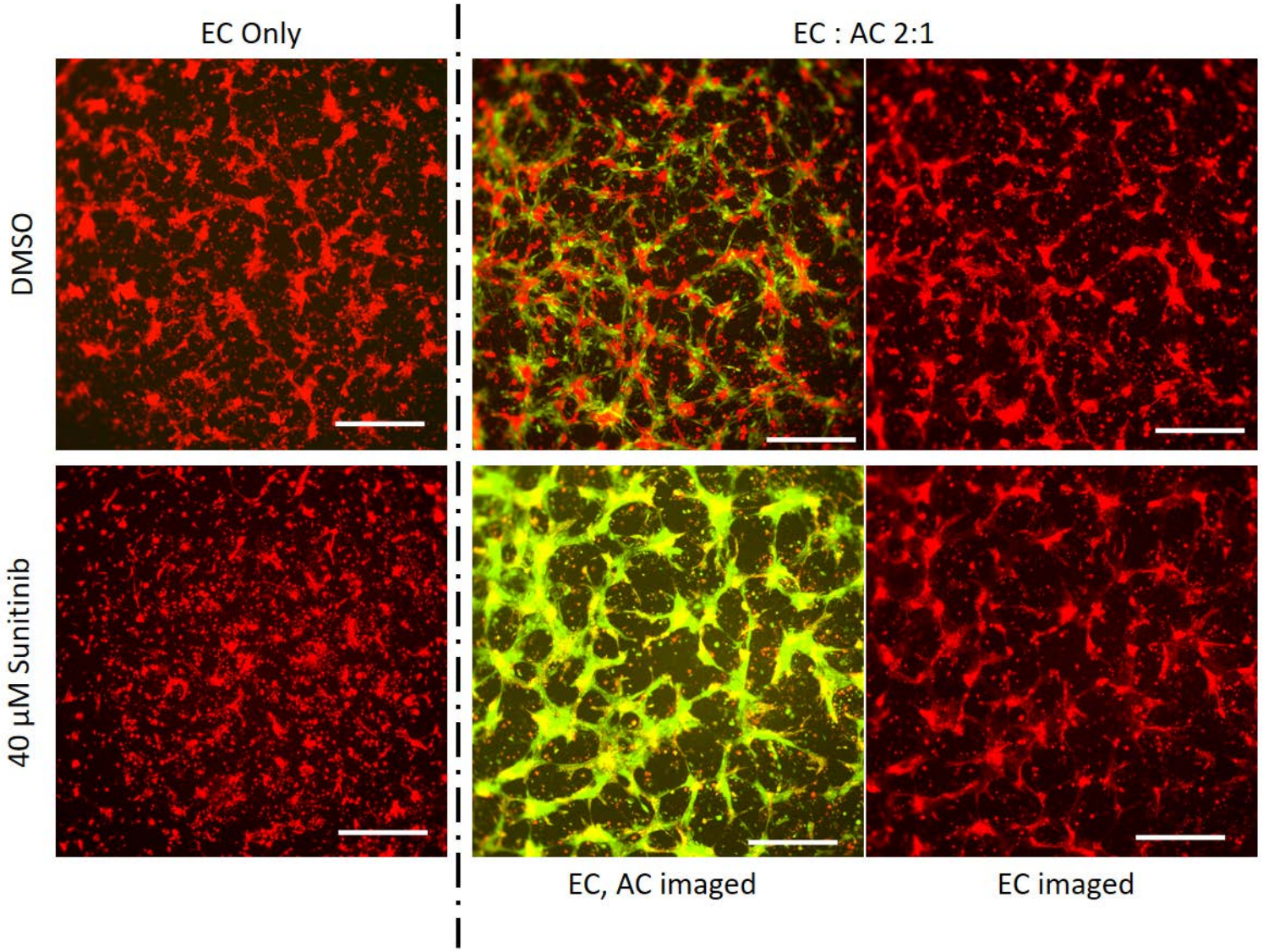
Red: EC Green: PC

Astrocytes prevent Sunitinib-mediated endothelial network disruption



	Sunitinib
Co-culture	**Disruption (+/-)**
EC only	+
EC-Pericyte	+
EC-Astrocyte	-
Triple – Low	
Triple – High	

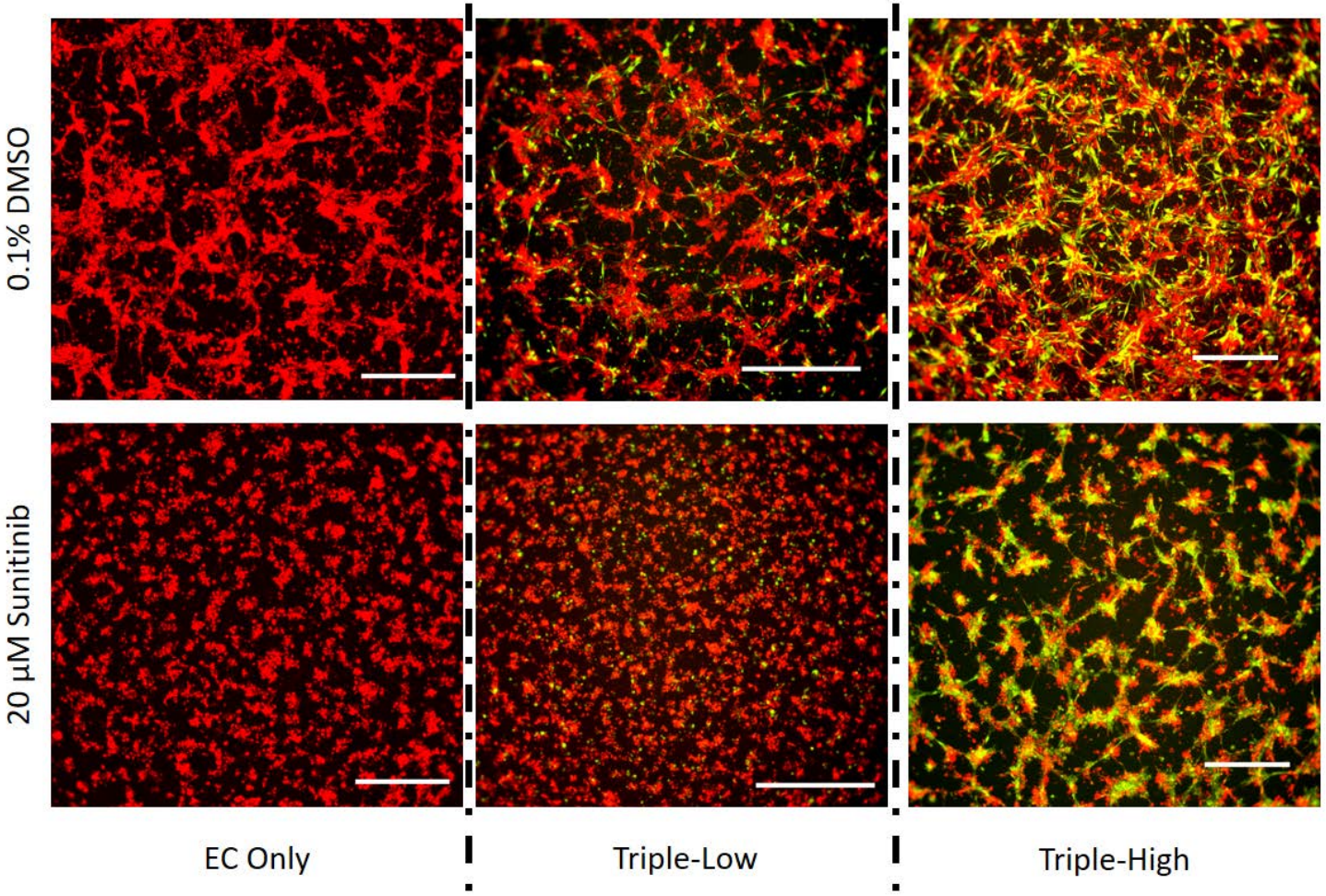
Astrocytes prevent Sunitinib-mediated endothelial network disruption



Scale Bar: 0.5 mm

Red: EC Green: AC

Sunitinib Treatment causes network reorganization but not disruption



	Sunitinib
Co-culture	**Disruption (+/-)**
EC only	+
EC-Pericyte	+
EC-Astrocyte	-
Triple – Low	+
Triple – High	-

Scale Bar: 0.5 mm

Red: EC Green: AC

Endothelial Network Area in the presence of VEGF inhibitor Sunitinib Malate

	Sunitinib
Co-culture	**Disruption (+/-)**
EC only	**+**
EC-Pericyte	**+**
EC-Astrocyte	**-**
Triple – Low	**+**
Triple – High	**-**

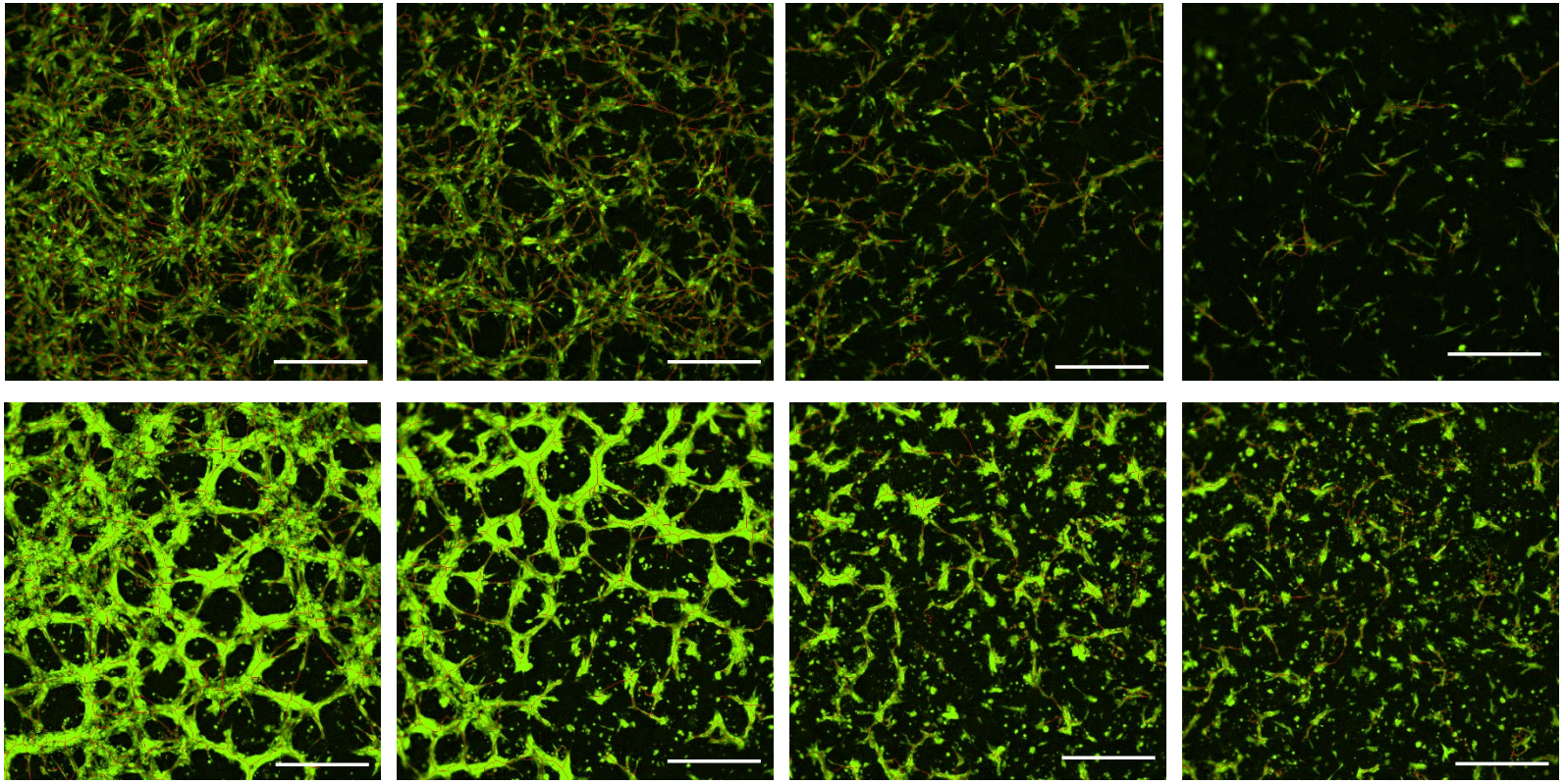
Sunitinib:

- Astrocytes prevent complete disruption of endothelial cell networks. Requires sufficient density of astrocytes in co-culture.

Sunitinib Treatment causes reorganization of astrocytes in co-culture

DMSO

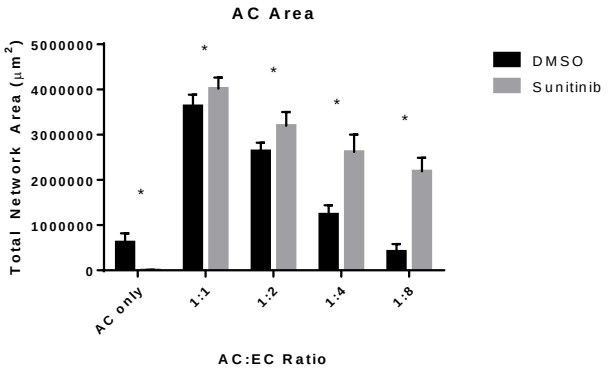
Sunitinib

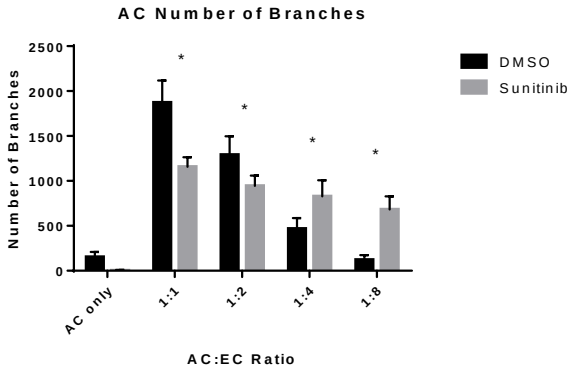


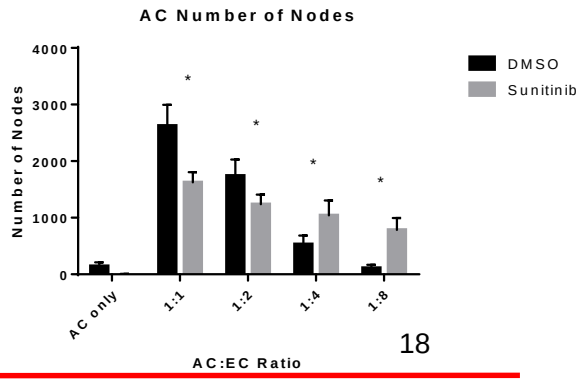
1:1 AC:EC | 1:2 AC:EC | 1:4 AC:EC | 1:8 AC:EC

Scale Bar: 0.5 mm

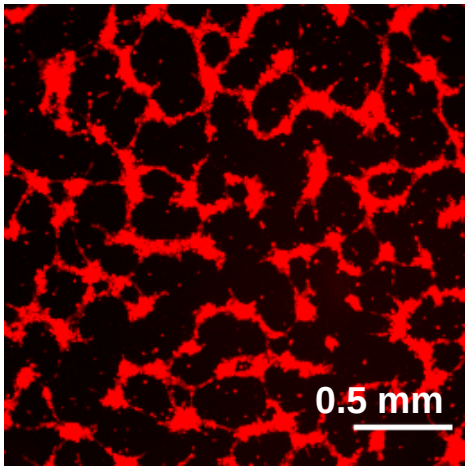
Green: AC



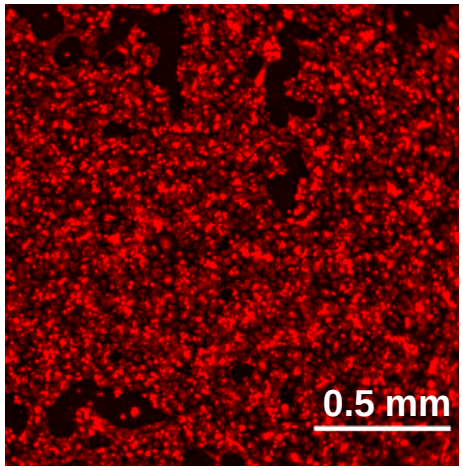




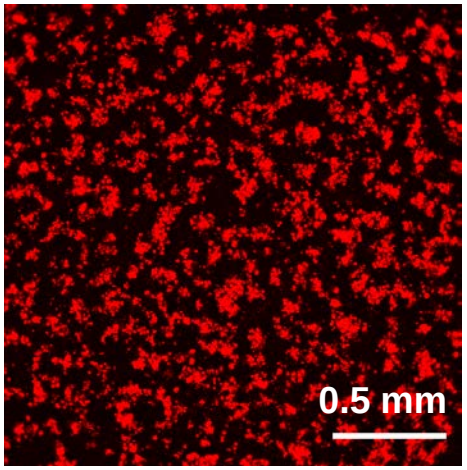
Tubulogenesis assay to screen blinded chemical compounds



DMSO



Above



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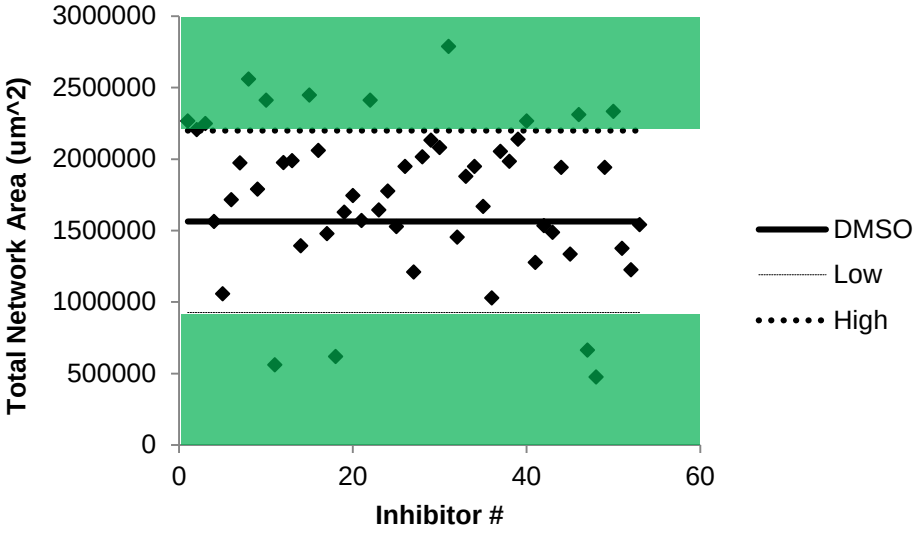
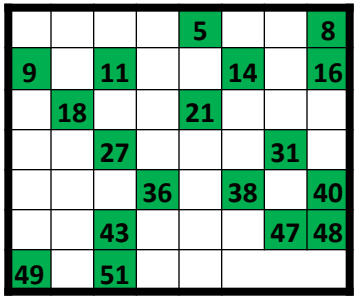
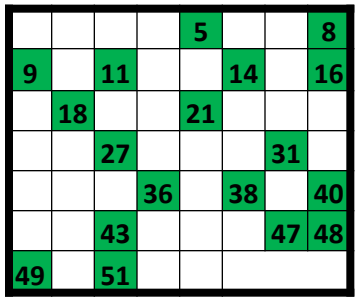




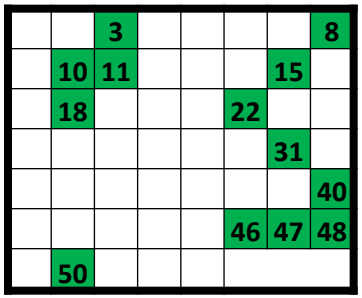
HUVECs

Neurovascular Unit to screen blinded chemical compounds

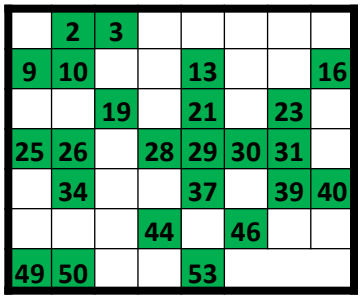
Endothelial Cells



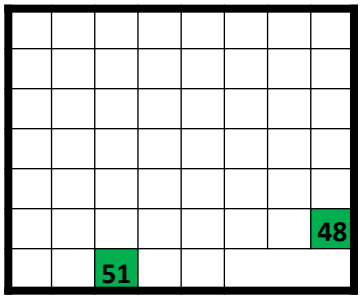
HUVECs



iPSC-ECs

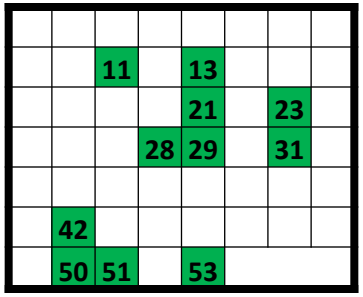


Triple - Low

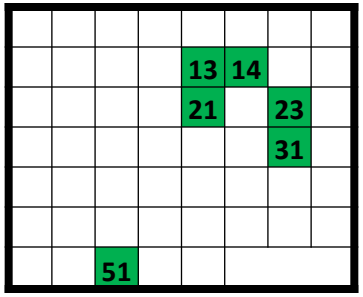


Triple - High

Astrocytes



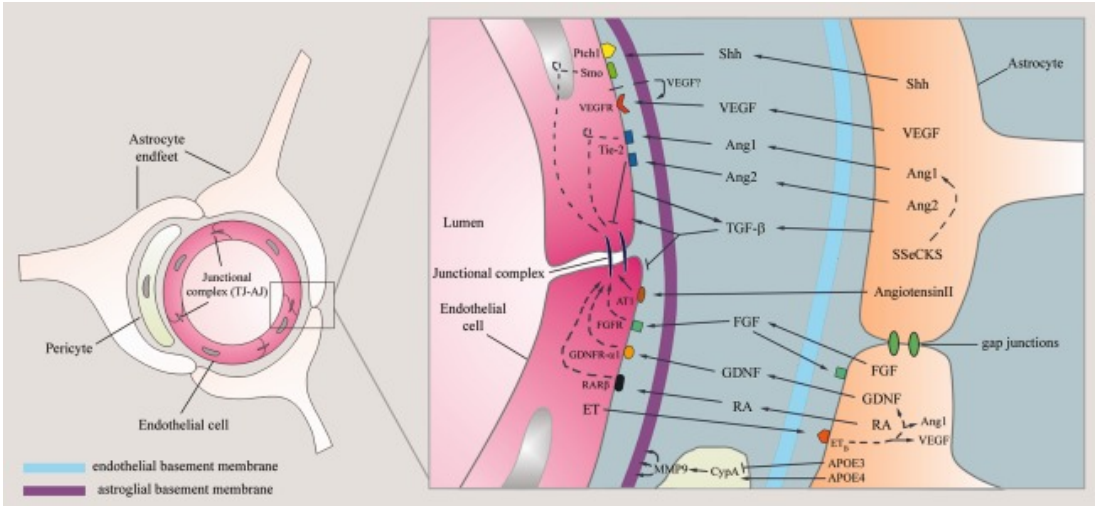
Triple - Low



Triple - High

Varying cell compositions generate different panels of vascular inhibitors

Conclusions



- Synthetic Hydrogels enable network formation by endothelial cells and co-cultures
- Co-cultures of endothelial cells, pericytes and astrocytes enables systematic identification of active and affected cell types in chemical exposure
- Co-cultures of endothelial cells detect differential inhibitory activity from panels of blinded chemical compounds

Alvarez et al, Glia 2013

Bioinspired Materials Laboratory

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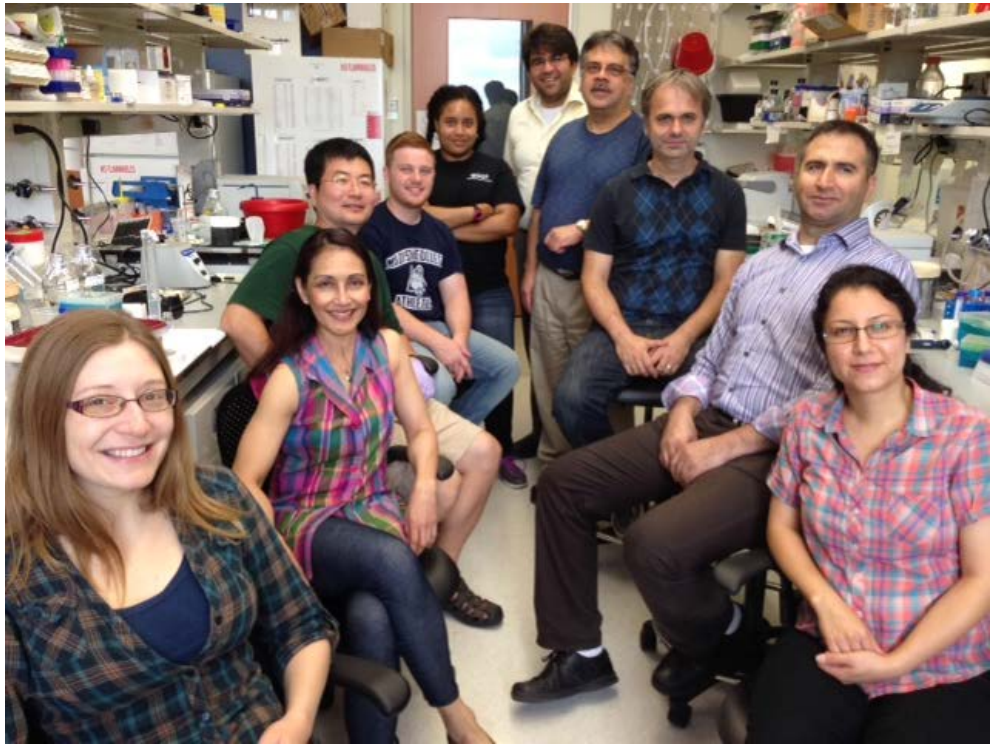

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WHERE DISCOVERIES BEGIN

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University of Wisconsin-Madison
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Cardiovascular Research Center

Wallace H. Coulter Foundation

Human MAPs Center

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McPherson Eye Research Institute
UNIVERSITY OF WISCONSIN–MADISON

Synthetic screening platform detects inhibition with high sensitivity

ANTI-VEGF

	Synthetic	Matrigel
Conc. (ug/ml)	Inhibition (+/-)	
1	+	-
0.5	+	-
0.25	+	-
0.125	+	-
0.062	+	-
0.031	+	-
0.015	+	-
0.008	+	-

Semaxinib (SU5416)

	Synthetic	Matrigel
Conc. (uM)	Inhibition (+/-)	
40	+	-
20	-	-
10	-	-
5	-	-
2.5	-	-
1.25	+	-
0.625	+	-
0.3125	+	-

sFLT-1

	Synthetic	Matrigel
Conc. (ug/ml)	Inhibition (+/-)	
1	+	-
0.5	-	-
0.25	-	-
0.125	+	-
0.062	+	-
0.031	+	-
0.015	+	-
0.008	-	-

Vatalaninb (PTK787)

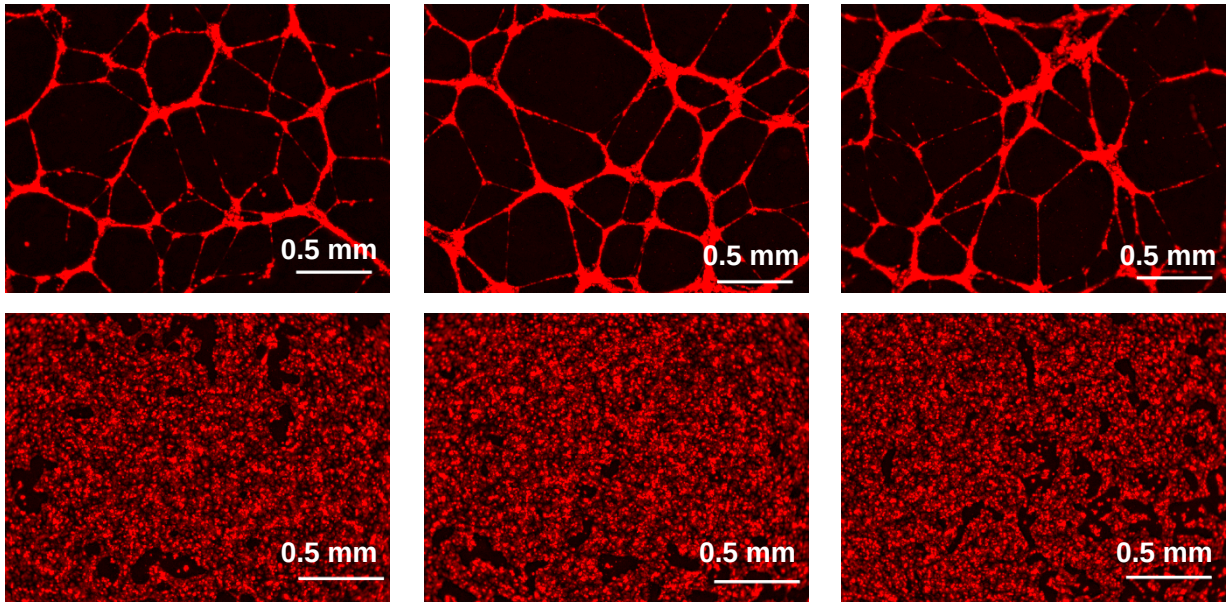
	Synthetic	Matrigel
Conc. (uM)	Inhibition (+/-)	
20	+	-
10	-	-
5	-	-
2.5	-	-
1.25	-	-
0.625	-	-
0.3125	-	-
0.1562	+	-

Sutent (Sunitinib)

	Synthetic	Matrigel
Conc. (uM)	Inhibition (+/-)	
40	+	+
20	+	-
10	+	-
5	+	+
2.5	+	-
1.25	+	-
0.625	+	-
0.3125	+	-

HUVECs on synthetic hydrogels demonstrate increased sensitivity to known VEGF inhibitors compared to Matrigel

VEGF-independent mechanisms of network inhibition



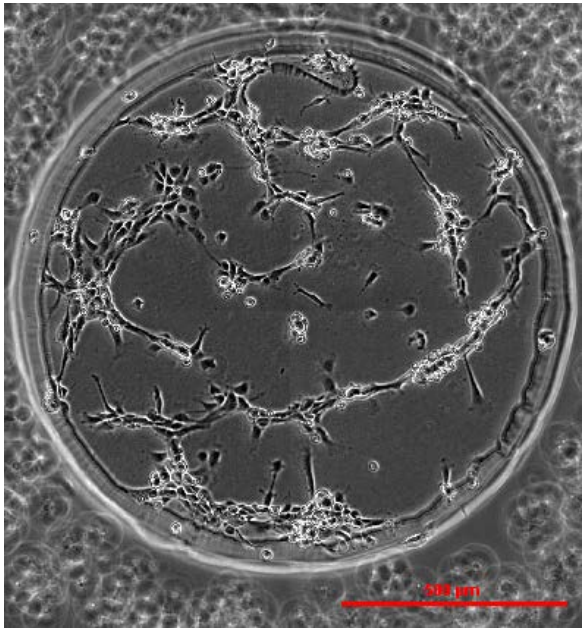
Prinomastat HCL

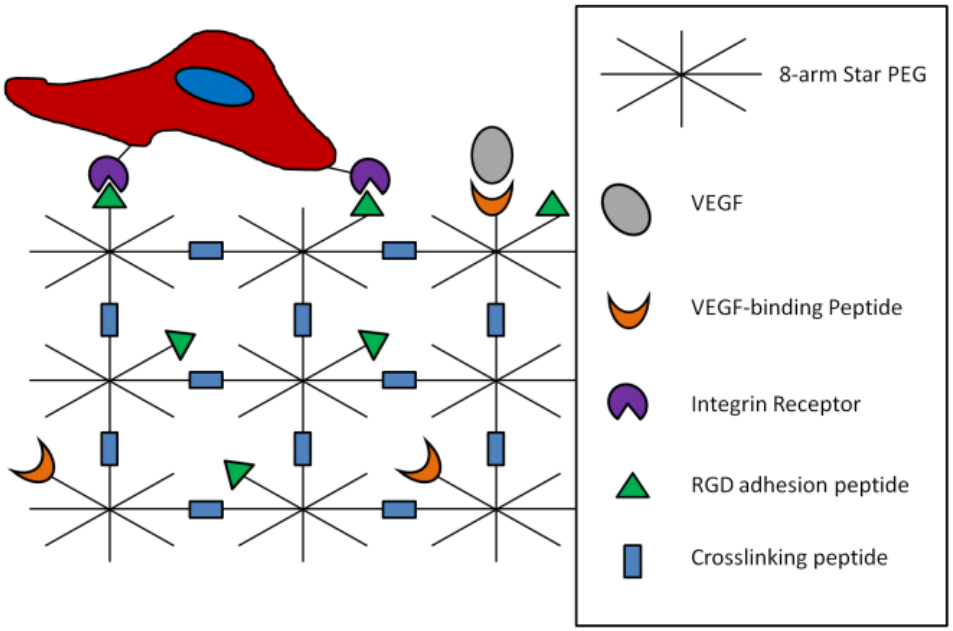
	Synthetic	Matrigel
Conc. (uM)	Inhibition (+/-)	
20	+	-
10	+	+
5	+	+
2.5	+	-
1.25	+	-
0.625	+	-
0.3125	+	+
0.1562	+	+

K**C**GGPQGIWGQG**C**K

Matrix metalloproteinase inhibition disrupts endothelial network formation on synthetic hydrogels but not Matrigel

Identifying culture conditions for network formation



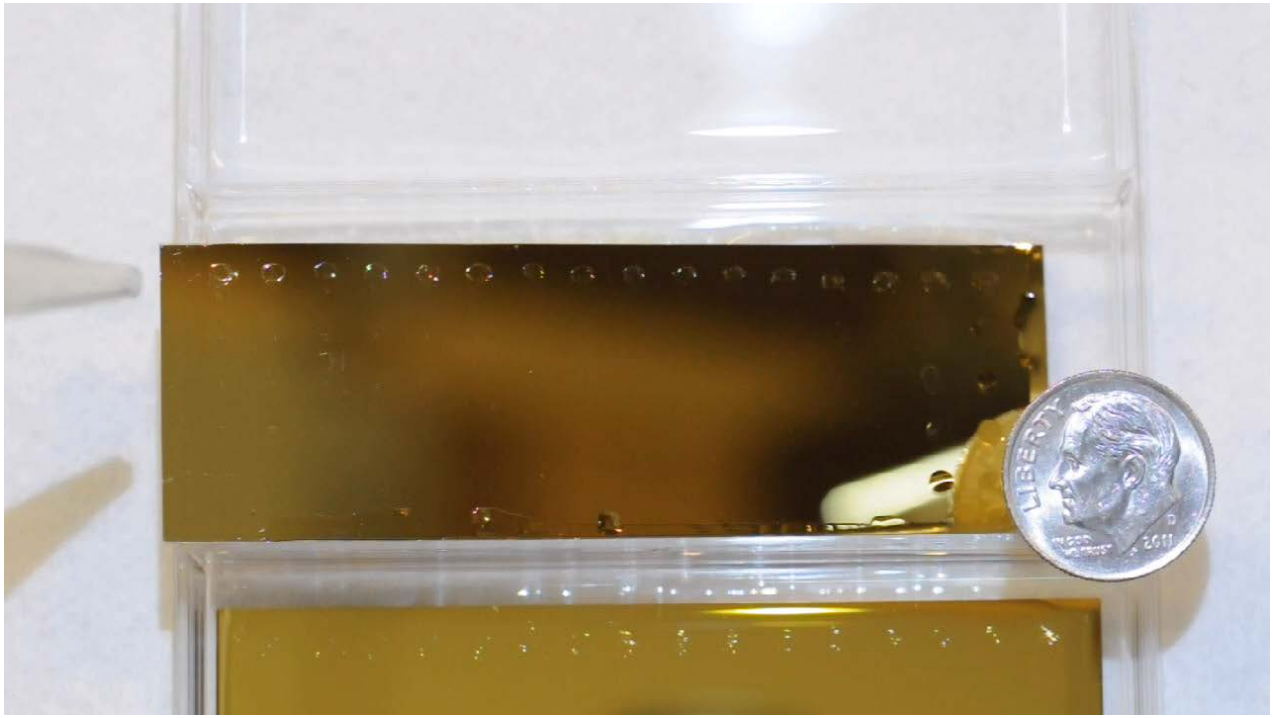


Chemically-defined PEG

- CRGDS, cyclic RGD
- 7 adhesion peptide concentrations
- 3 stiffness levels
- 3 VEGF concentrations

Use a material discovery system that defines hydrogel properties and VEGF concentration in media to discover environments to comparable to Matrigel-based environments.

Enhanced-throughput material discovery

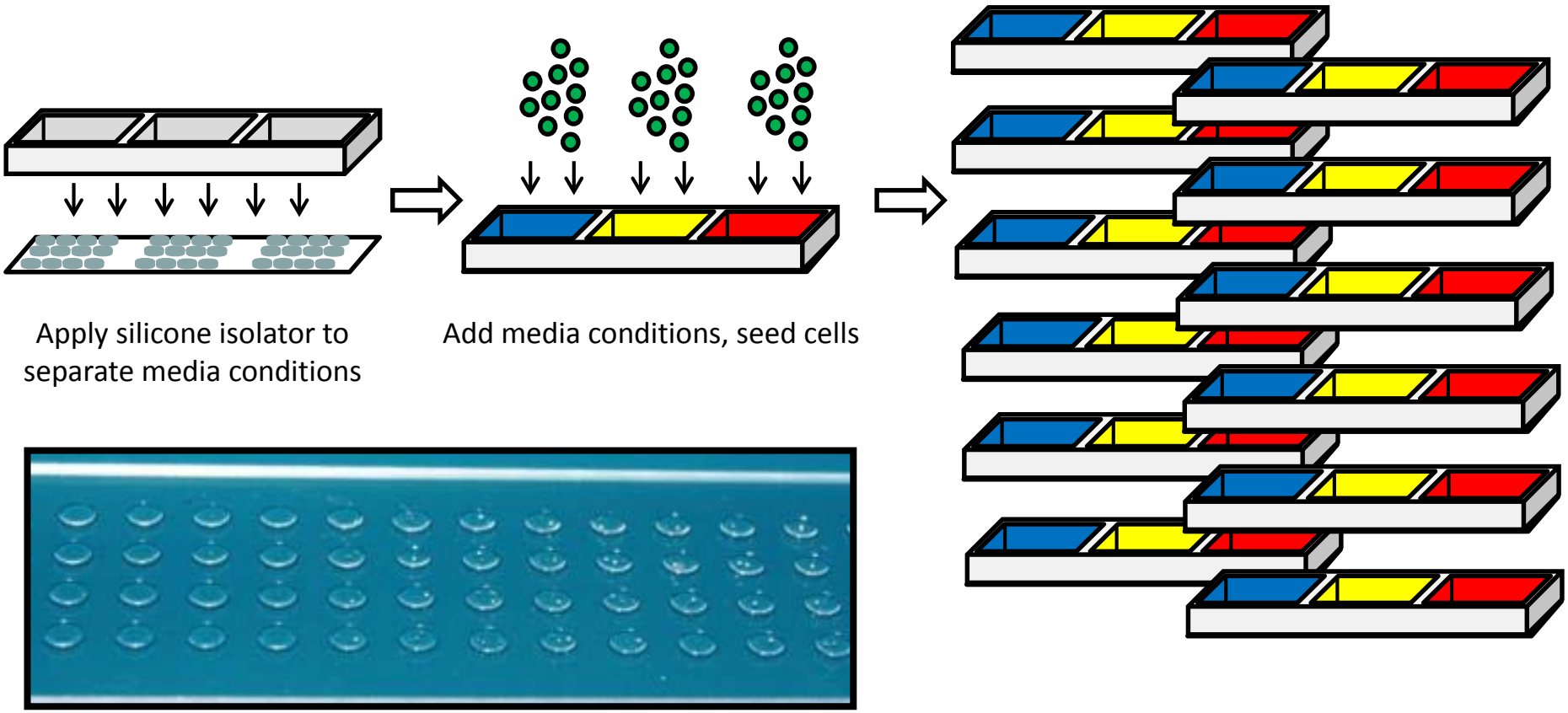


Enhanced throughput experimentation is necessary for efficient exploration of pro-angiogenic materials

Ack: Ngoc Nhi Le, William Daly, David Belair

Enhanced-throughput material discovery

Objective: Optimize hydrogels for 2D tubulogenesis and toxicity screening



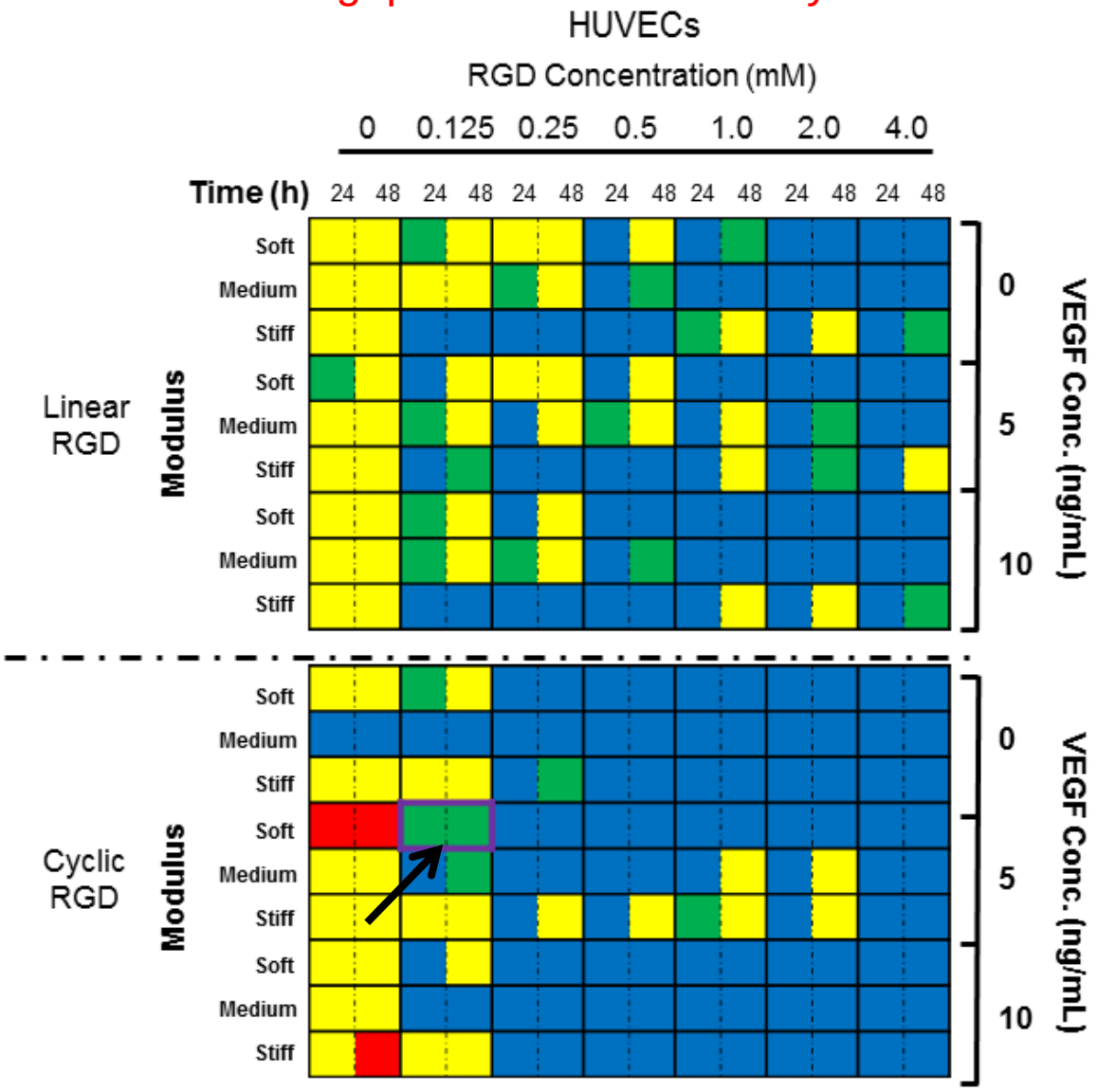
Apply silicone isolator to separate media conditions

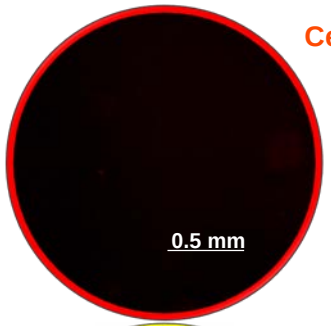
Add media conditions, seed cells

> 300 conditions tested

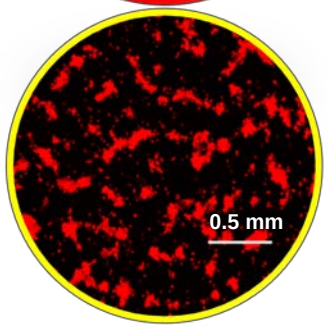
Ack: Ngoc Nhi Le, William Daly, David Belair

Enhanced-throughput material discovery

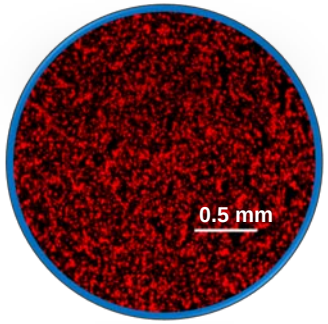




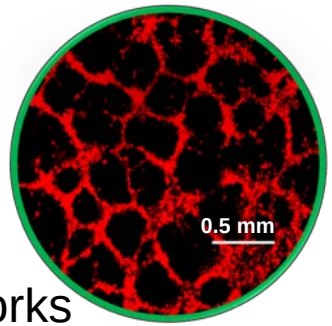
No Adhesion



Low Adhesion



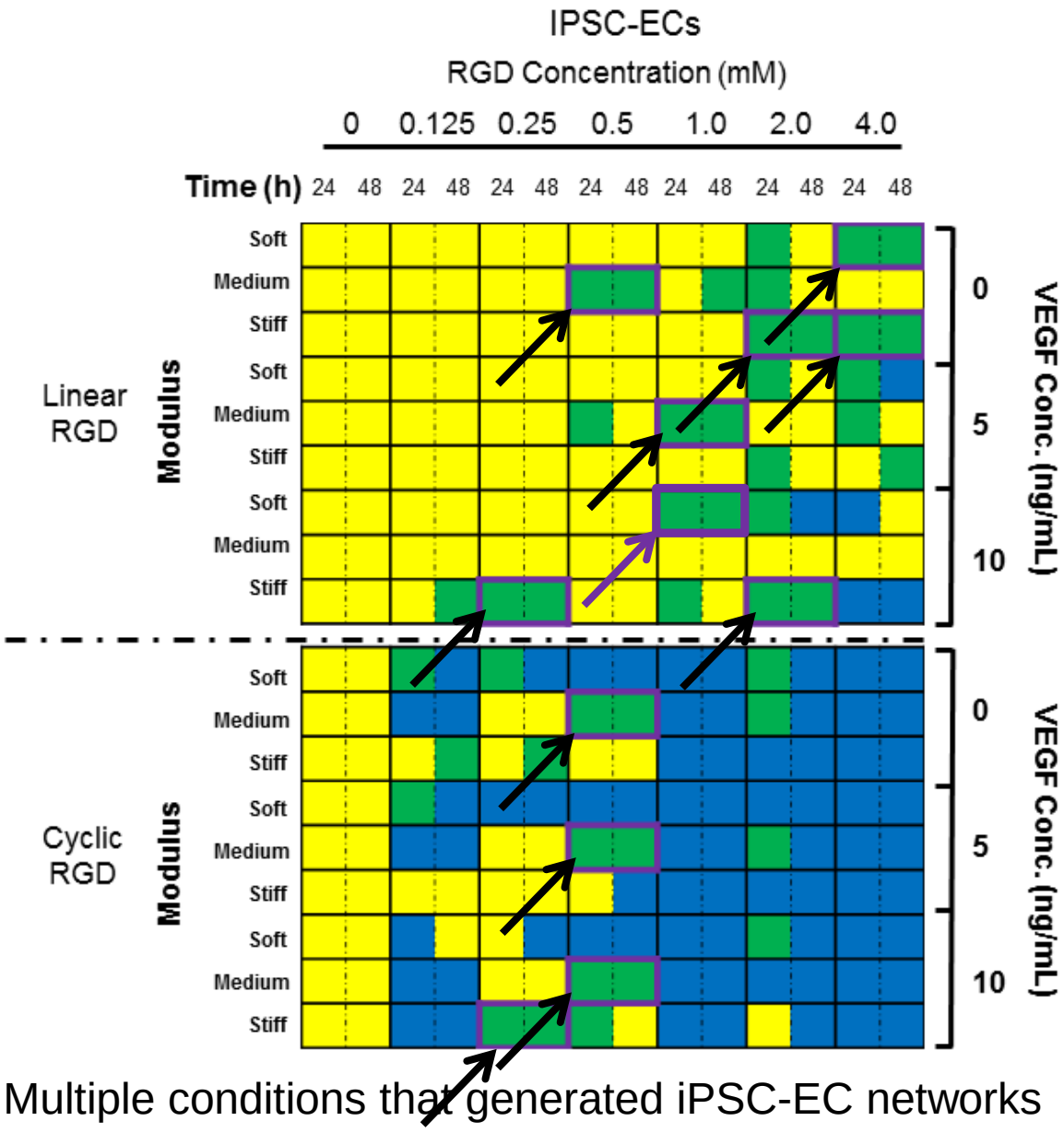
Monolayer

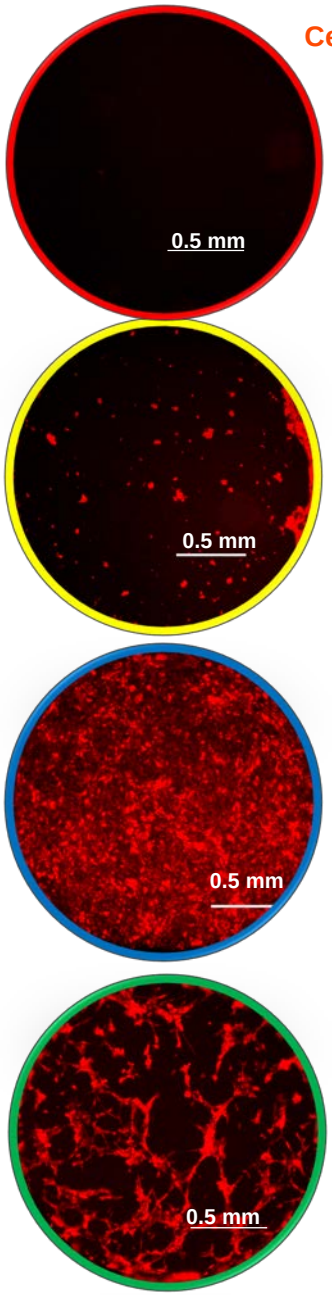


Network

Screening revealed 1 condition that generated HUVEC networks

Enhanced-throughput material discovery – iPSC-ECs





No Adhesion

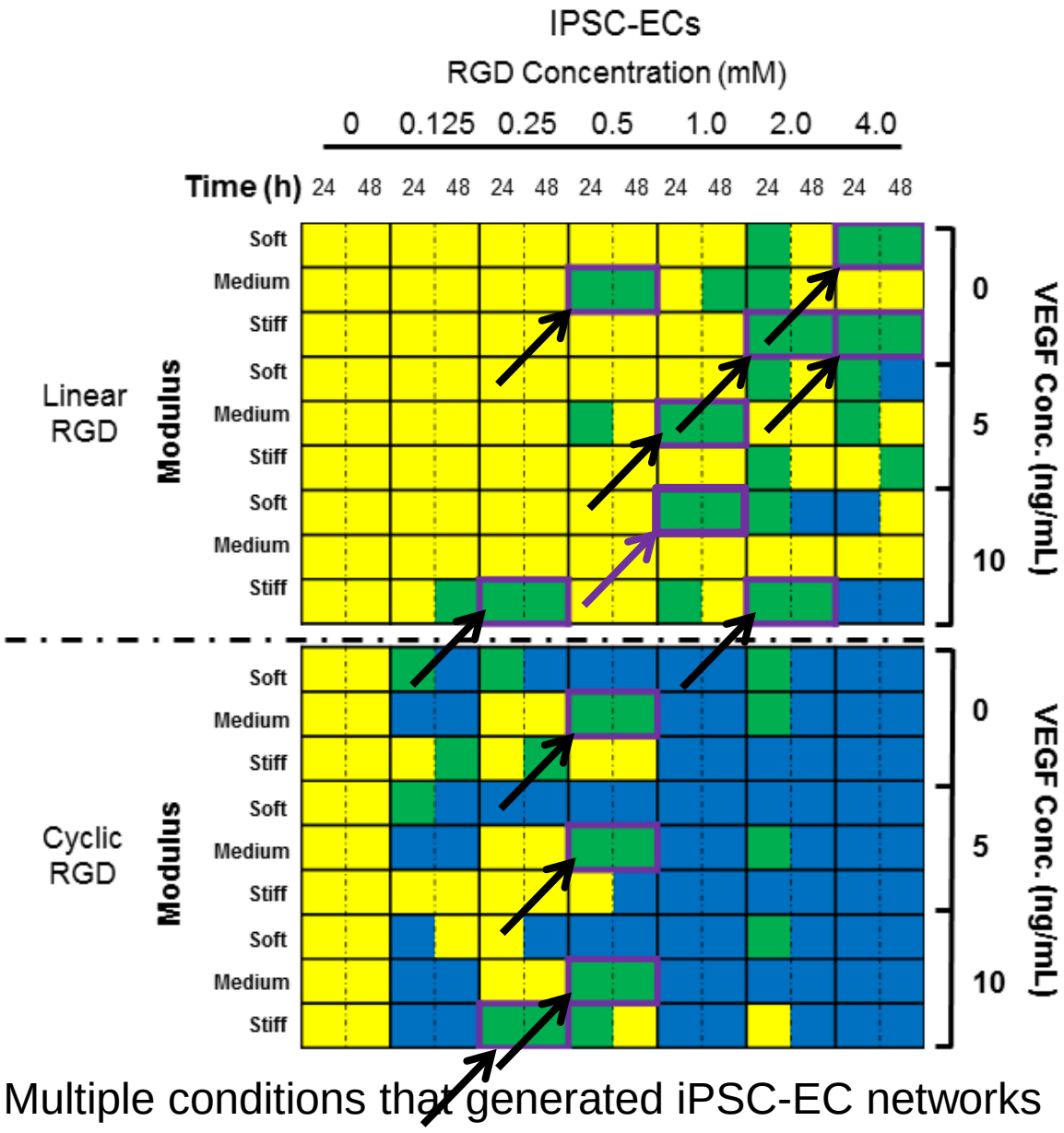
Low Adhesion

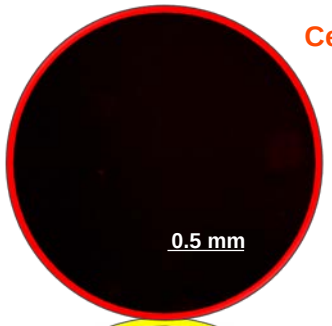
Monolayer

Network

Multiple conditions that generated iPSC-EC networks

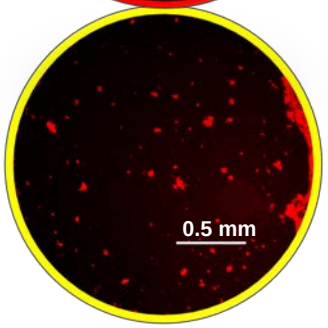
Enhanced-throughput material discovery – iPSC-ECs



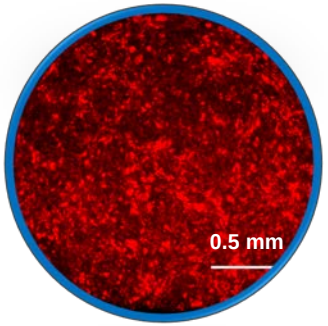


Cell Tracker Red

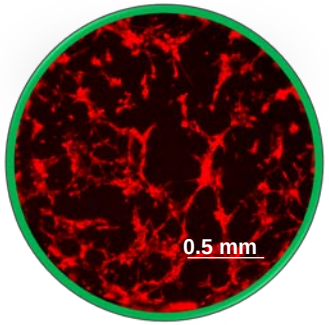
No Adhesion



Low Adhesion



Monolayer

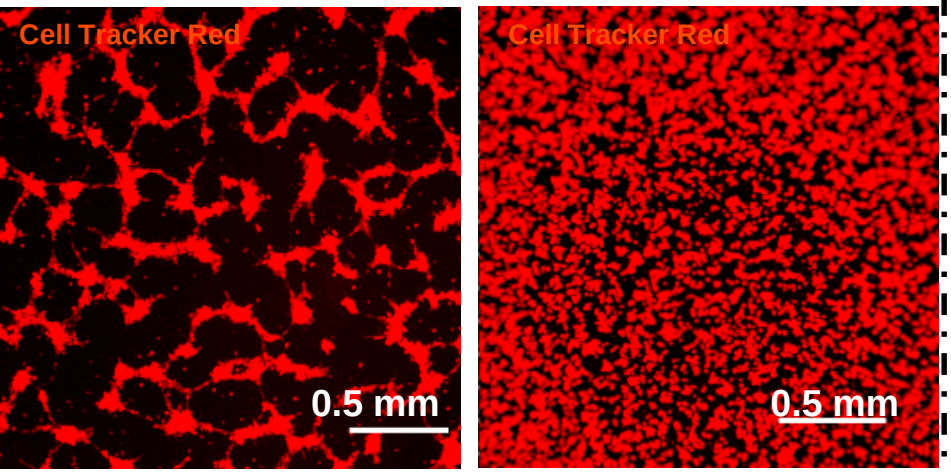


Network

Multiple conditions that generated iPSC-EC networks

Synthetic screening platform detects VEGF inhibition with high consistency

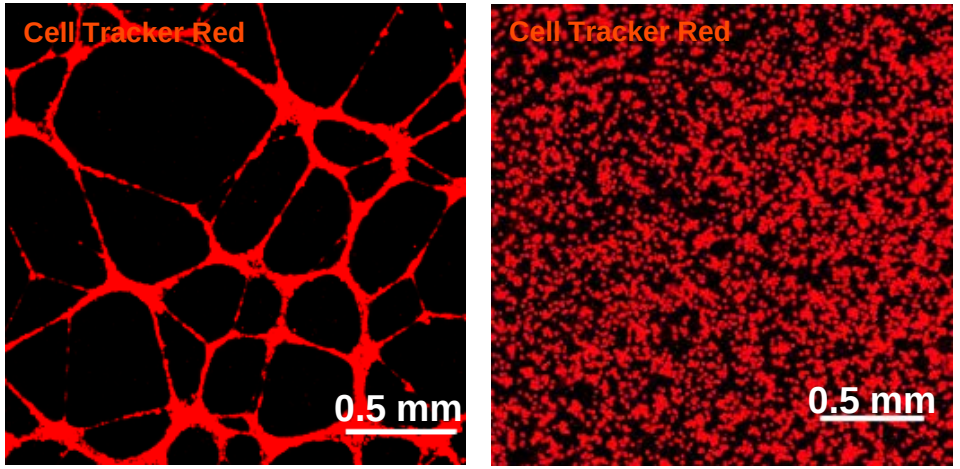
PEG Hydrogel



Negative Ctrl: 0.2% DMSO

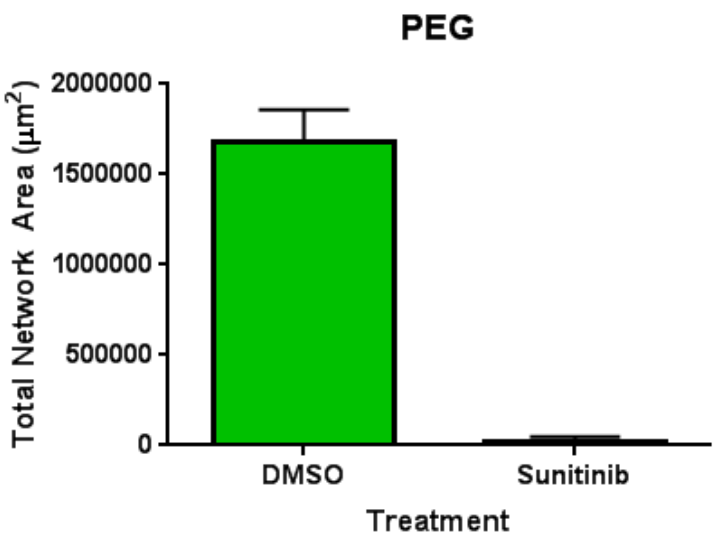
Positive Ctrl: 20 µM Sunitinib

Matrigel

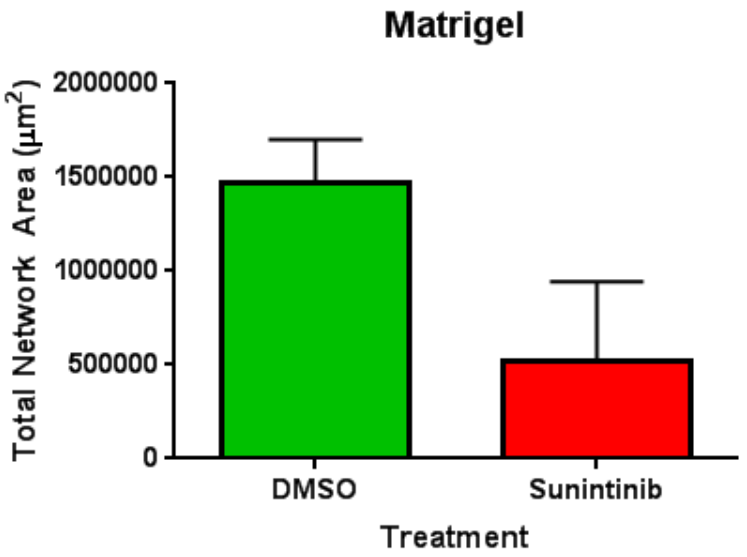


Negative Ctrl: 0.2% DMSO

Positive Ctrl: 20 µM Sunitinib



PEG Hydrogel Z' : 0.66



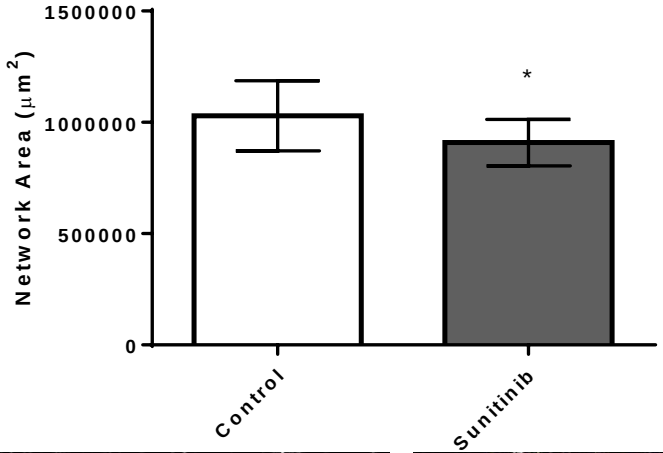
Matrigel Z' : -0.74

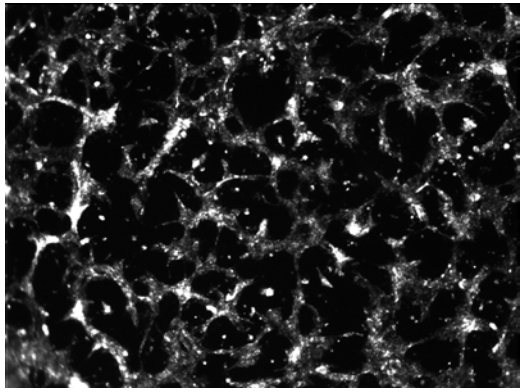
Conclusions

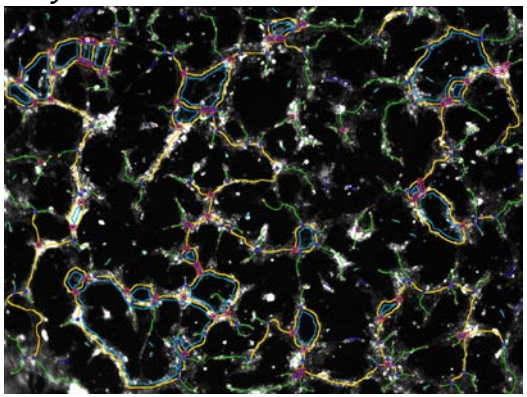
- Enhanced-throughput experimentation to co-vary cell-hydrogel adhesion, mechanical stiffness and soluble VEGF detected culture conditions to promote 2D endothelial network formation

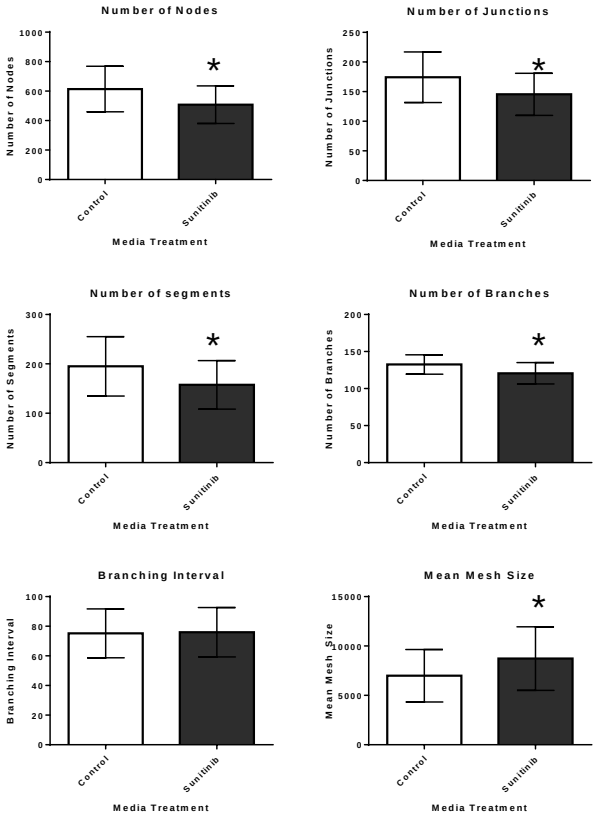
- Fundamental advantages in synthetic cultures versus Matrigel
  - Consistency between replicates
  - Sensitivity to chemical compounds

Sunitinib Malate Treatment – Triple co-cultures



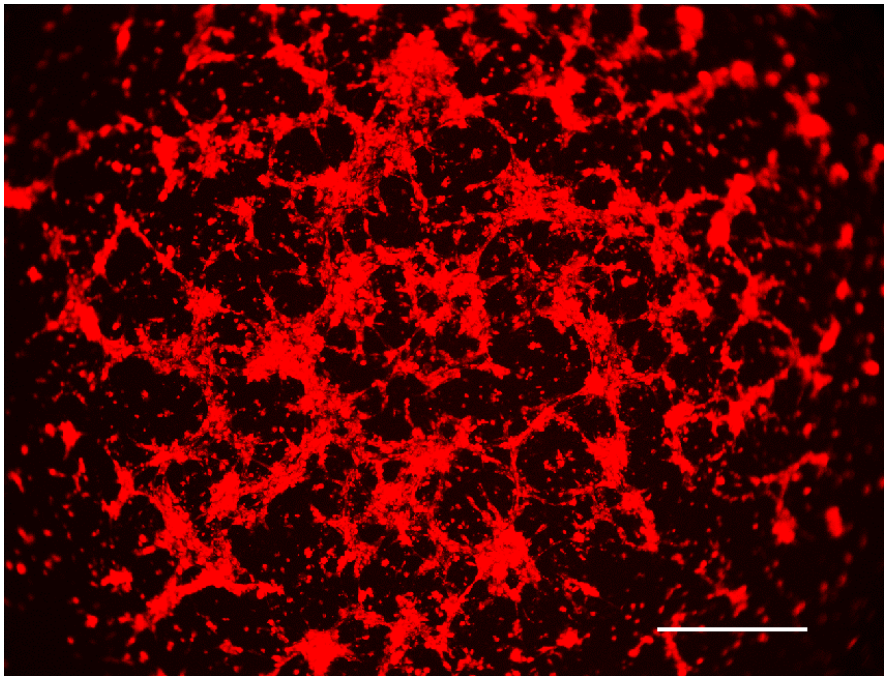




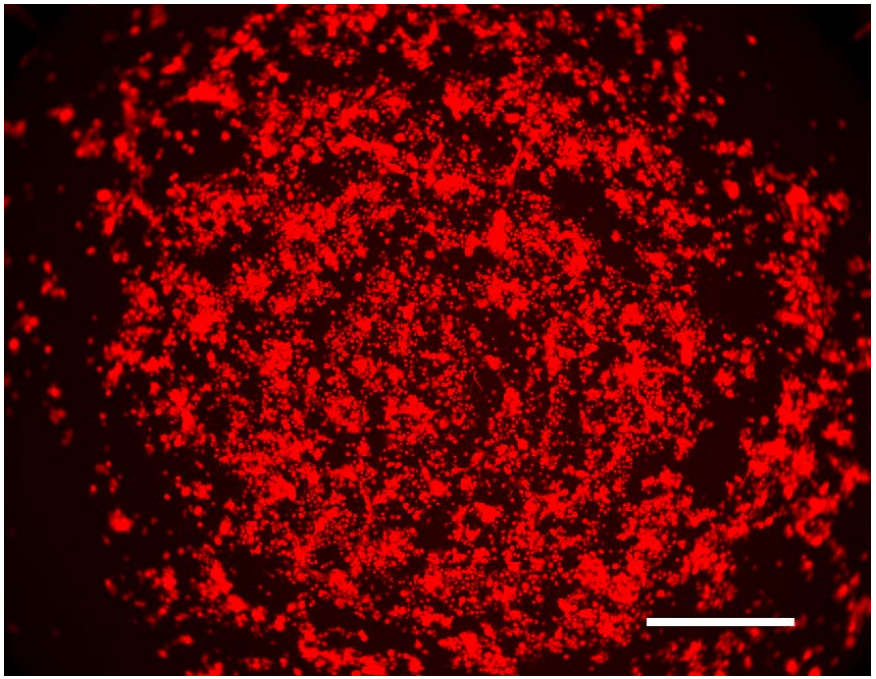


Sunitinib Treatment causes network reorganization but not disruption

Compound 31 – ECs Only



DMSO

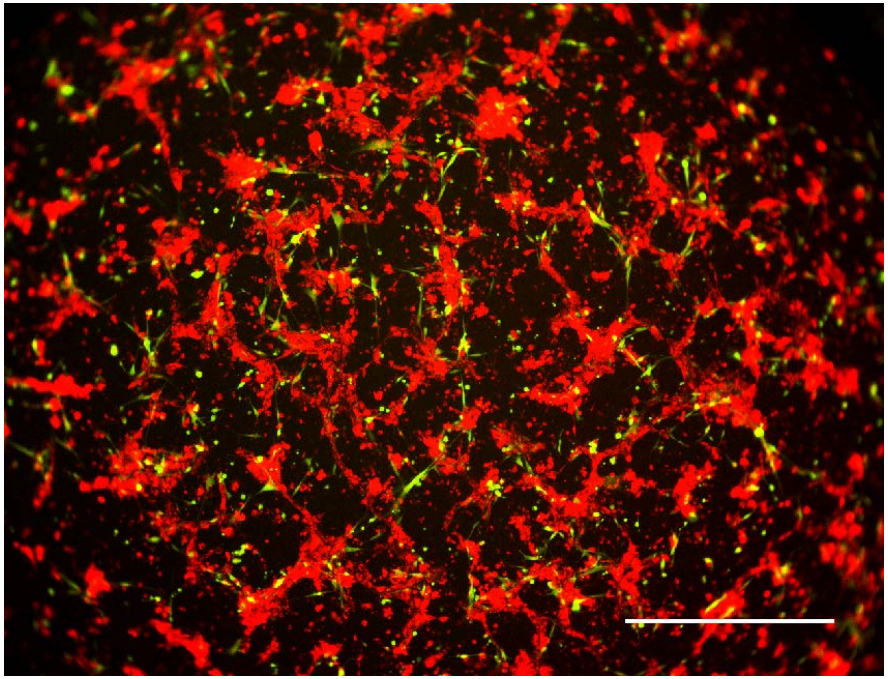


Compound 31

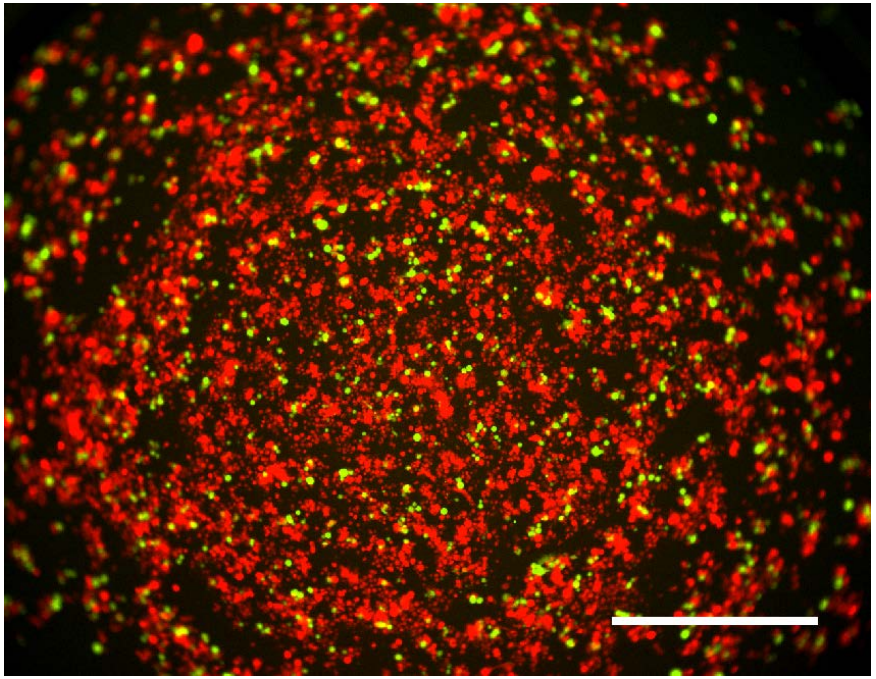
Compound 31 results in inhibited EC spreading and cell clumping

Scale Bar: 0.5 mm

Compound 31 – NVU, lo support density



DMSO

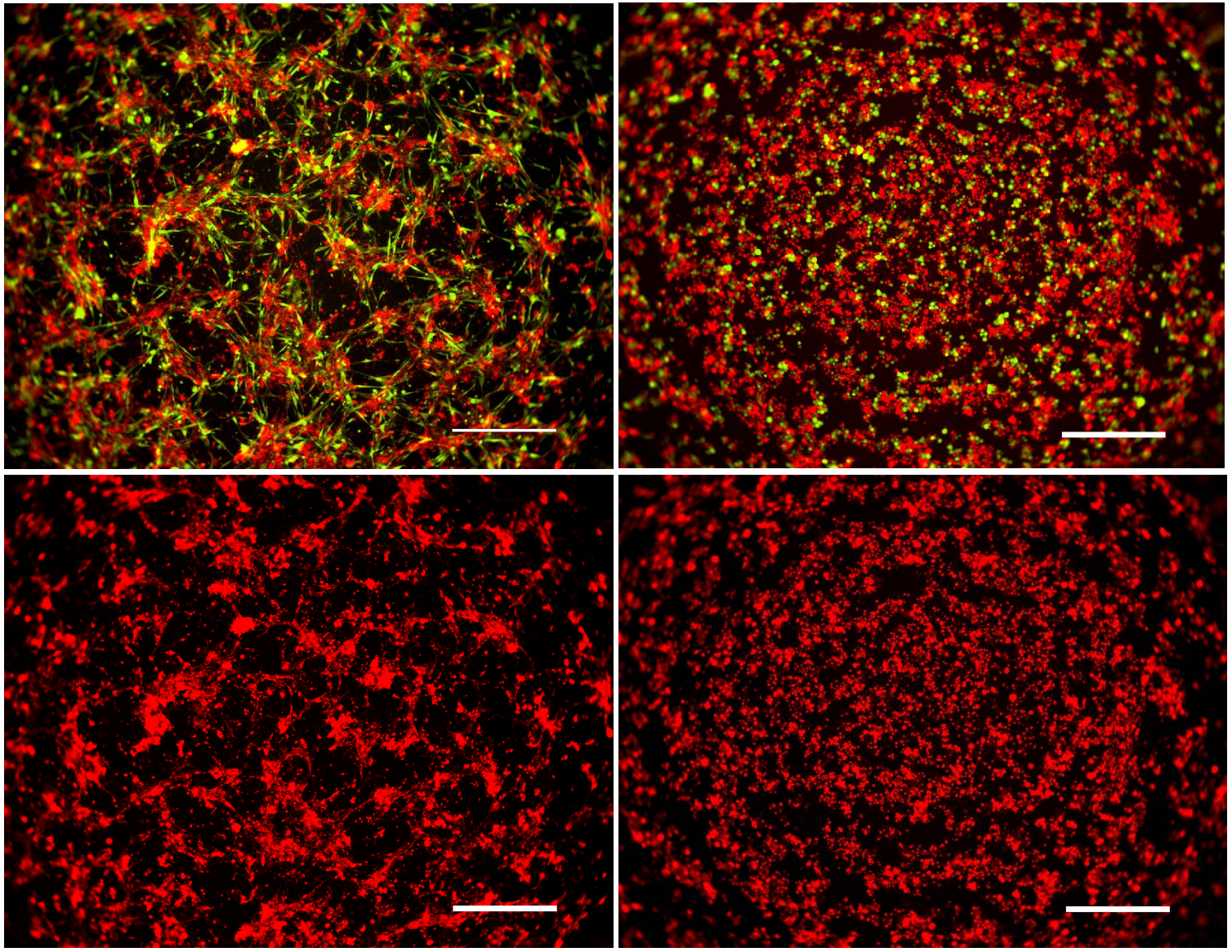


Compound 31

Compound 31 results in inhibited EC spreading and cell clumping

Scale Bar: 0.5 mm

Compound 31 – NVU, hi support density – Not quantitatively detected

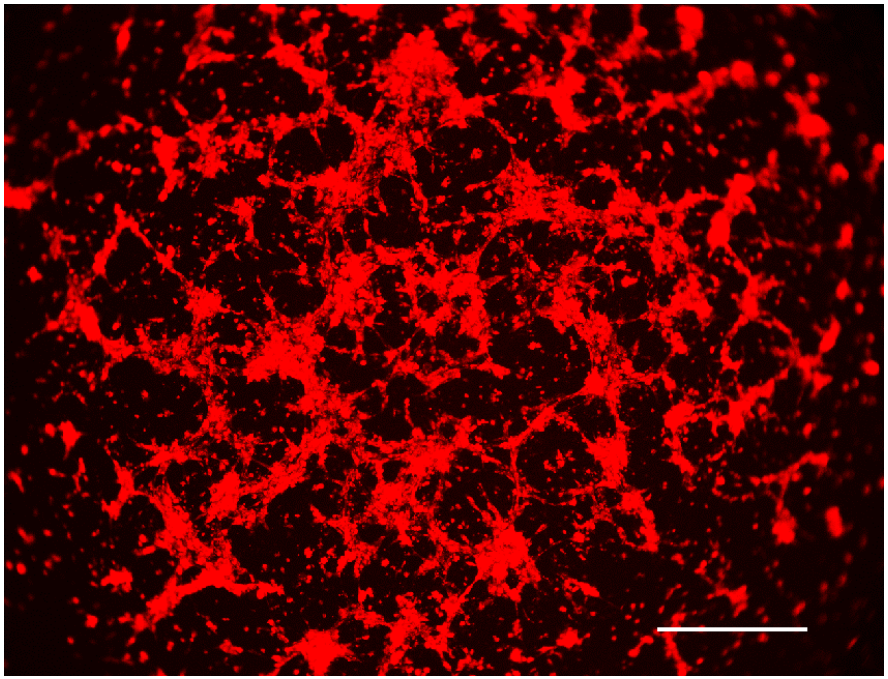


DMSO

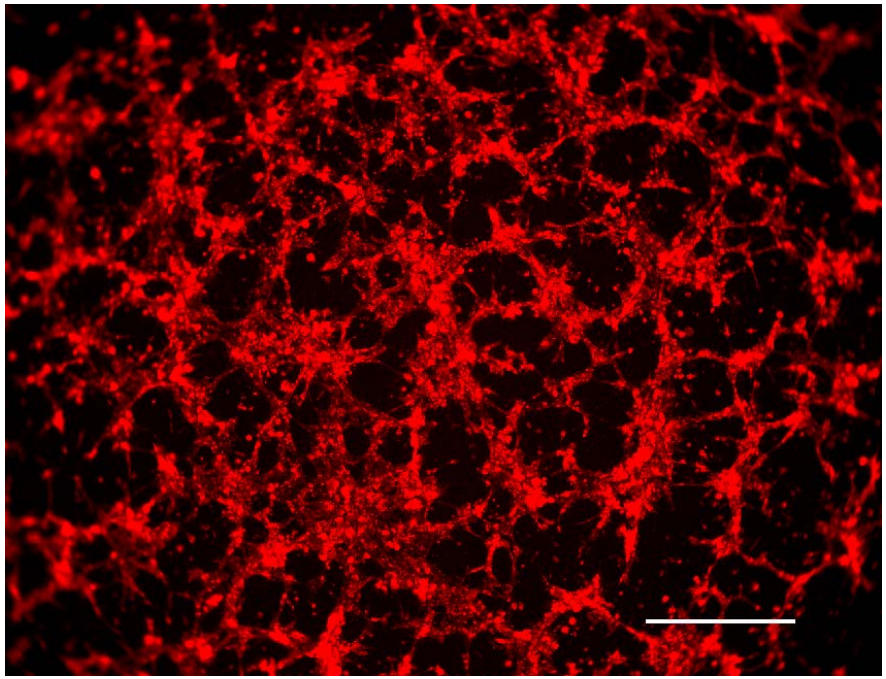
Compound 31

Scale Bar: 0.5 mm

Compound 40 – ECs Only



DMSO

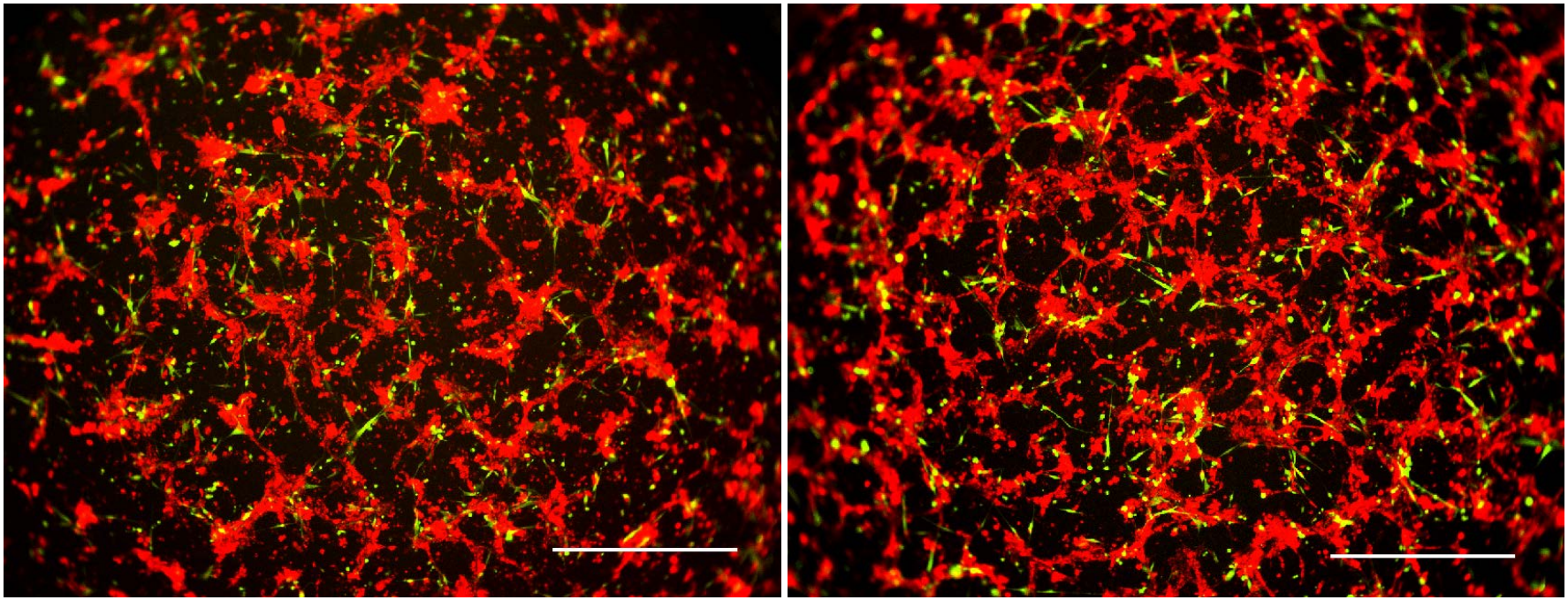


Compound 40

Compound 40 results in enhanced network connectivity

Scale Bar: 0.5 mm

Compound 40 – NVU, lo support density



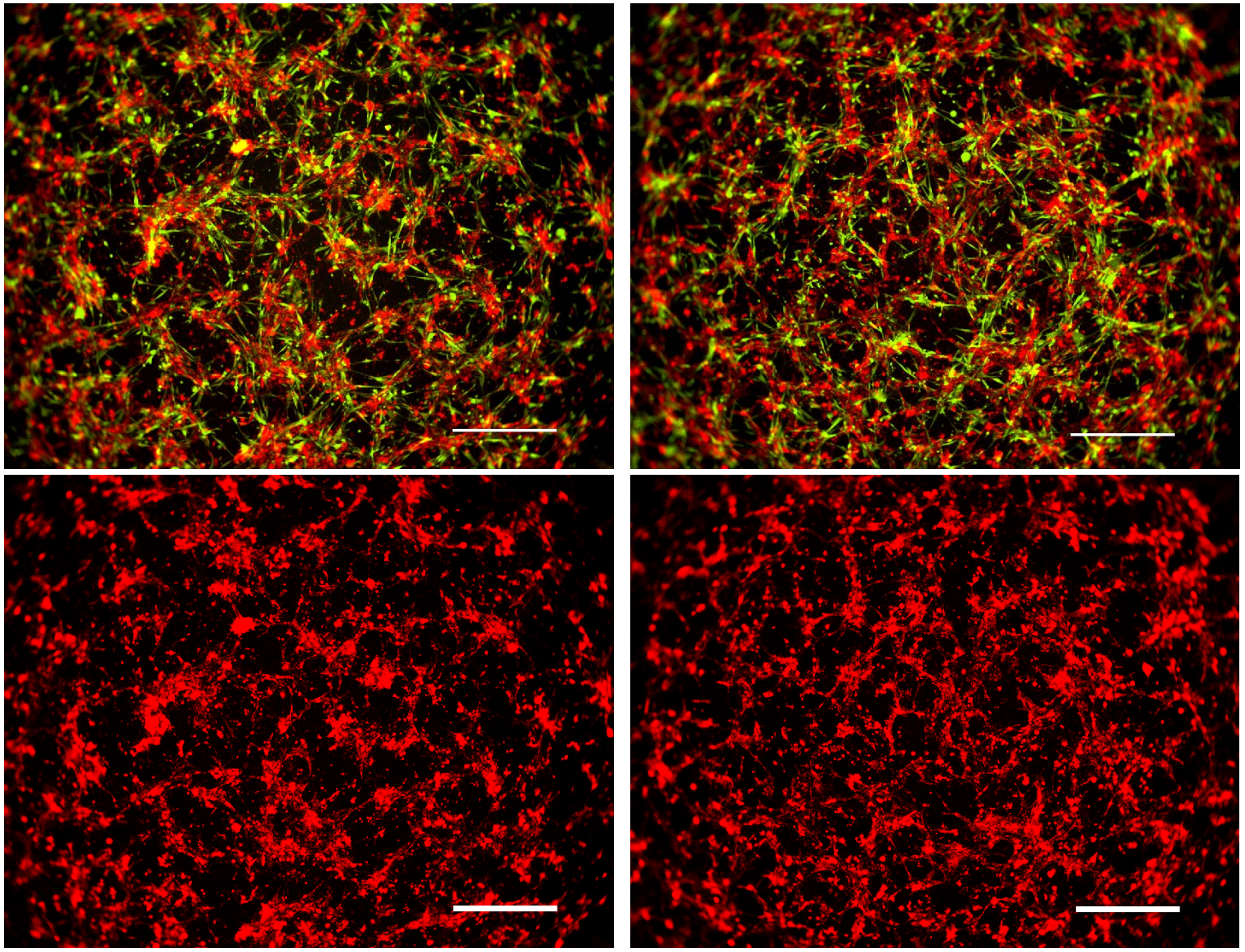
DMSO

Compound 40

Compound 40 results in enhanced network connectivity

Scale Bar: 0.5 mm

Compound 40 – NVU, hi support density – Not quantitatively detected

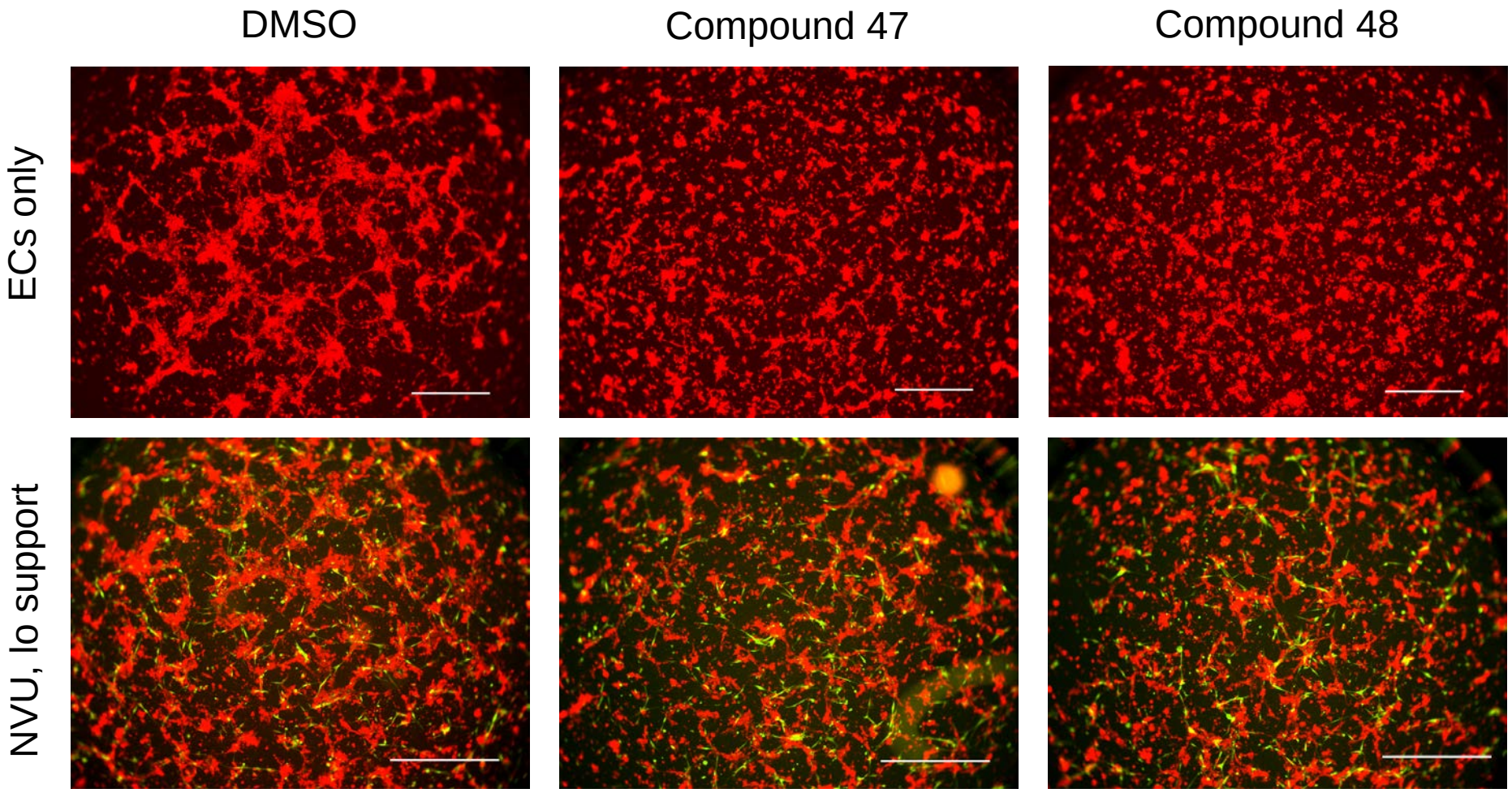


DMSO

Compound 40

Scale Bar: 0.5 mm

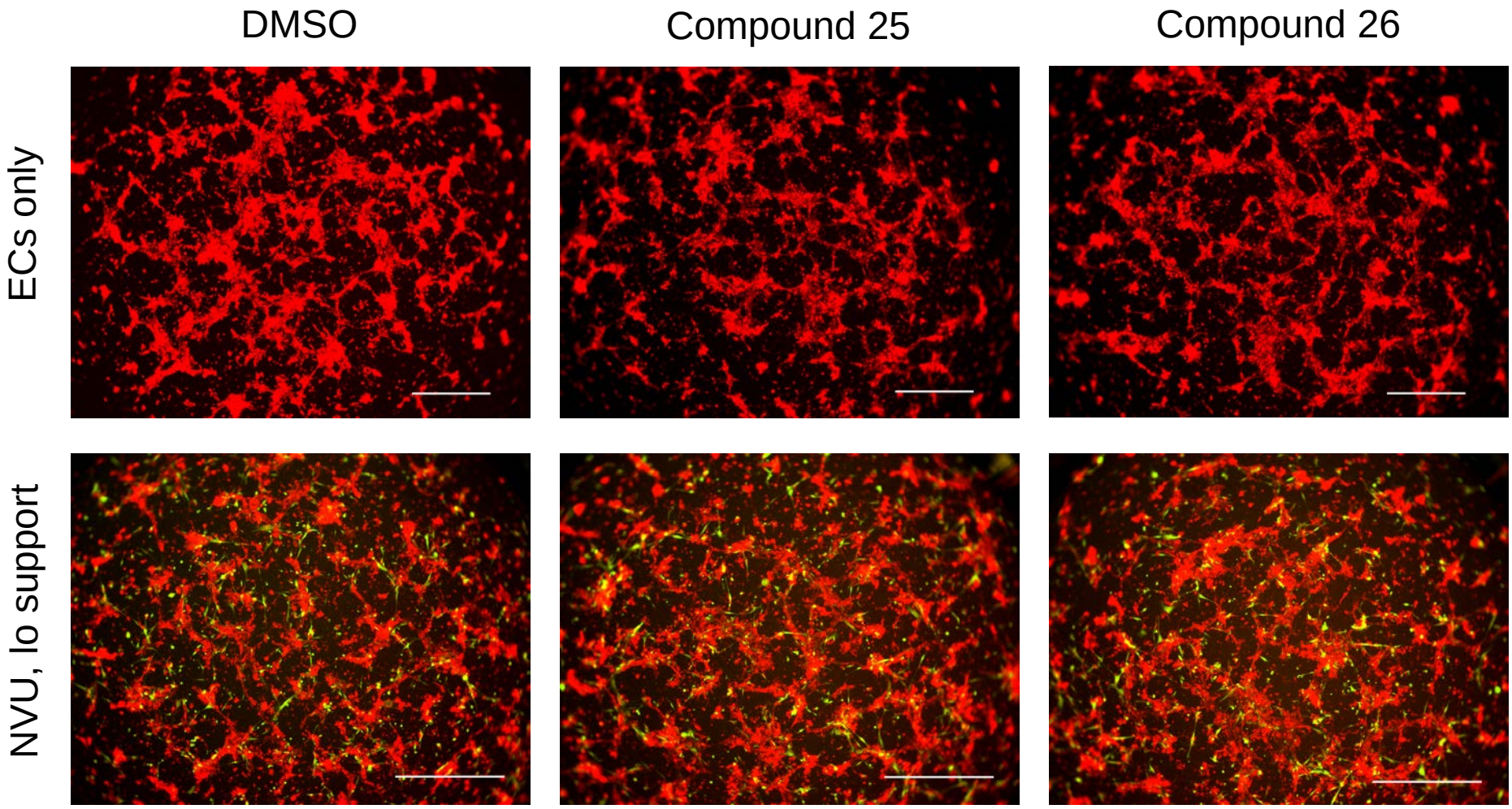
Compounds 47, 48



NVU Support cells help endothelial network to resist breakage by compounds 47 and 48

Scale Bar: 0.5 mm

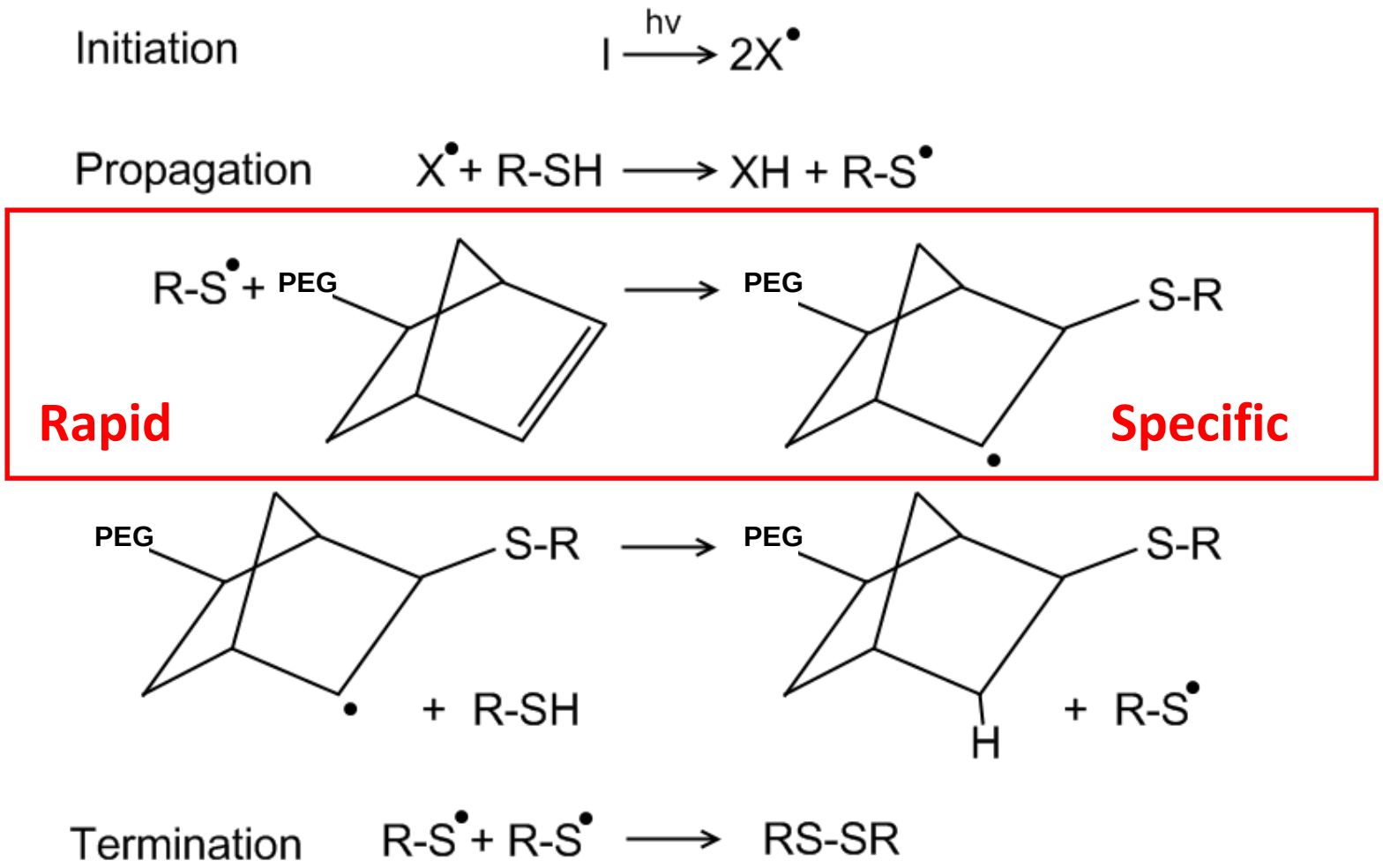
Compounds 25, 26



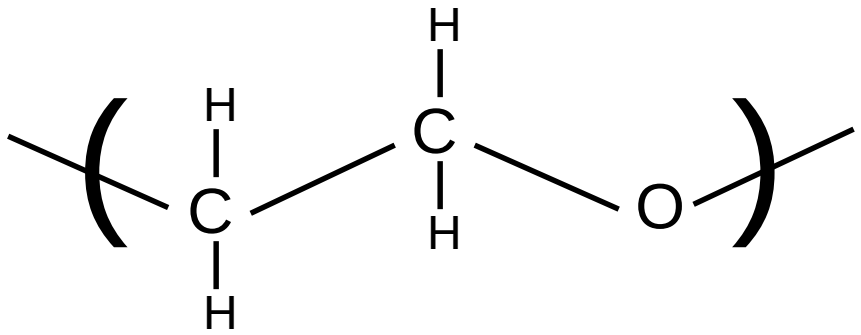
Compounds 25 and 26 broaden endothelial networks when NVU support cells are present

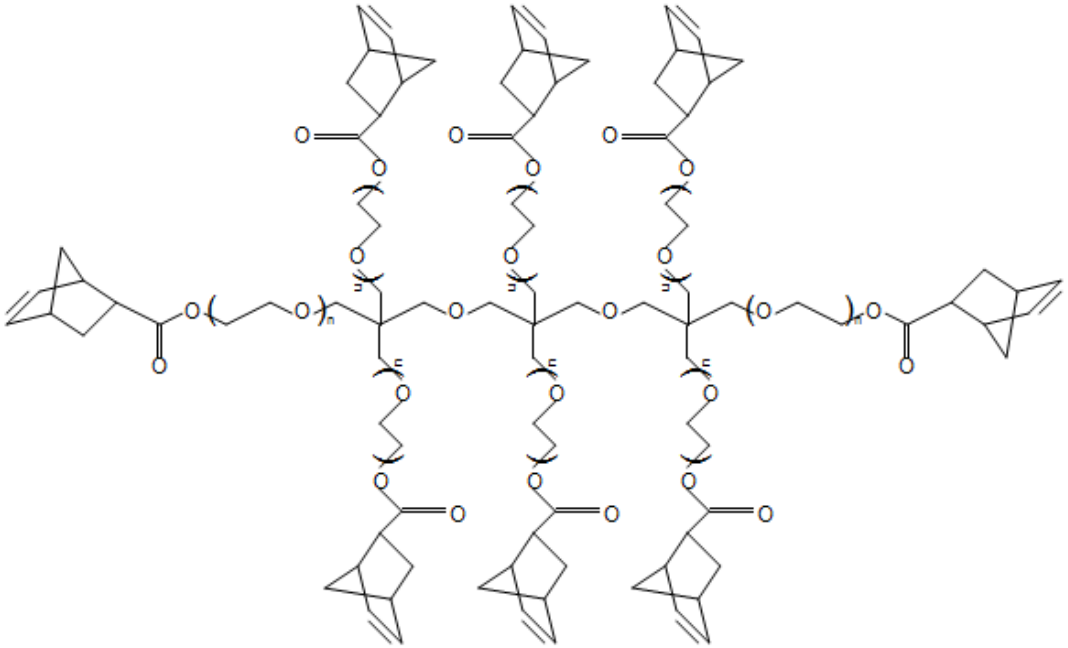
Scale Bar: 0.5 mm

Thiol-ene “click” reaction



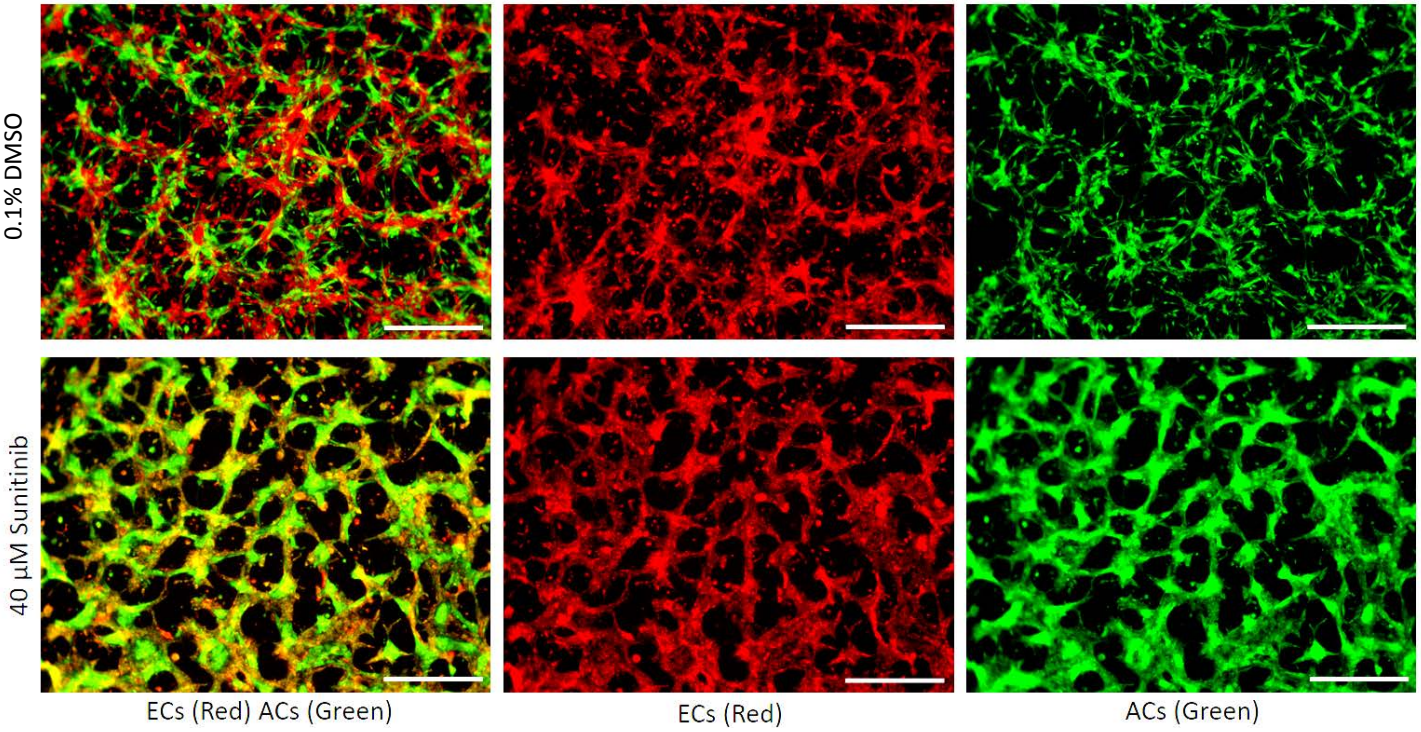
Poly(ethylene glycol) (PEG)





Schwartz *et* al.2009, *Integrative Biology.*

Sunitinib Malate Treatment – Triple co-cultures, high density support cells



Co-culture	Sunitinib Disruption (+/-)
EC only	+
EC-Pericyte	+
EC-Astrocyte	-
Triple – Low	+
Triple – High	-

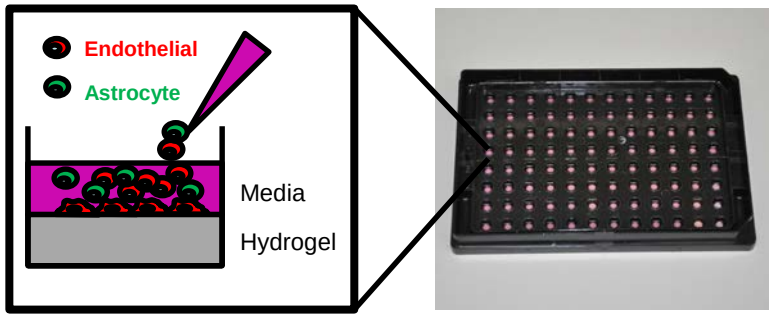
Sunitinib Treatment causes network reorganization but not disruption

Scale Bar: 0.5 mm

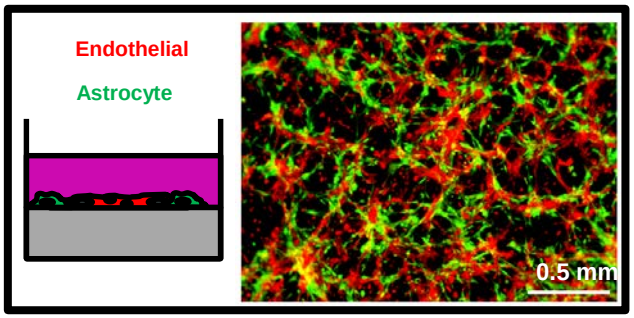
Red: EC Green: AC

Overview

2-Dimensional Tubulogenesis Assay

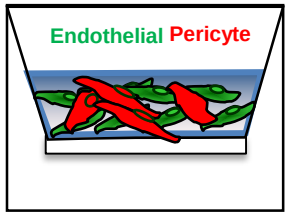


2-dimensional cell seeding

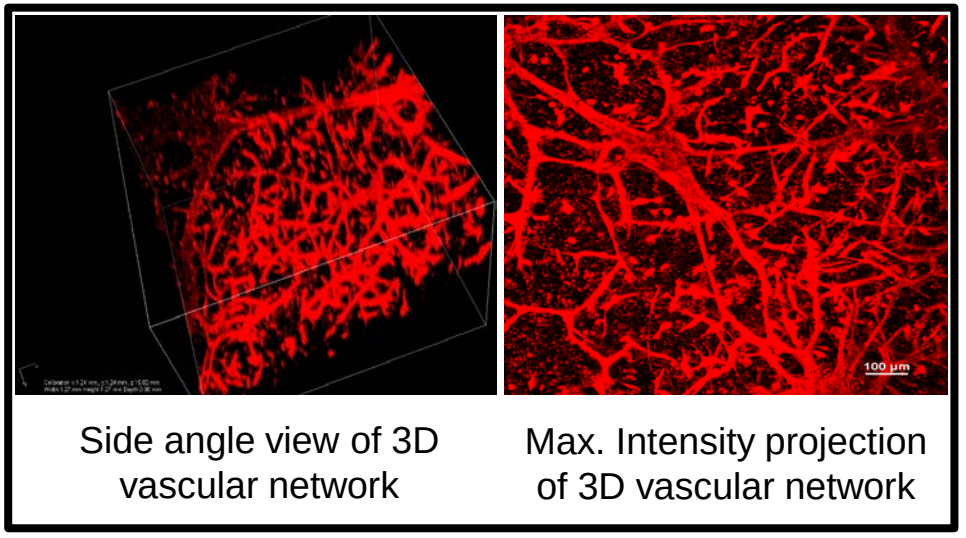


24-hour cell culture

3-Dimensional Organoid Culture



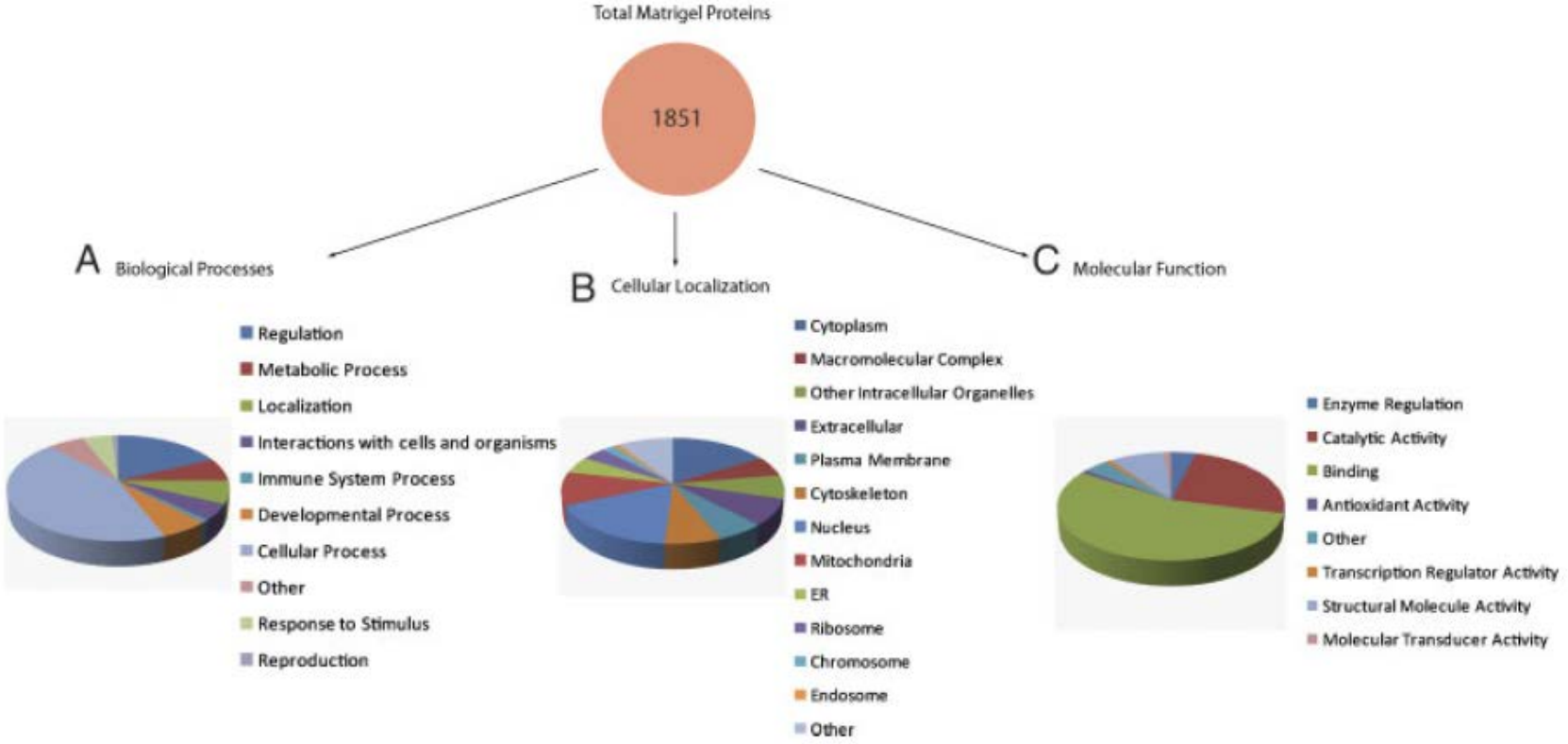
Encapsulated cell seeding



Side angle view of 3D vascular network

Max. Intensity projection of 3D vascular network

The extracellular matrix: Matrigel



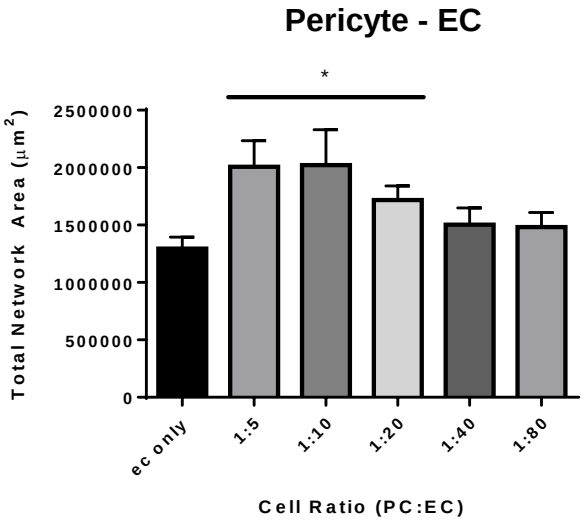
Matrigel: ECM derived from mouse Englebreth-Holm-Swarm tumors, commonly used to study angiogenesis.

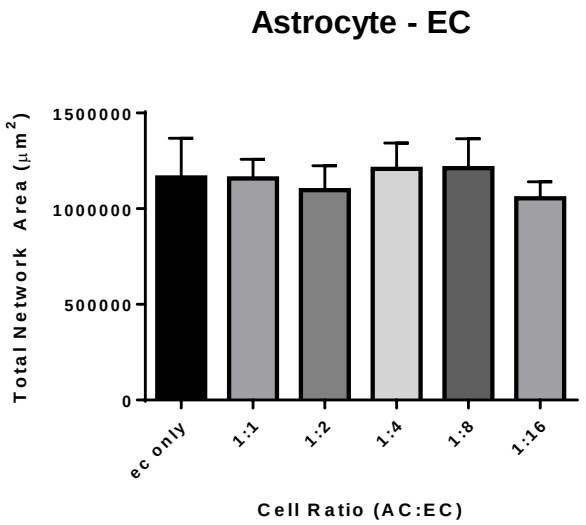
Complex material composed of thousands of proteins and biomolecules

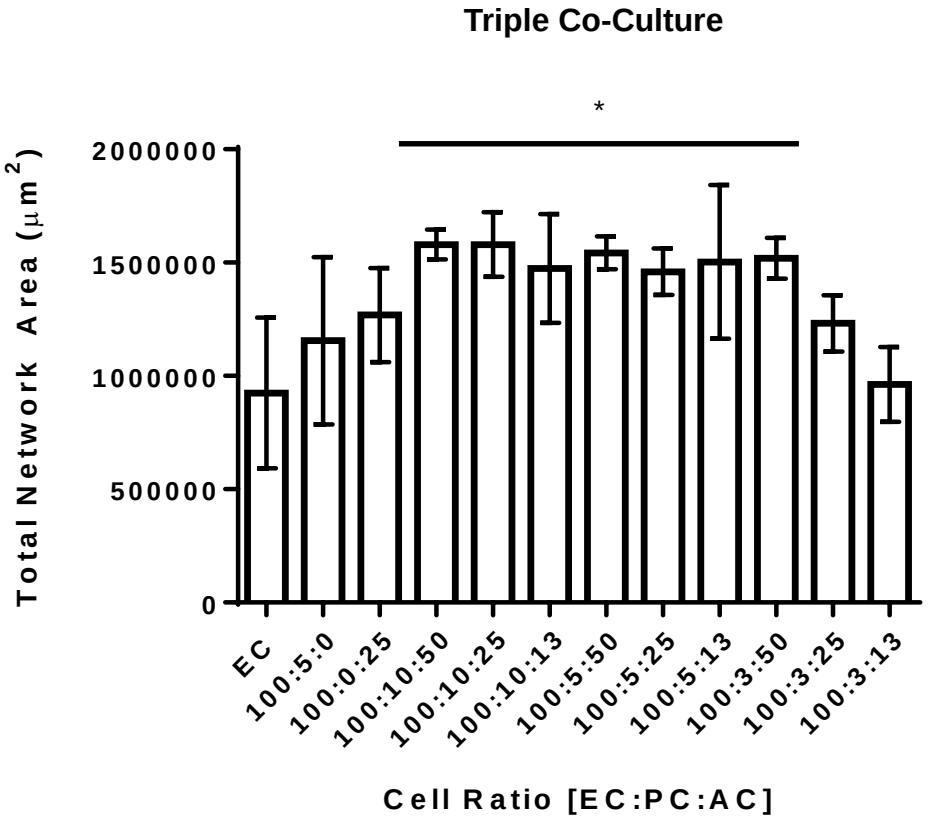
Difficult to customize or define, batch-batch variation, extraneous growth factors

Hughes *et al* 2010, *Proteomics,* 10, 1886-1890.

Total Endothelial Network Area – Pericyte and Astrocyte co-cultures

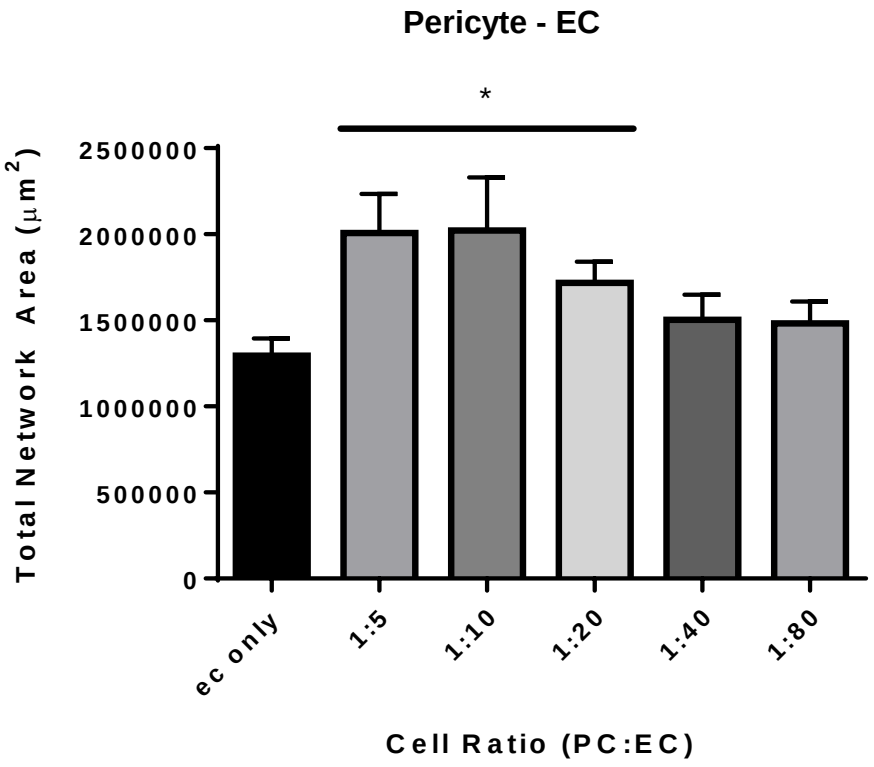


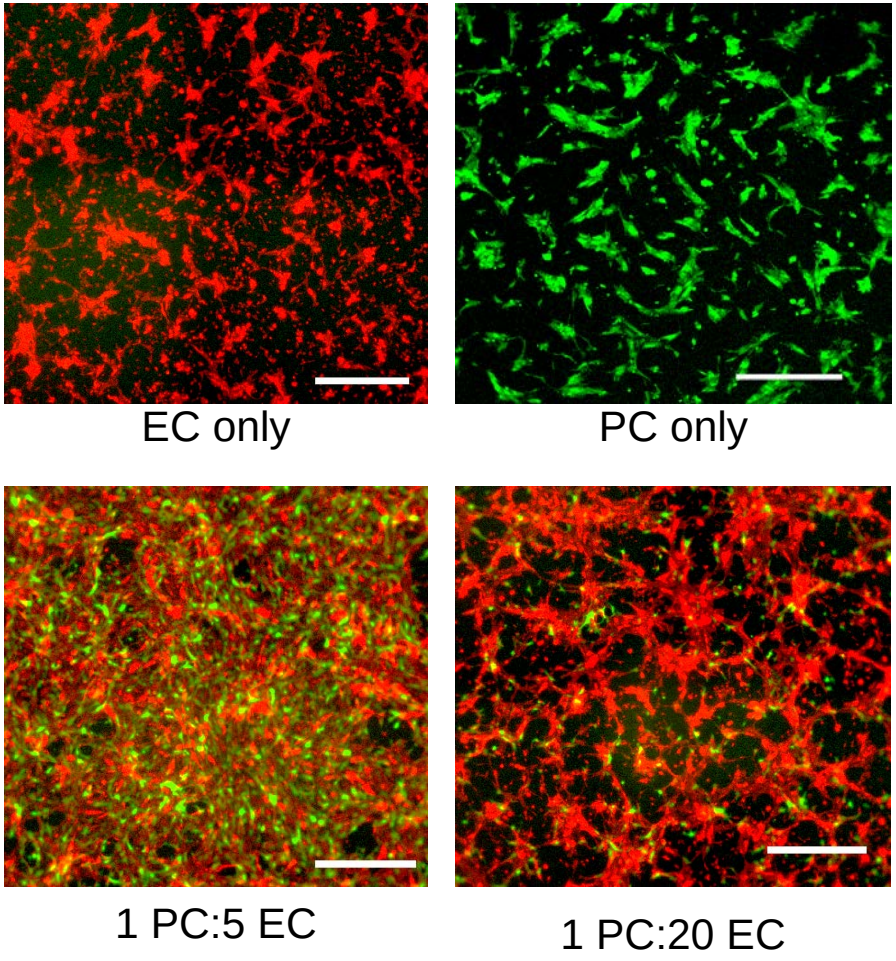




Triple Co-culture: Increase network area differentially from duo co-cultures

Total Endothelial Network Area – Pericytes increase network area

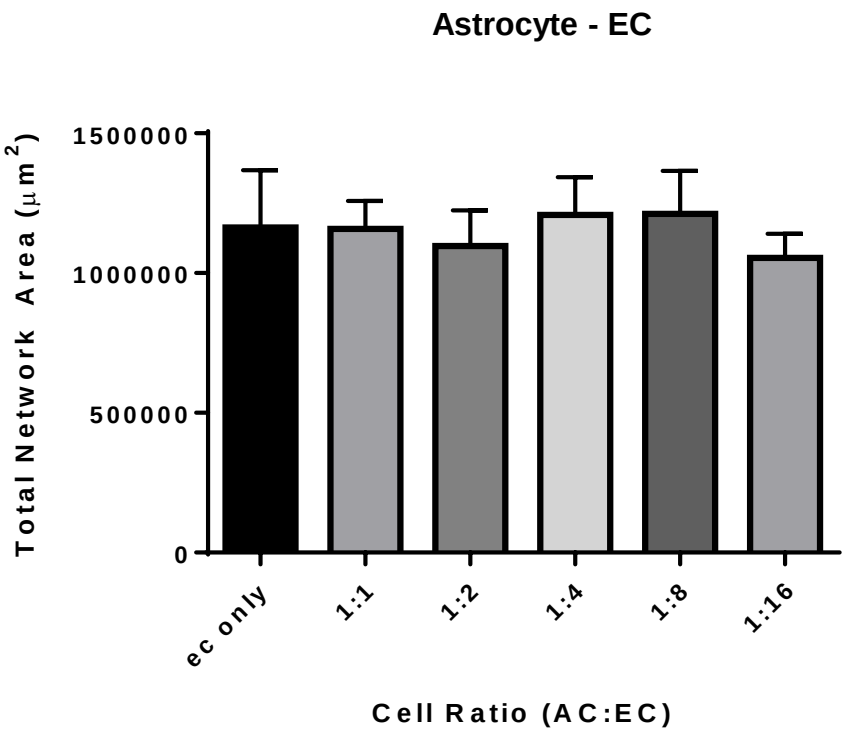


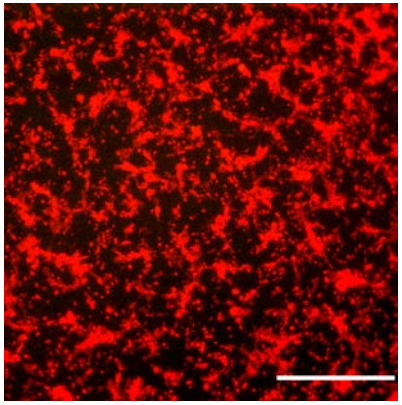


Red: EC Green: PC

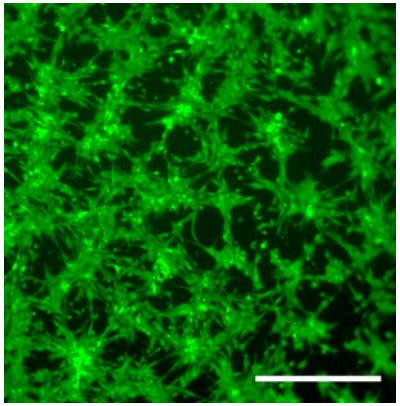
Scale Bar: 0.5 mm

Total Endothelial Network Area – Astrocytes do not affect network area

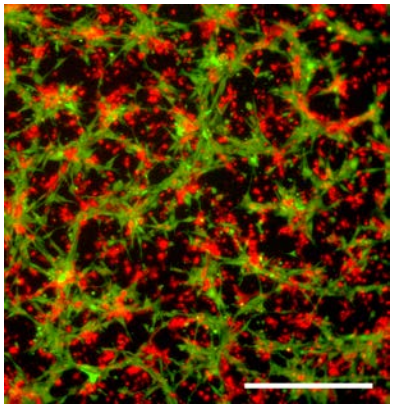




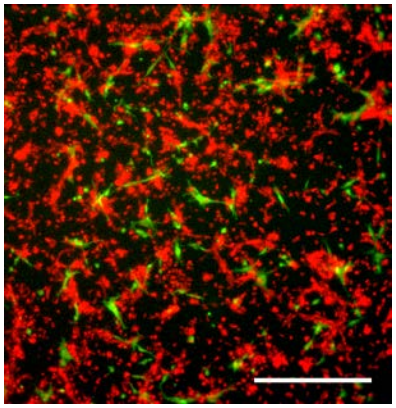
EC only



AC only



1 AC: 2 EC

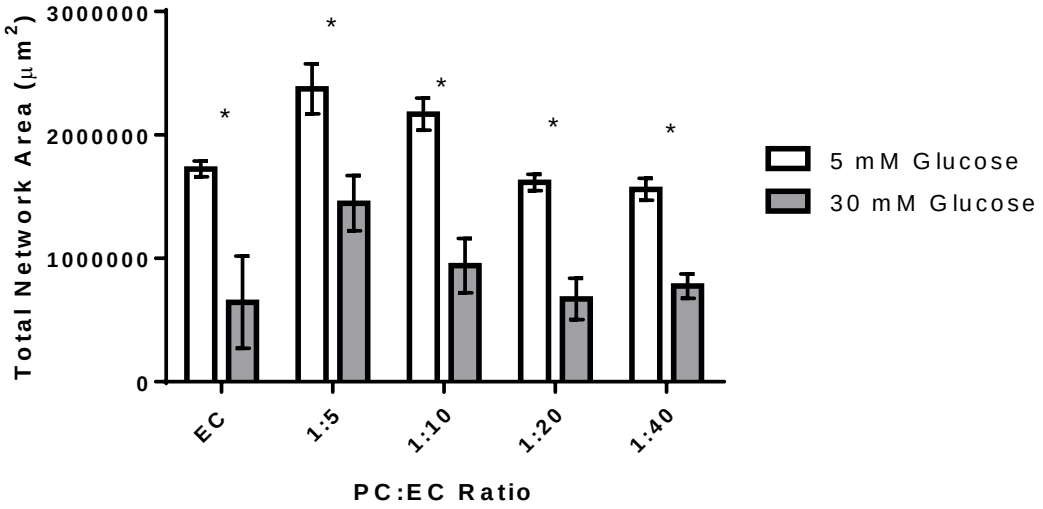


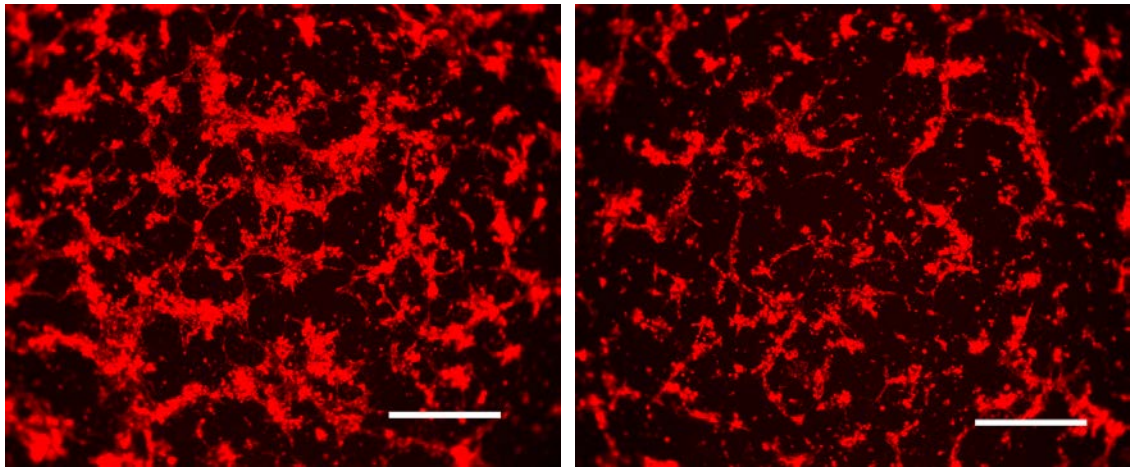
1 AC: 16 EC

Red: EC Green: AC

Scale Bar: 0.5 mm

Osmotic Stress leads to network disruption in EC-Pericyte co-cultures



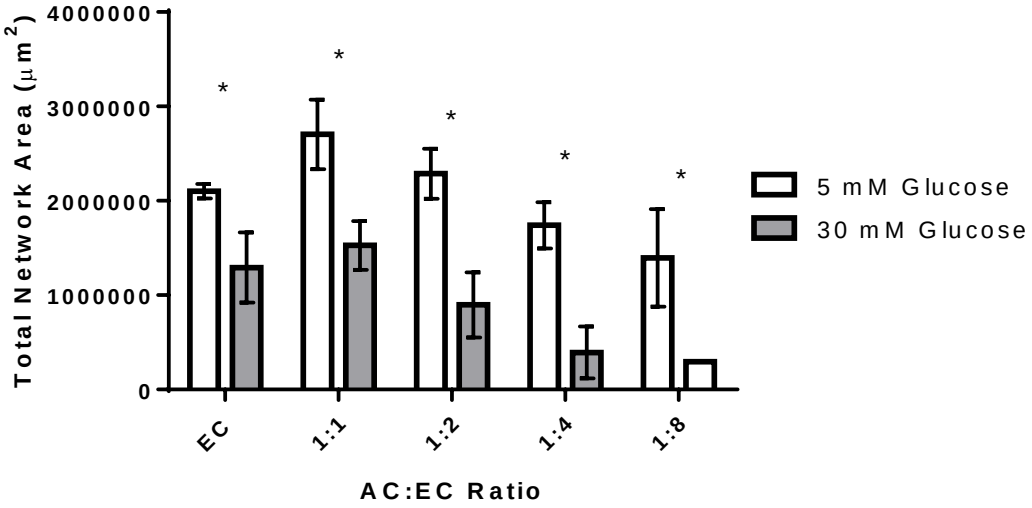


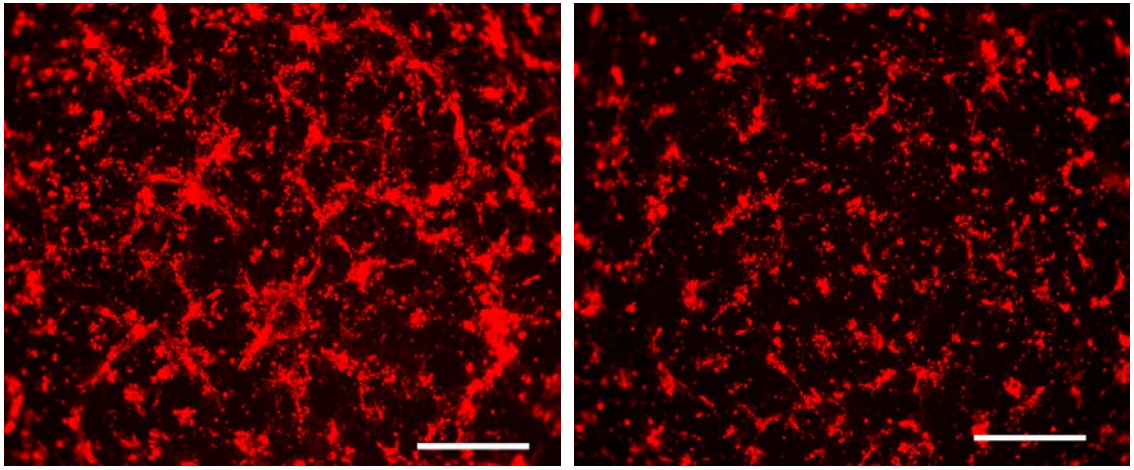
5 mM Glucose **30 mM Glucose**

	High Glucose
Co-culture	**Disruption (+/-)**
EC only	+
EC-Pericyte	+
EC-Astrocyte	
Triple – Low	
Triple – High	

Scale Bar: 0.5 mm

Osmotic Stress leads to network disruption in EC-Astrocyte co-cultures



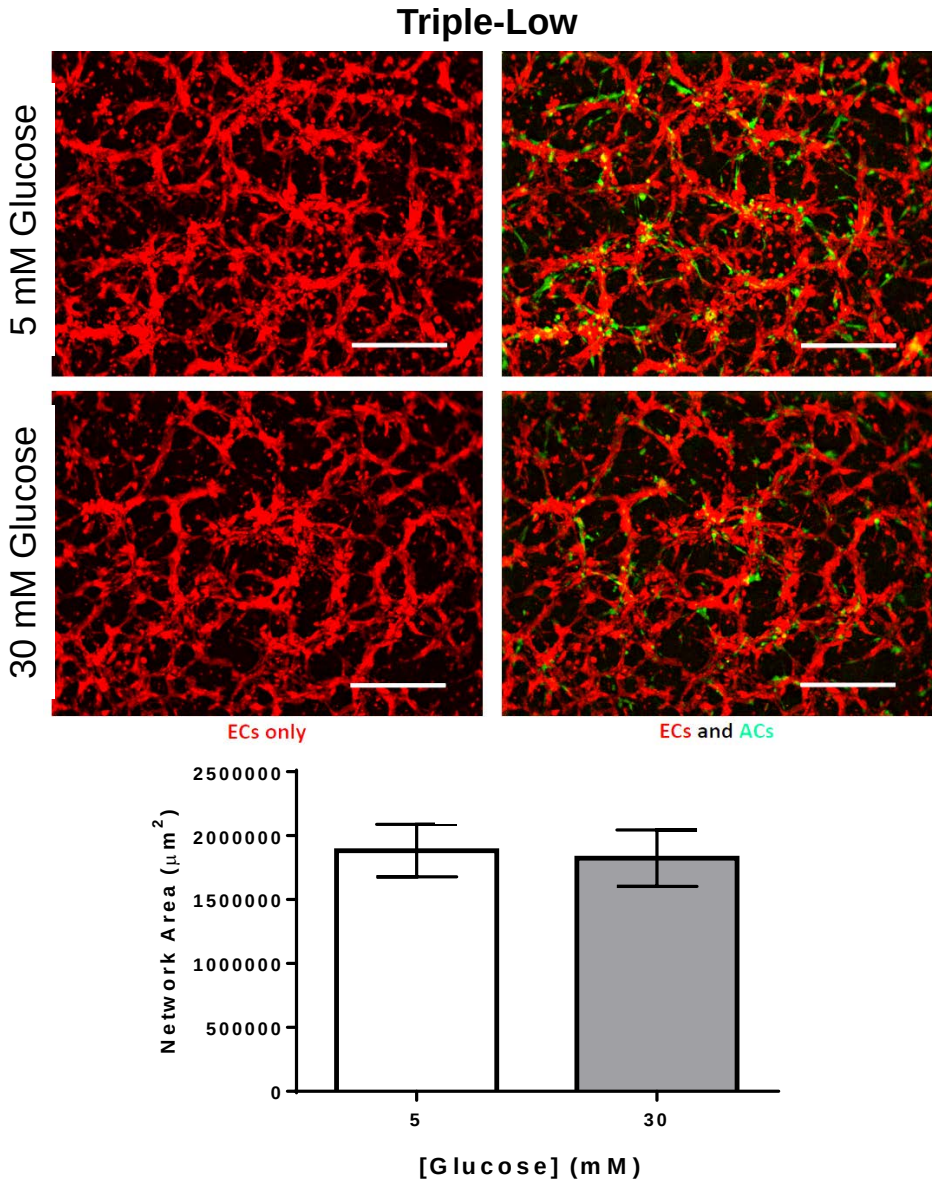


5 mM Glucose **30 mM Glucose**

	High Glucose
Co-culture	**Disruption (+/-)**
EC only	+
EC-Pericyte	+
EC-Astrocyte	+
Triple – Low	
Triple – High	

Scale Bar: 0.5 mm

High D-glucose does not disrupt endothelial networks in the presence of Astrocytes and Pericytes

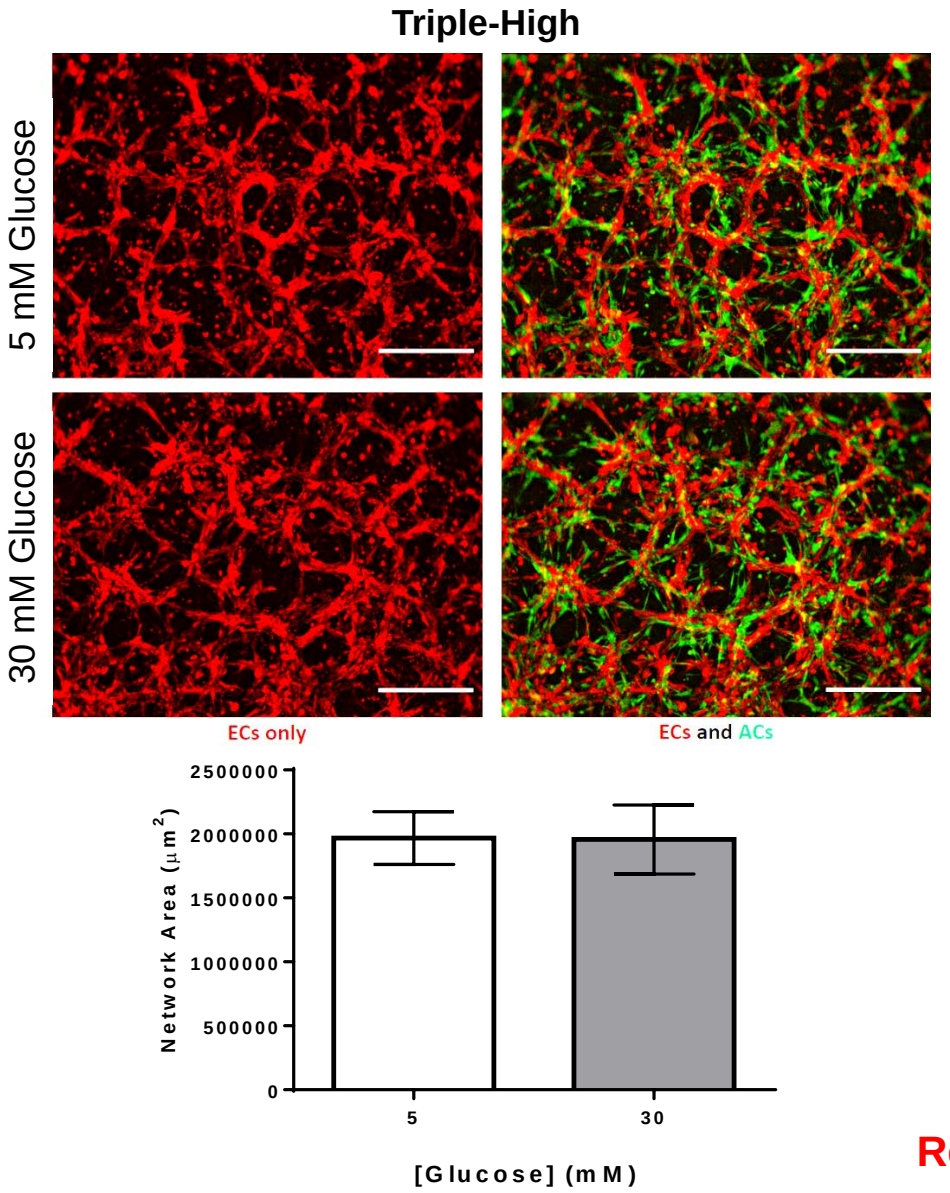


	High Glucose
Co-culture	**Disruption (+/-)**
EC only	+
EC-Pericyte	+
EC-Astrocyte	+
Triple – Low	-
Triple – High	

Scale Bar: 0.5 mm

Red: EC Green: AC

High D-glucose does not disrupt endothelial networks in the presence of Astrocytes and Pericytes



	High Glucose
Co-culture	**Disruption (+/-)**
EC only	+
EC-Pericyte	+
EC-Astrocyte	+
Triple – Low	-
Triple – High	-

Scale Bar: 0.5 mm

Red: EC Green: AC

Astrocytes and Pericytes affect Endothelial Network coherence in the presence of high glucose and VEGF inhibitor Sunitinib Malate

	Sunitinib	High Glucose
Co-culture	**Disruption (+/-)**	
EC only	+	+
EC-Pericyte	+	+
EC-Astrocyte	-	+
Triple – Low	+	-
Triple – High	-	-

Astrocyte and Pericyte functions:

- Apoptosis
- Tight junction
- Proliferation/Cell cycle
- Reactive Oxygen Species
- GFAP
- Glut-1 transporter
- Growth factor production

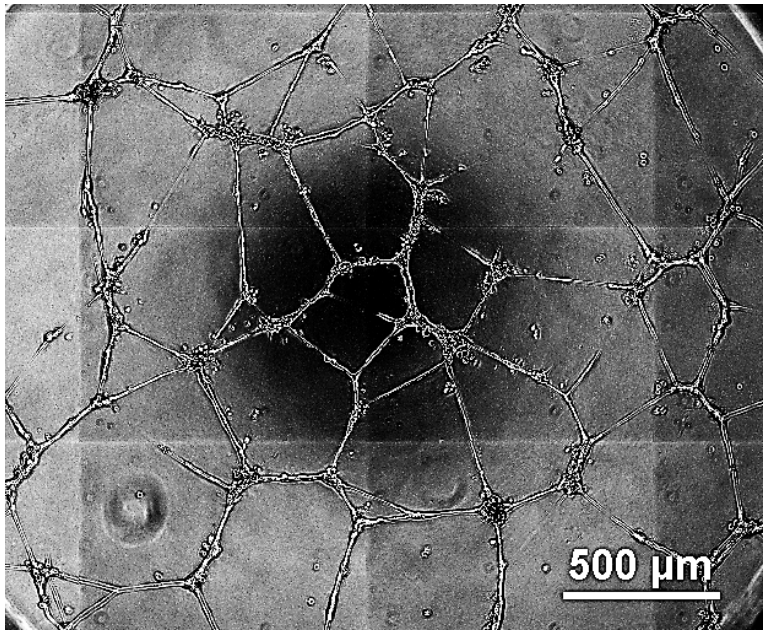
Astrocytes and Pericytes affect Endothelial Network coherence in the presence of high glucose and VEGF inhibitor Sunitinib Malate

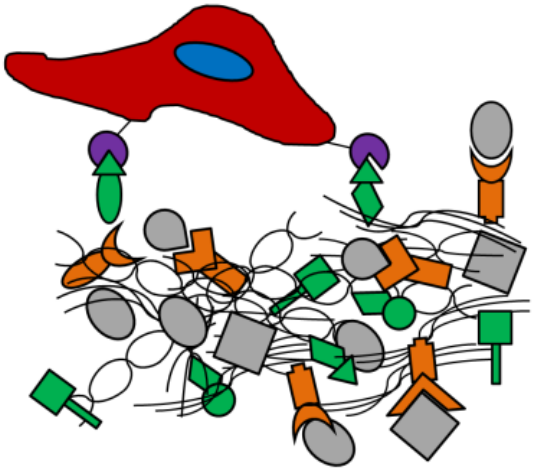
	High Glucose
Co-culture	**Disruption (+/-)**
EC only	**+**
EC-Pericyte	**+**
EC-Astrocyte	**+**
Triple – Low	**-**
Triple – High	**-**

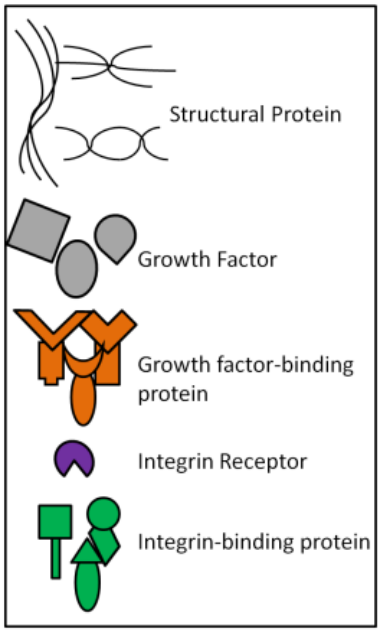
Glucose:

- Pericytes and Astrocytes prevent complete disruption of endothelial cell networks. Both support cell types must be present in culture.

Matrigel and the tubulogenesis assay







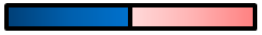
Matrigel's complexity leads to limited:

Consistency
Sensitivity

Hughes *et al* 2010, *Proteomics,* 10, 1886-1890.

Synthetic screening platform identifies vascular inhibitors in blinded screen

Chemical Name:	Matrigel	Synthetic	pVDC score
1,2,4-Trichlorobenzene	0	0	0.000
Decane	0	0	0.000
Tris(2-chloroethyl) phosphate	0	0	0.000
1,2,3-Trichloropropane	0	0	0.002
Pymetrozine	0	0	0.002
Methimazole	0	0	0.002
Diethanolamine	0	0	0.002
Imazamox	0	0	0.007
D-Mannitol	0	0	0.007
Methylparaben	0	0	0.010
Valproic acid	0	0	0.016
2,4-Diaminotoluene	0	0	0.069
Bisphenol A	0	0	0.146
Haloperidol	0	0	0.177
Tris(2-ethylhexyl) phosphate	0	0	0.182
Tris(1,3-dichloro-2-propyl)phosphate	0	0	0.188
Cladribine	0	0	0.196
TNP-470	0	0	0.238
Oxytetracycline dihydrate	0	0	0.260
Celecoxib	0	1	0.269
Docusate sodium	0	0	0.304
C.I. Solvent Yellow 14	0	1	0.306
Reserpine	0	0	0.307
Quercetin	0	1	0.309
Phenolphthalein	0	0	0.327
5HPP-33	1	0	0.327
tert-Butylhydroquinone	0	0	0.336
Triclocarban	1	1	0.362
Triclosan	0	1	0.372
Pyridaben	0	1	0.379
1-Hydroxypyrene	1	1	0.386
Sodium dodecylbenzenesulfonate	0	0	0.429
Disulfiram	1	1	0.432
Fluazinam	1	1	0.434
Octyl gallate	0	1	0.450
Bisphenol AF	0	1	0.457
PFOS	0	0	0.460
4-Nonylphenol, branched	0	0	0.461

pVDC Score: Non-Inhibitory  Inhibitory

Blinded screen of toxic compounds reveals increased sensitivity of synthetic hydrogels

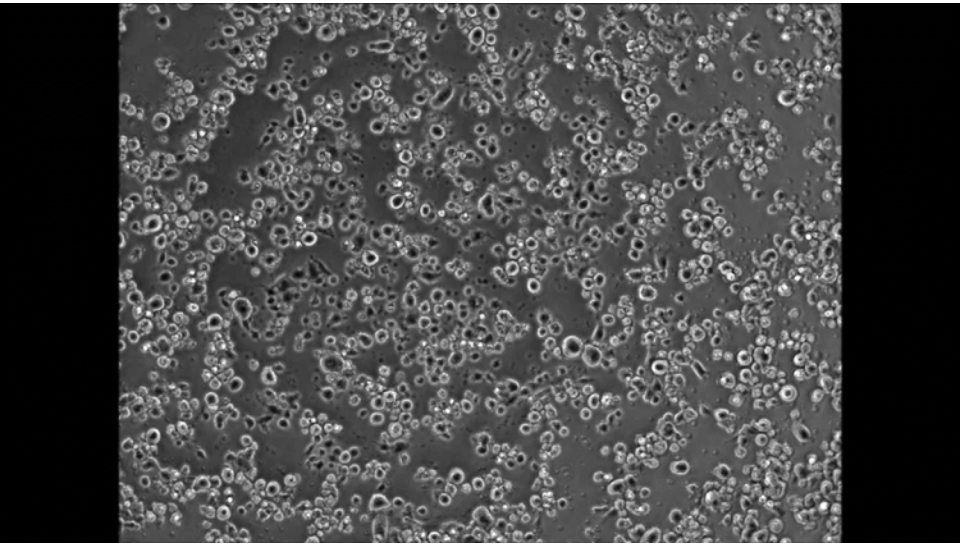
Accuracy:

Synthetic – 61%
Matrigel – 45%

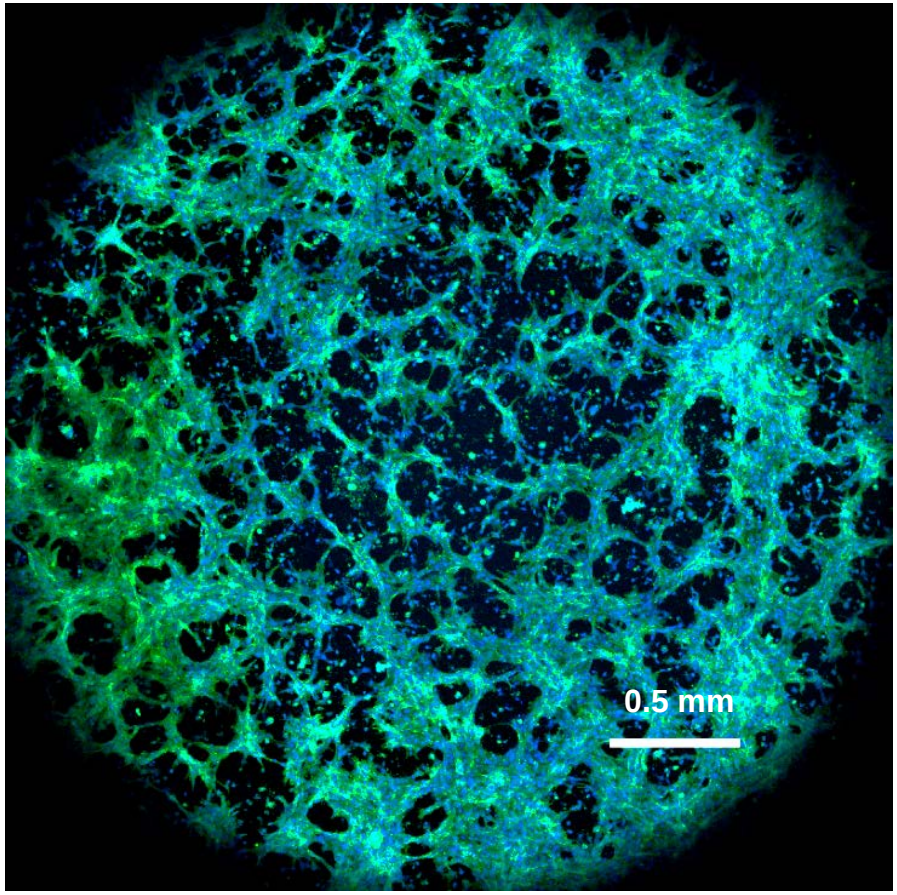
Sensitivity:

Synthetic – 42%
Matrigel – 19%

IPSC-ECs form interconnected networks on specified hydrogels



IPSC-ECs

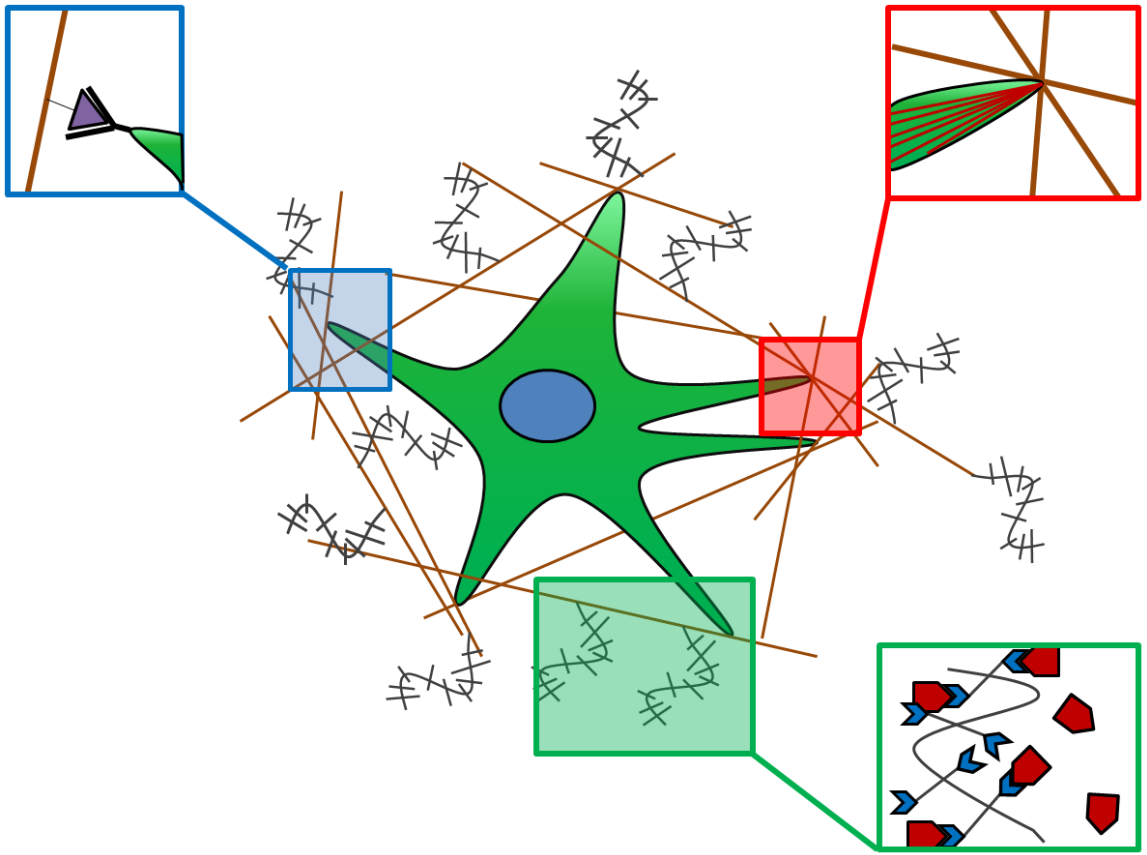


Green: CD31 **Blue:** DAPI

The extracellular matrix (ECM)

Adhesion Ligands

Integrin-binding molecules



Matrix Stiffness

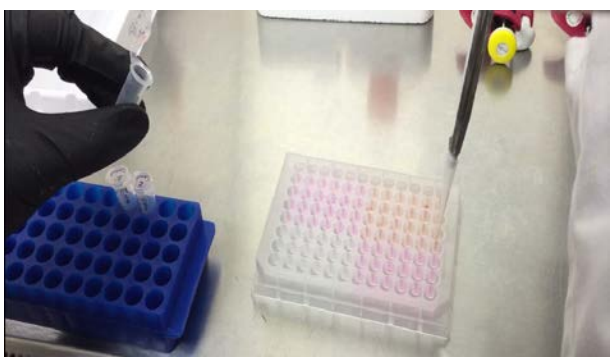
Fiber strength, pore-size, crosslinking density, swelling

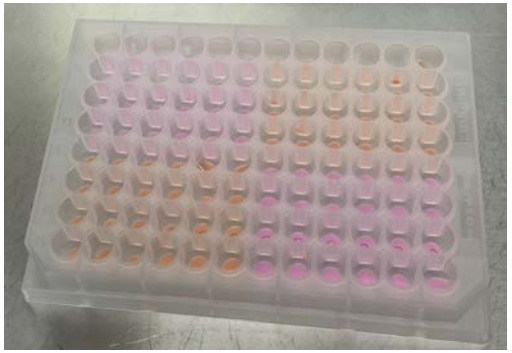
Growth Factor Sequestration

Proteoglycans

ECM modulates cell fate with multiple cues

ECs form networks on PEG and Matrigel





Synthetic