MIMETAS
the organ-on-a-chip company

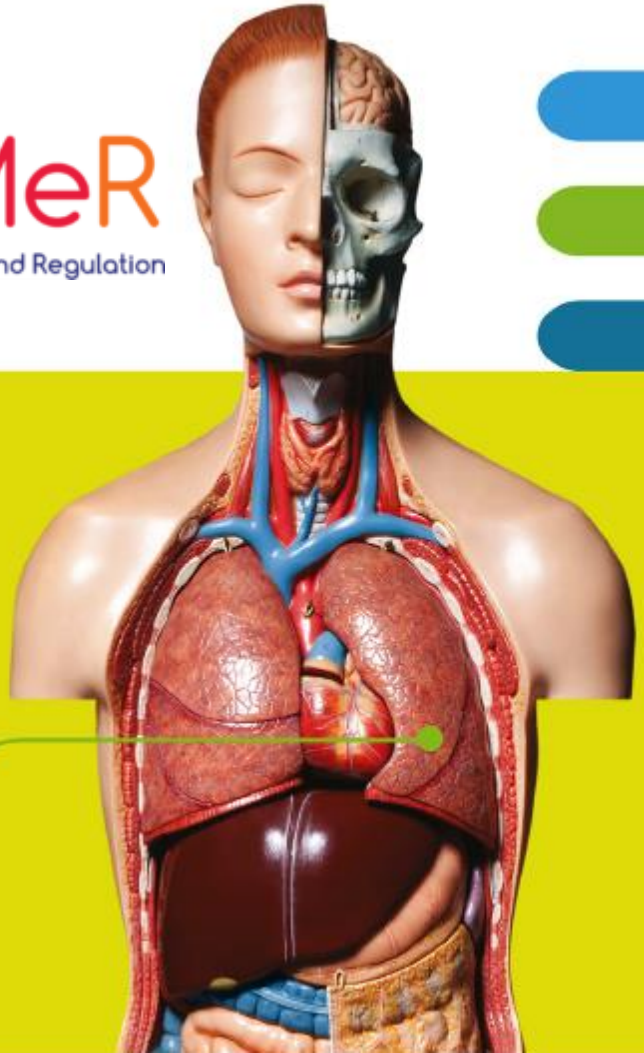
PoLiMeR
Polymers in the Liver - Metabolism and Regulation

Human Tissue Models for Better Therapies

Dorota Kurek

PoLiMeR lecture

09-09-2012



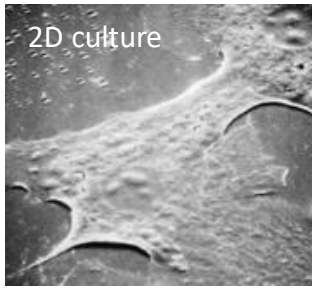
Lecture overview

- In vitro tissue culture
- Limitations of current in vitro models
- Introduction to the 3D organ-on-a-chip
- Building 3D tissues:
  - Modeling gut in vitro
  - Adding complexity

A leap forward in physiological relevance

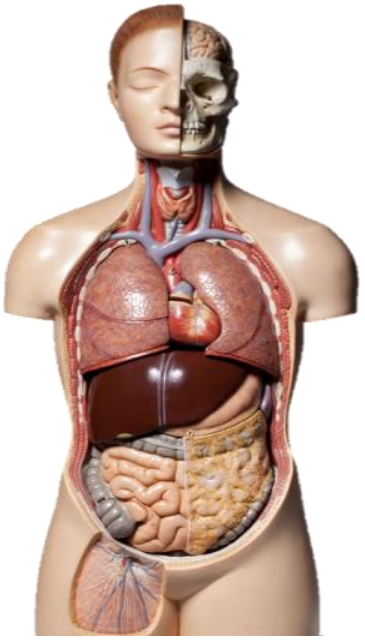
The challenge of reductionism:

"Make things as simple as possible, but not simpler", Albert Einstein

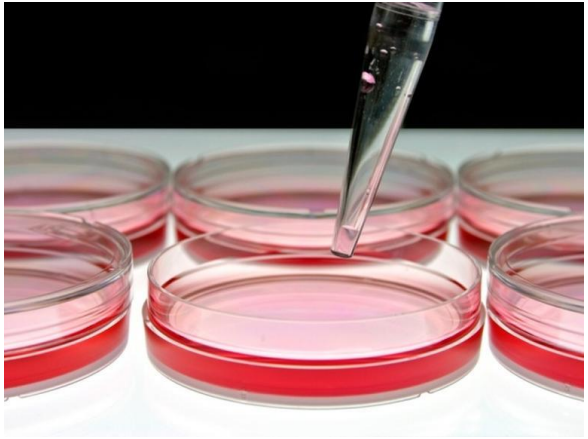


Animal testing

Human Organotypic Models



Cell culture last decades



3D culture techniques

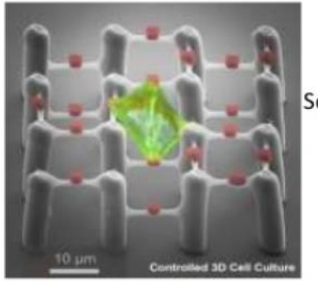
a) Scaffold Based

- Polymeric Hard Scaffolds
- Biologic Scaffolds
- Micropatterned Surface Microplates

b) Non Scaffold Based

- Hanging Drop Microplates
- Spheroid Microplates containing Ultra-Low Attachment (ULA) coating
- Microfluidic 3D cell culture

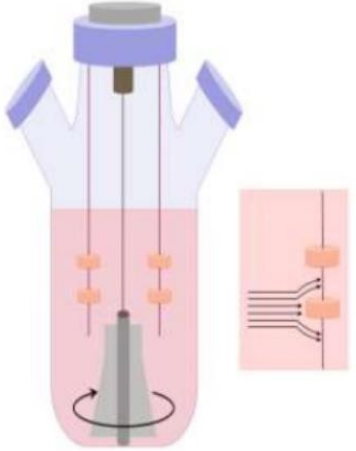
c) Bioreactors

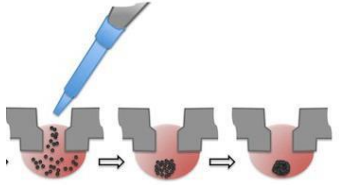


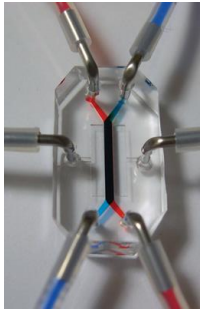
Scaffold Based


Non Scaffold Based

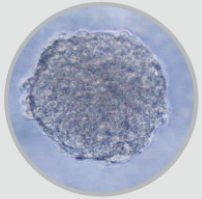

3D Cell Culture Gels


3D Cell culture Bioreactor





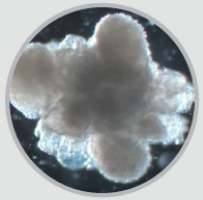
3D Microtissues – Terminology and Definitions



Spheroid: HepG2

Spheroid

A spheroid is a 3D cellular aggregate composed of one or more cell types that grow and proliferate, and may exhibit enhanced physiological responses but do not undergo differentiation or self-organization. They are typically derived from primary tissues or immortalized cell lines.



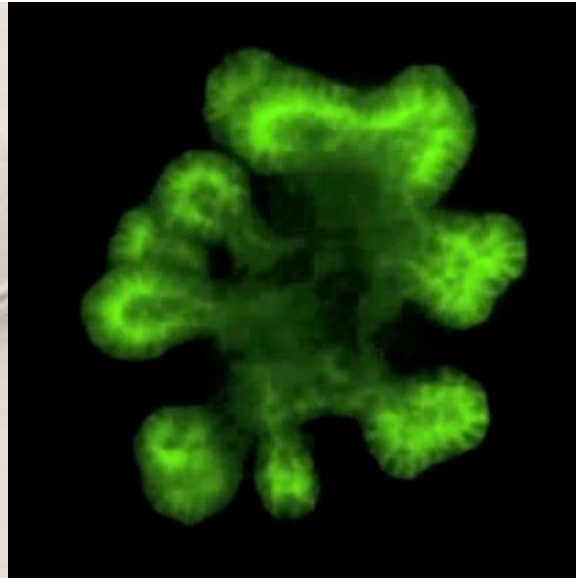
PSC-derived organoid

Organoid

"An 'organoid' is a 3D structure derived from either pluripotent stem cells (PSCs), neonatal tissue stem cells, or adult stem and progenitor cells, in which cells spontaneously self-organize into properly differentiated functional cell types and progenitors, and which resemble their *in vivo* counterpart and recapitulate at least some function of the organ."

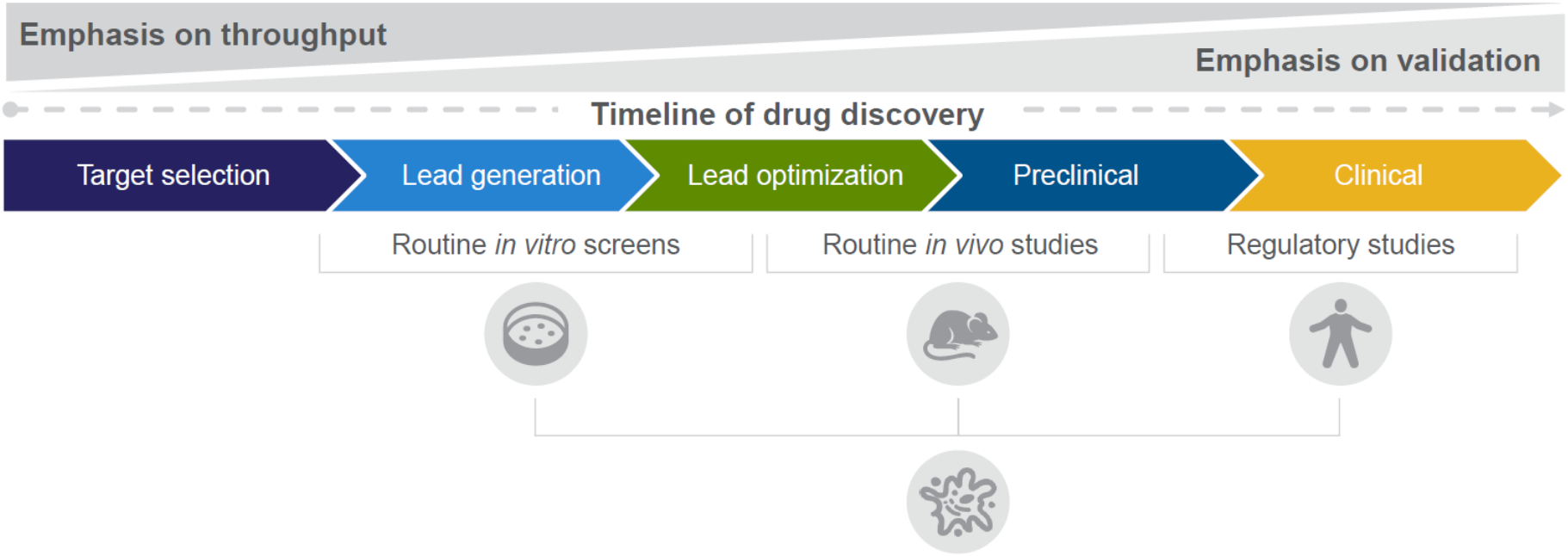
Huch M, Koo BY (2015) Modeling mouse and human development using organoid cultures. *Development.*

3D cell culture



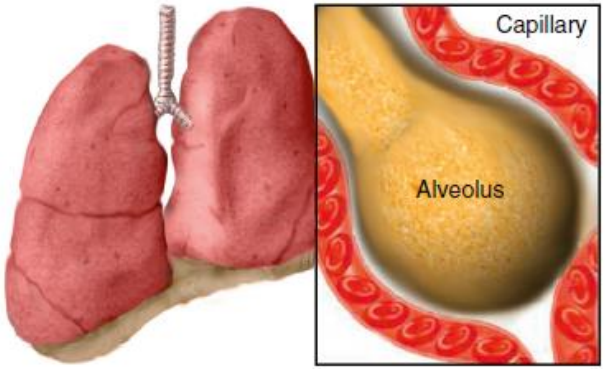
Why do we need better 3D models?

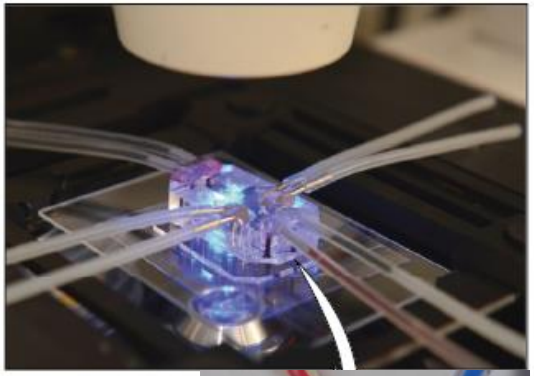
Maximizing the Drug Discovery Pipeline With 3D Models

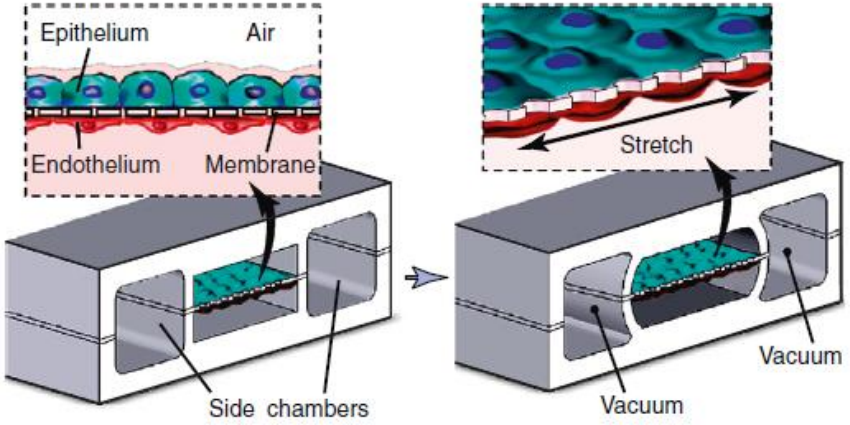


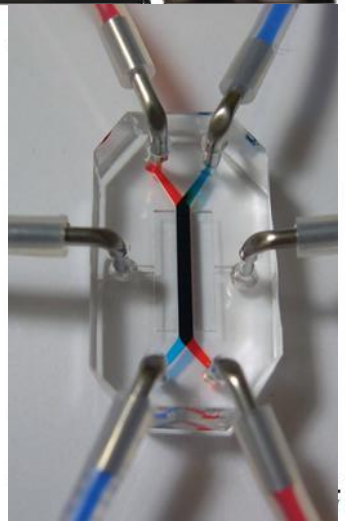
- Bring biological relevance upstream in the process
- Improve *in vitro* predictivity/problem-solving capability
- Use 3D cultures to confirm 2D culture results
- Recapitulate human physiology more closely
- Reduce number of *in vivo* investigative studies

Organ-on-a-Chip



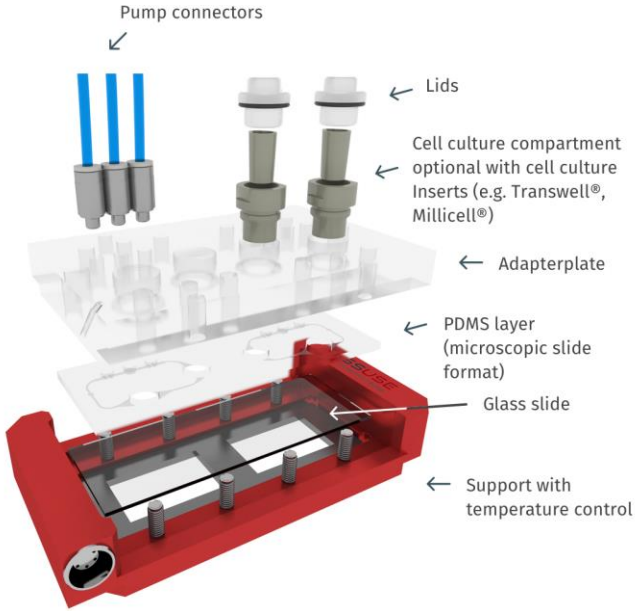


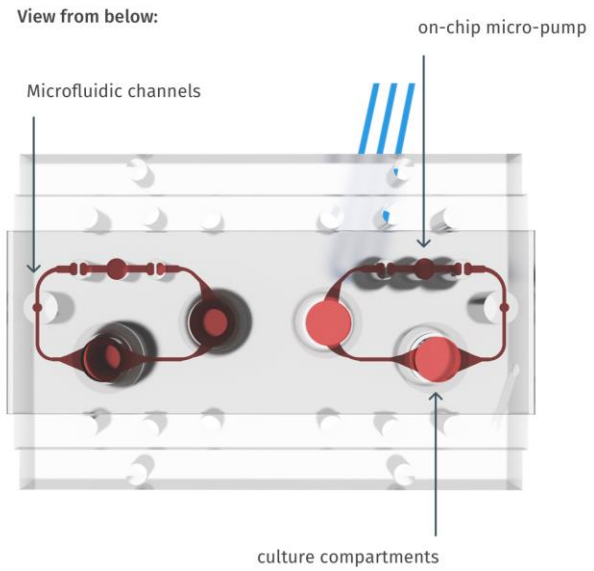




Dan Huh et al. Science 2010

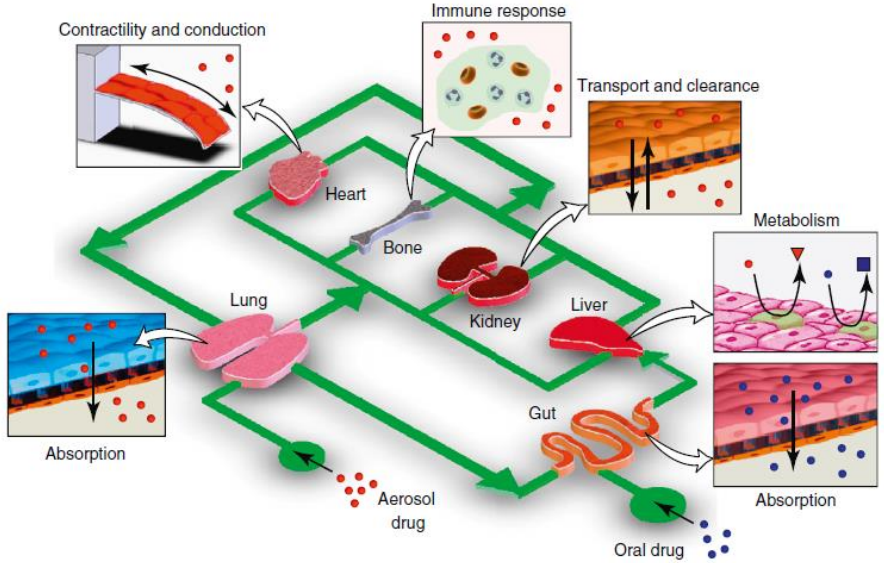
Multi-organ Chip

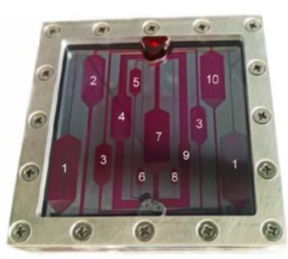


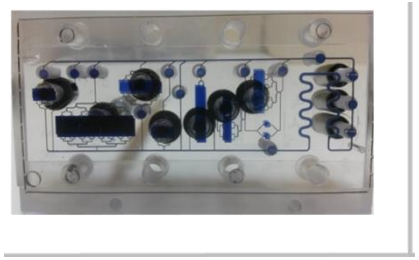


www.tissuse.com

Towards a Human-on-a-Chip?

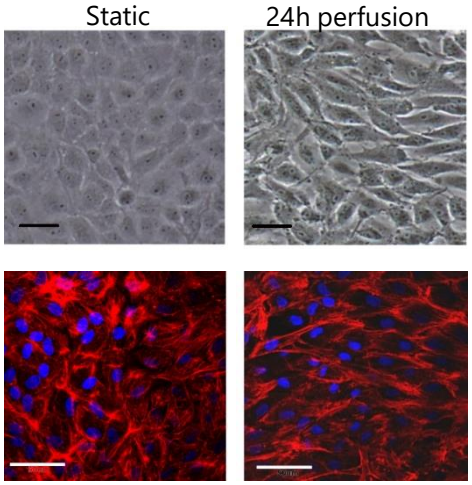




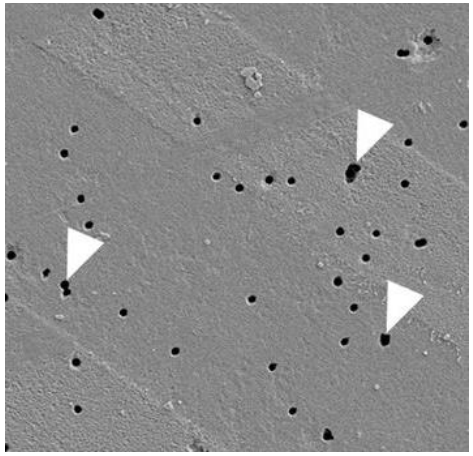


What are the limitations of the current models?

Flow



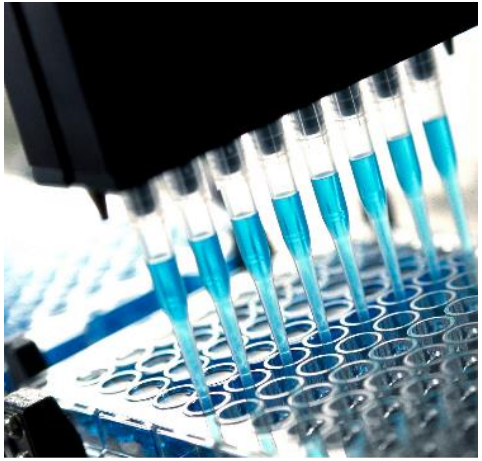
Membranes



Imaging

What are the limitations of the current models?

Throughput

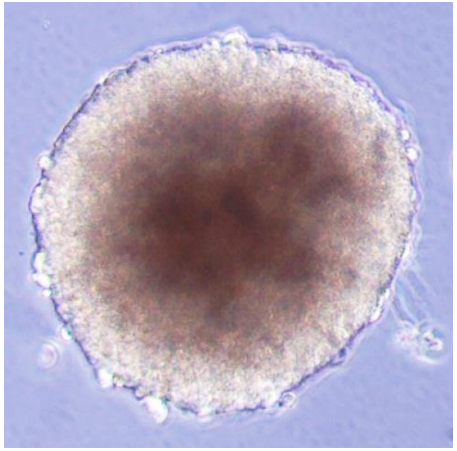


Reagent consumption

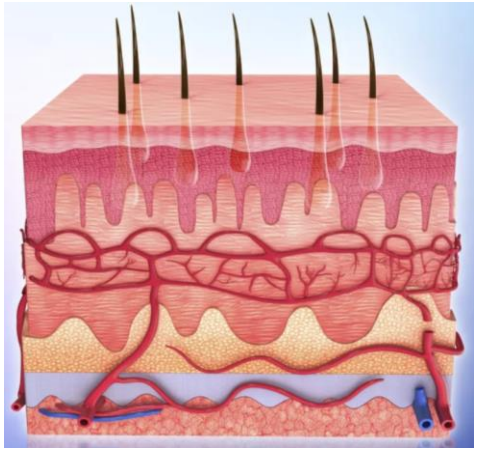
Compatibility

What are the limitations of the current models?

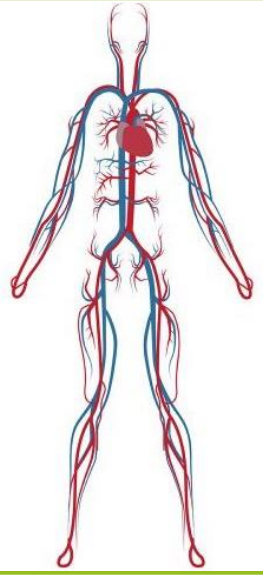
Diffusion limits



Tissue complexity



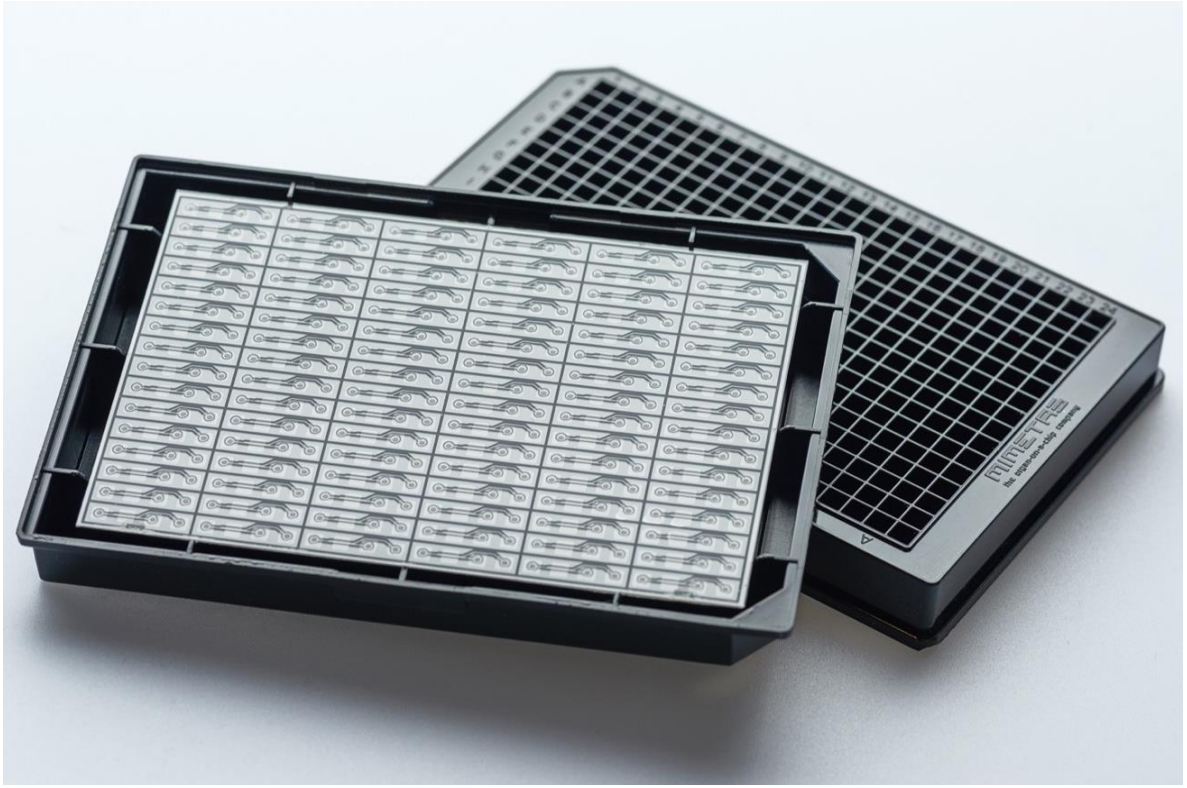
Lack of vessels



Model development: relevant parameters

- Culture vessel type
- Readout assays
- Tissue selection
- Tissue complexity
- Cell source
- Extracellular matrix
- Seeding density
- Medium composition
- Seeding orientation
- Flow settings

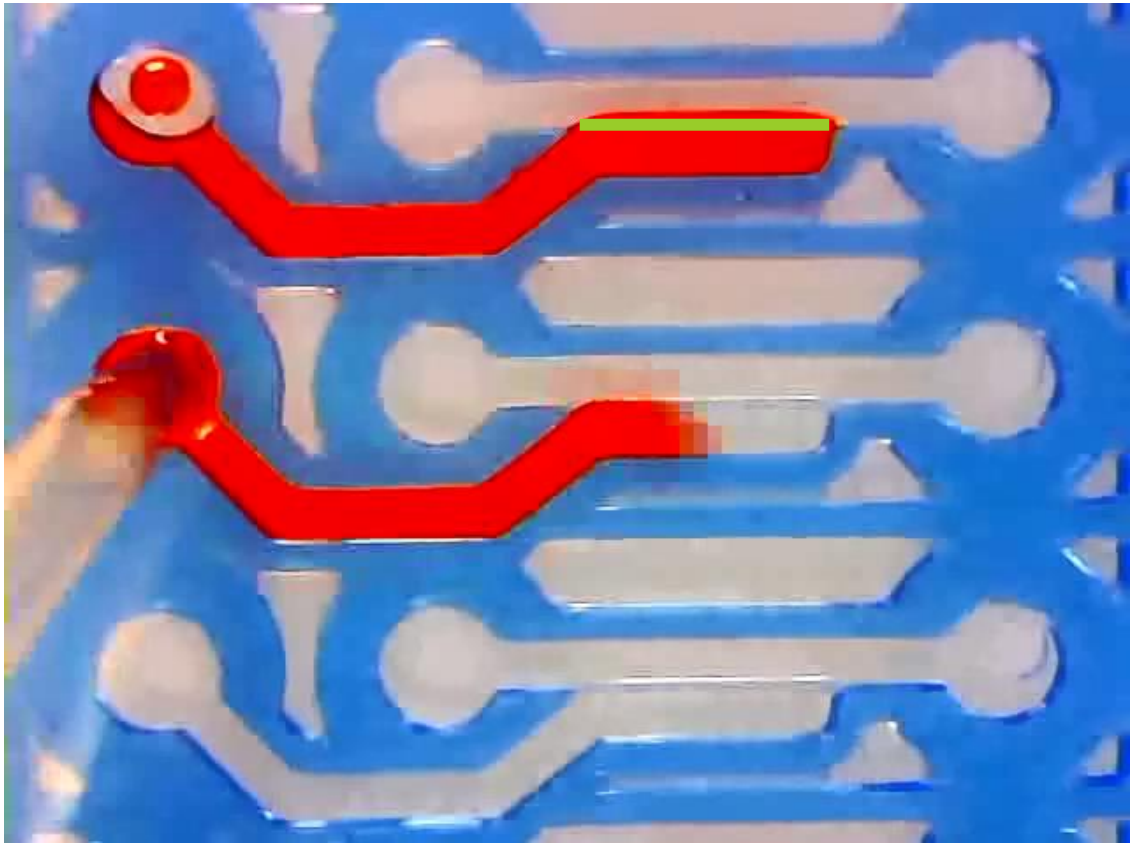
Microfluidics in OrganoPlate®



- 3-Dimensional culture
- Flow
- Multiple cell types
- Fully compatible

PhaseGuide™ Technology

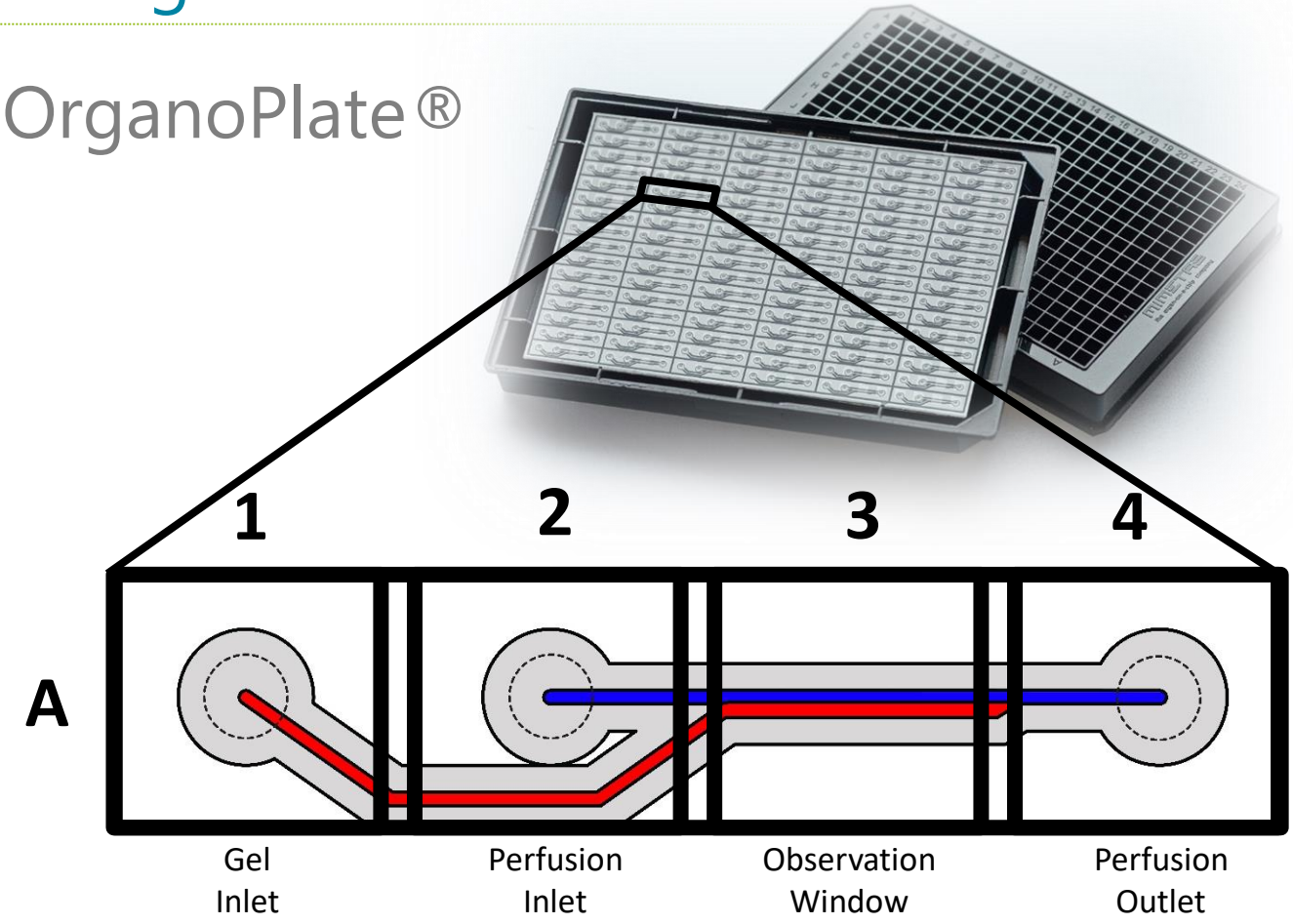
PhaseGuide™



Vulto et al 2011 Phaseguides: a paradigm shift in microfluidic priming and emptying. *Lab Chip* 11(9) 1596-1602

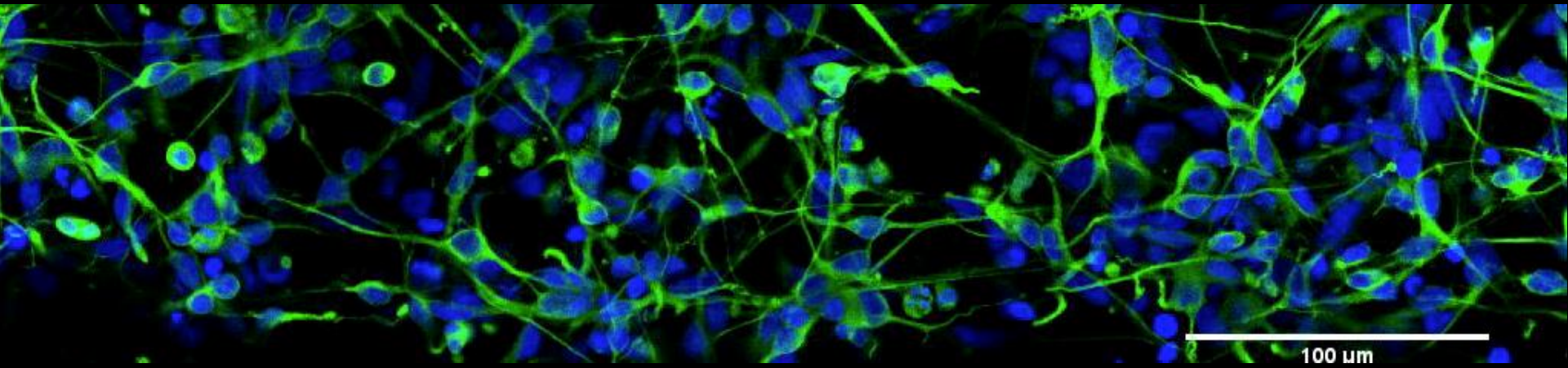
The 2-lane OrganoPlate®

- 96x chips per OrganoPlate®
- Single chip

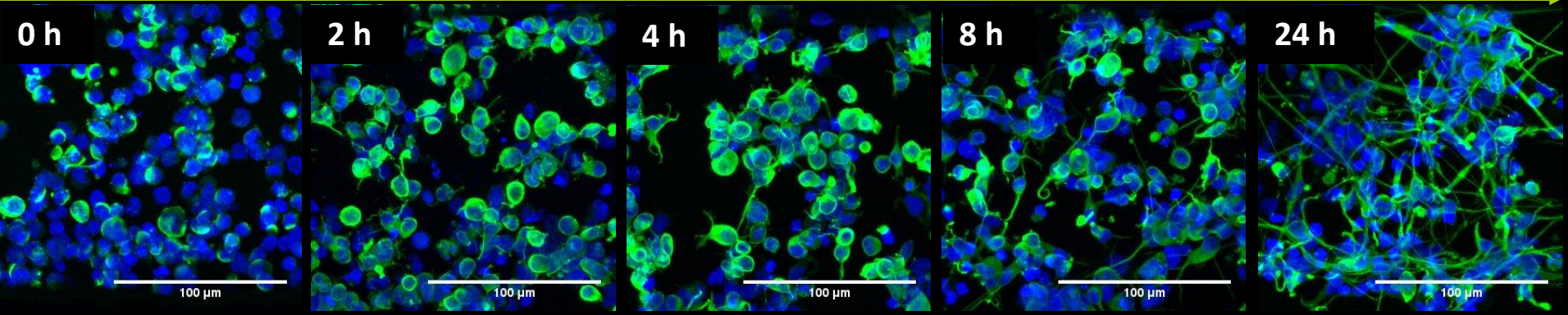


https://www.youtube.com/watch?v=BhFETKQqJY0

iPS-derived 3D neuronal networks

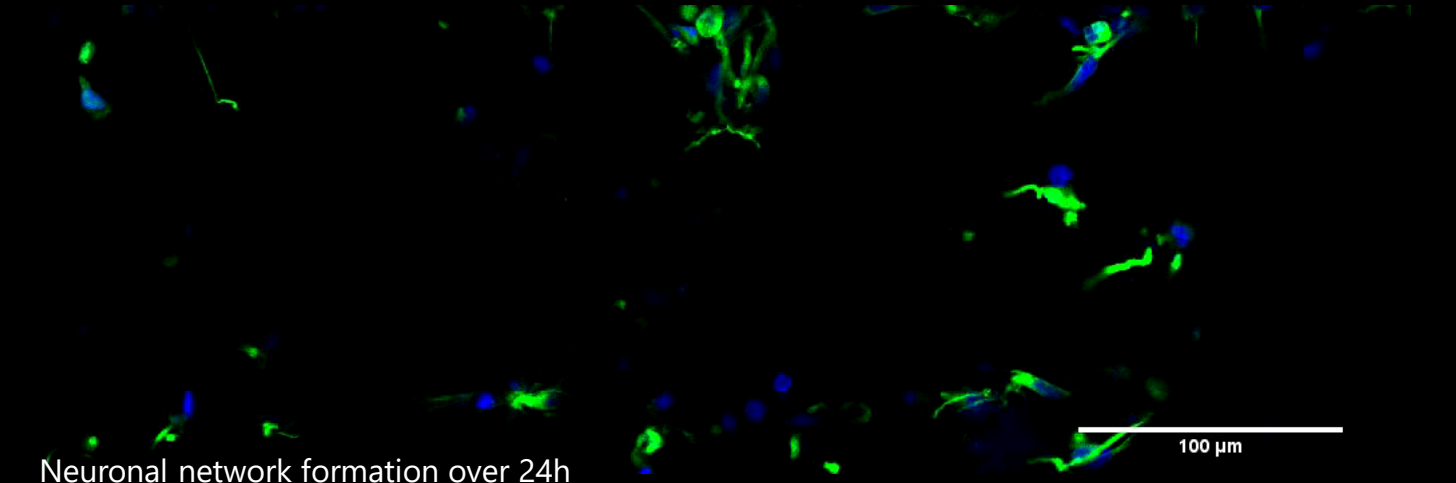


Rapid network formation directly after seeding in OrganoPlate®

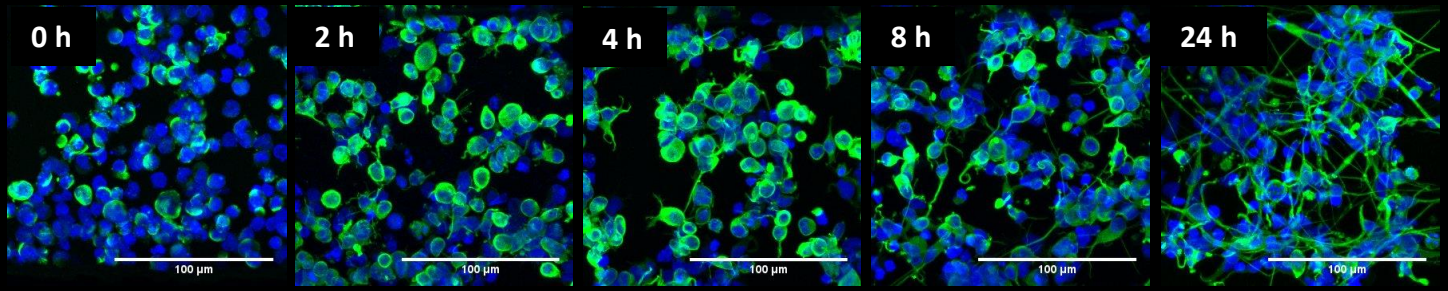


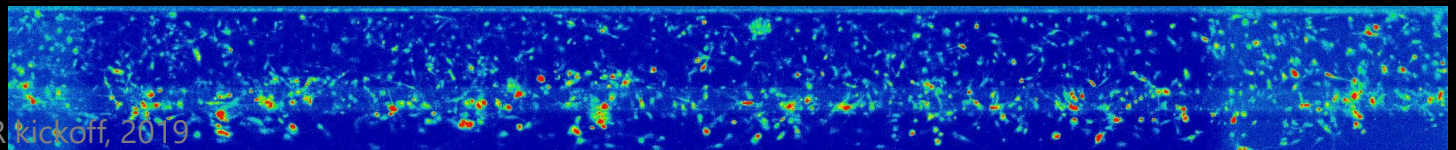
Wevers, N. R. *et al. Sci. Rep.* **6,** 38856 (2016).

iPSC-derived 3D neuronal networks

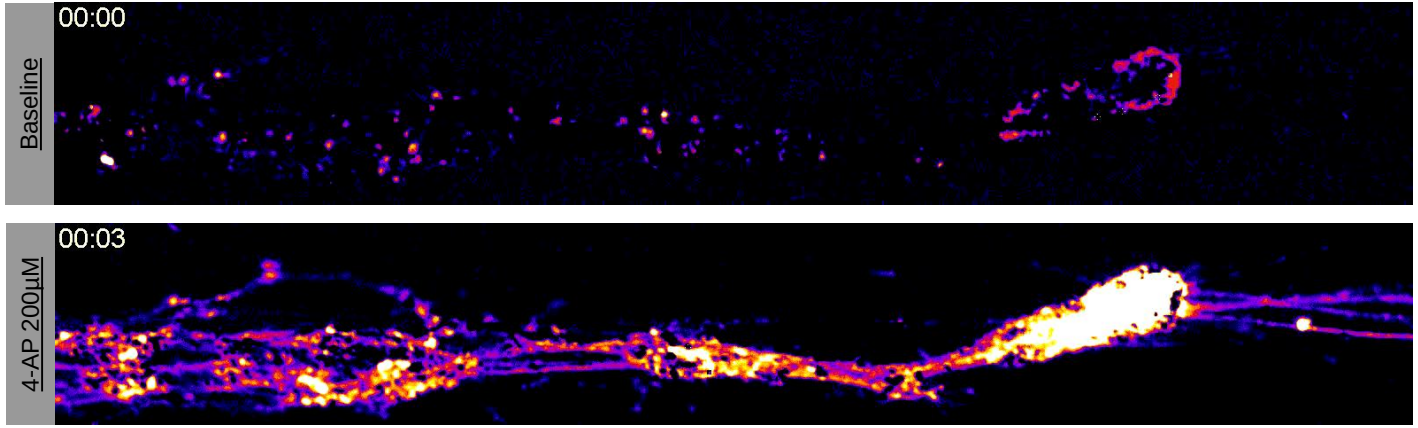


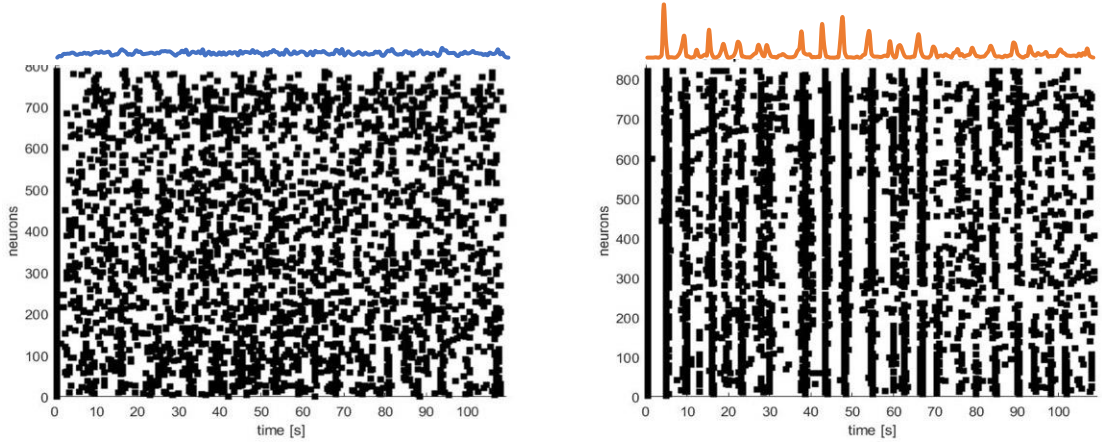
Neuronal network formation over 24h





Inducing seizure

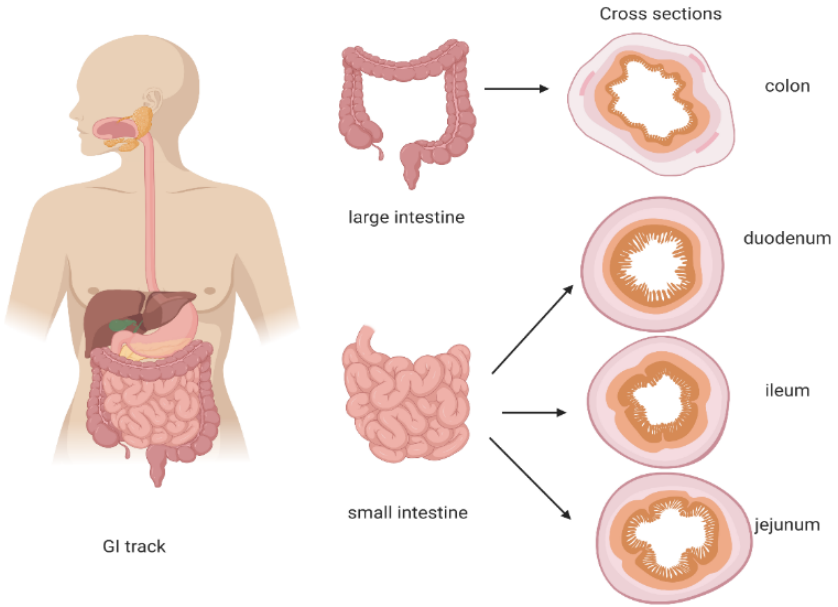




- Synchronicity observed in 4-AP exposed cultures (day 19)

How to set up 3D organotypic culture models?

Model development - In vitro Gut

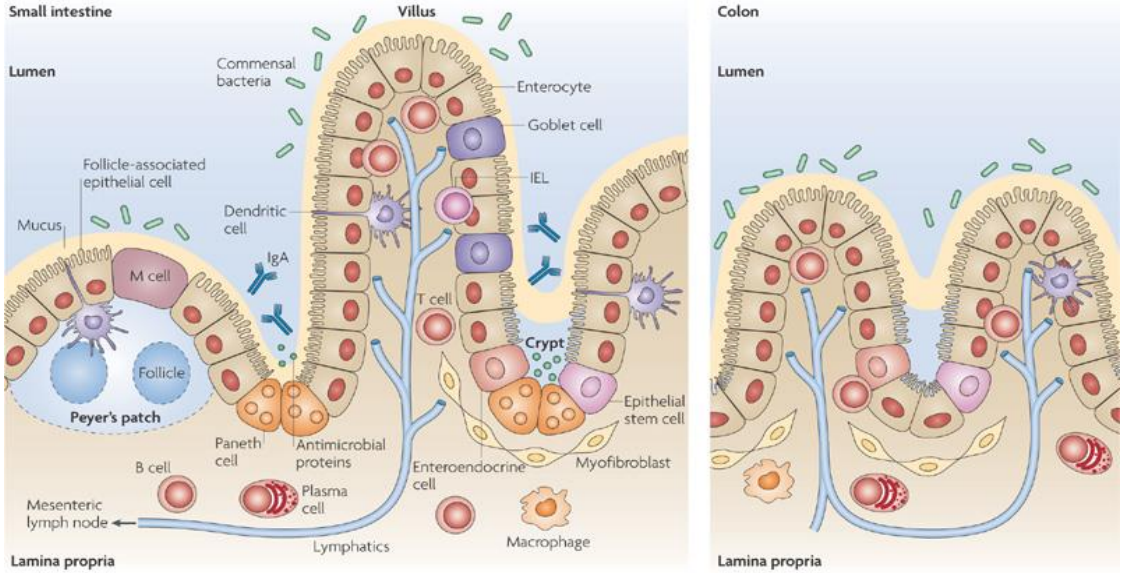


- The gut is part of the gastrointestinal tract
  - Large intestine (cecum, colon, rectum, and anus)
  - Small intestine (duodenum, ileum, and jejunum)
- Important for absorption of orally delivered drugs
- Dysfunction involved in many different diseases
  - Inflammatory Bowel Disease (IBD)
  - Acute and chronic enteritis/ colitis
  - Peptic ulcers (affecting the duodenum)
  - Coeliac disease
  - Whipple's disease
  - Cancer
  - &&&

Model development: required organ complexity

"Make things as simple as possible, but not simpler", Albert Einstein

- Depends on your research question
  - E.g. Colon barrier integrity upon toxicant stimuli
  - Or Disease modelling: Inflammatory Bowel Disease (IBD)

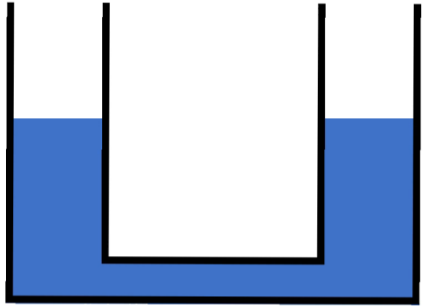


Nature Reviews | Immunology

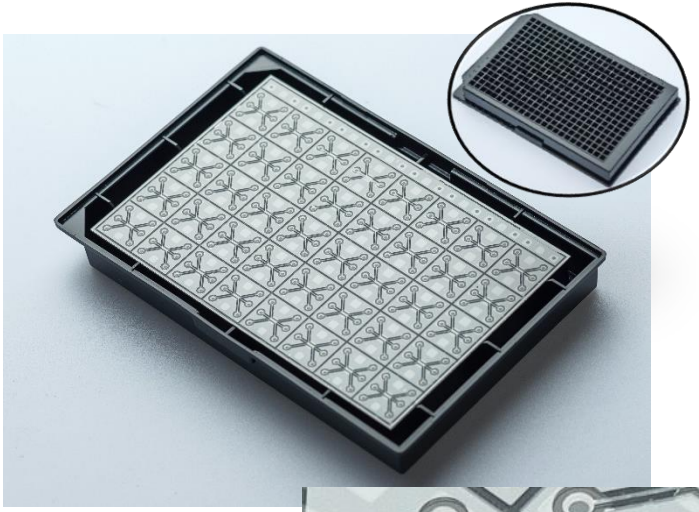
Abreu et al 2010 Nat Rev Immu

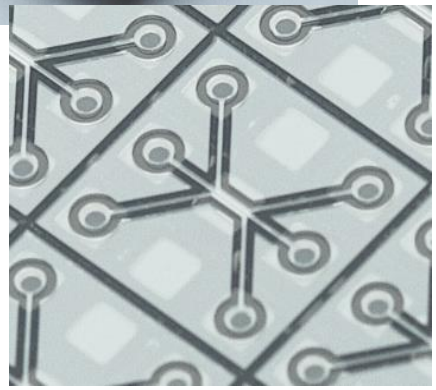
Continuous passive perfusion (bidirectional)

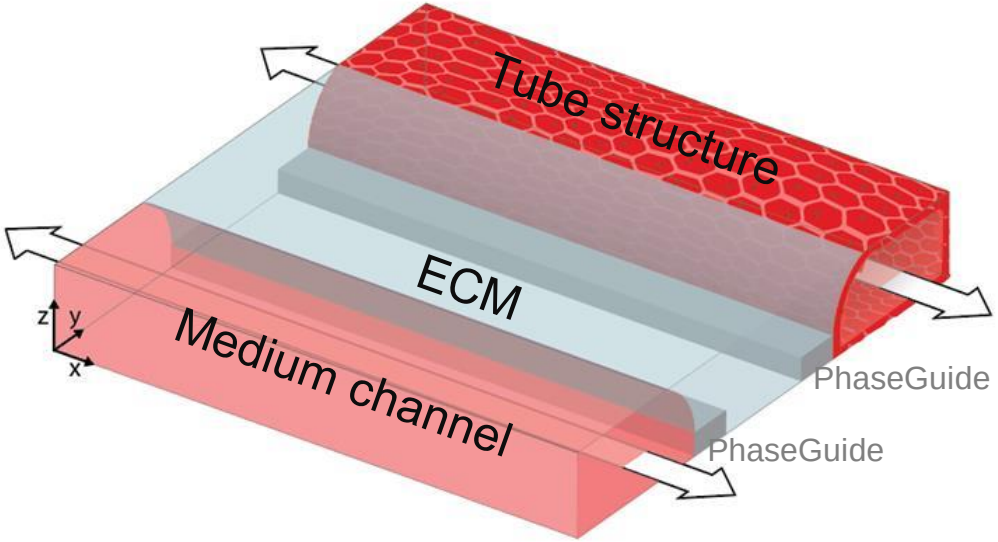
- OrganoPlates® are placed on top of the rocker platform inside the incubator



Growing Tubules in the OrganoPlate® 3-lane

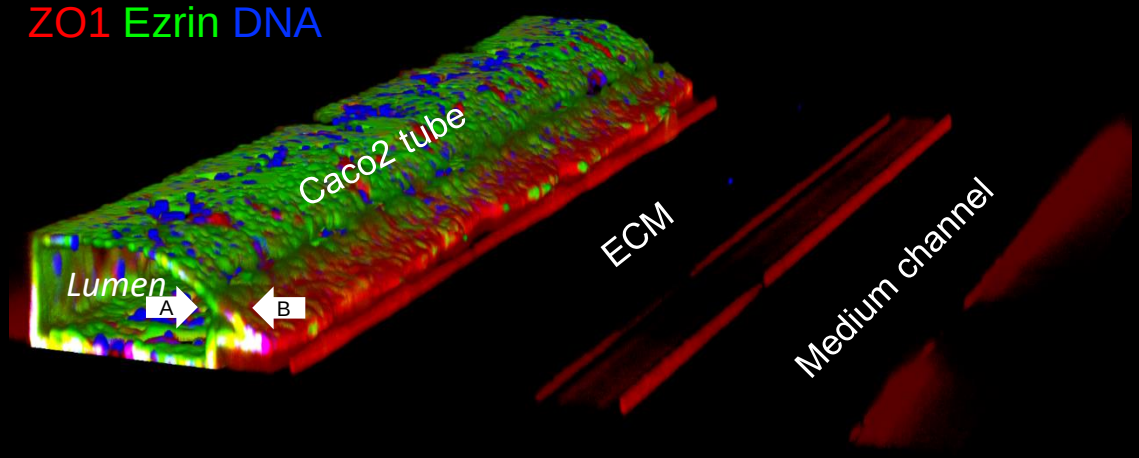


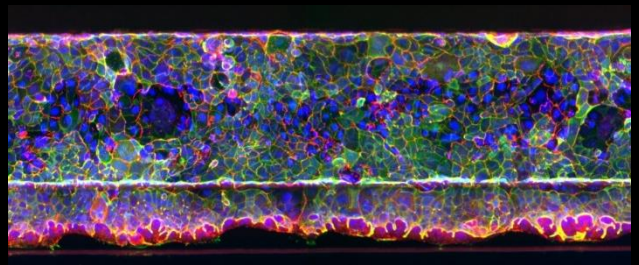


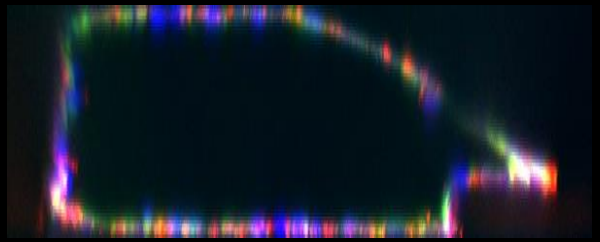


Growing Caco-2 gut tubules

- **Caco-2 intestinal model** forms tubular structures with accessible apical and basal sides, expressing various drugs transporters





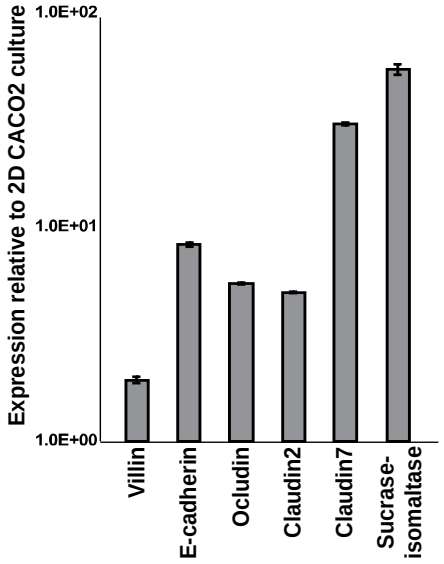


Trietsch et al., *Nature Comm., 2017*

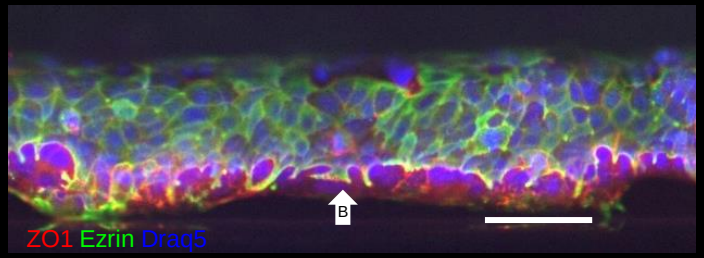
nature COMMUNICATIONS

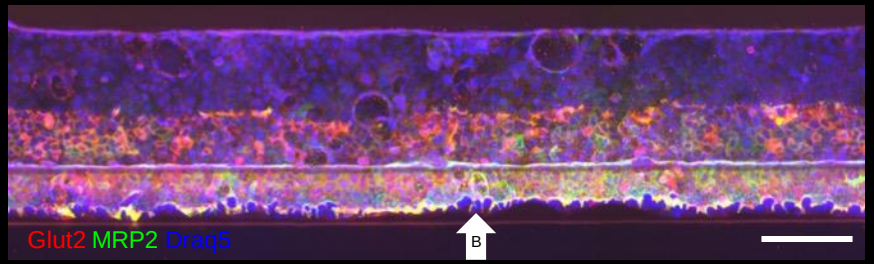
Gut - 2D vs 3D gene expression

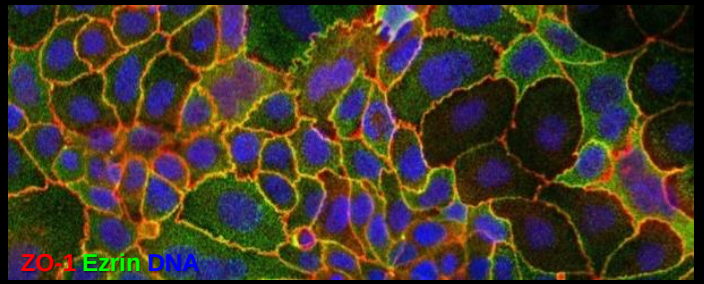
- Caco-2 cells cultured in the OrganoPlate® show upregulation of a myriad of gut markers compared to Caco-2 cells cultured in 2D

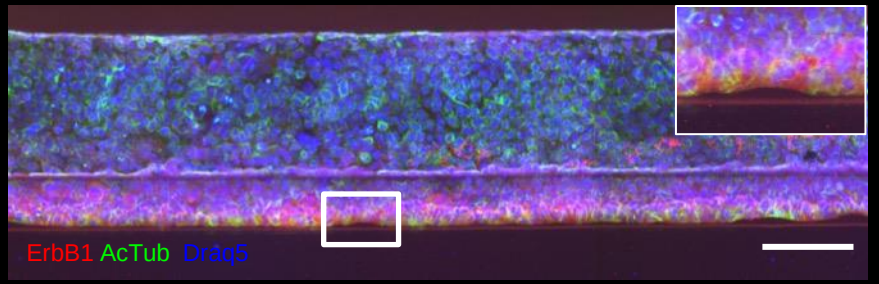


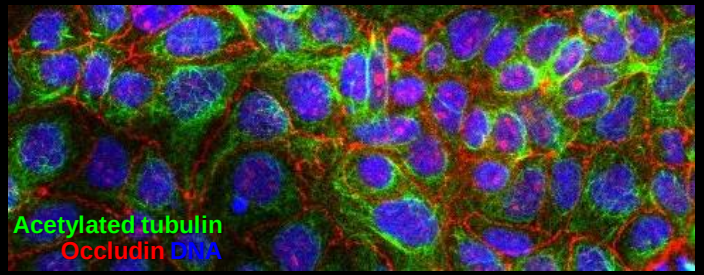
Caco-2 gut tubes in OrganoPlates®

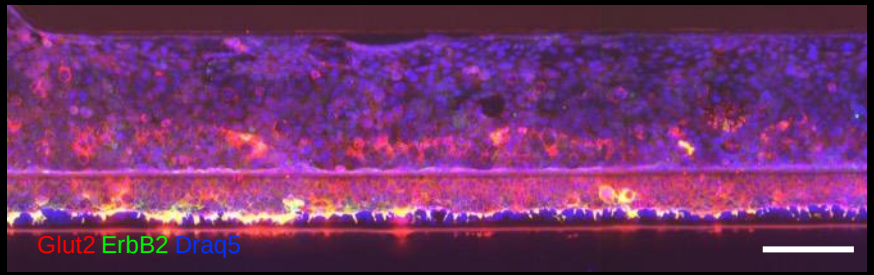






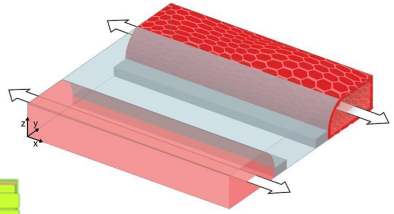


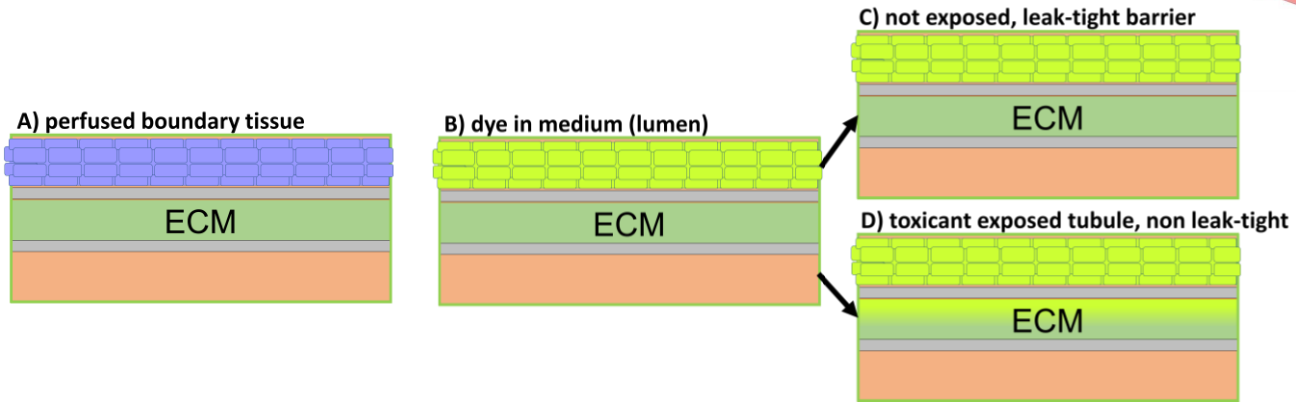


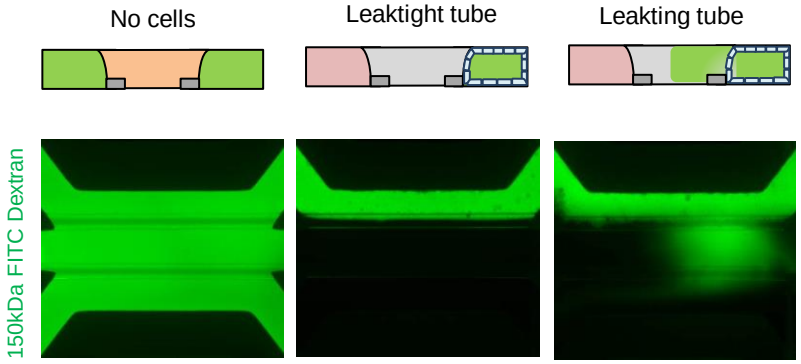


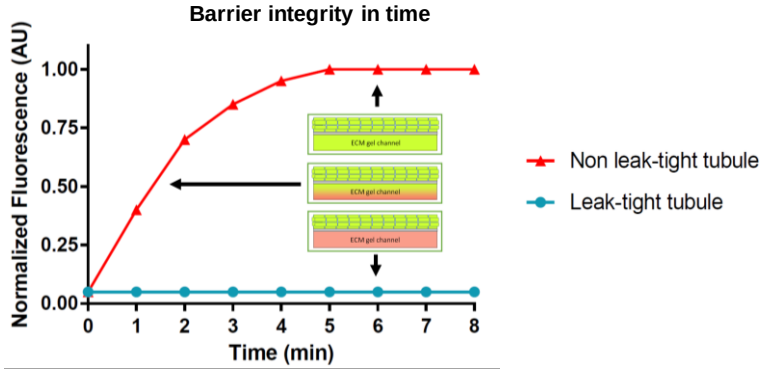
Trietsch et al., *Nature Comm., 2017*

Real-time barrier integrity assay



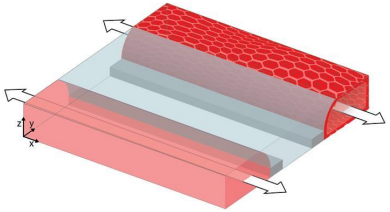


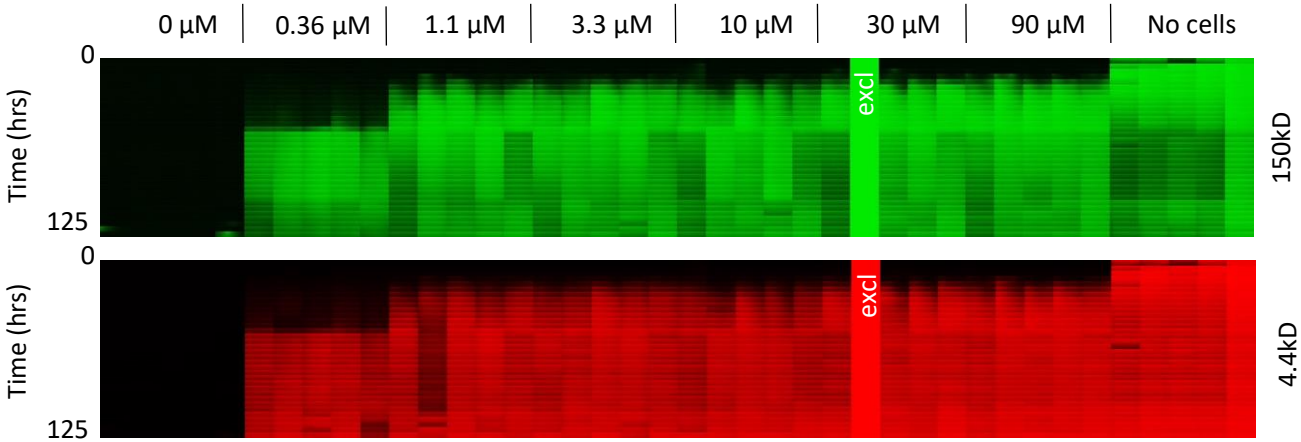


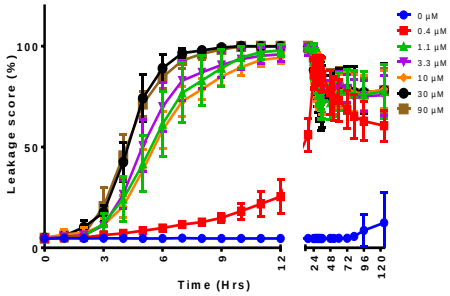


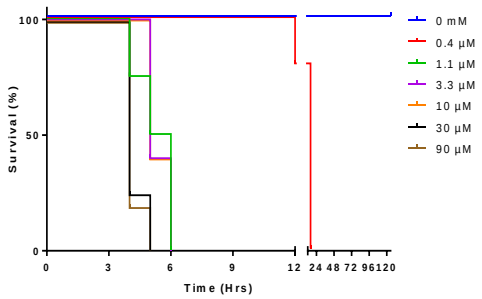
Trietsch et al., *Nature Comm., 2017*

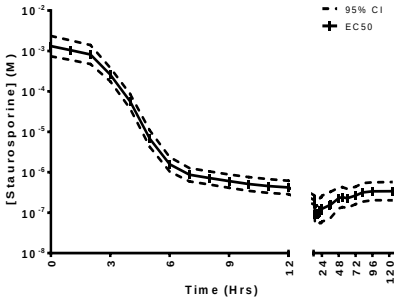
Staurosporine-induced gut toxicity





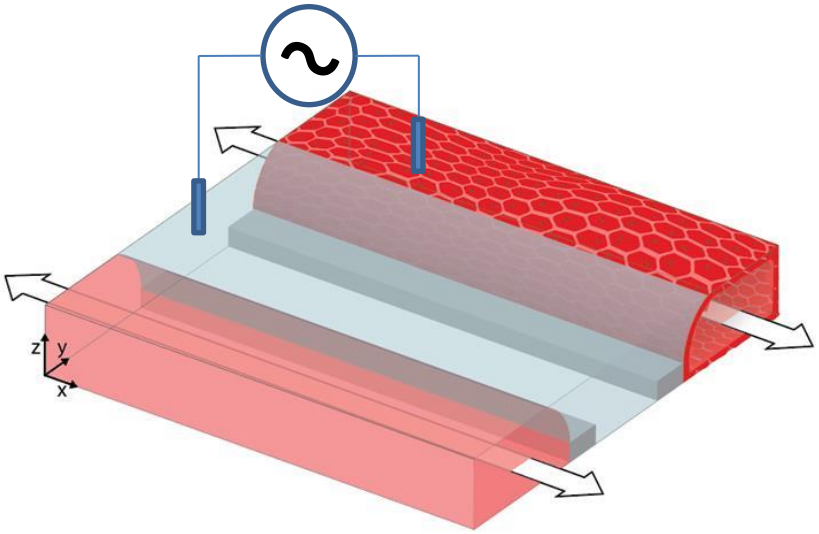


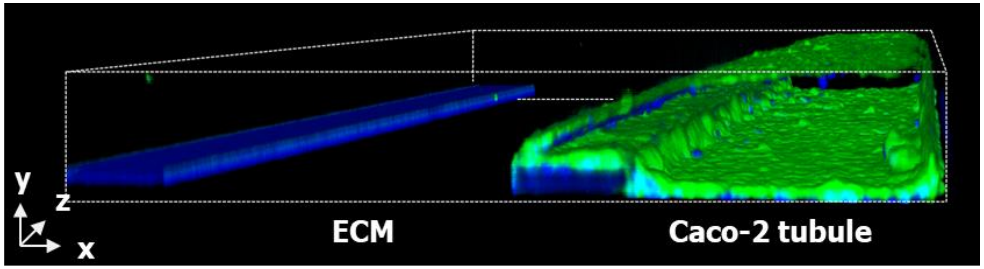




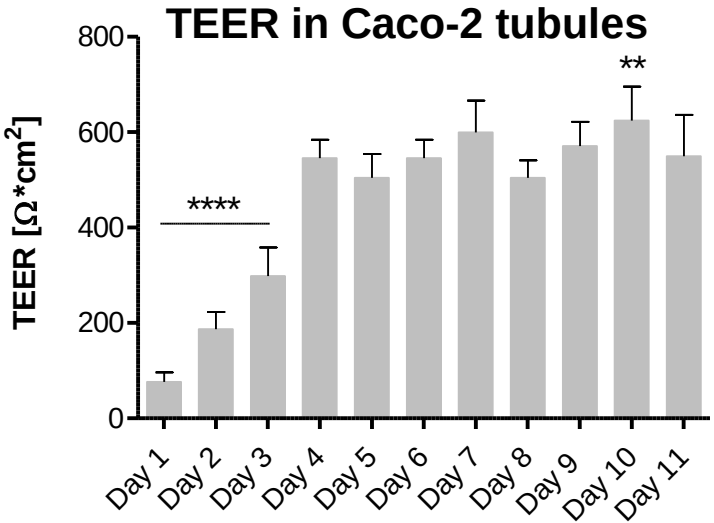
Trietsch et al., *Nature Comm., 2017*

OrganoTEER – barrier assessment





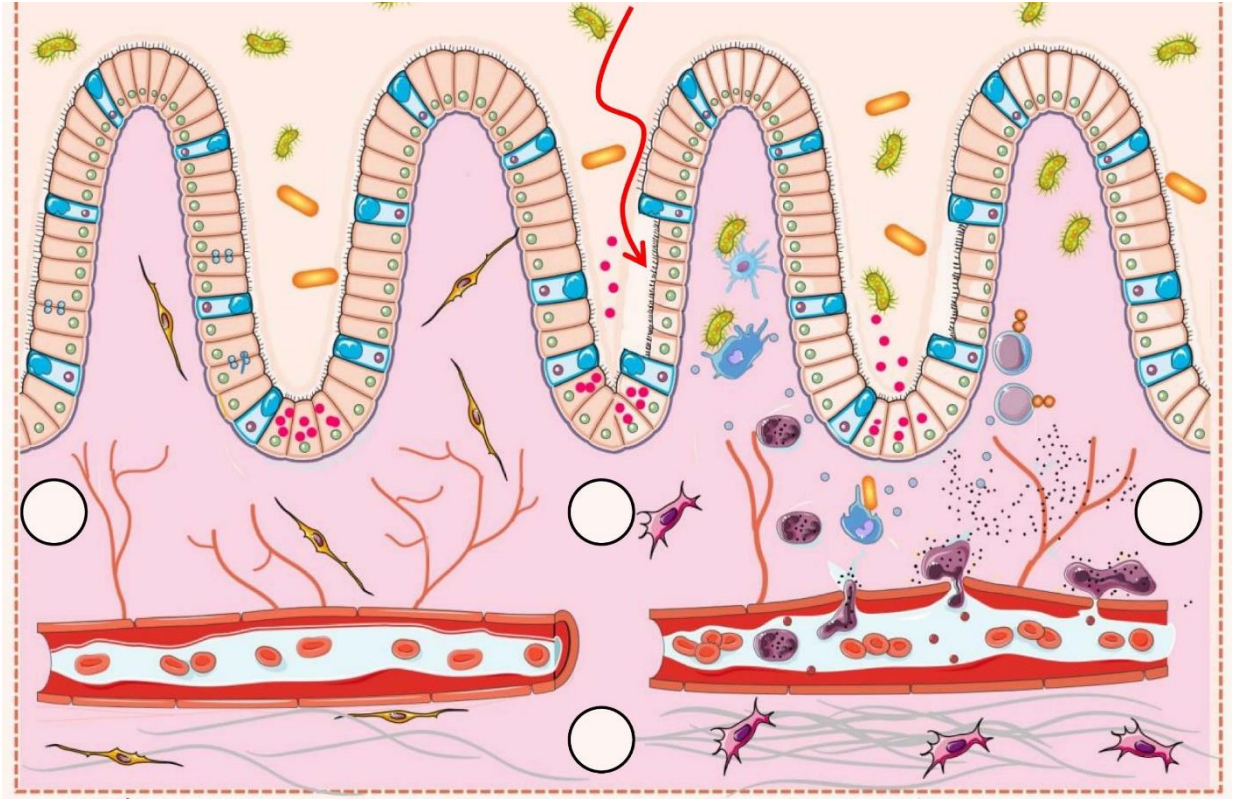
- Viability
- Toxicity
- Efficacy
- Quality control



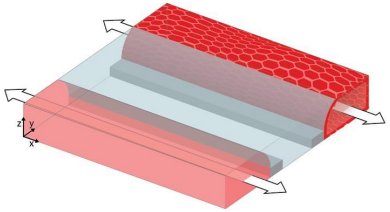
Disease modelling – gut inflammation

Inflammatory Bowel Disease (IBD)

Healthy vs diseased



Disease modelling – gut inflammation



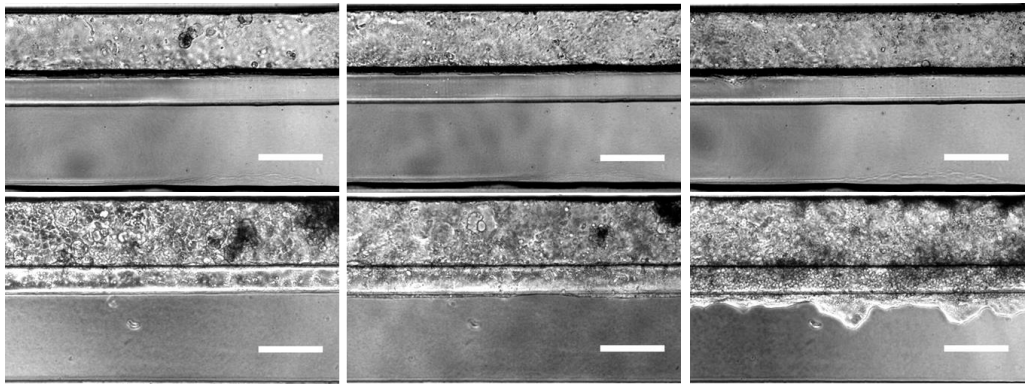
Beaurivage et al 2019 Int J Mol Sci

Galápagos

Day 4 | Day 7 | Day 11

Control gut tubules

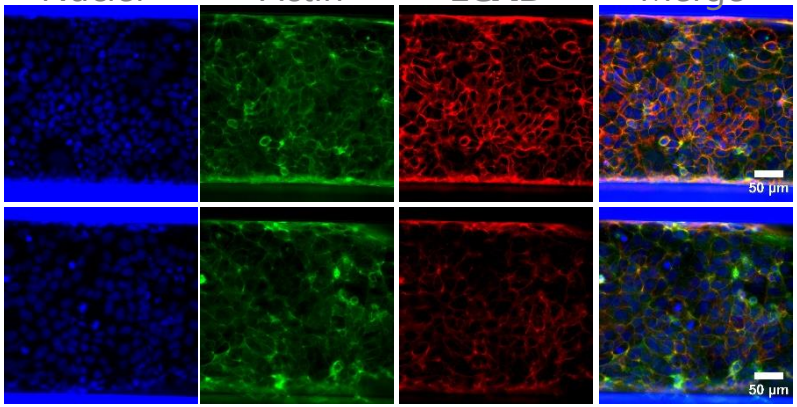
+ inflammatory trigger (cytokines)

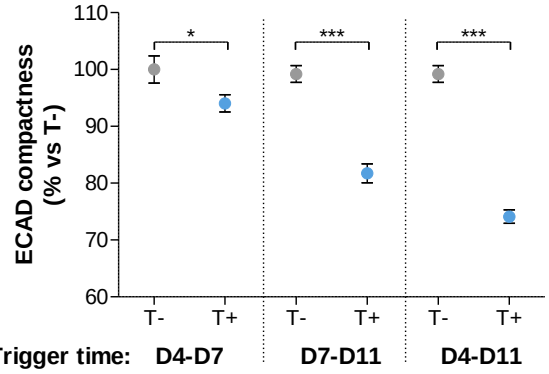


Nuclei | Actin | **ECAD** | Merge

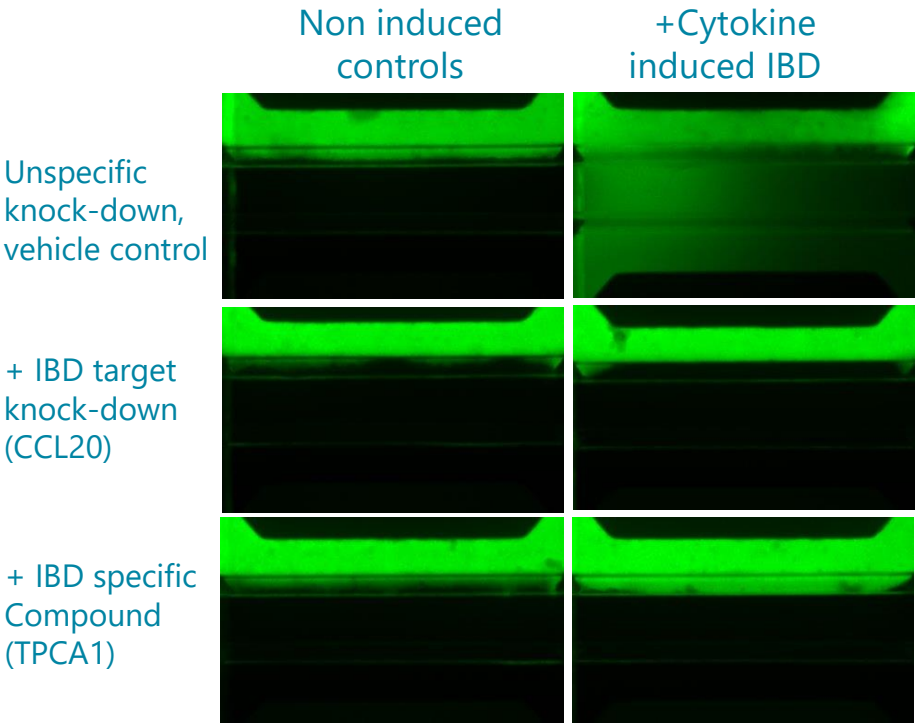
Control gut tubules

+ inflammatory trigger 72h



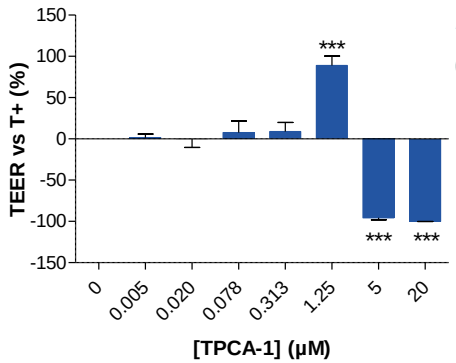


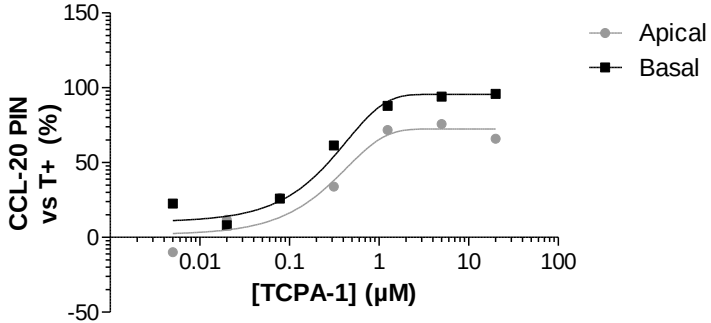
Inflamed Gut-on-a-Chip Model for Drug Discovery



Beaurivage et al 2019 Int J Mol Sci

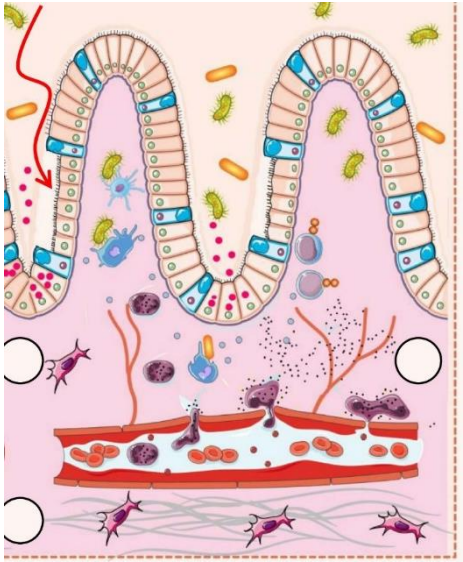
Galápagos

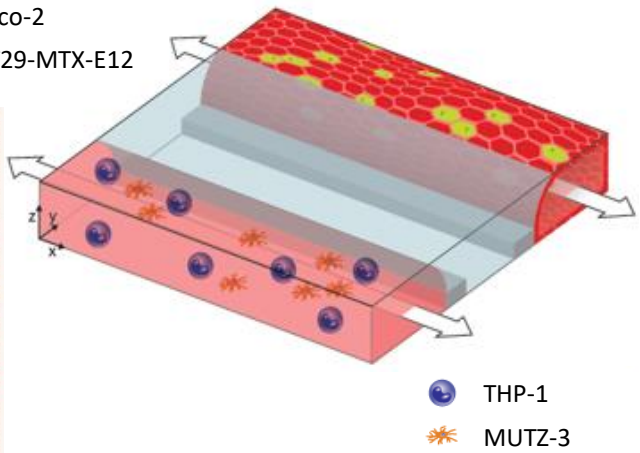


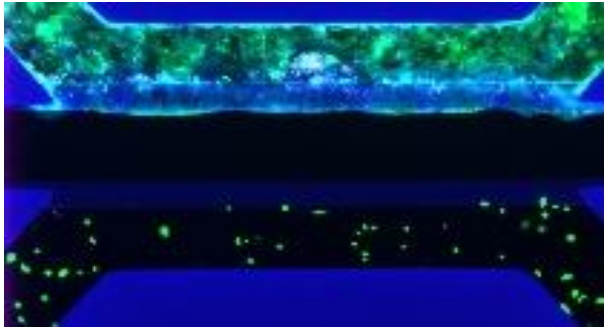


- Adenoviral Knockdown of Inflammatory Effectors Prevents IBD-like Phenotype in Caco-2
- Exposure to anti-inflammatory TPCA-1 Prevent the Inflammatory State of Caco-2 Tubules

An intestine-on-a-chip model of plug and play modularity to study inflammatory processes





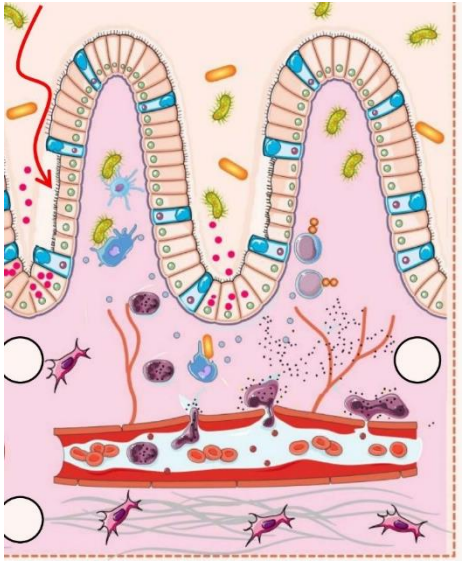


Gijzen et al 2020 SLAS Tech

Adding more complexity to gut model

Disease characteristic | Multiple cell interactions and readouts

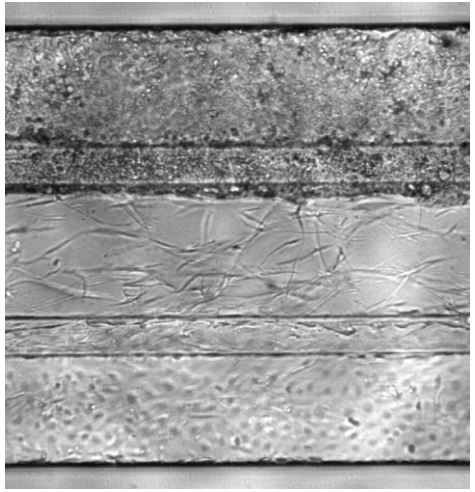
Gut model with intestinal tube, stroma and endothelial vessel containing immune components



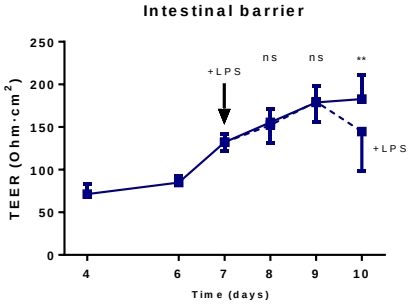
Gut tubule (enterocytes and goblet cells)

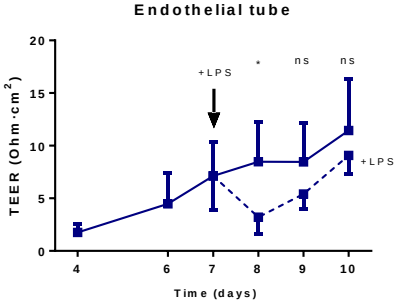
Intestinal myofibroblasts

Vasculature with immune cells (macrophages and dendritic cells)

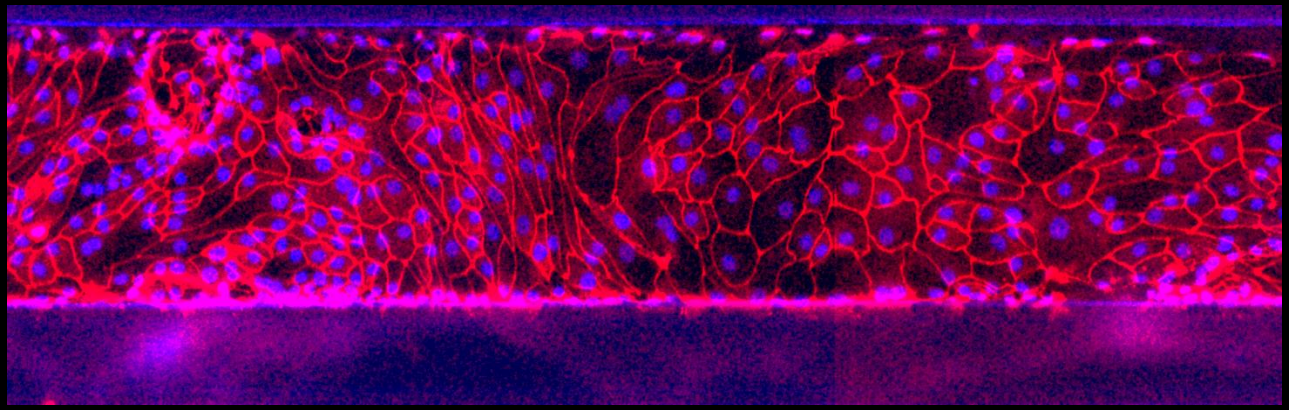


Intestinal epithelial and endothelial barrier assessment in OrganoPlate

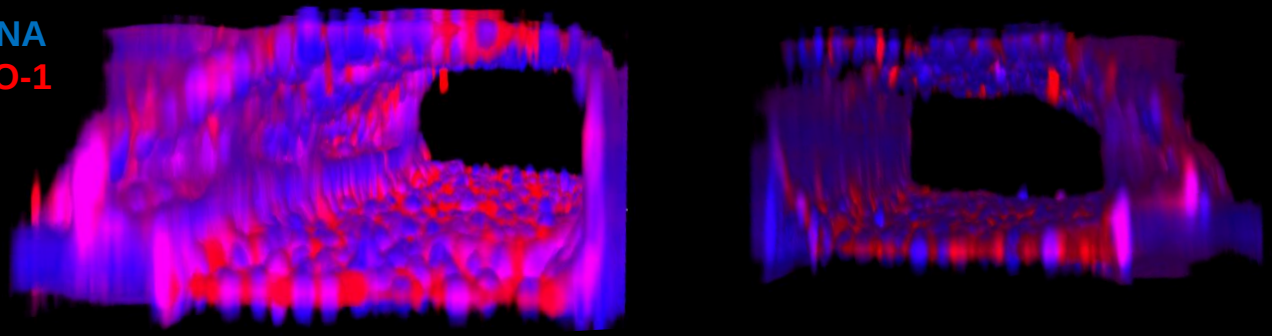




Human Colon Organoid tubules in OrganoPlate



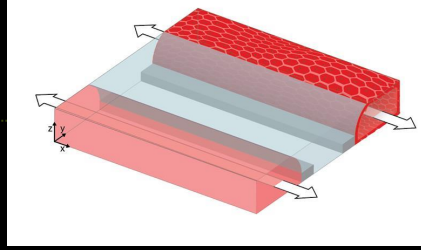
DNA
ZO-1

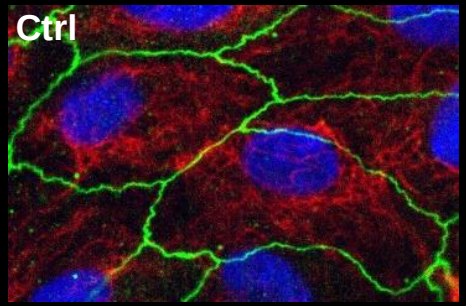


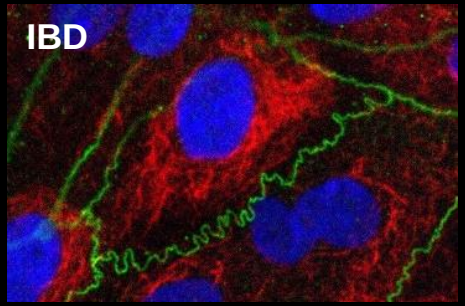
- 3D perfused hC tubule formation with apical and basal access

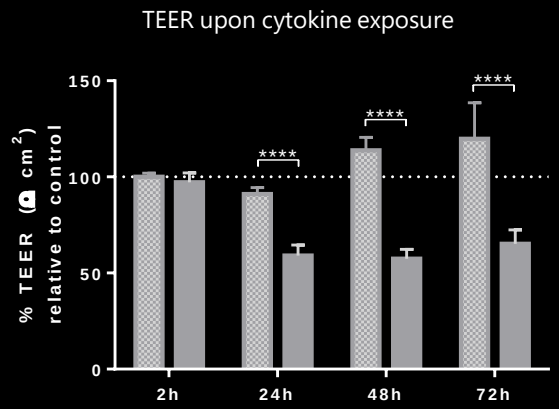
Hubrecht Institute

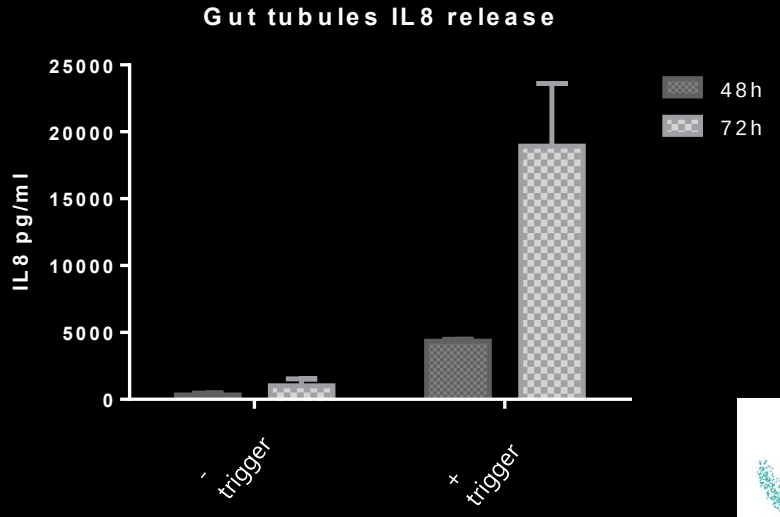
IBD modelling in colon organoid tubules





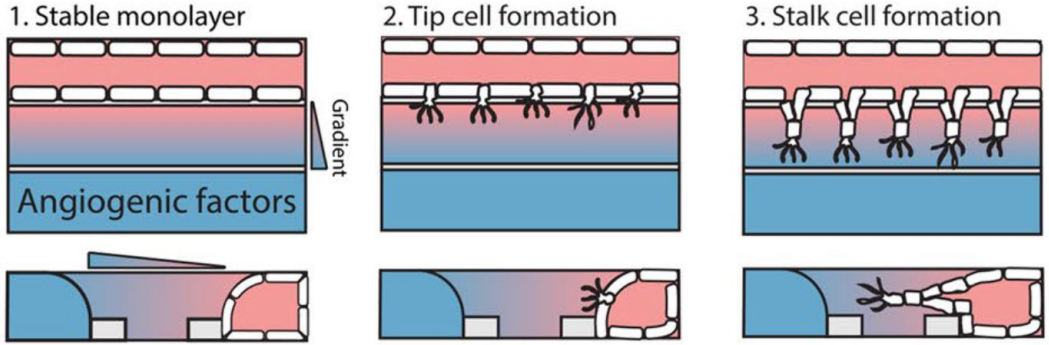




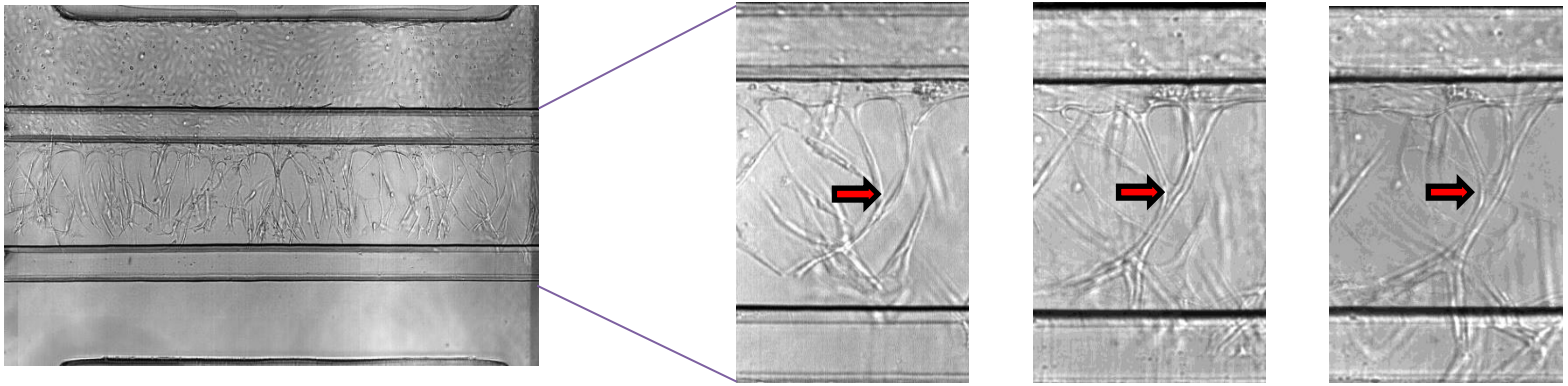


Hubrecht Institute

Blood vessel in the OrganoPlate® 3-lane



Exposure to a gradient of pro-angiogenic factors induces vascular sprouting from endothelial vessels



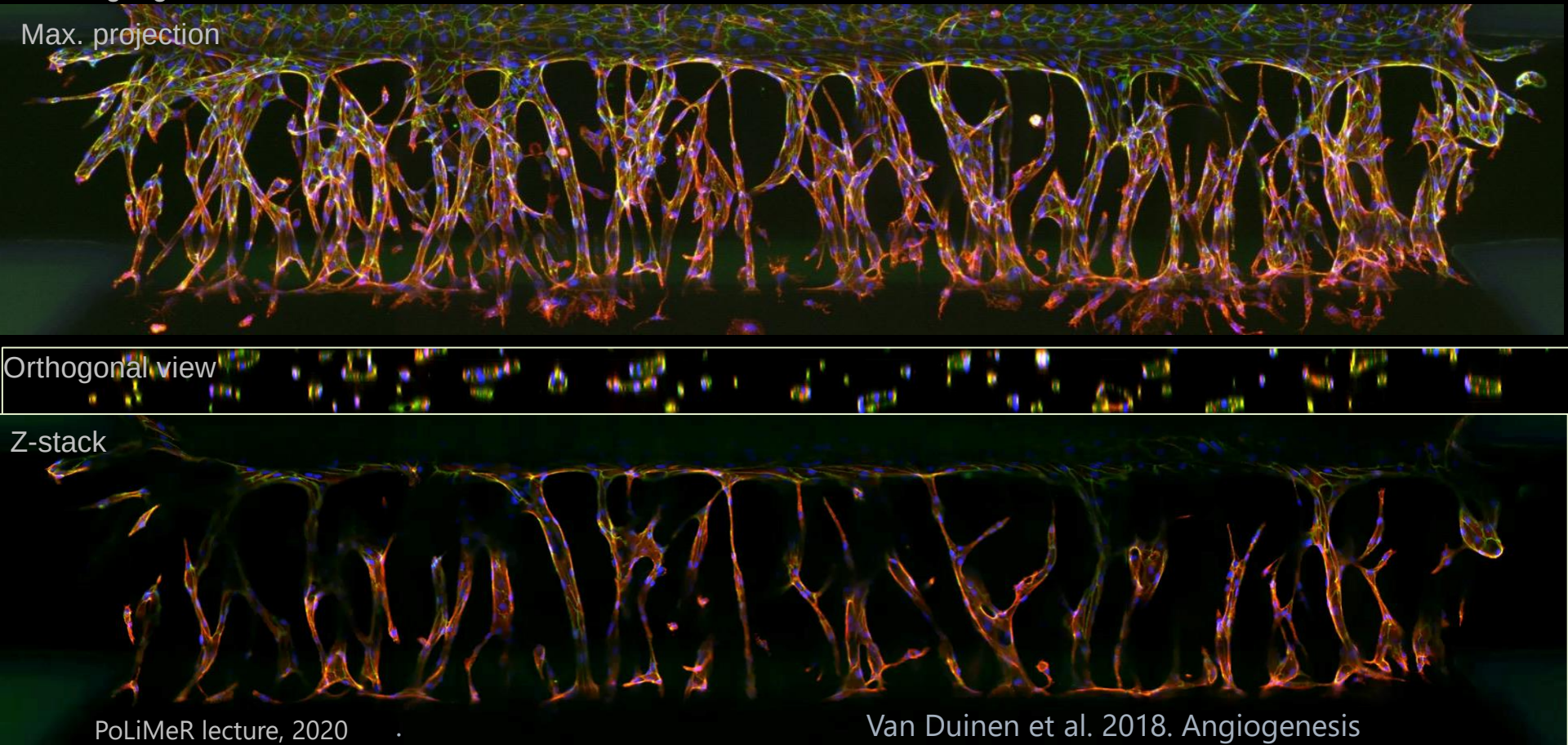
Day 2
Angiogenic sprouting and merging

Day 3
Fusion of capillaries and lumen formation

Day 4
Lumen widening

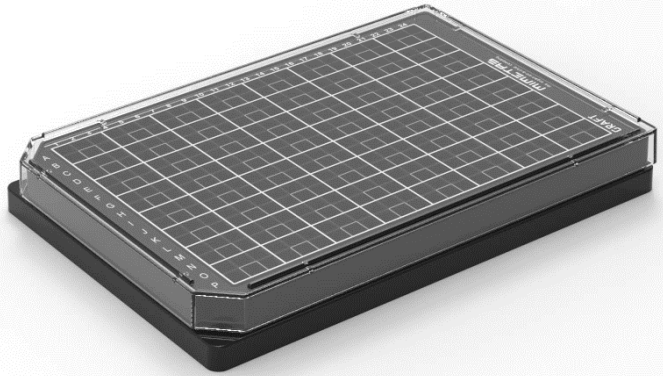
Angiogenic Sprouts

Staining legend: DNA VE-Cadherin Actin

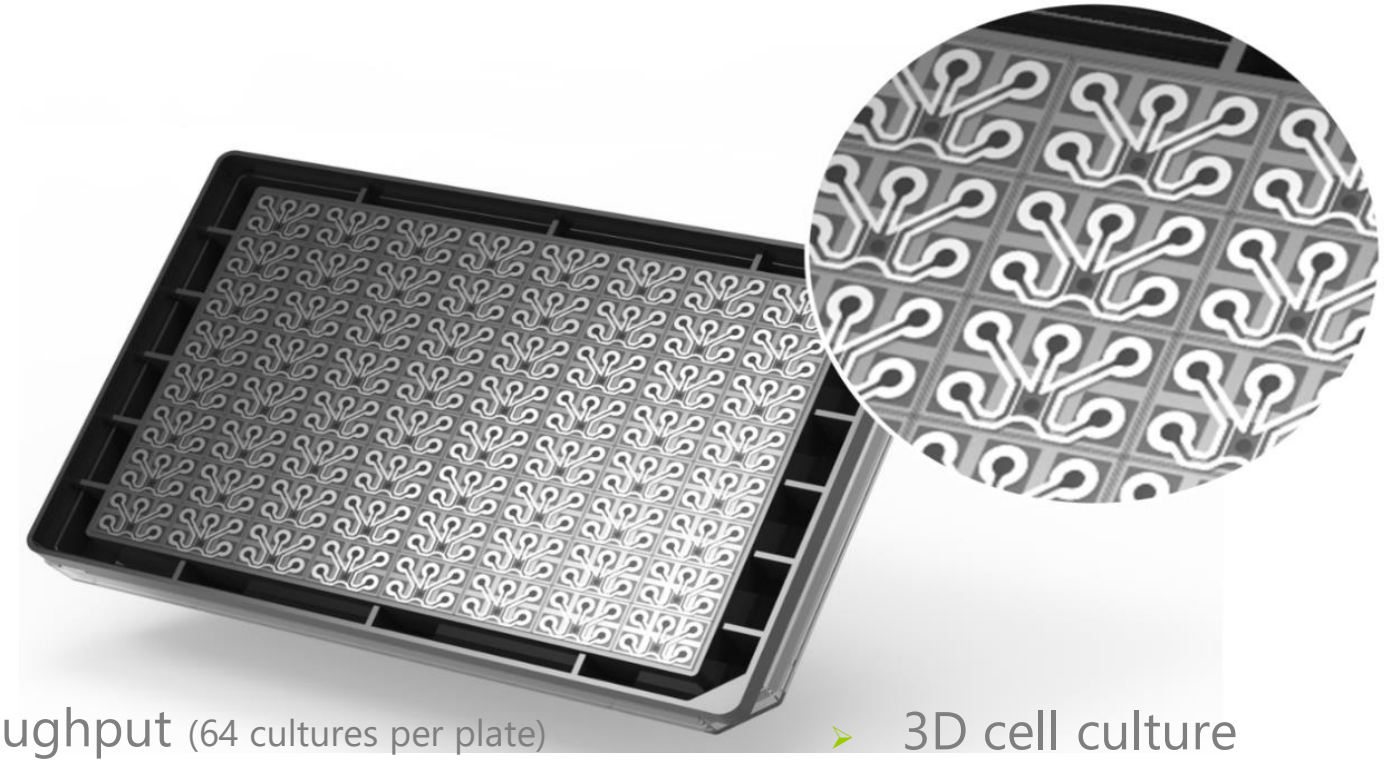


Van Duinen et al. 2018. Angiogenesis

OrganoPlate® Graft

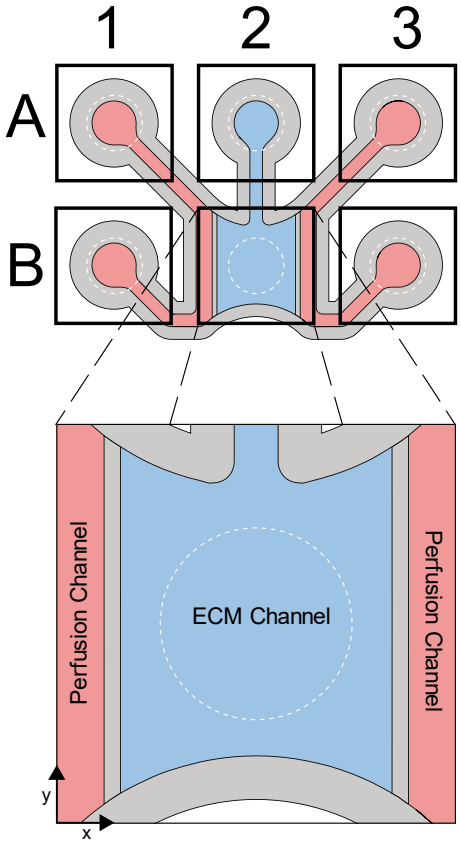


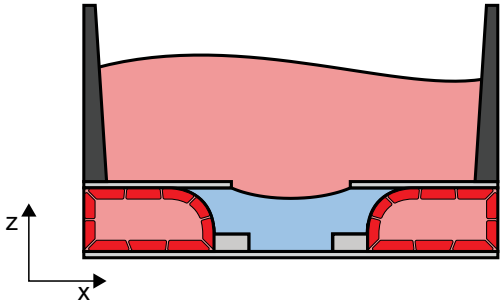
OrganoPlate® Graft

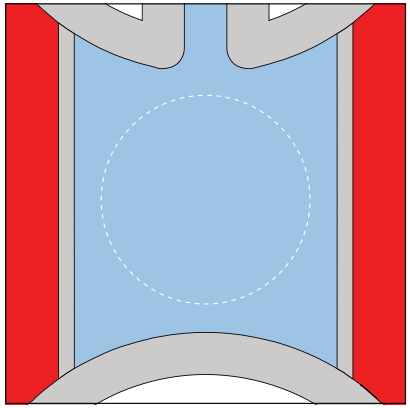


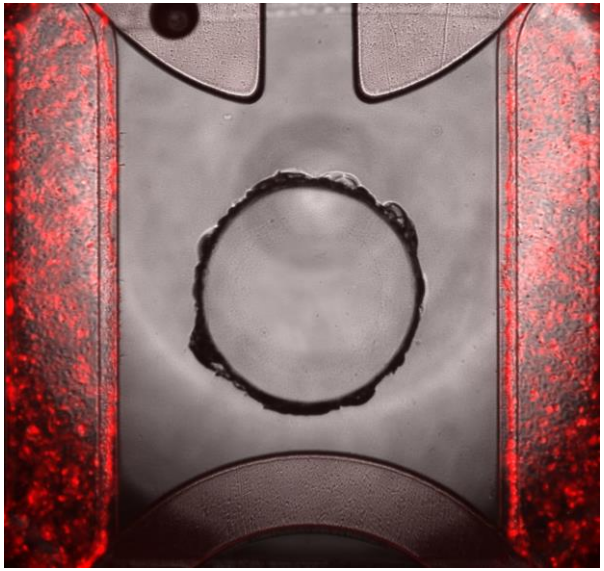
- Throughput (64 cultures per plate)
- Membrane-free
- Pump-free perfusion
- 3D cell culture
- Complex co-cultures
- Excellent imaging

Phase 1: *3D tube formation with Endothelial Cells*

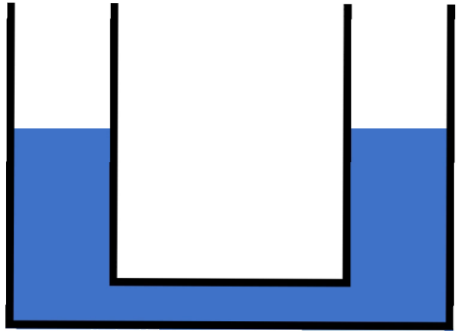


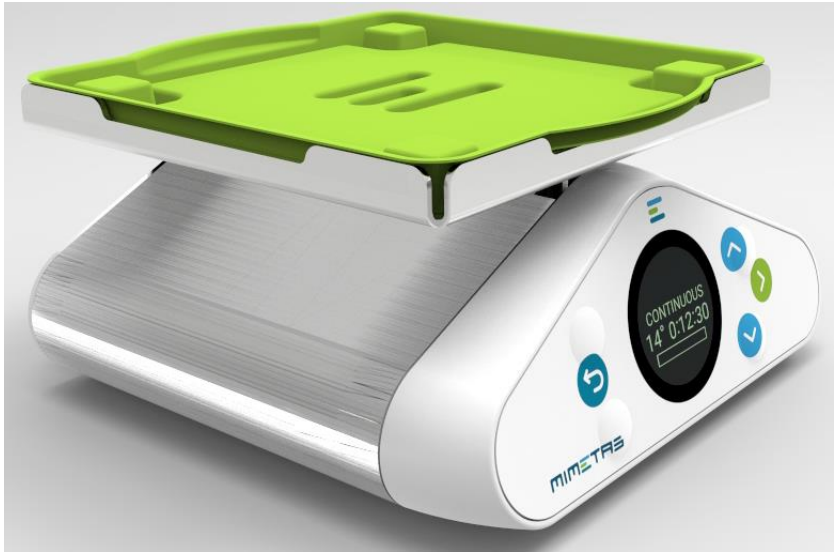




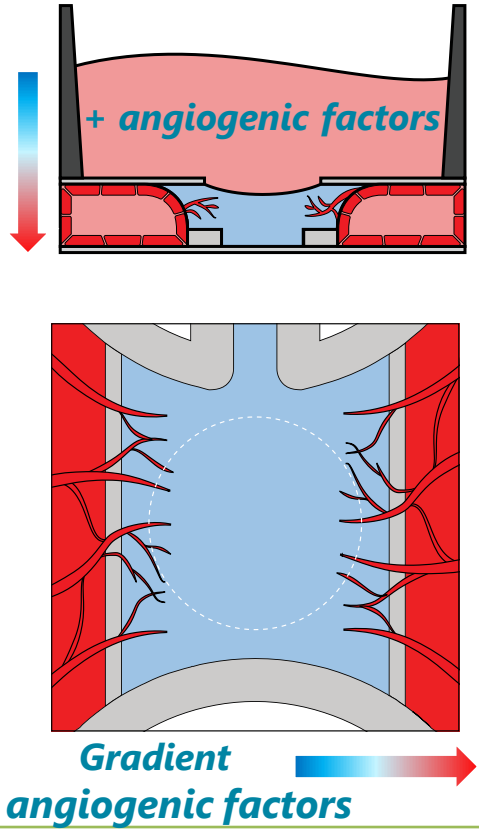


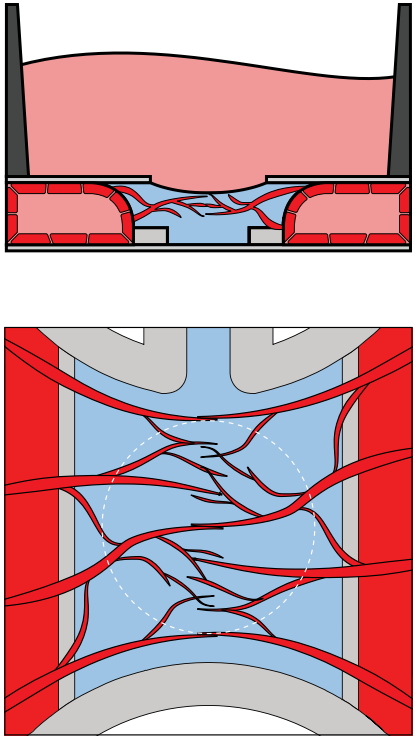
Continuous perfusion

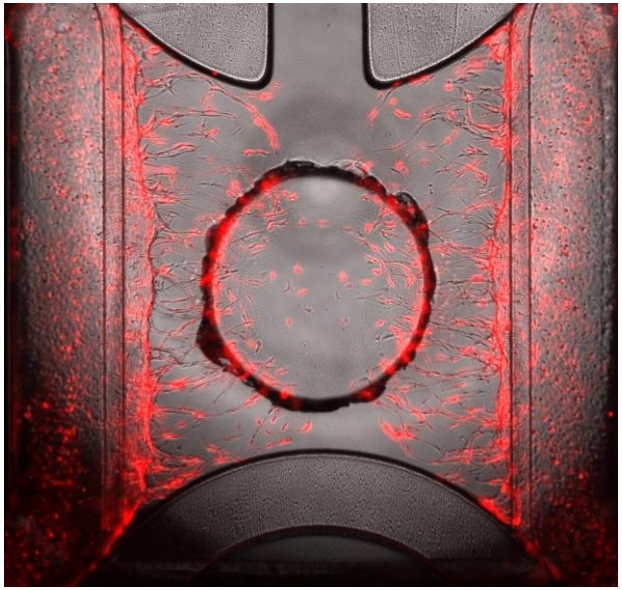




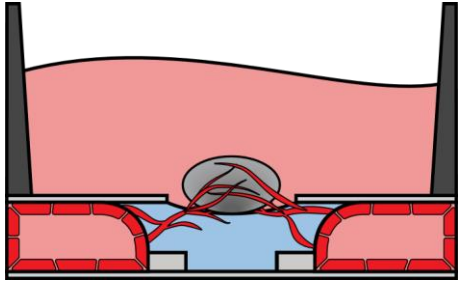
Phase 2: *Vascular bed formation*

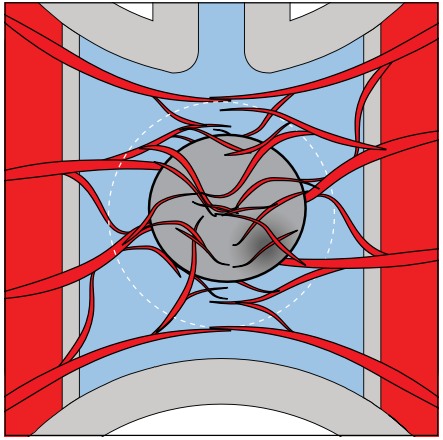


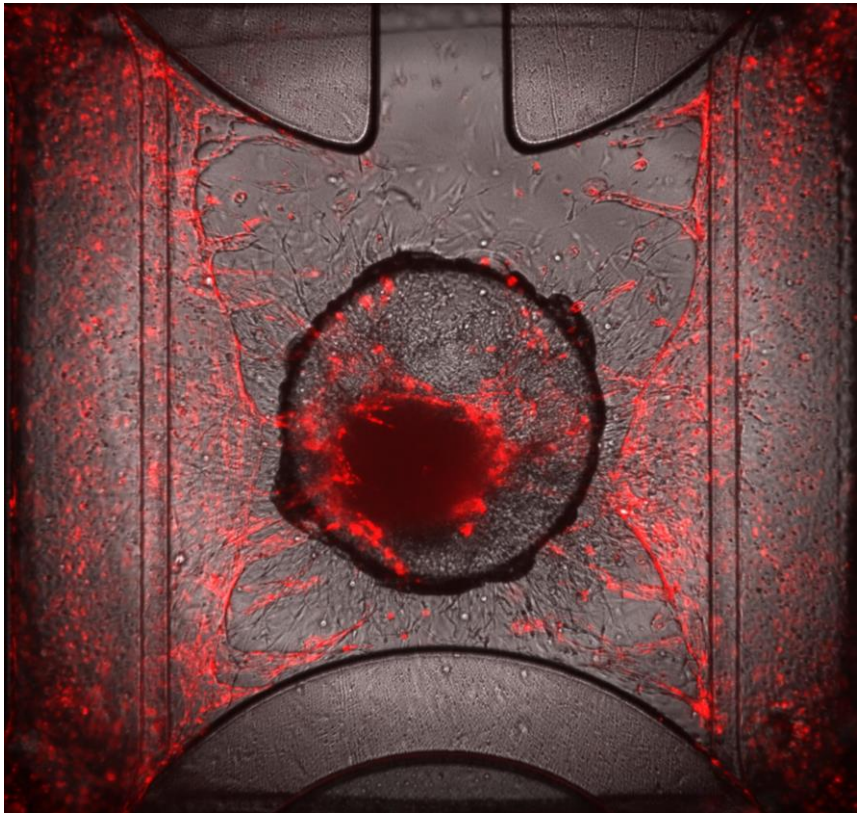




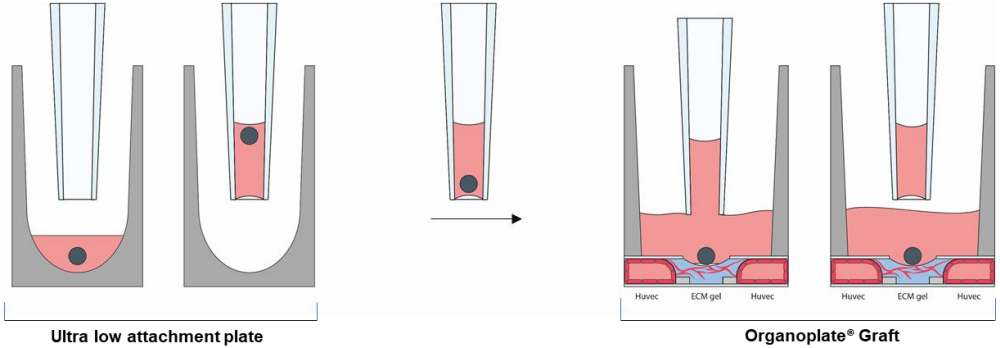
Phase 3: *Tissue transplantation*

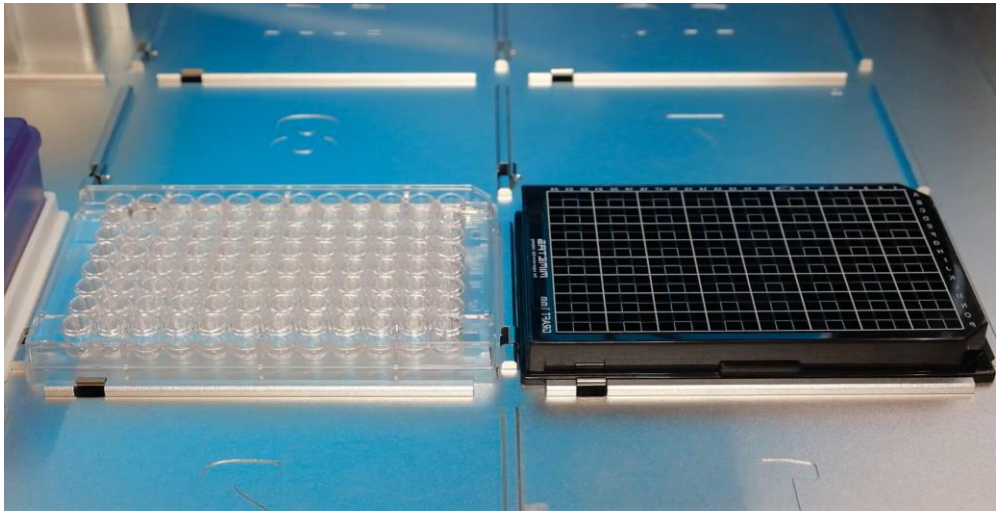




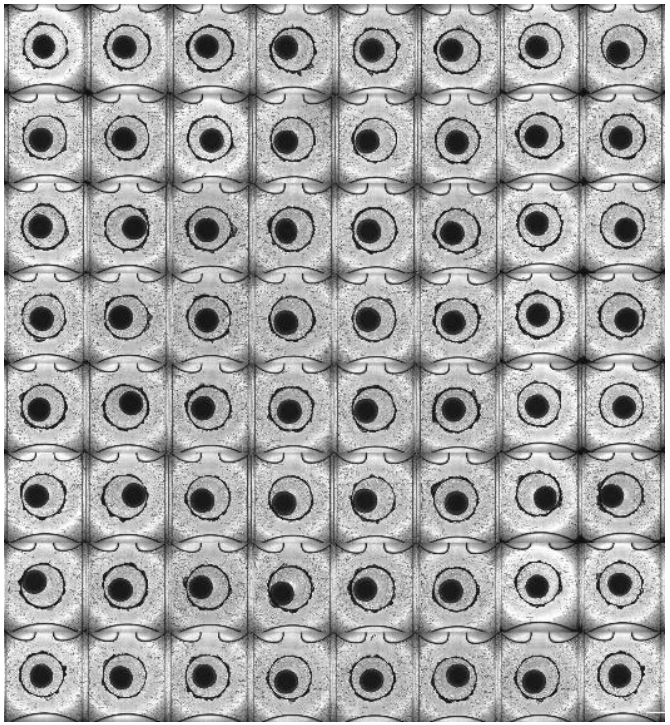


Compatible with automation

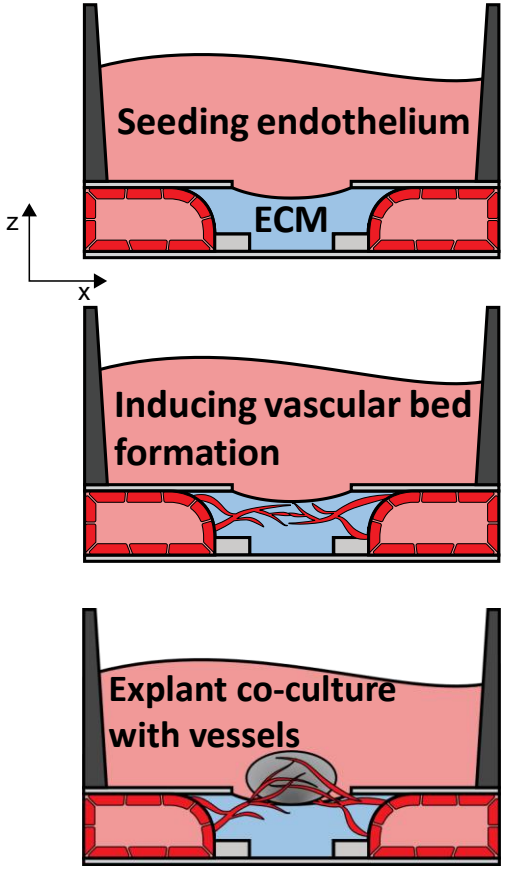


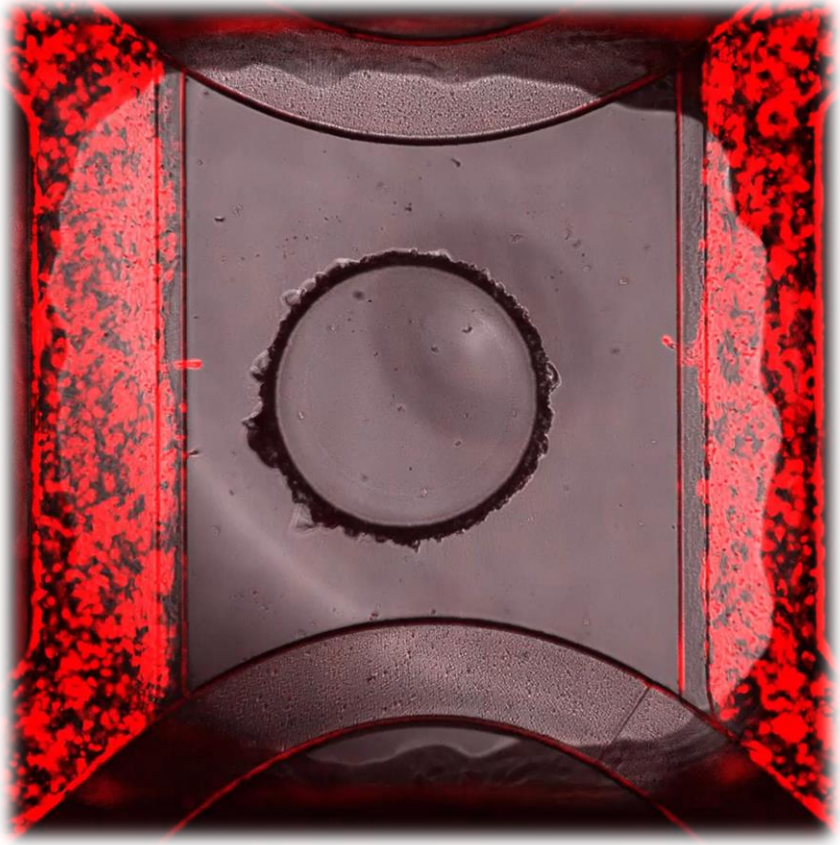


64 individual microtissues ready for vascularization

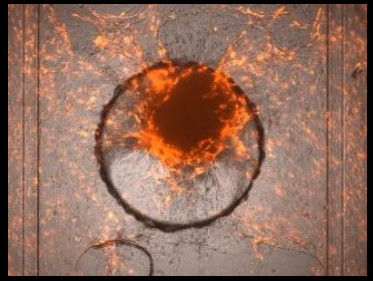


Tissue vascularization in OrganoPlate Graft®

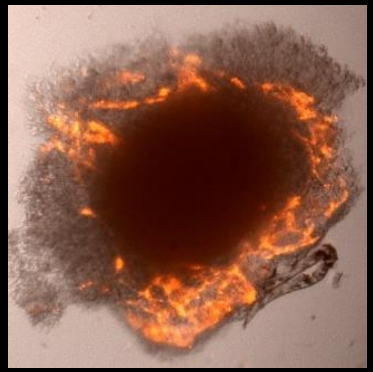




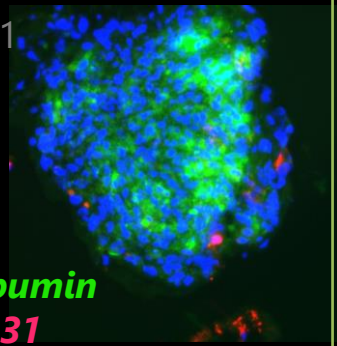
Liver microtissue vascularization in OrganoPlate Graft



RFP

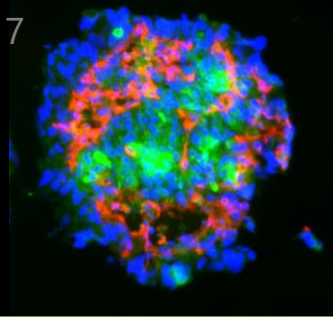


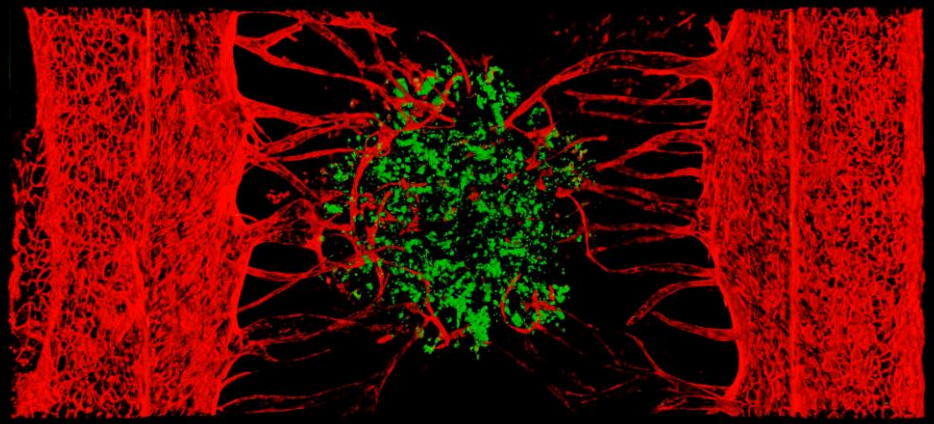
Day 1



Albumin
CD31
Nuclei

Day 7

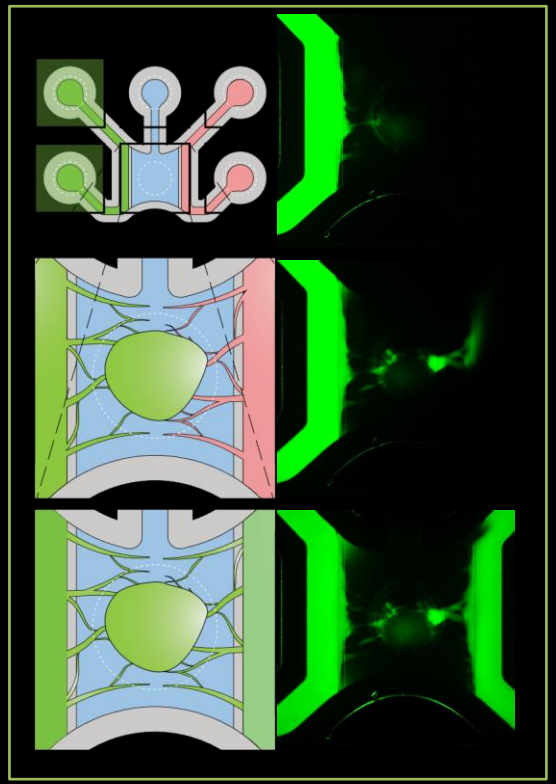


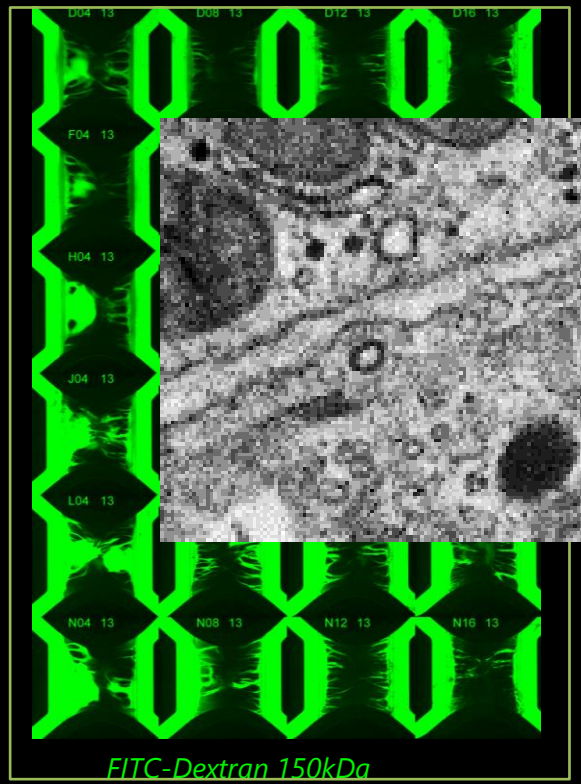


Albumin
CD31

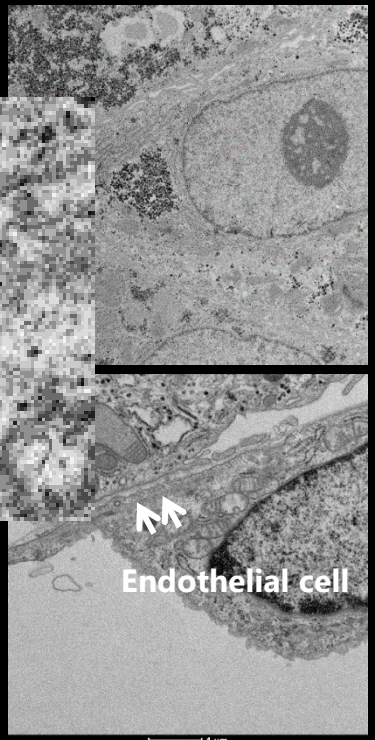
- Sectioning of liver microtissues recovered from OrganoPlate Graft and 3D imaging show vessels penetrating liver microtissues

Vessel stabilization in co-culture with microtissues





FITC-Dextran 150kDa



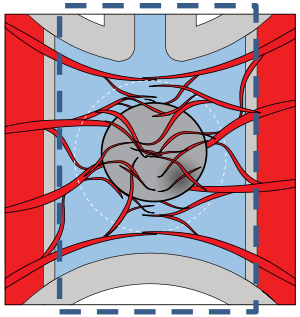
TEM of Hepatocyte Organoids recovered from OrganoPlate Graft

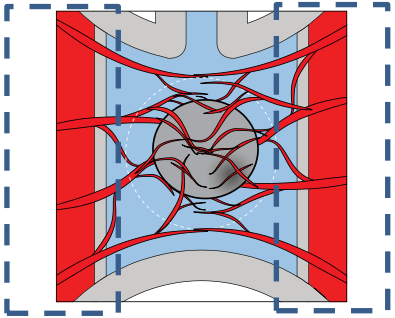
Maastricht University

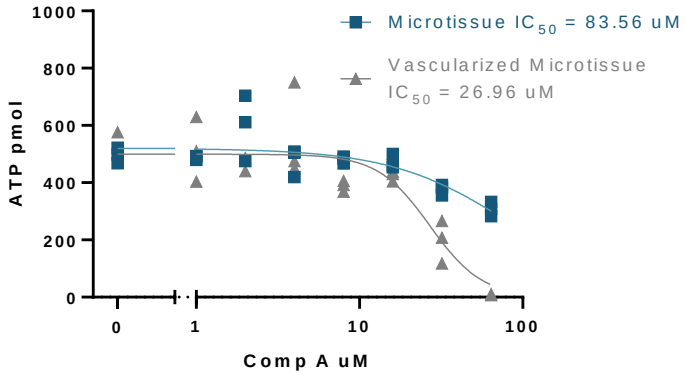
Hubrecht Institute

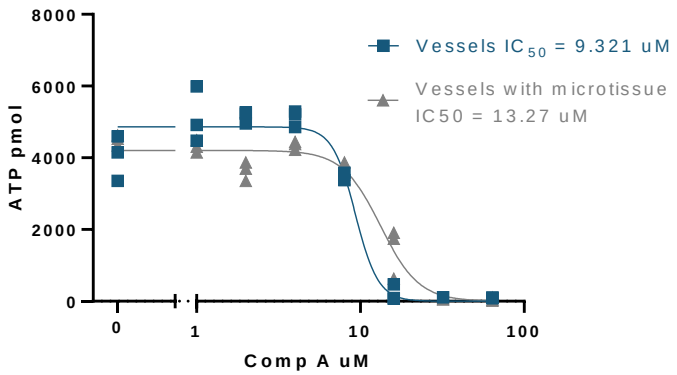
- Reproducibly stable vessels over the subsequent 21 days of co-culture with microtissues

DILI in vascularized liver tissue





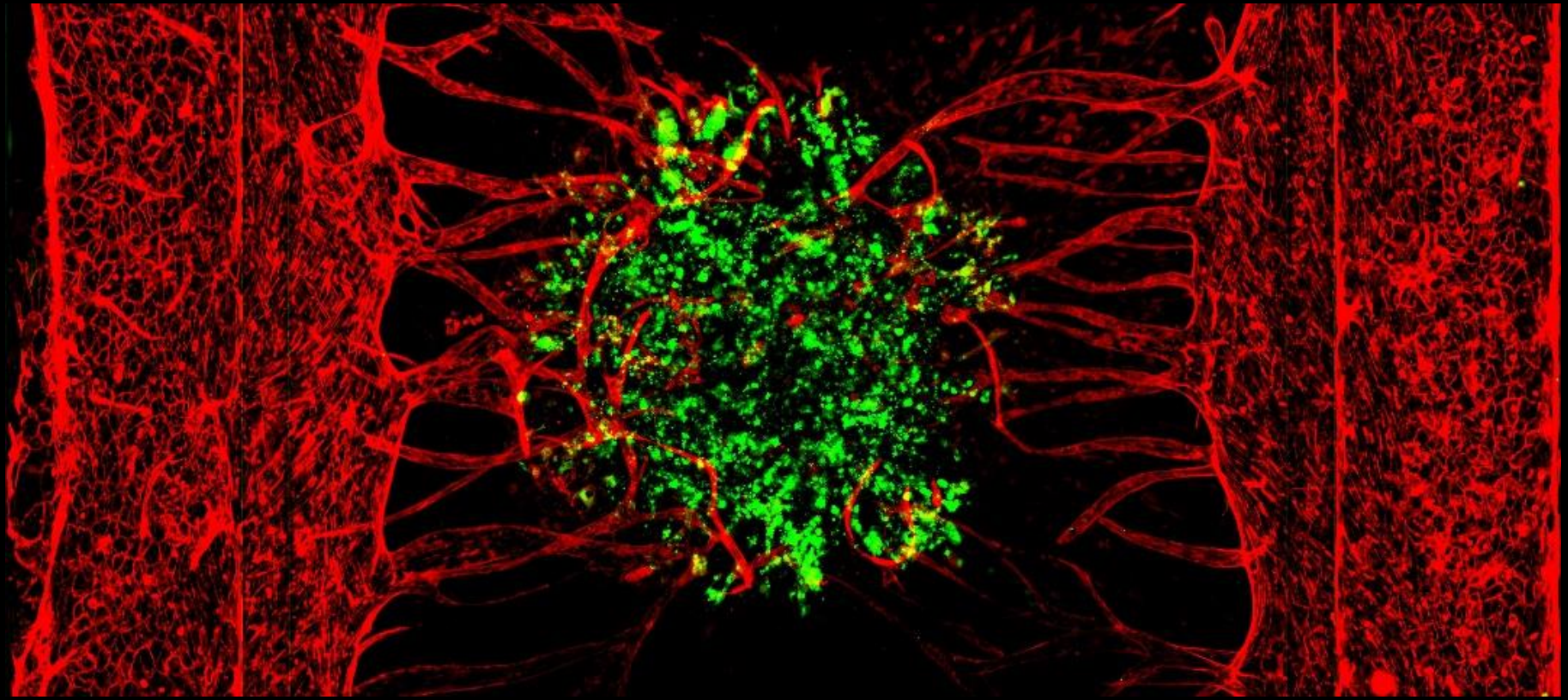




- Vascularised liver microtissue is more sensitive to compound A
- Protective function liver microtissue on vascular tox

Connecting patient tissue with human vasculature

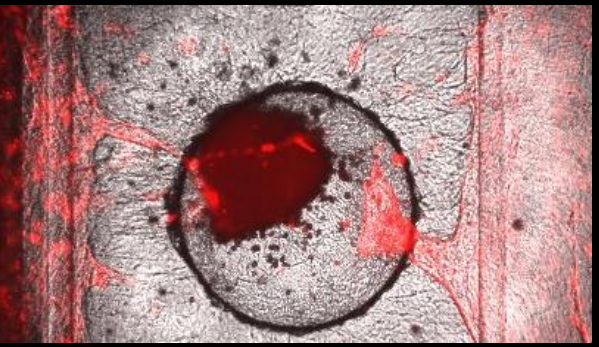
- Applications: vascularizing xenografts, organoids, spheroids



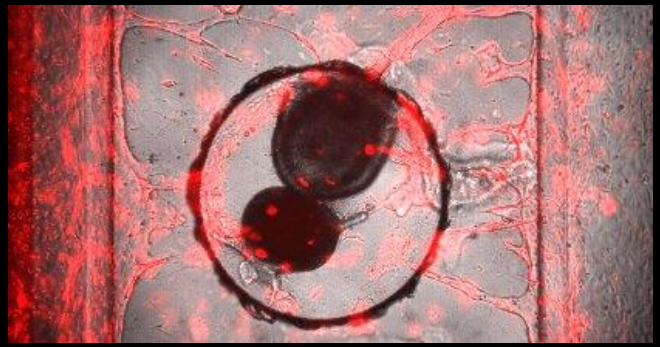
Vessels Microtissue

Tumor tissues in the OrganoPlate Graft

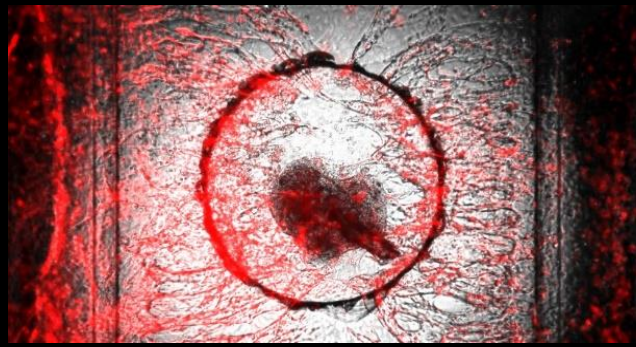
Glioma spheroids



HNSCC organoids



Ovary Cancer explant



- Glioma OMS interact with vascular bed, cells migrating into the ECM
- Head-and-neck organoids and vascular bed interaction
- Ovary cancer explant shows massive interaction with vascular beds.

Erasmus MC
Universitair Medisch Centrum Rotterdam

Hubrecht Institute

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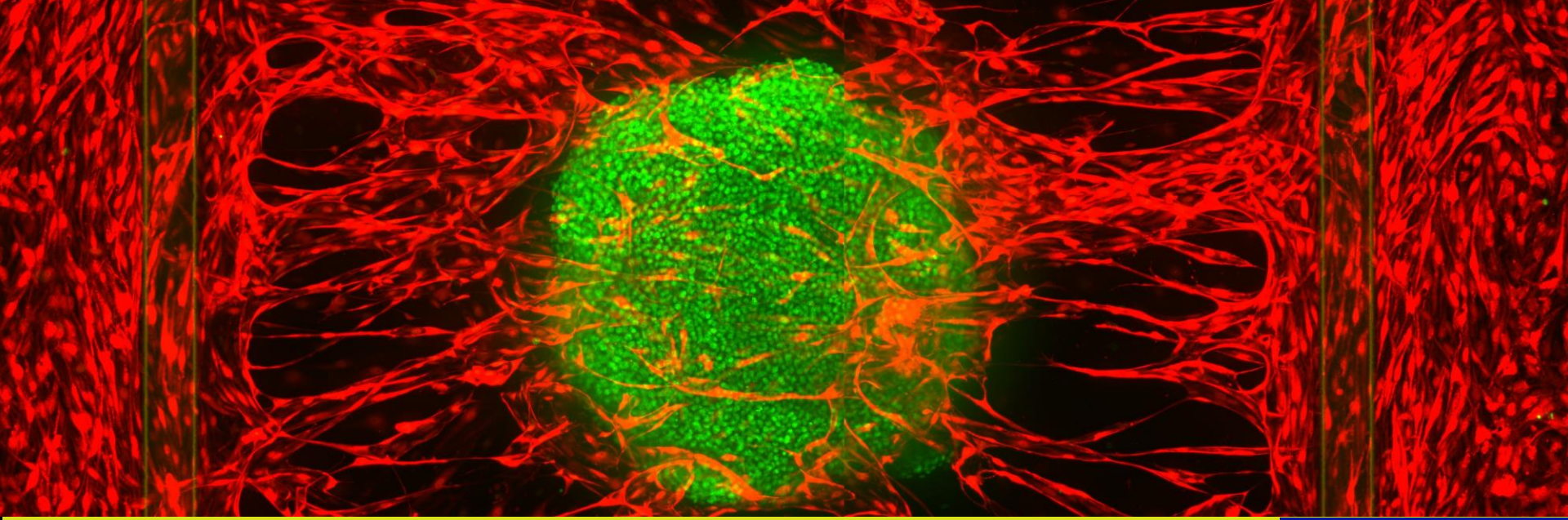
BIOTECHNOLOGY

Take home message

- Organotypic models recapitulate human physiology
- Improve in vitro predictivity
- Reduce number of in vivo investigative studies



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MIMETAS
the organ-on-a-chip company

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