

UNIVERSITY OF TWENTE.

Introduction to Organ-on-Chip (OoC) models

Séverine Le Gac

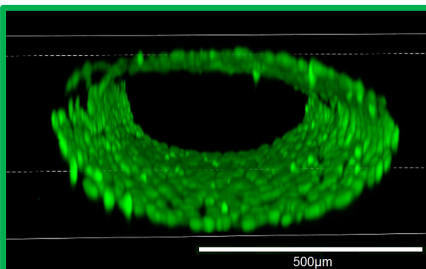
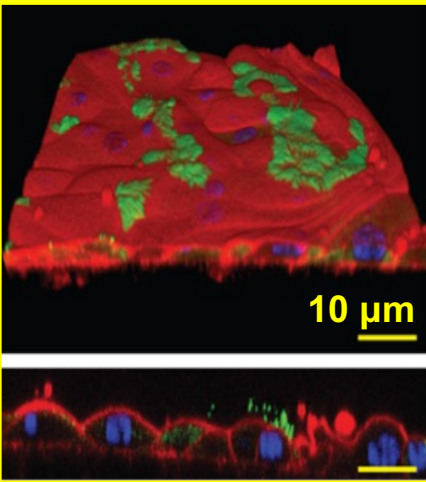
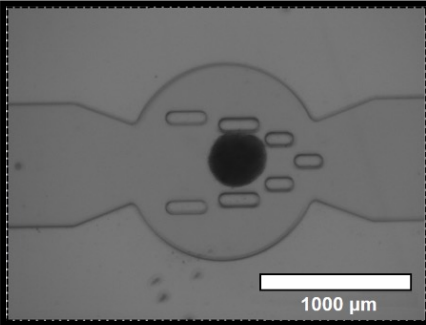
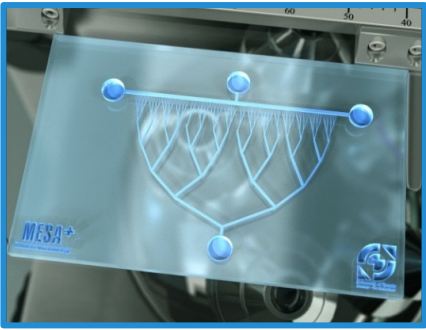
**Applied Microfluidics for BioEngineering Research
University of Twente, The Netherlands**

s.legac@utwente.nl

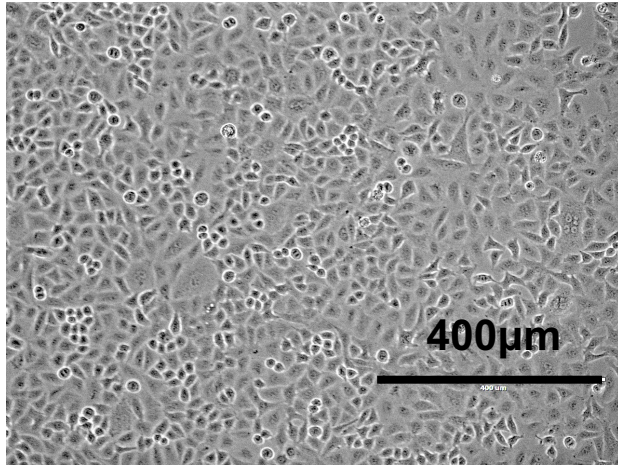


Scope and content

- Introduction
 - Background / motivation
 - **Microfluidic technology** & key characteristics
 - **Organ-on-chip** platforms
- **Approaches to develop organ-on-chip models / Classification**
 - **Microbioreactor** approach
 - How to model the tissue?
 - Importance of oxygen
 - **Physiological barriers**
 - Biomimetic approach to support the epithelium
 - **Bottom-up** approach
 - Tissue vascularization
 - Tissue (mechanical) actuation
- **Critical analysis:** Opportunities and challenges



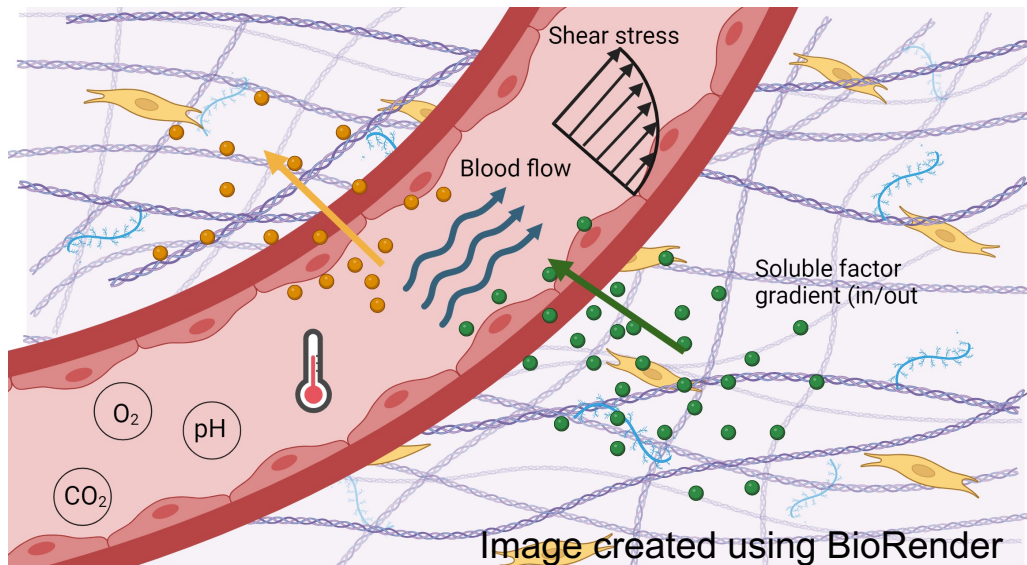
OoC modeling - Background & Motivation



Current models

- No good models of the *in vivo* human situation
- *In vitro*: oversimplified; changes in cell phenotype in 2D.
- *In vivo*: differences in physiology, genetic background, size & loading; ethical limitations (3R's); no HTS.

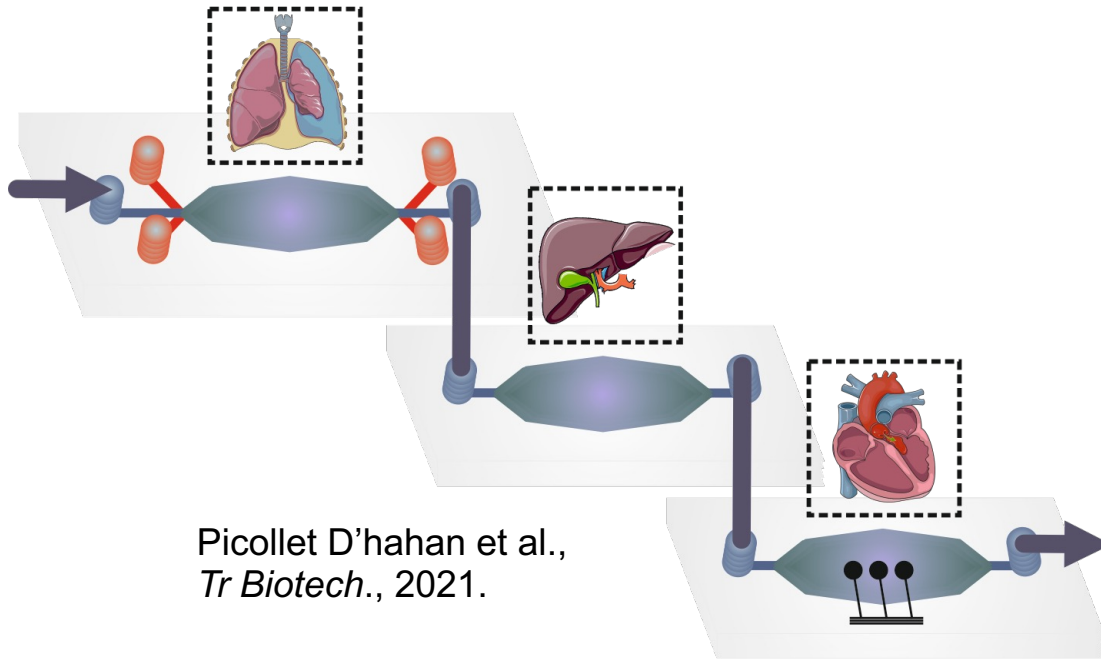
⇒ Poor to no translational value



The *in vivo* cellular microenvironment

- Various cell types
- Cell-cell & Cell-ECM interactions
- Cellular physical and chemical **microenvironment**: controlled and tunable
- **Dynamic** environment: incl. perfusion, cell mechanical, electrical, and biochemical stimulation
- And... vascularization, immune system, innervation

Organ-on-chip models



Picollet D'hahan et al.,
Tr Biotech., 2021.

- **Miniaturized devices (“chip”)** providing high control on the cell microenvironment
- **Hybrid models** combining cells and microfabricated structures aiming at reproducing the physical architecture and cell interactions found in a specific organ.
- **Multi-OoC** platforms for systemic studies

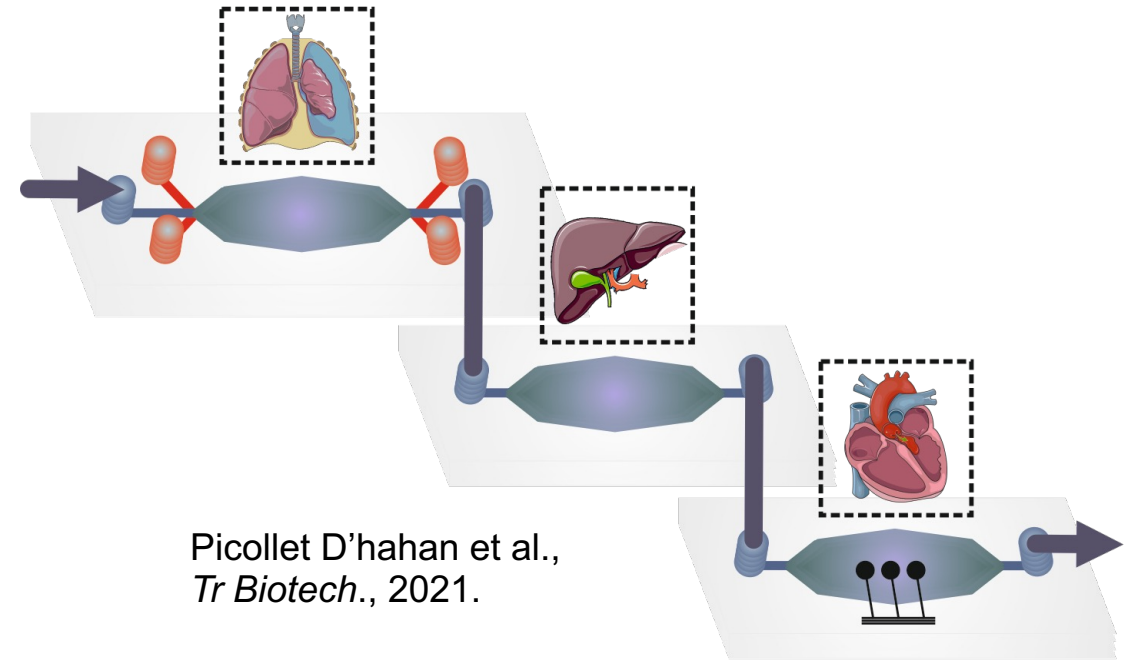
- ⇒ Experimentation under **physiological conditions**
- ⇒ Amenable to parallelization / automation
- ⇒ Compatible with standard detection techniques (offline / online analysis)
- ⇒ **Next generation of *in vitro* models**
- ⇒ **Powerful alternative to animal experimentation?**

How to approach the design of an OoC model?

- **Which organ to model?** What are the main features of this organ?

Features: Cell type(s), extra-cellular matrix (nature, (bio)chemical/physical properties), physiological characteristics, microenvironmental parameters ($T^{\circ}\text{C}$, O_2 %), pH, etc.), architectural elements, need for cell/tissue stimulation? etc.

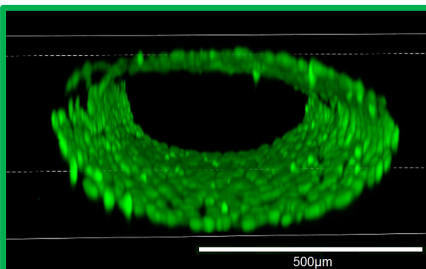
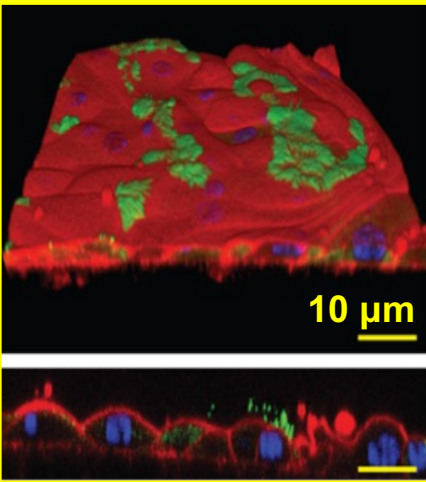
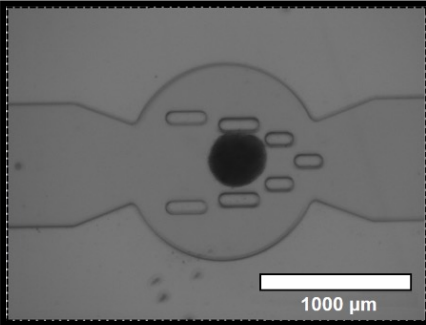
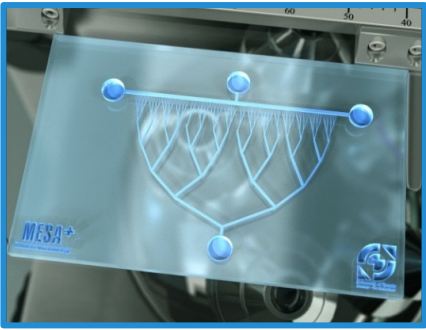
- **Which targeted application?**
- What are the **organ-specific features essential** for this **application**?
- Which **readout(s)** (assays/techniques) to be implemented?



⇒ Find a compromise between simplicity of use/engineering and complexity to address the biological/medical question

Scope and content

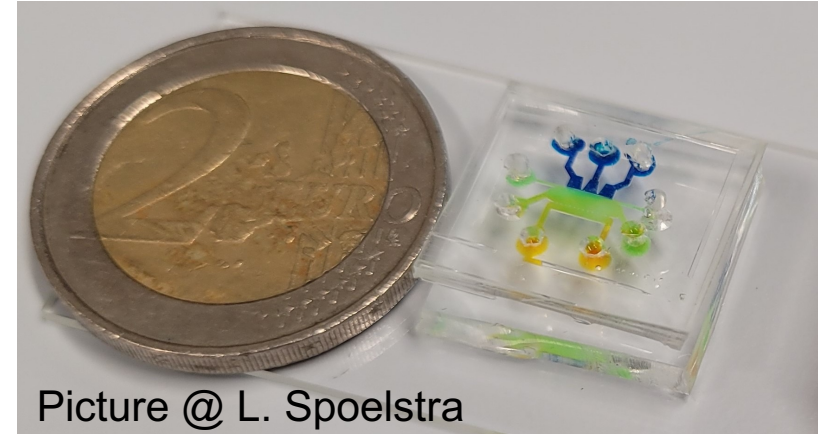
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OoC Classification (1) – Microbioreactor

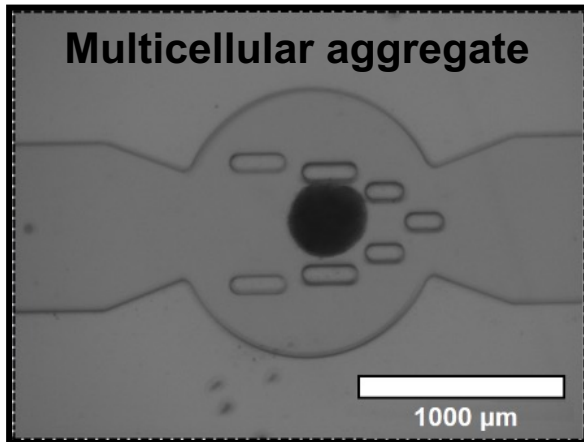
3D cell culture in a microfluidic bioreactor

- Continuous medium perfusion; vascular-like system
- Supply in nutrients and oxygen & removal of waste
- On-demand and controlled exposure to soluble factors
- Controlled cell microenvironment

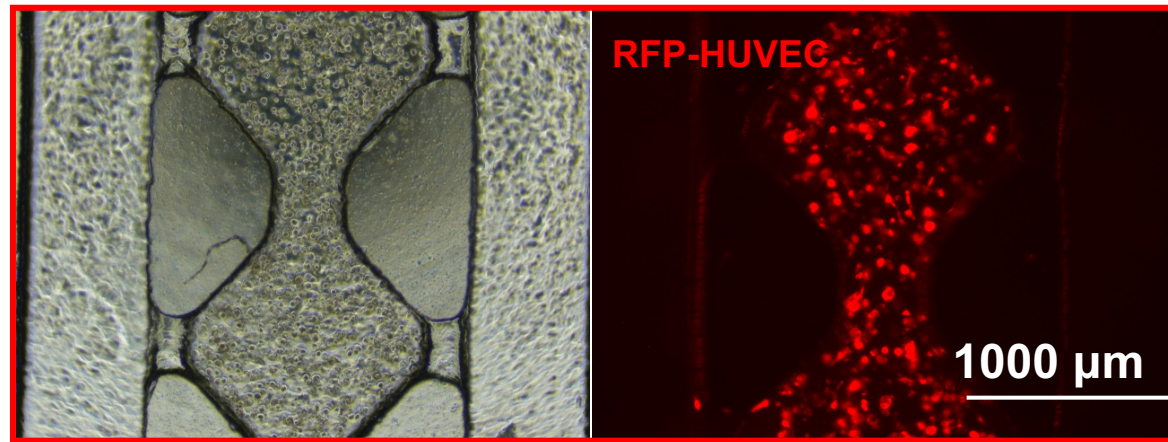


3D cellular model

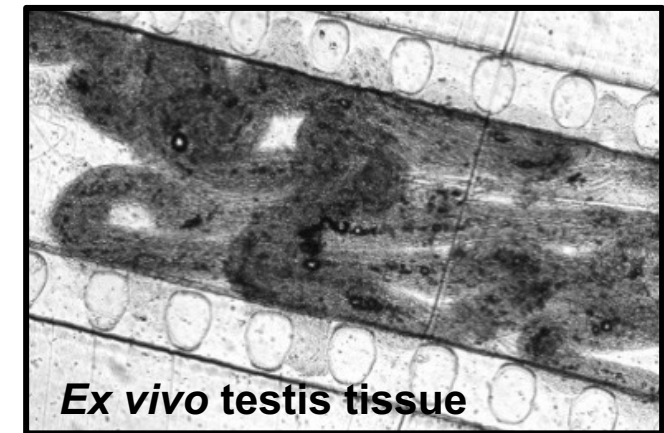
- Bioengineered cell constructs: 3D cellular aggregate or 3D cell culture in an ECM-like hydrogel
- *Ex vivo* tissue



Jean-Baptiste Blondé



Utku Devamoglu



Sharma, Venzac et al., 2022.

BioEngineering approach – Tumor-on-chip

TUMOR MICROENVIRONMENT

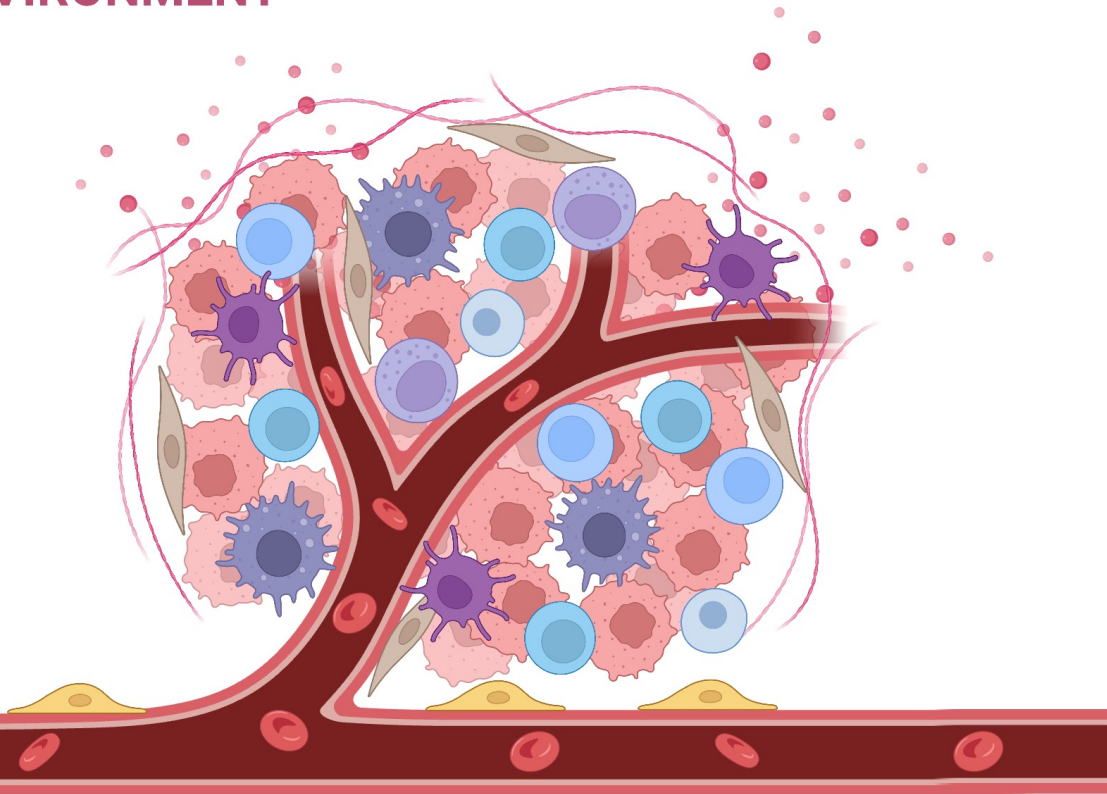


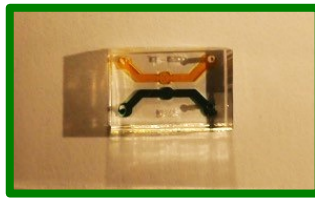
Image created using BioRender

- **Heterogeneous cell aggregates**
- Cancer cells... but also...
- Stromal cells such as fibroblasts (cancer associated fibroblasts), immune cells, endothelial cells, etc.
- Extra-cellular matrix (ECM)
- **“Irregular” vasculature**

⇒ Complex tumor environment playing a role for **drug penetration** and tumor response to **treatments**.

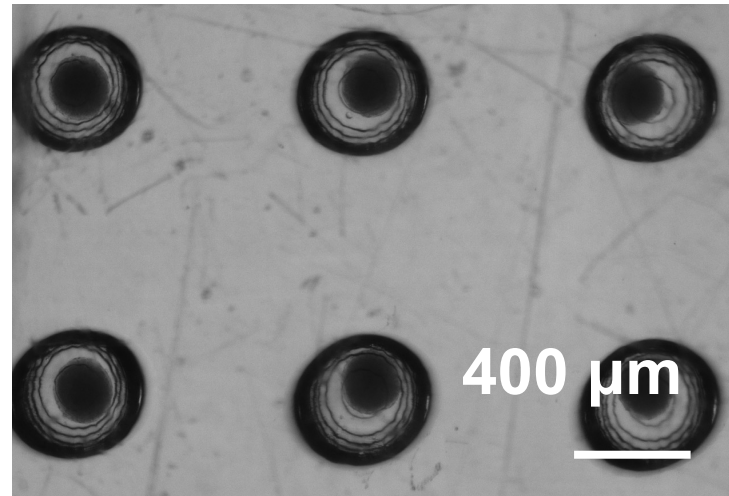
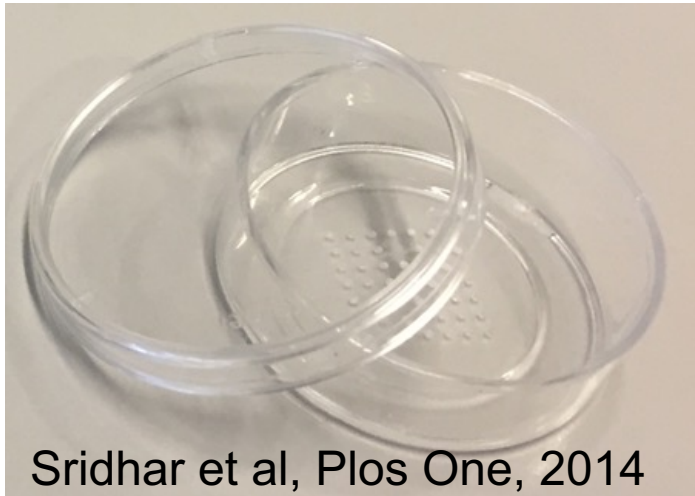
⇒ **Barriers** to cross for drug delivery: endothelium & stroma.

BioEngineering approach – Tumor-on-chip



■ Bioengineered Multicellular tumor spheroids (MCTS)

- Microwell array in a Petri dish
- Control on MCTS size and cellular composition

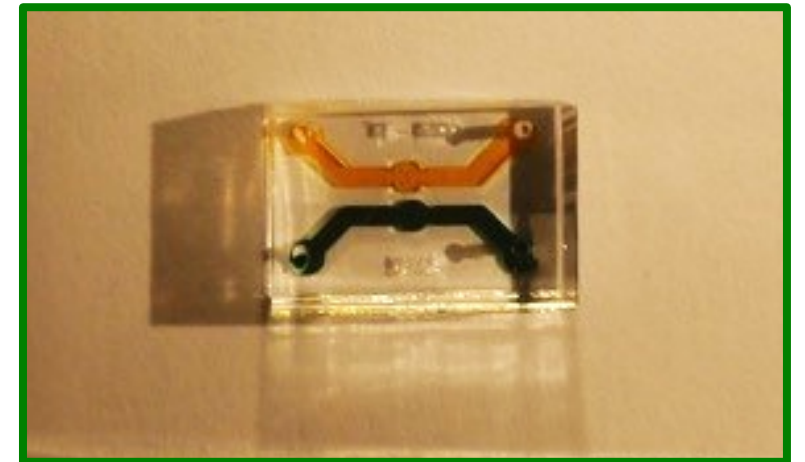
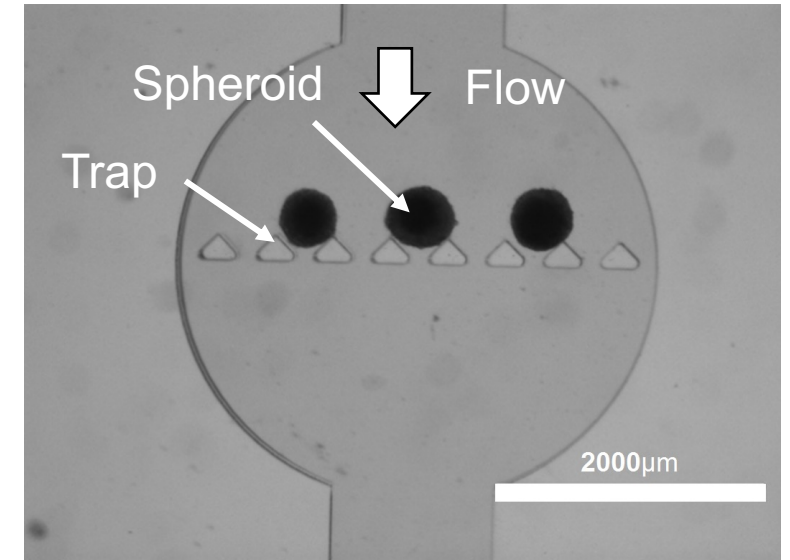


- Mono-culture MCTS: breast tumor cells only
- Co-culture MCTS: fibroblasts and breast tumor cells (5:1 ratio) to mimic stroma

Priwitaningrum et al., *J. Control Release*, 2016; doi: 10.1016/j.jconrel.2016.09.004

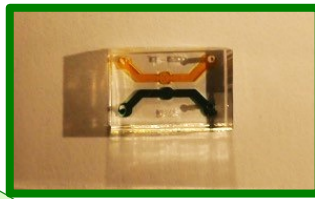
Blondé et al., MicroTAS proc., 2016 and 2018; *in preparation*

■ Microfluidic platform



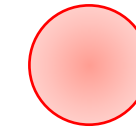
Collaboration Prof. Prakash, UTwente

BioEngineering approach – Tumor-on-chip



■ Nanomedicine penetration assay

- Fluorescently labeled silica nanoparticles
- Dye-loaded liposomes (100 nm)
- Dynamic exposure (0.1 – 1 – 10 $\mu\text{L}/\text{min}$) for 24 or 48 h
- *In situ* confocal microscopy



30 nm Si

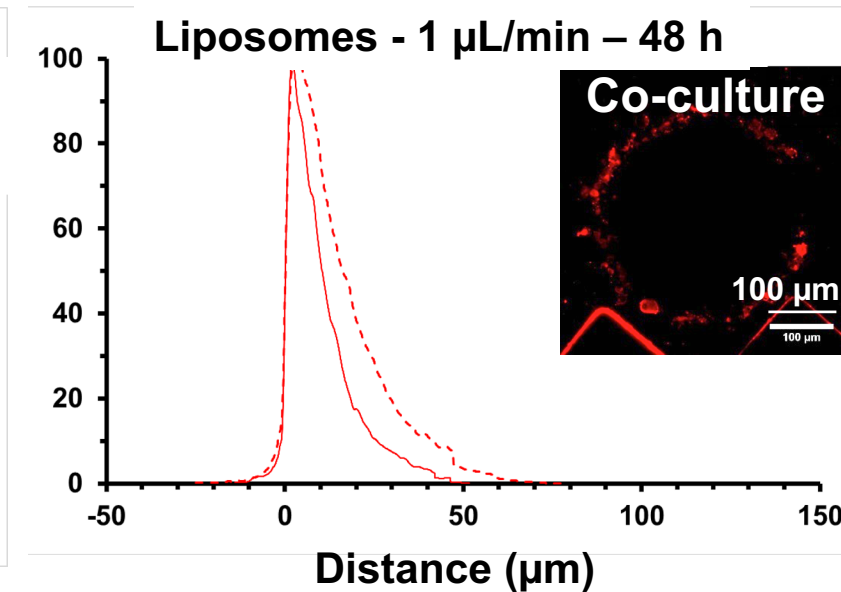
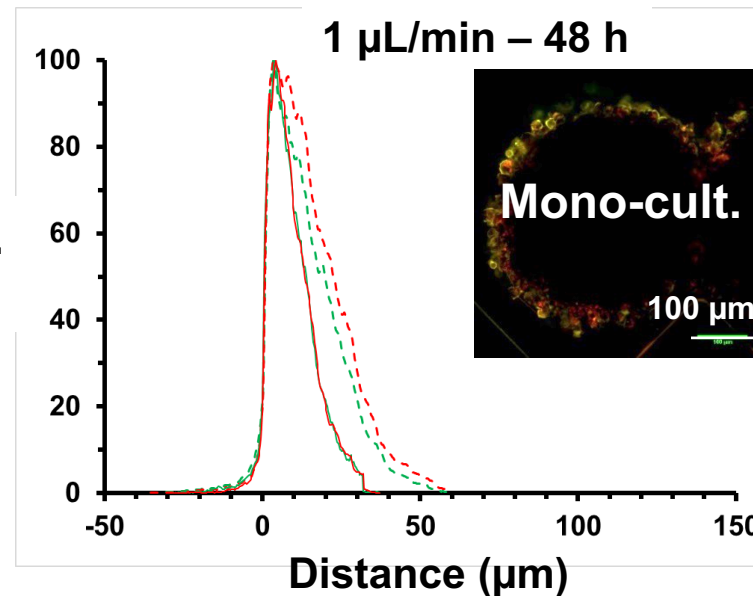
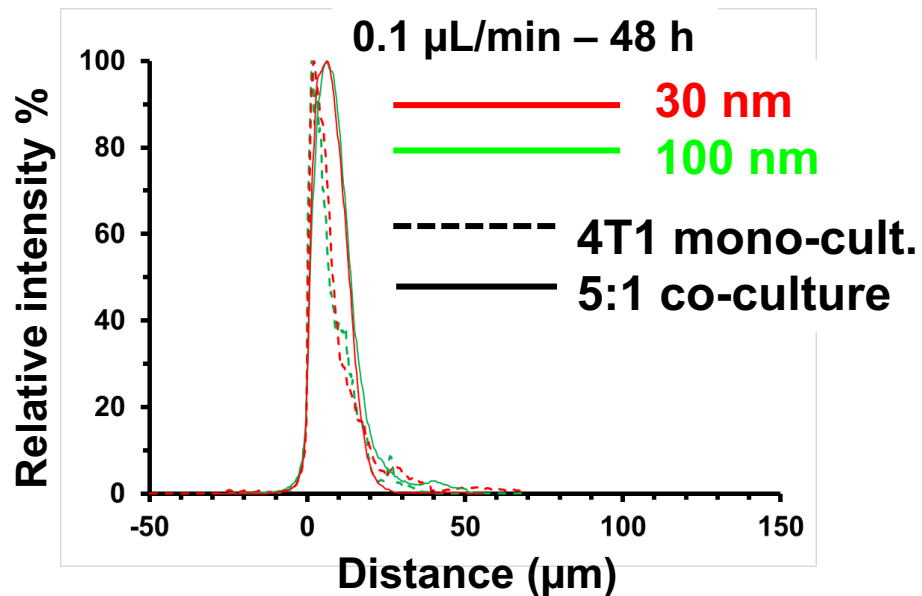
λ_{exc} : 569 nm,
 λ_{emi} : 585 nm

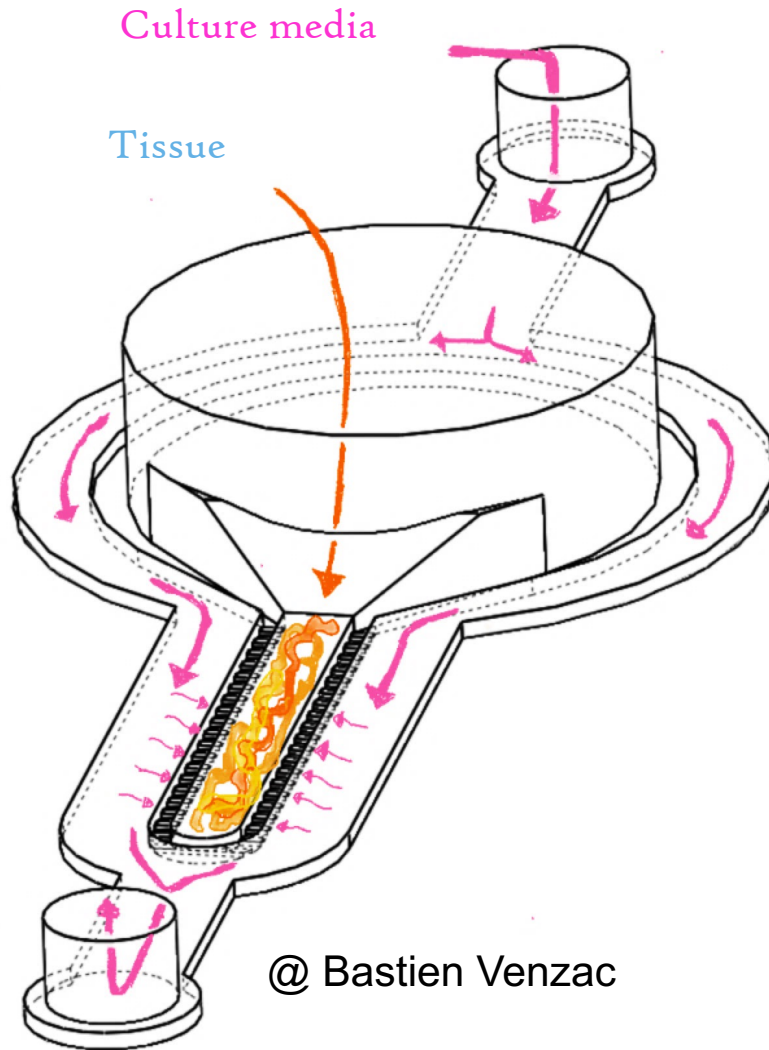
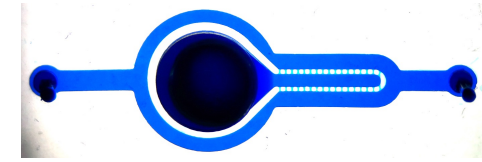
(ξ = - 40 mV)



100 nm Si

λ_{exc} : 488 nm,
 λ_{emi} : 501 nm





@ Bastien Venzac

Ex vivo samples

- Inherent tissue complexity (adequate cell ratio/organization, right ECM, preserved architecture, mature tissue)
- Patient-specific studies!
- Tumor “cuboids”; tissue slices; dissected “intact” structures
- **Culture of human testis tissues** (seminiferous tubules)
 - Gametogenesis *in vitro*: fundamental study & production of sperm for infertile patients;
 - Toxicity screening (e.g., endocrine disruptors, « plastics »).

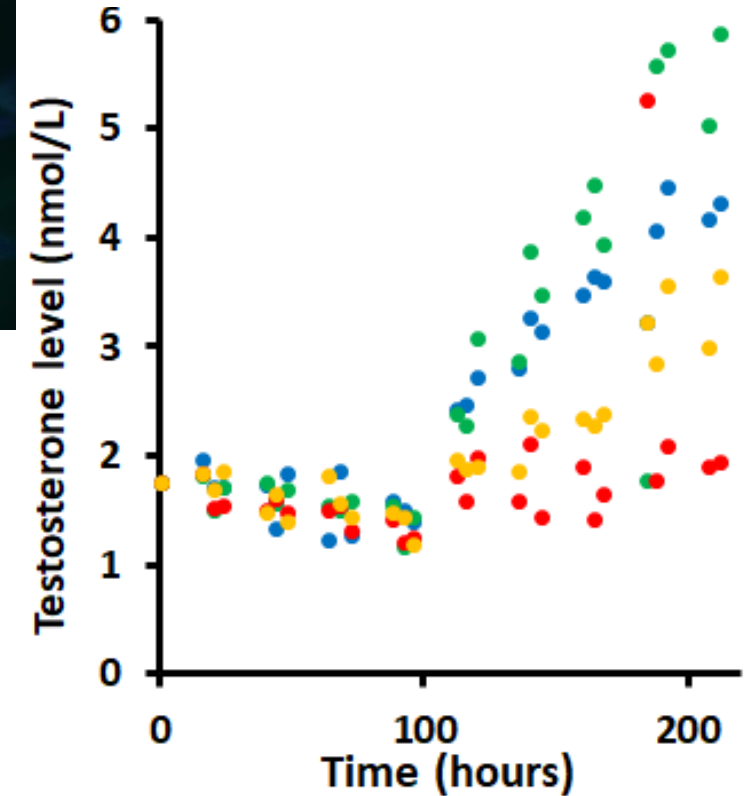
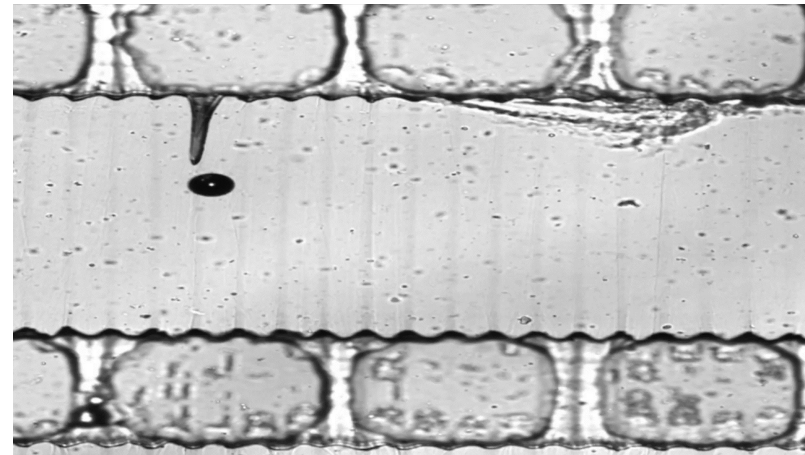
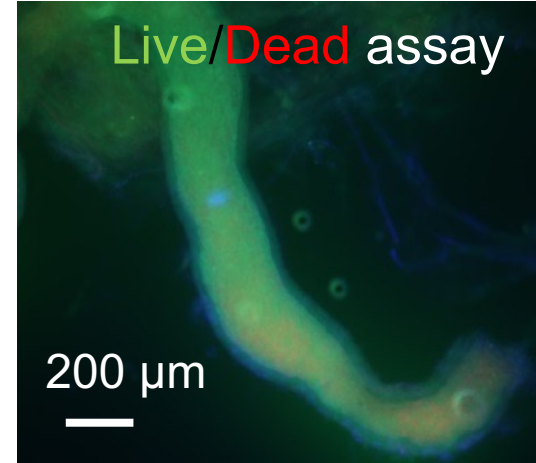
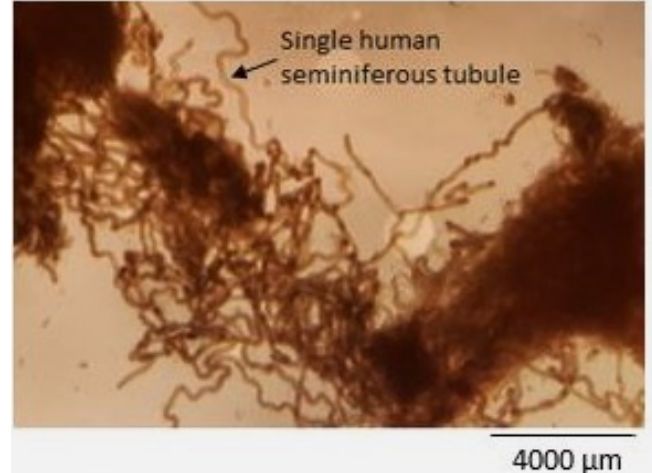
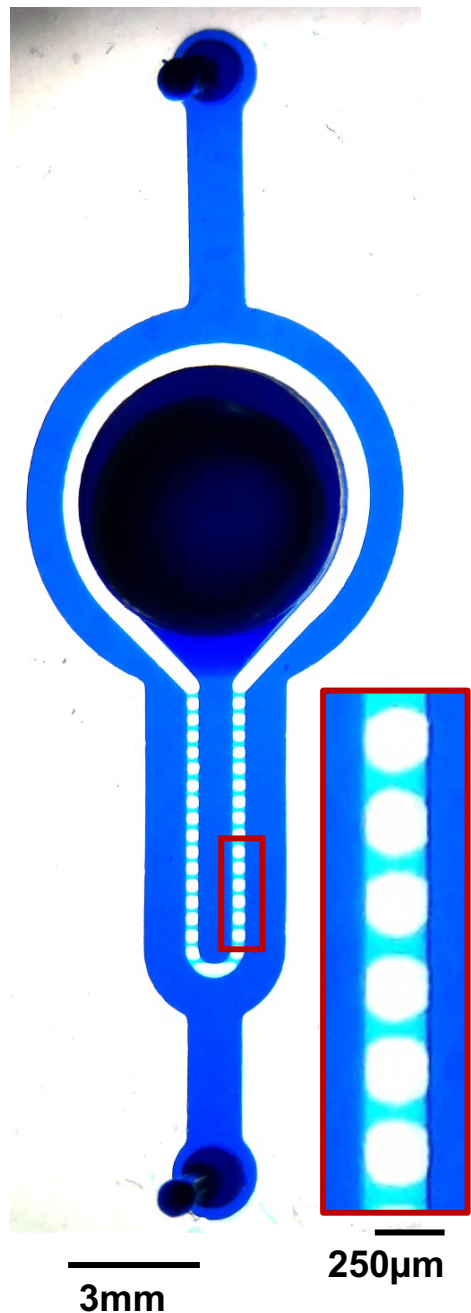
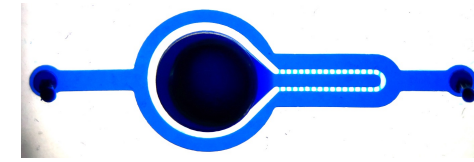
Added value of microfluidics

- Better preservation of the tissue microenvironment;
- Nutrient and oxygen delivery via a vascular-like system;
- *Ad hoc* and *in situ* hormonal stimulation;
- *In situ* monitoring of tissue viability, and function (hormone production & spermatogonial stem cell differentiation).

Reviewed by McLean et al., 2018, Lab Chip; doi.org/10.1039/C8LC00241J

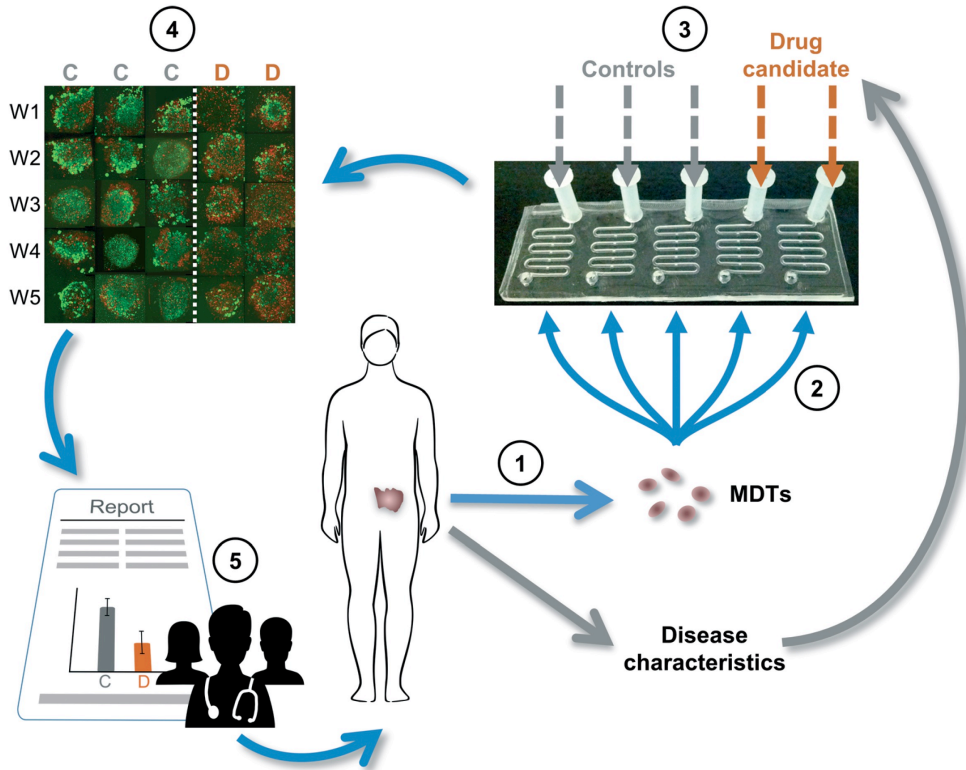
Collaboration: Prof. Schlatt, CeRA

Ex vivo approach - Testis-on-chip

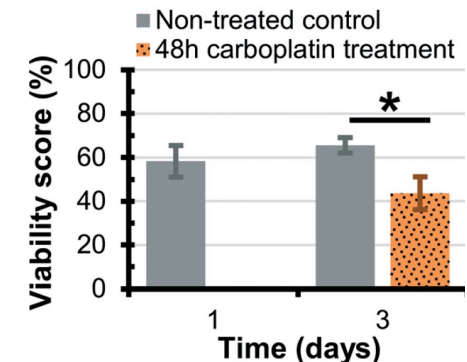
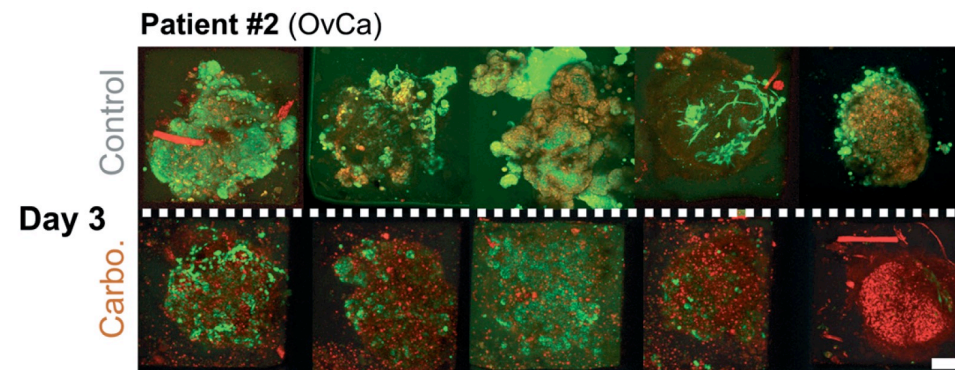
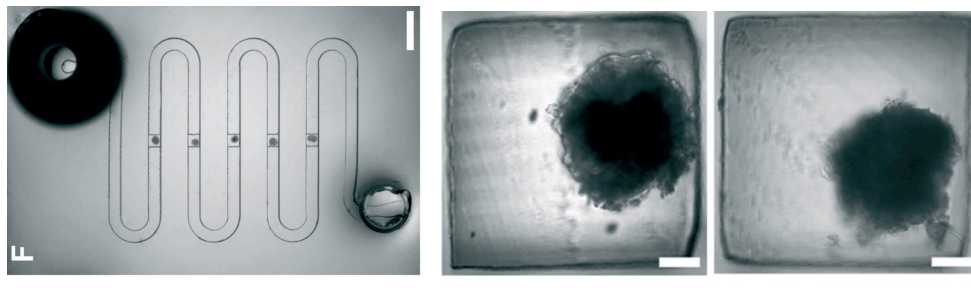


- Tissue (human/non-human primate) viability & integrity for ~10 days (perfusion 80 $\mu\text{L/h}$)
- Tissue hormonal stimulation & recording of testosterone production: marmoset tissue sensitive to hormonal stimulation in a dose-dependent manner

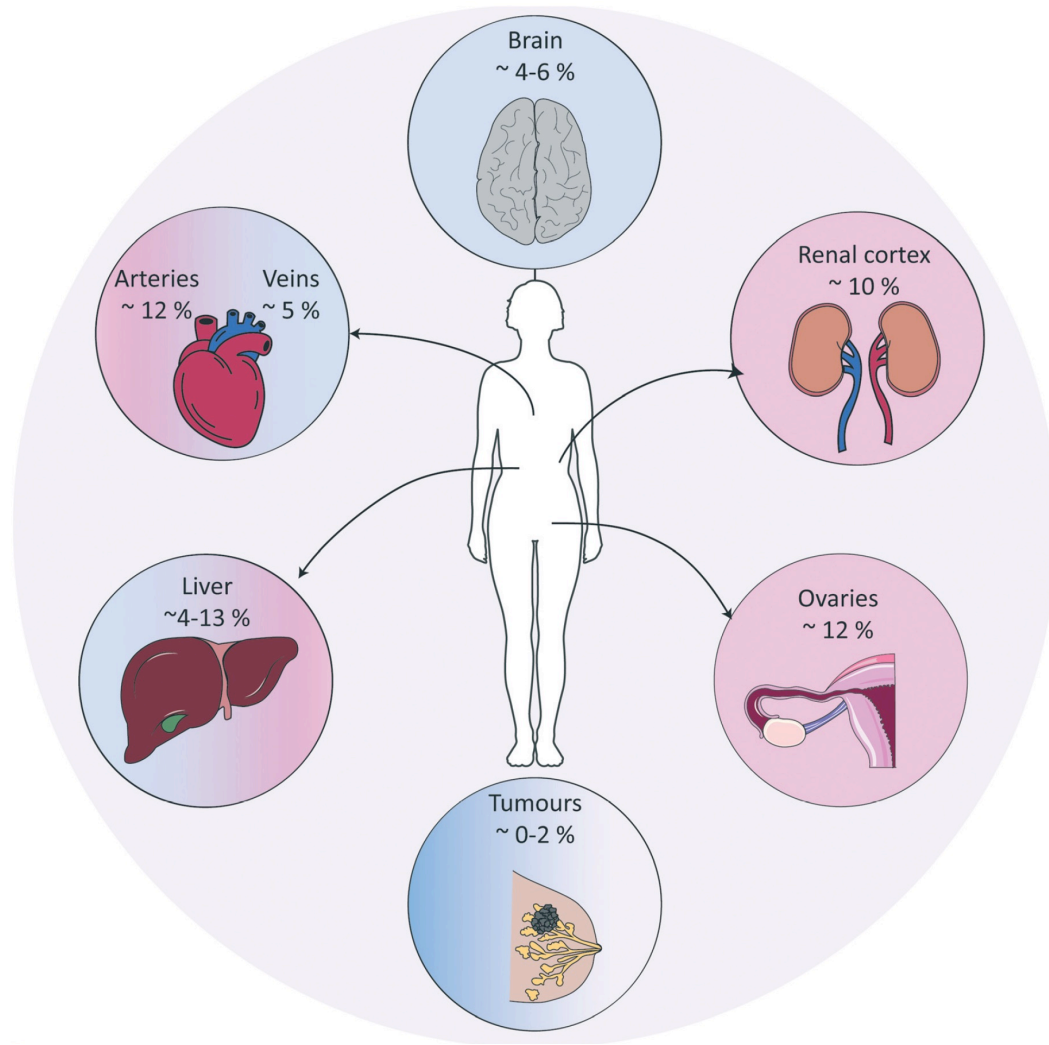
Ex vivo approach - Micro-dissected tumor tissues on chip



- Biopsy/surgery-based isolation of small tissues
- Sectioning in smaller microtissues (<500 μm in size)
- Low-shear perfusion (no tissue damage) for dynamic drug delivery
- Long-term tissue viability (~10 days)
- Early drug testing (carboplatin; ovarian cancer)



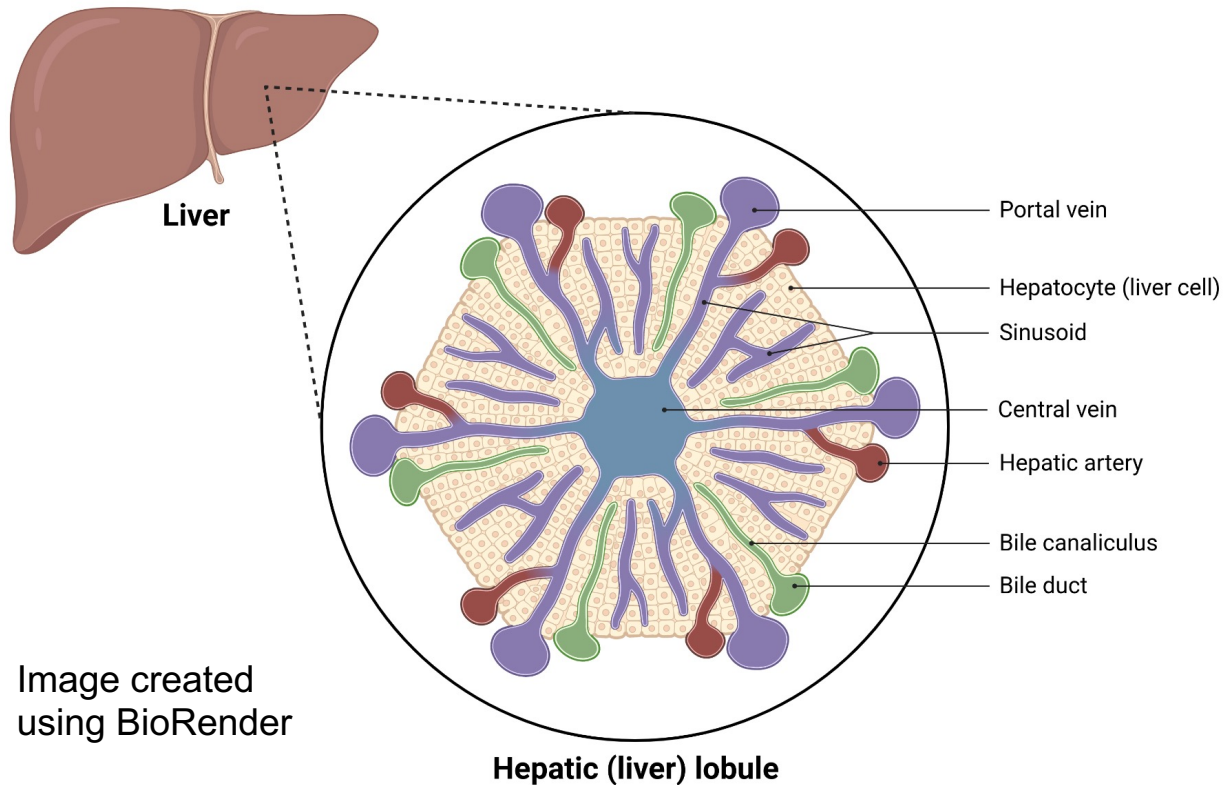
Oxygen in the human body and a cell's microenvironment



- Almost all organs/tissues/cells in the human body are at an oxygen tension below 15% (vs. 21% in the atmosphere).
- Oxygen tension essential for the phenotype and function of cells (e.g., impact on cell metabolism).
- Presence of gradients of oxygen in some tissues/organs.
- Changes in oxygen tension correlating with disease onset and/or progression.

Anoxia	Exposure to extremely low levels of oxygen (< 0.02%), which often leads to necrosis
Hypoxia	Exposure to low levels of oxygen that activate adaptive responses of the cells or tissues. Depending on the tissue, this usually occurs below 3-5% oxygen
Physioxia	Exposure to normal levels of oxygen as happens inside the body, ranging from ~4-12% depending on the tissue
Normoxia	Exposure to atmospheric levels of oxygen (~20%). This is often hyperoxic for tissues in <i>in vitro</i> conditions
Hyperoxia	Exposure of cells and tissues to oxygen levels that are supraphysiological

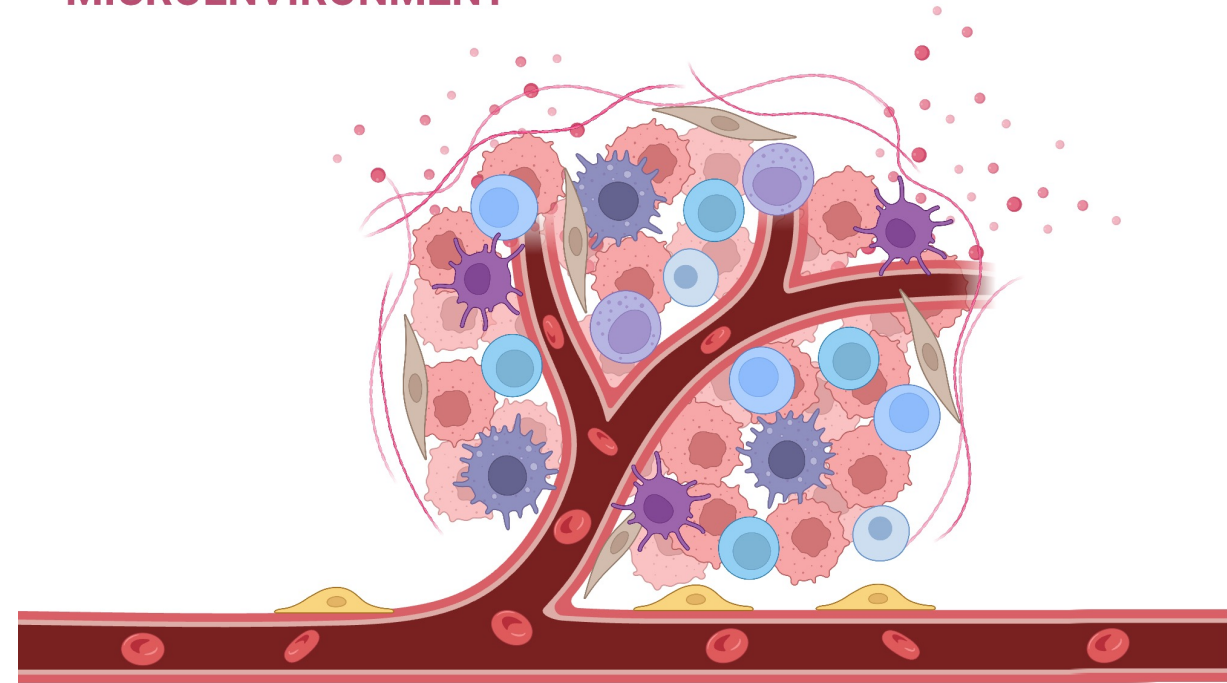
Oxygen in the human body and a cell's microenvironment



Liver sinusoid

- Gradient of oxygen – liver zonation
- Three zones with various oxygen levels (~4 to 13%)
- Impact on cell metabolism, function, and secretion profile (cytokines)

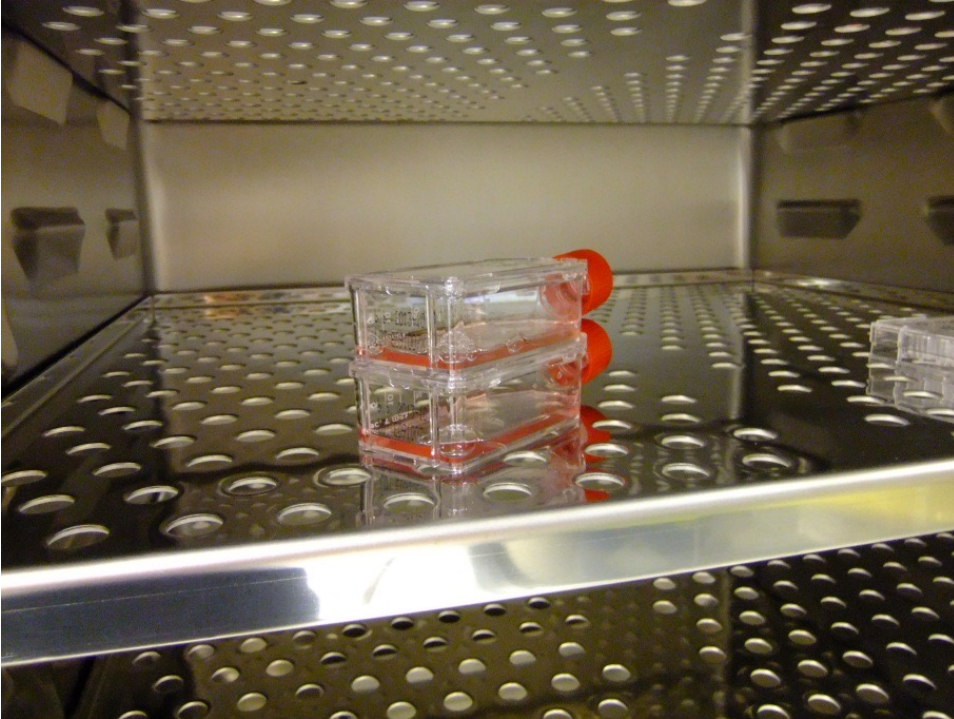
TUMOR MICROENVIRONMENT



- **Tumor tissue: hypoxic environment**
 - Impact on cell phenotype, tumor progression, response to treatments (chemotherapeutics, radiation, etc.)

Oxygen tension and cell culture

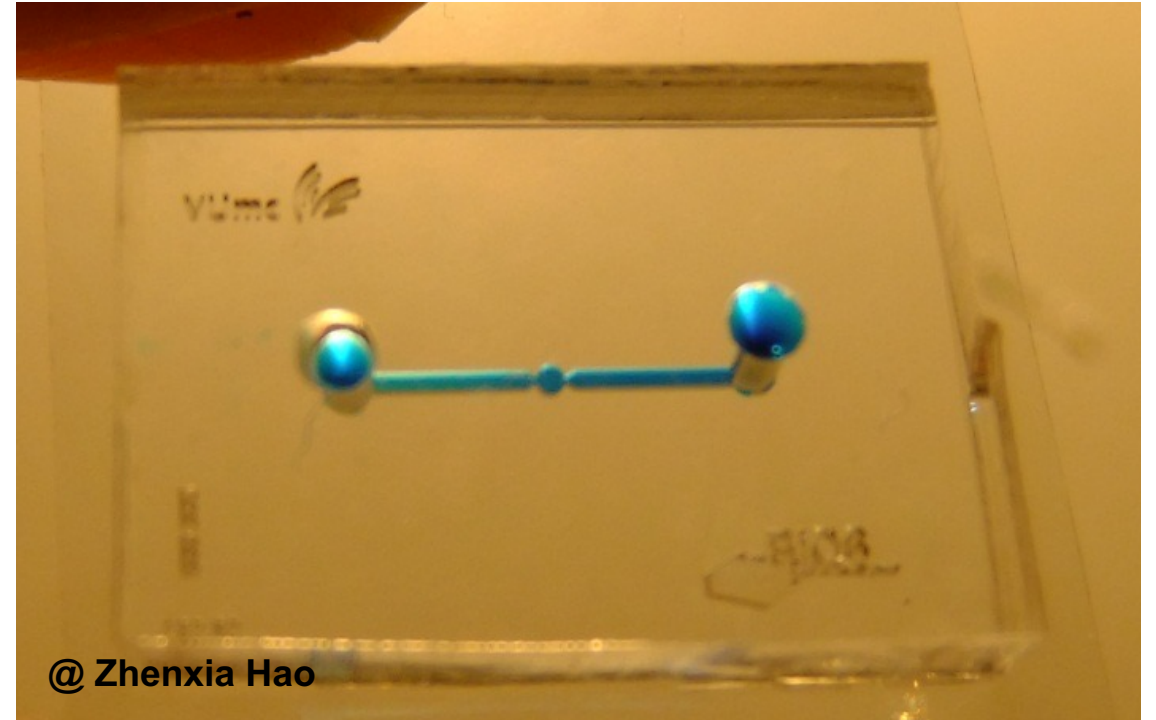
Conventional cell culture



- Cell tension set at the incubator level (atmospheric vs. 5% (hypoxic conditions))
- Variations across the entire incubator (no micrometer-scale /cell-scale control)

⇒ Non physiological conditions!!

Microfluidic cell culture

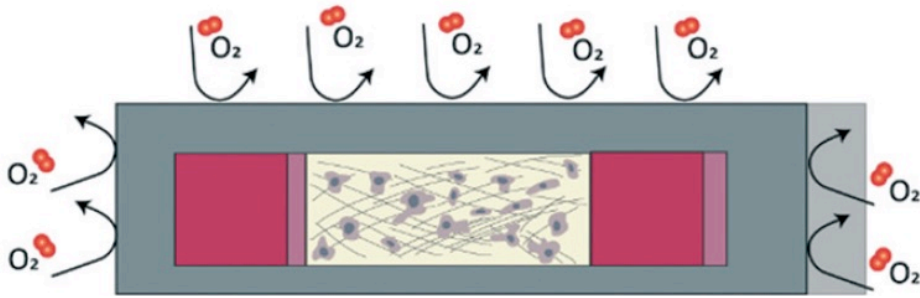


- **High surface-to-volume ratio**
 - ⇒ Efficient surface exchange phenomena
 - ⇒ Possibility to create gradients in oxygen across tissue models

⇒ More physiological conditions!!

Controlling and monitoring oxygen in OoC models

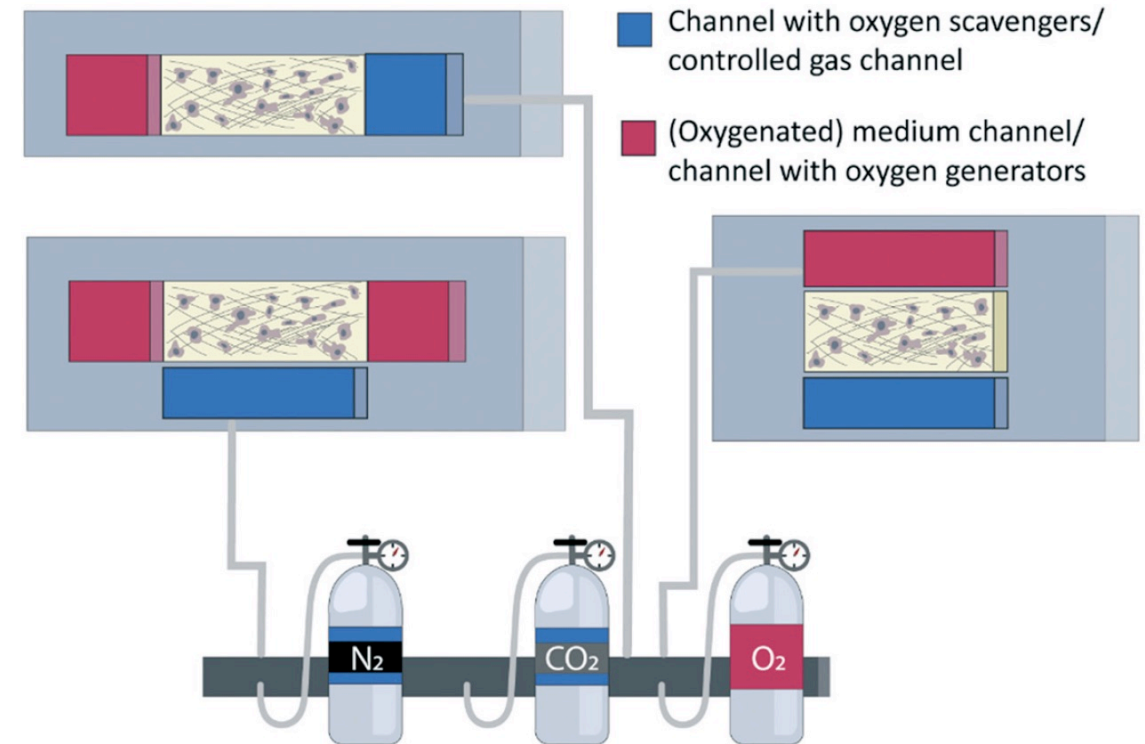
- **Approach 1:** Use of gas-impermeable materials to build the device - possible perfusion of medium at the right oxygen level



Glass, SU-8, polystyrene (PS), cyclic olefin copolymer (COC)

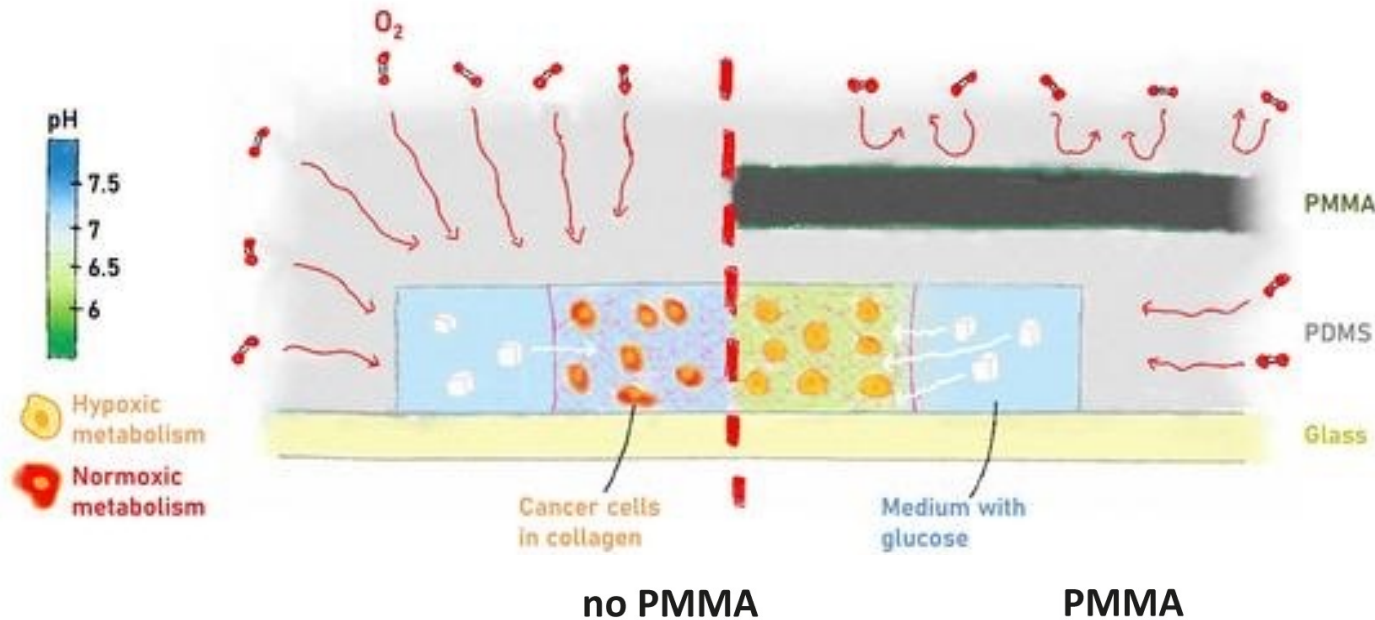
- Dynamic regulation of the oxygen tension via perfusion
- Oxygen tension in the device set by the cells and their metabolism
- Possibility to create gradients of oxygen concentration across a cellular model

- **Approach 2:** Implementation of additional layers/channels in which fluids (gas/liquids) at a given oxygen level or supplemented with oxygen-producing/-scavenging chemicals, are perfused

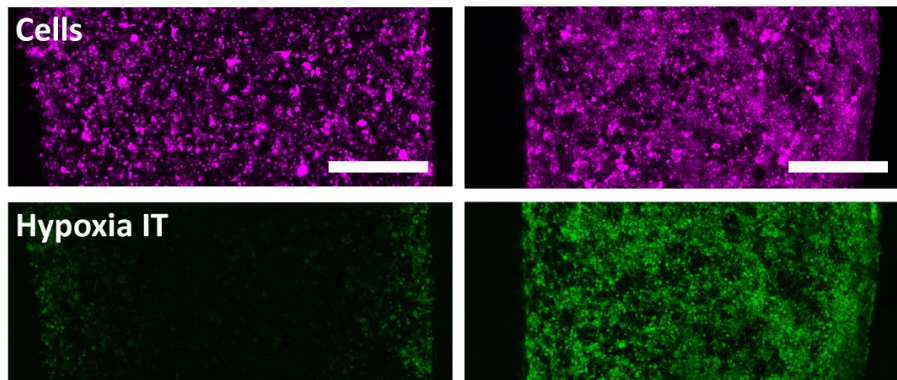
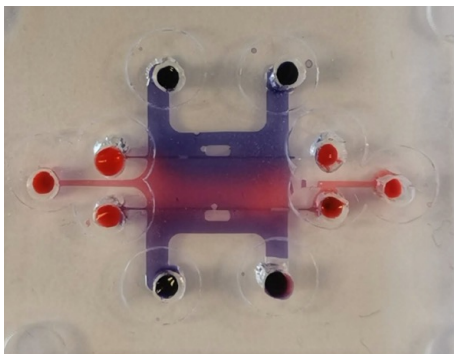
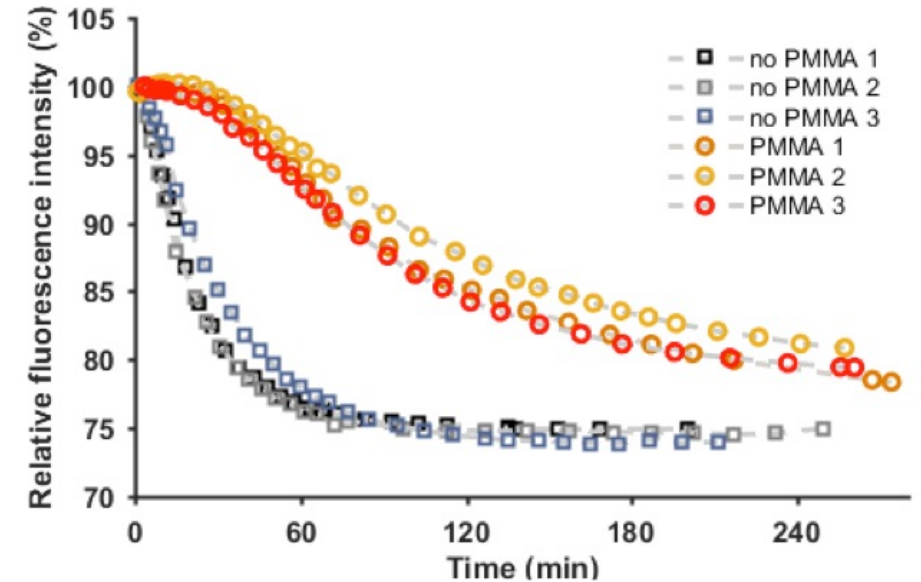


Tumor-on-chip – Hypoxic environment

Establishing a hypoxic environment in a tumor-on-chip model

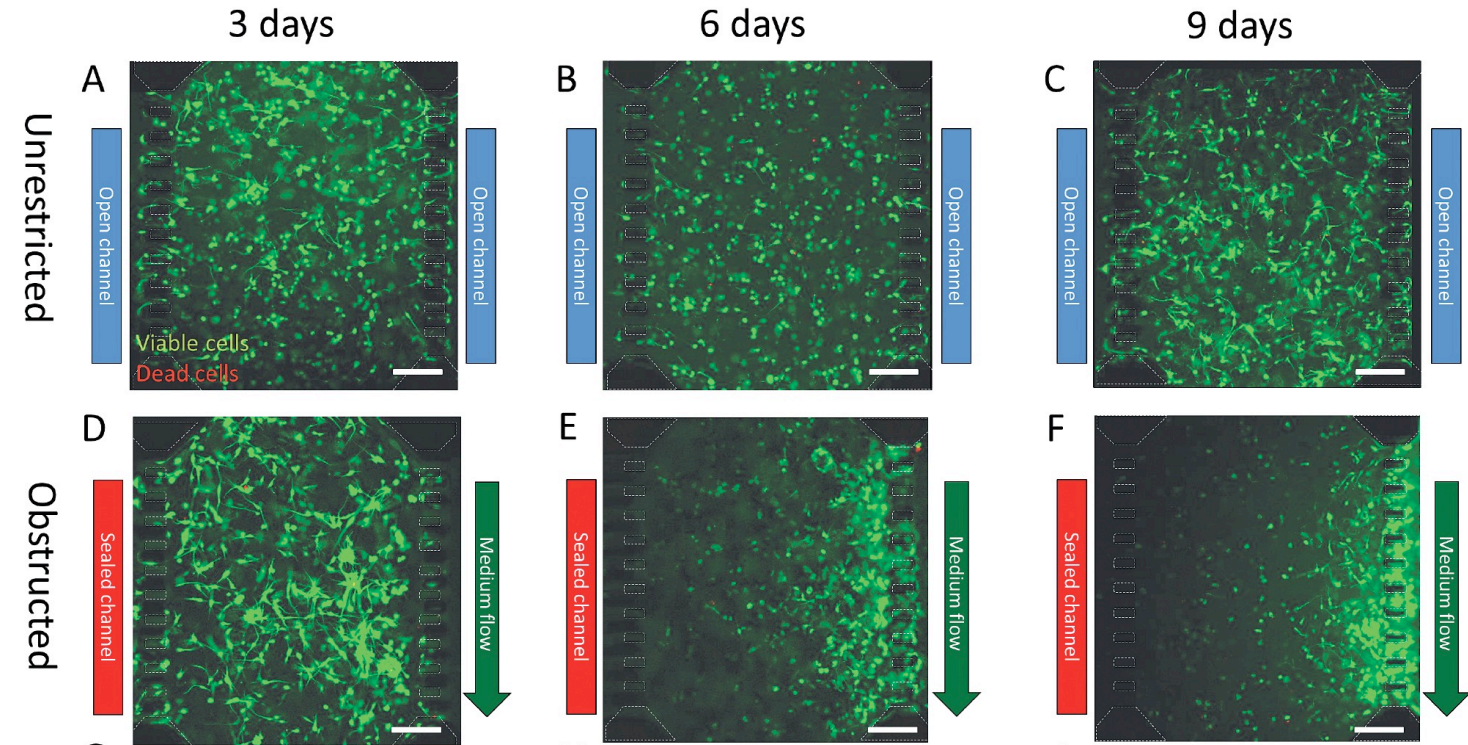
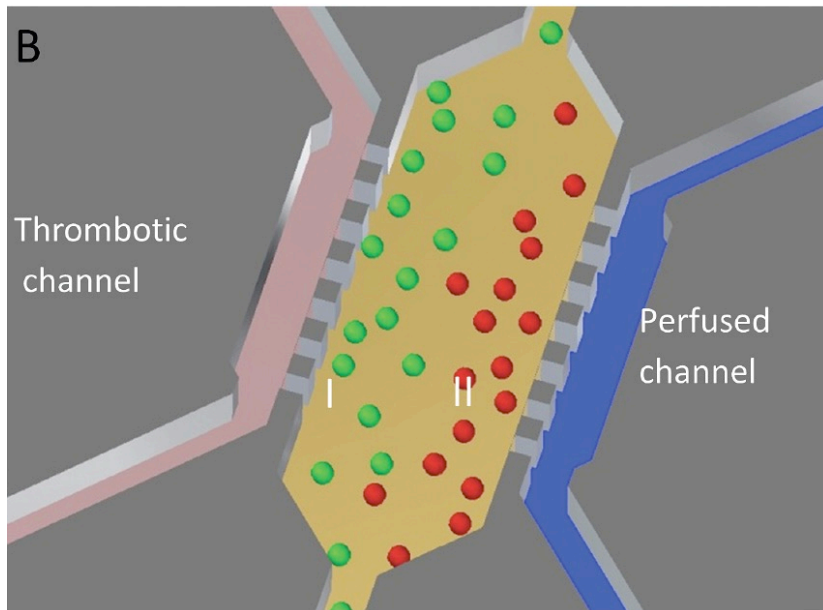
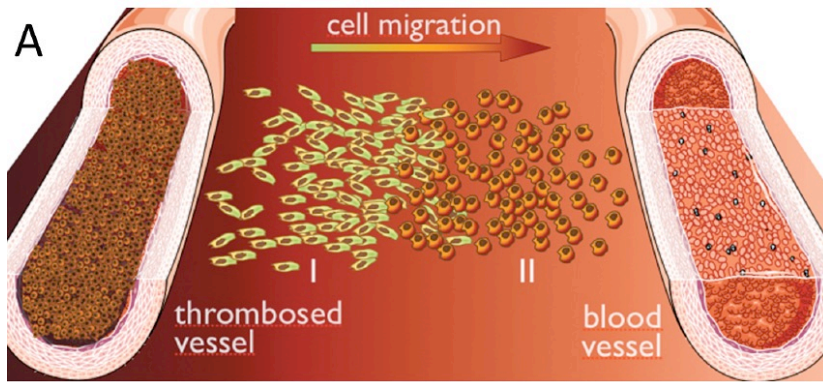


Characterization using oxygen-sensitive dyes



- Blocking O_2 diffusion through the PDMS
- Culture of U-251 MG cells in collagen matrix
- Successful creation of hypoxic conditions
- Cell metabolic switching under hypoxic culture conditions

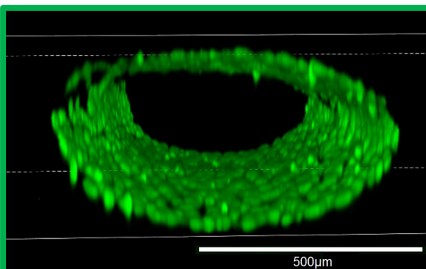
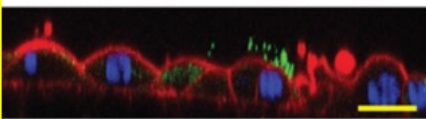
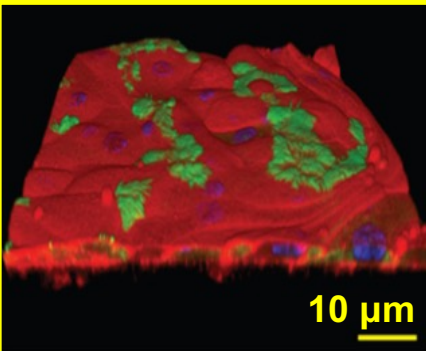
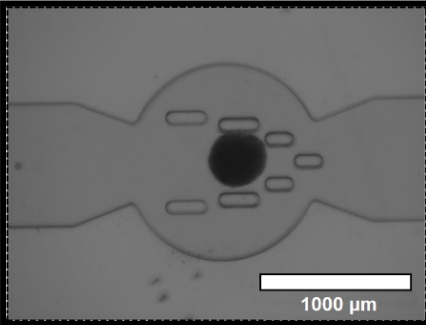
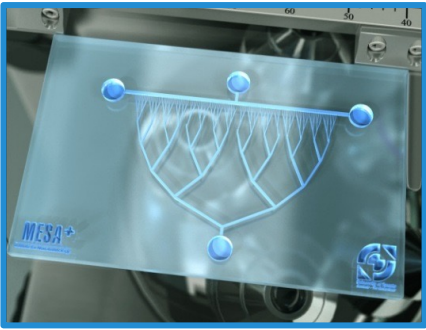
Tumor-on-chip – Hypoxia-induced cell migration



- Device fabricated from an oxygen-impermeable material (SU-8)
- Culture of glioblastoma U251-MG cells in collagen
- Medium perfused in one channel only
- Strong migratory behavior of cells in presence of hypoxia (Pseudo-lapalisade)

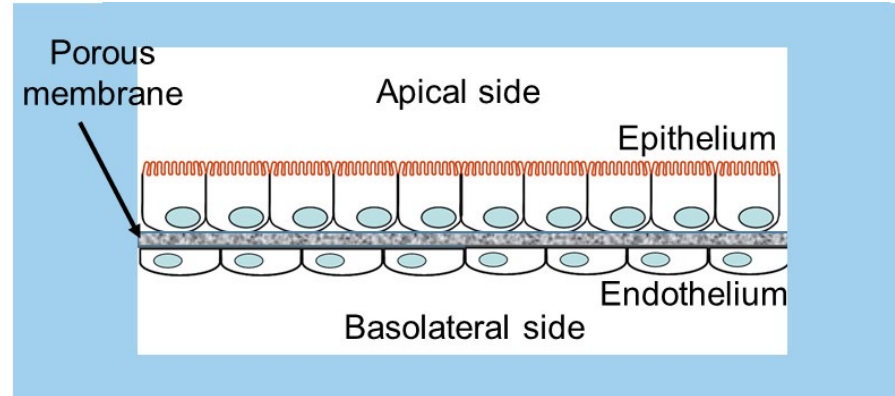
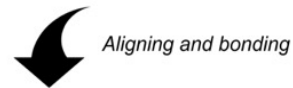
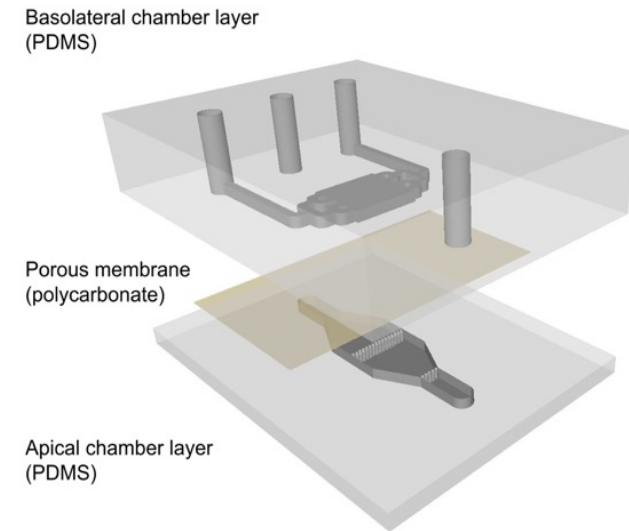
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Organ-on-chip models – Classification (2)

Compartmentalized device in a microfluidic format

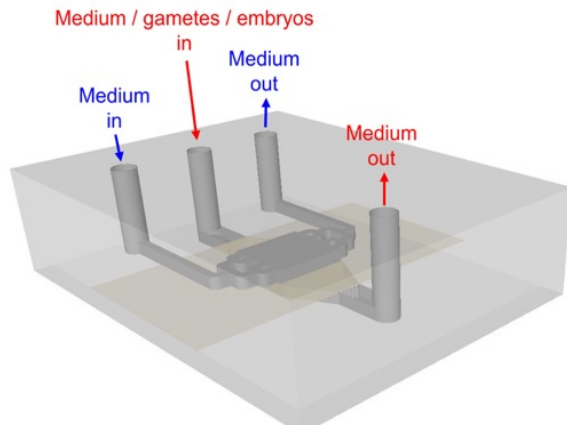
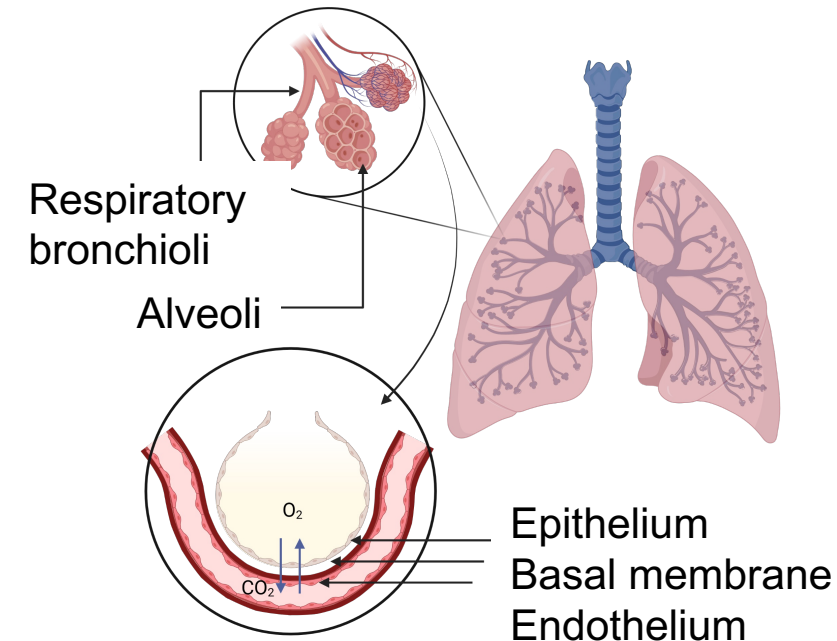


Two channels (compartments) separated by a horizontal membrane (or a vertical “porous” wall)

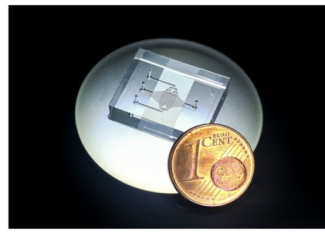
Goals

- to study **cell** chemical **communication**
- to model **physiological barriers** (kidney, gut, lung, oviduct, BBB, etc.)

Porous membrane + flow $\Rightarrow$ Polarization and differentiation of epithelial cells

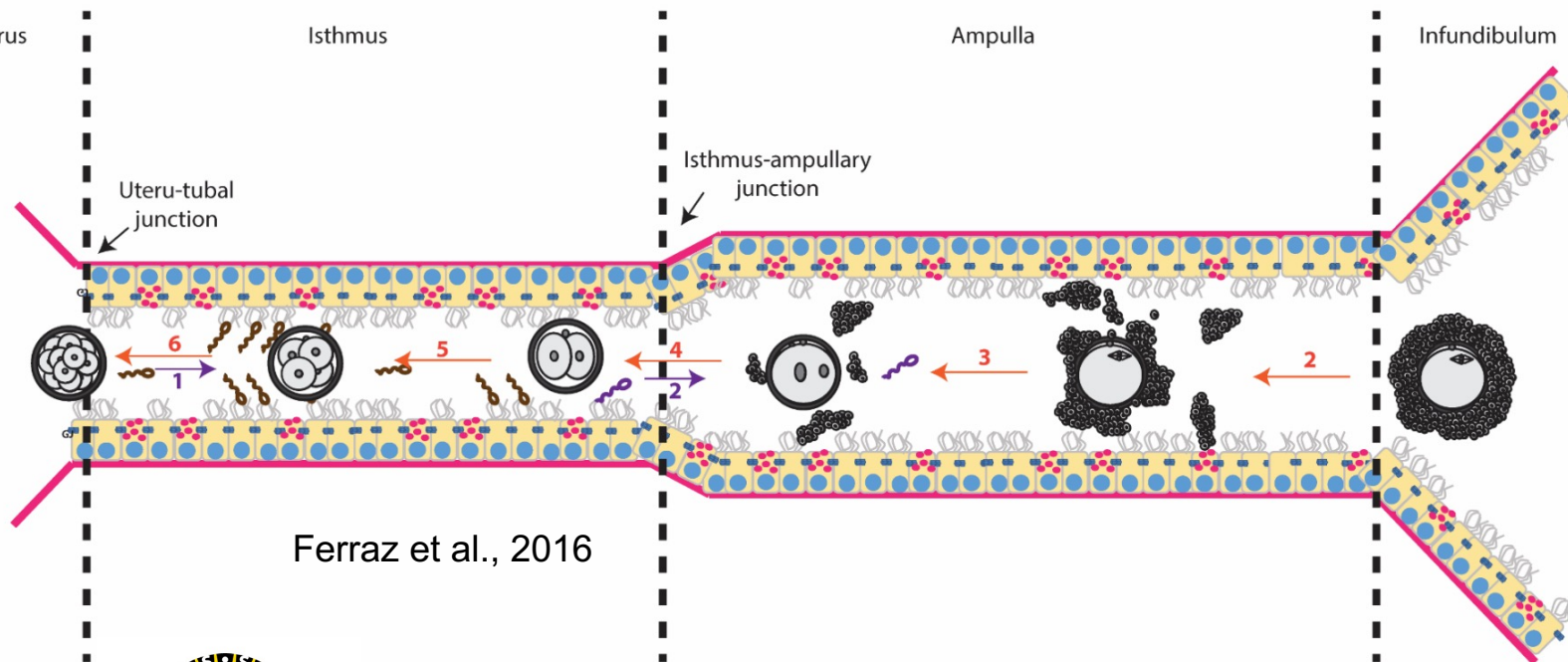
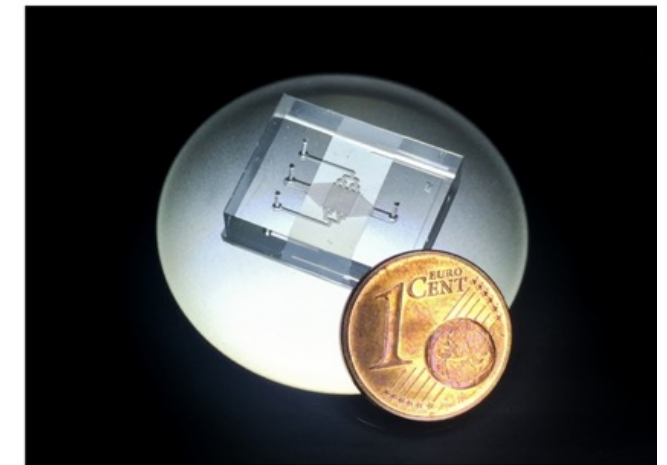


Compartmentalized approach - Oviduct-on-chip

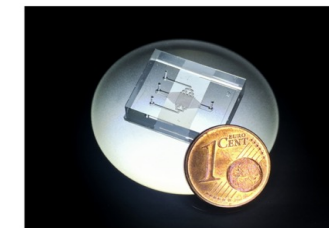


- Embryos produced using conventional ART:
 - Developmental arrest** *in vitro*;
 - Molecular aberrations** at early-stage development (epigenetic level);
 - Increased incidence for certain **diseases and disorders**: e.g., metabolic and cardio-vascular diseases, cancer, etc.

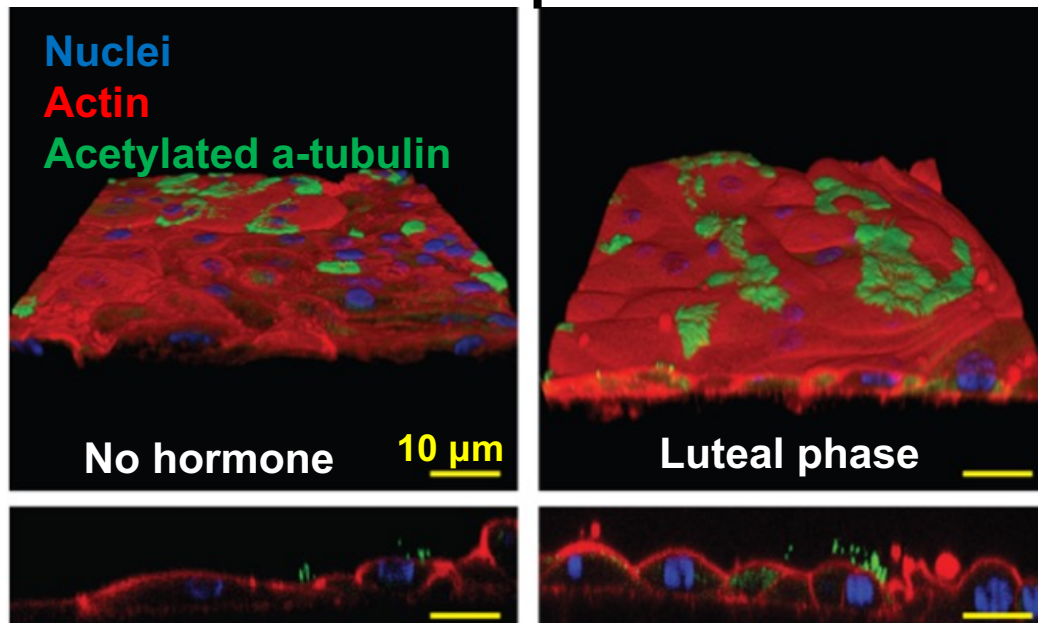
- Use of a **biomimetic environment** for the *in vitro* production and culture of bovine embryos.



Compartmentalized approach - Oviduct-on-chip

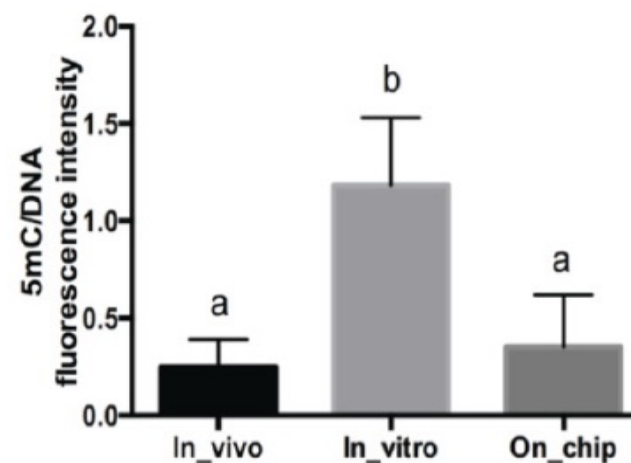
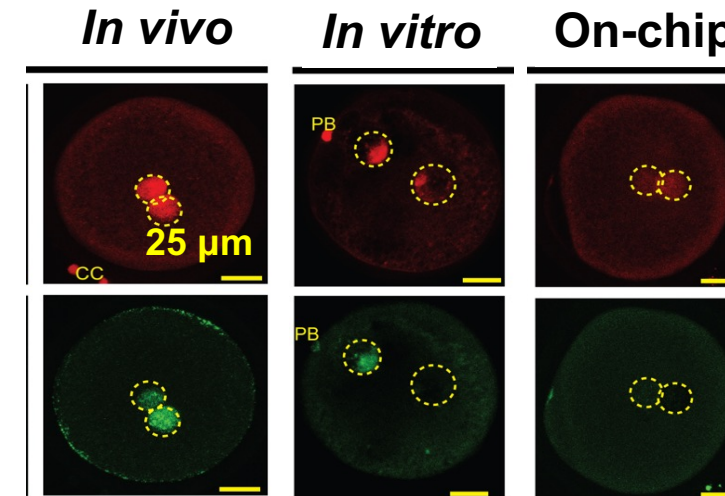


- Functional and differentiated bovine oviduct epithelium



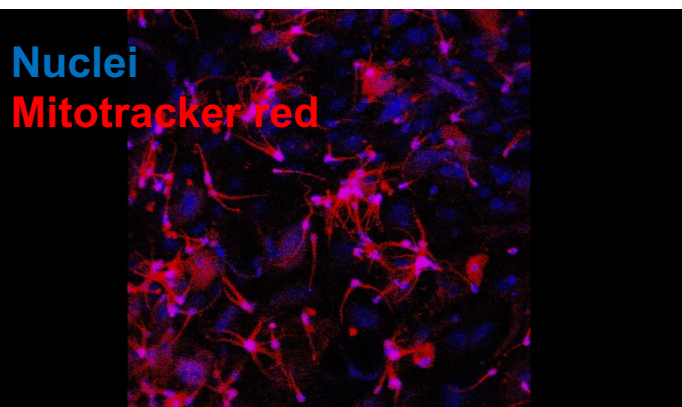
- In vitro* produced embryos: molecular characterization (zygote stage)

Propidium iodide (DNA)
5mC (global methylation)



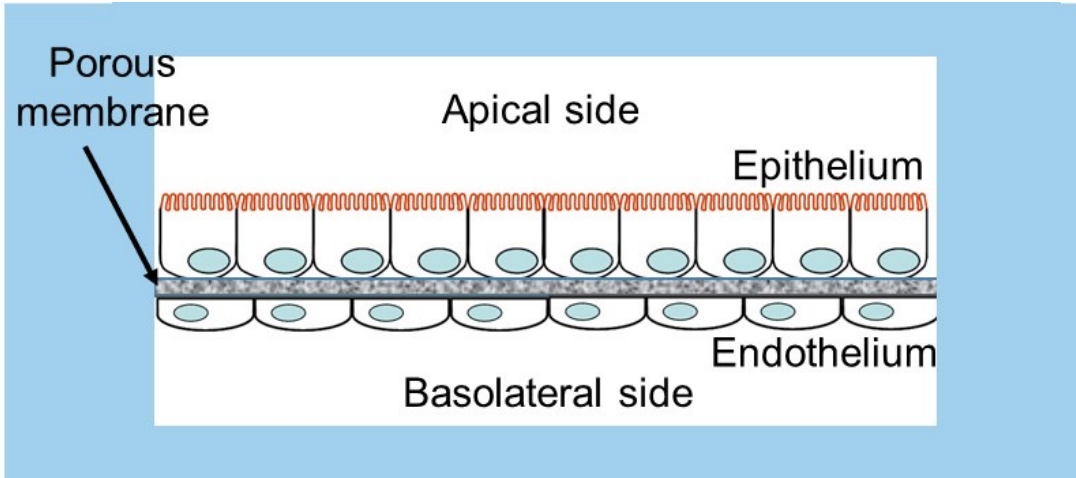
- On-chip produced embryo's: methylation patterns closer to the *in vivo* situation.
- Reduced stress?

Ferraz et al., *MicroTAS* 2017; *Nat. Comm.*, 2018



- Tight monolayer
- 3D morphology
- Responsive to hormonal treatment
- Cilia structures
- Interaction with sperm

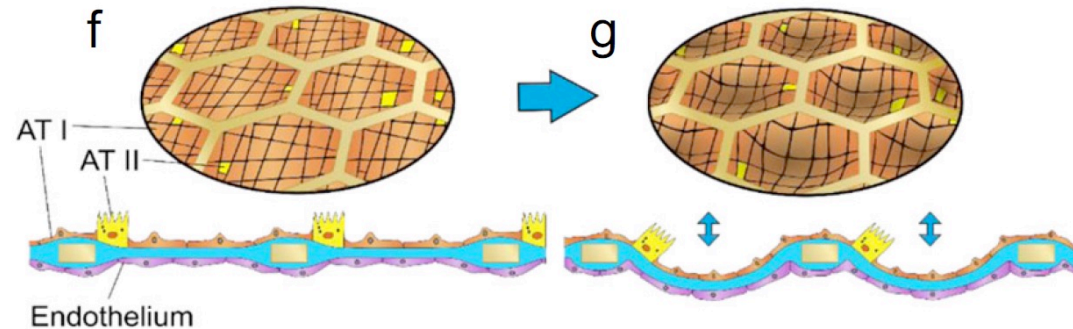
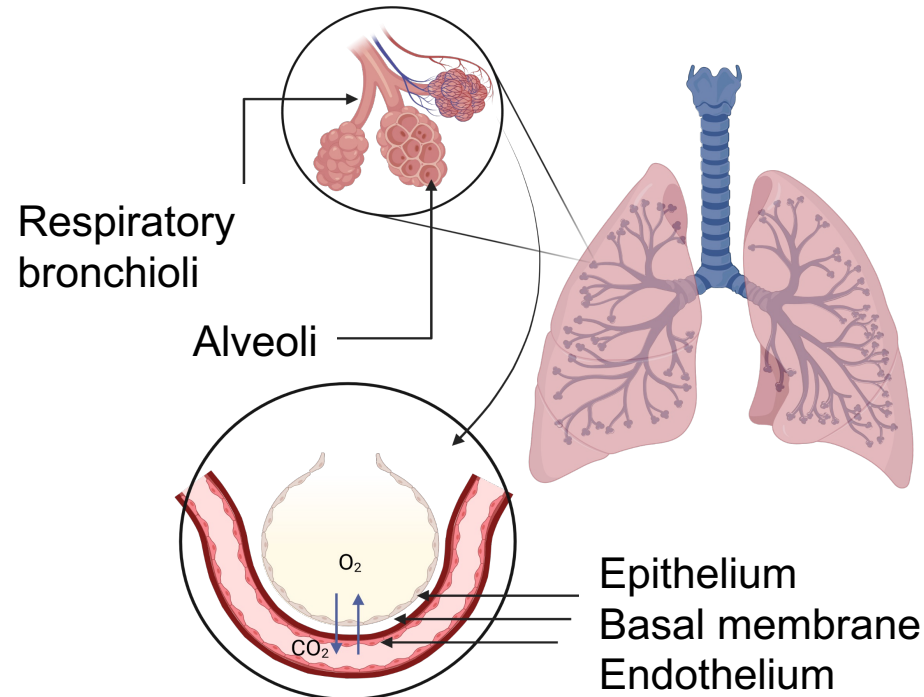
Compartmentalized approach - Limitations



In vitro vs. in vivo

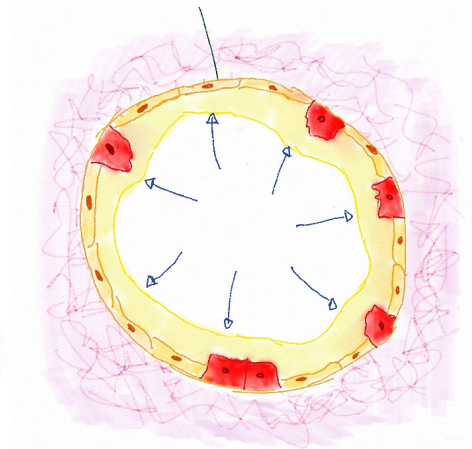
- Flat vs. curved
- Hard vs. soft
- Thick vs. thin
- Protein-rich vs. synthetic plastic

⇒ **Biomimetic?**



Collagen-elastin mixture on a metal mesh

Zamprogno et al., Comms Biol, 2021
doi.org/10.1038/s42003-021-01695-0

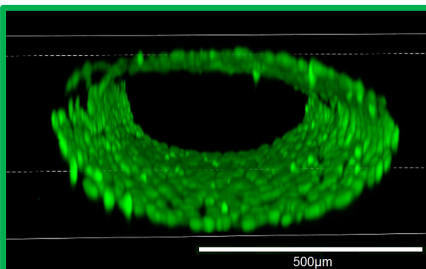
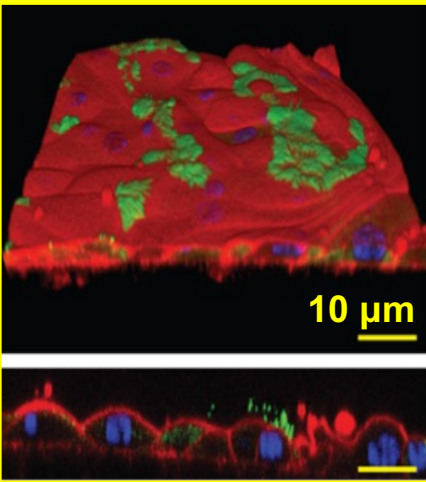
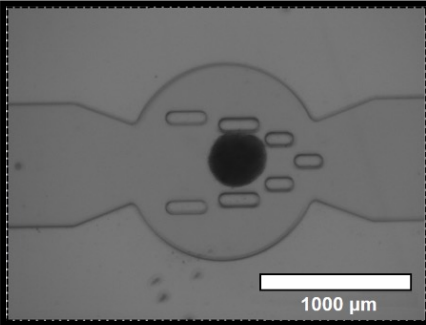
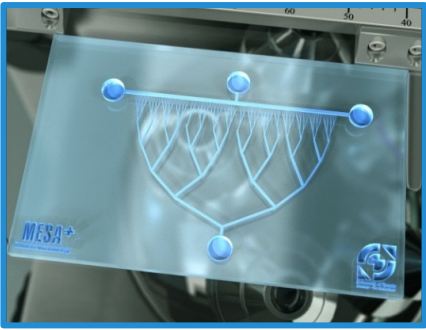


Cylindrical lumen in a soft ECM-like matrix

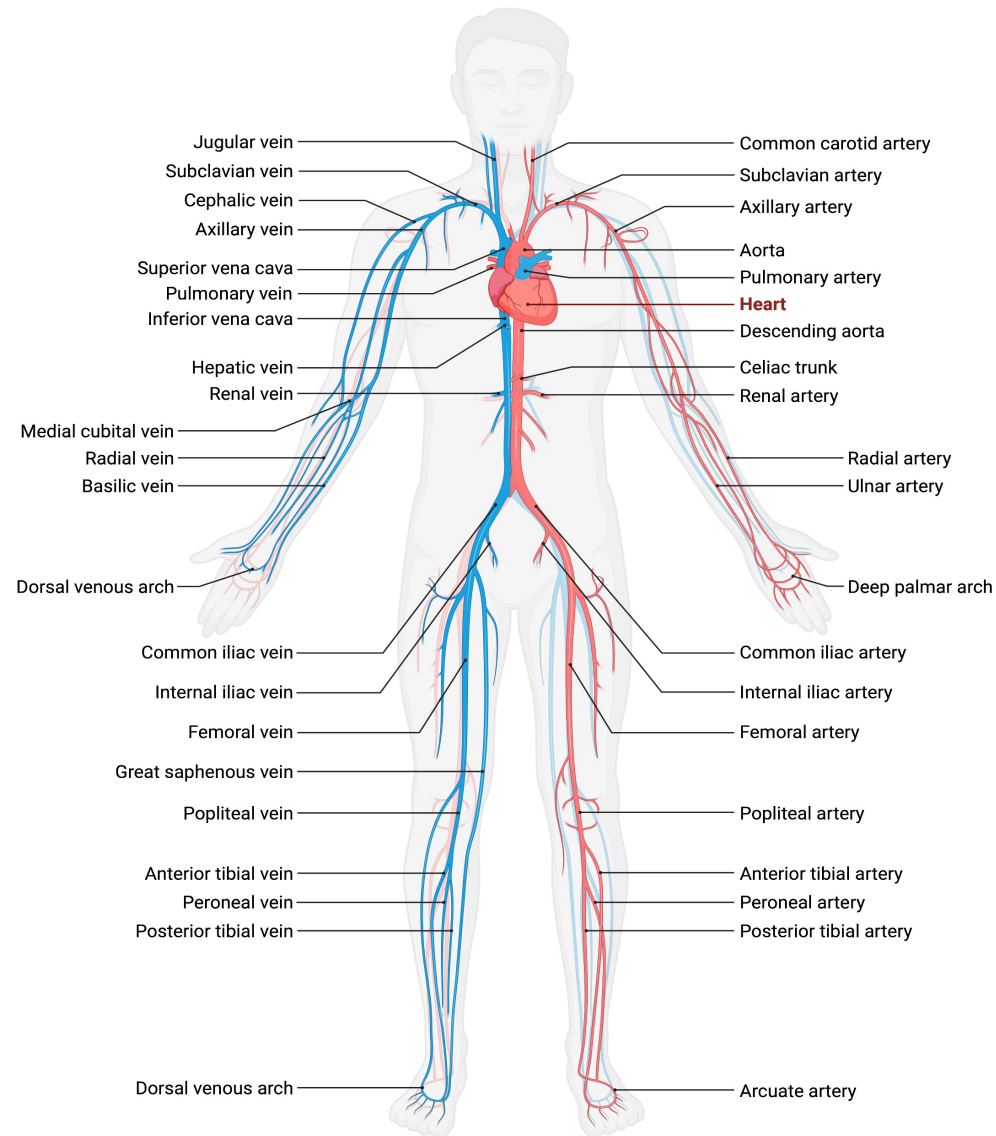
*Unpublished results;
@ Bastien Venzac*

Scope and content

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- **Approaches to develop organ-on-chip models / Classification**
 - **Microbioreactor** approach
 - How to model the tissue?
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 - Tissue (mechanical) actuation
- **Critical analysis:** Opportunities and challenges



Tissue vascularization



Roles in physiological/healthy conditions

- Providing nutrients and oxygen
- Removing waste products
- Supporting inter-organ communication
- Maintaining homeostasis of the body ($T^{\circ}\text{C}$, hormones)

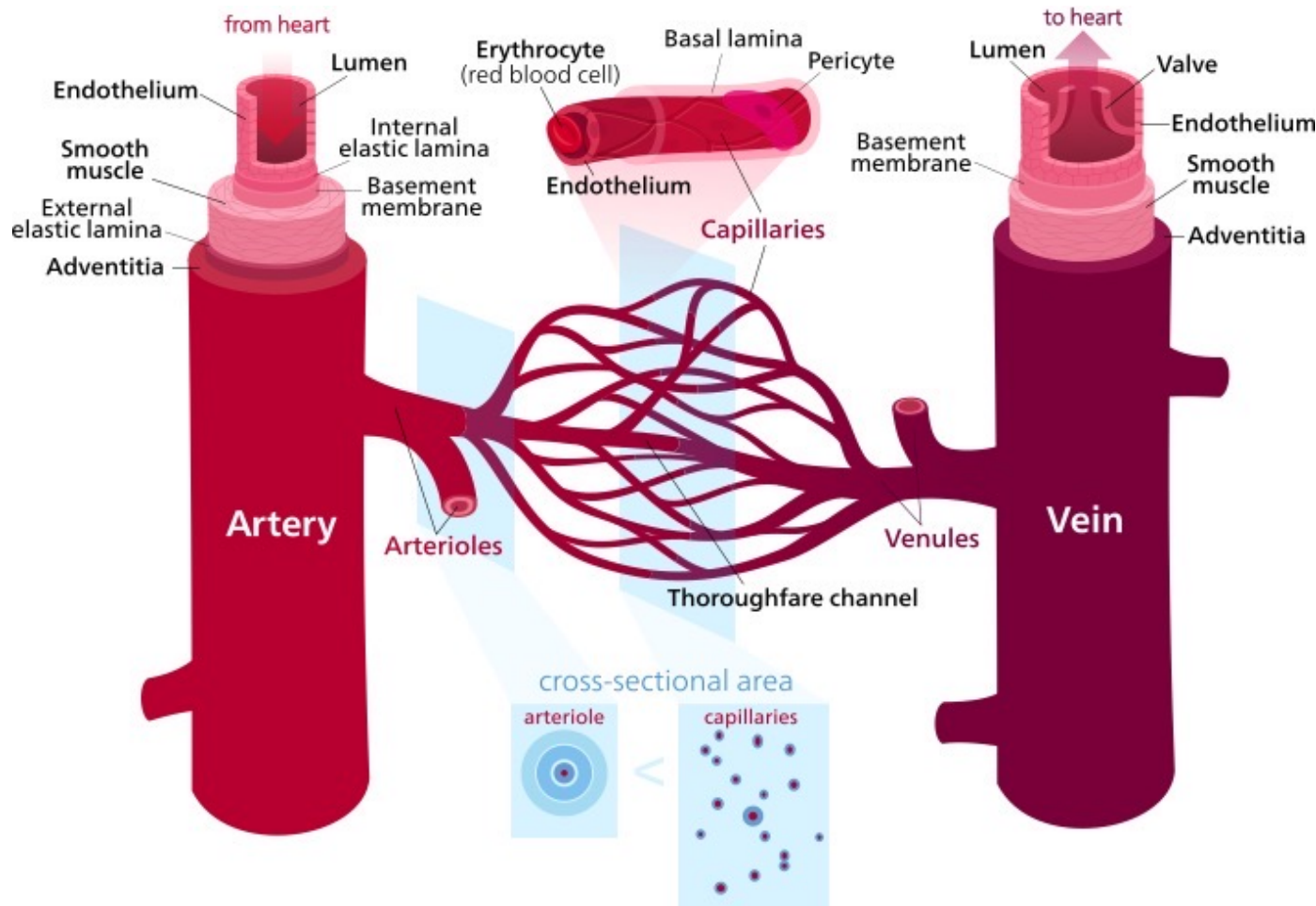
⇒ All cells in the body at $< 200\ \mu\text{m}$ of a blood vessel!!

- Essential in immune cell interactions
- Endothelium: barrier to be crossed by drugs, exogenous substances, etc.
- Specialized endothelium in all organs – organ-specific
- Liver: selective passage of nutrients (fenestrae)

Roles in pathological conditions

- Cardio-vascular diseases – alterations in vasculature
- Thrombosis
- Cancer: deformation and higher permeability
- Diabetes, etc.

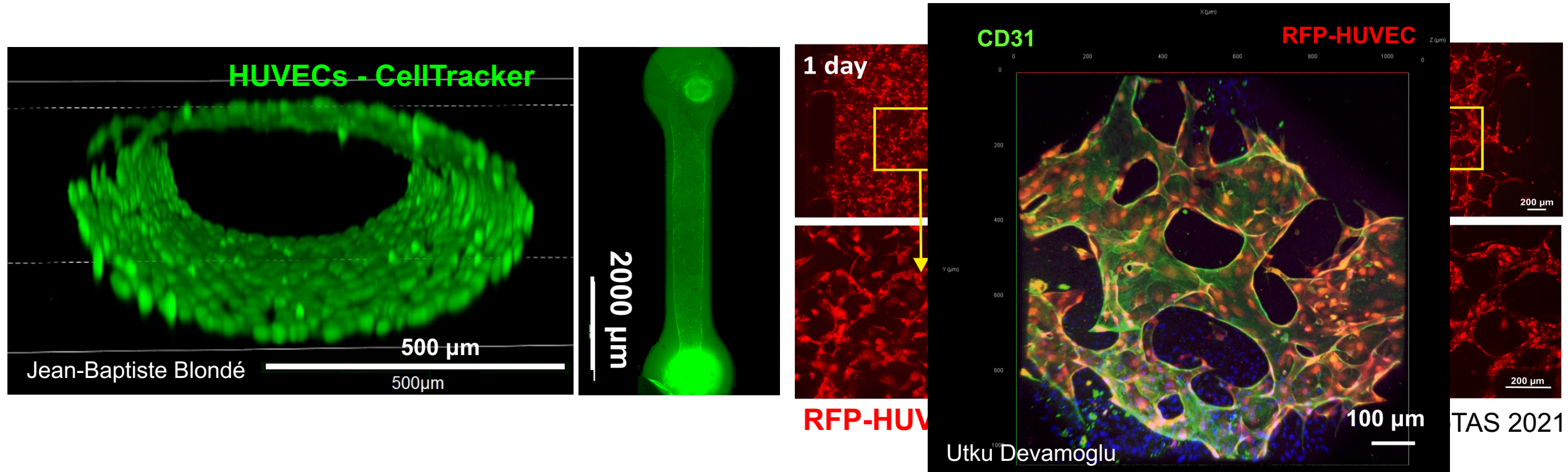
Tissue vascularization



■ Vascular system:

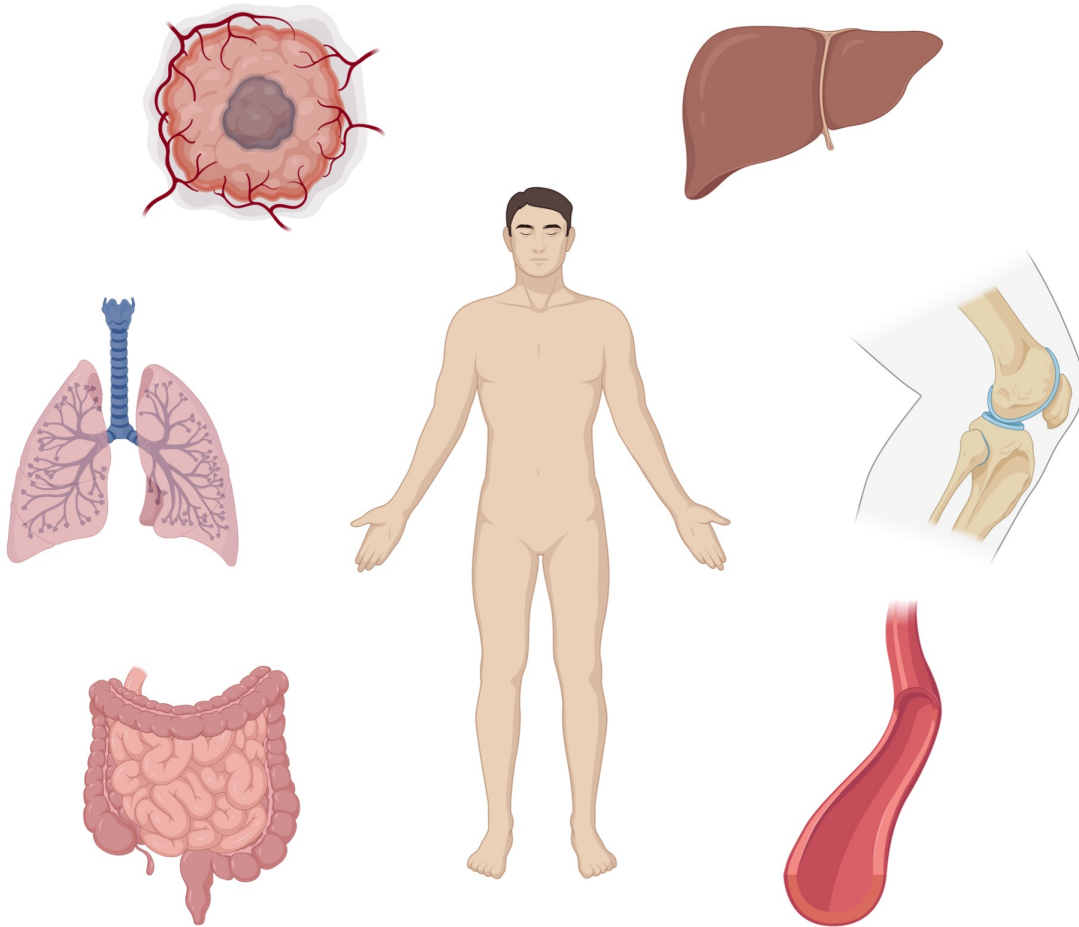
- Total length: 100,000 km.
- Blood vessels with different characteristics dimensions: from artery (25 mm) to capillary ($\sim 8 - 10 \mu\text{m}$).
- Lined with endothelial cells (organ-specific) and surrounded by supporting cells.

Tissue vascularization



- **Large blood vessels** in separate channels
 - Channel lined with endothelial cells (physiological barrier)
 - Patterning of a lumen in a hydrogel material
- **Smaller blood vessels (network):**
 - Endothelial cell self-assembly and/or migration induced by growth factors or a co-culture with fibroblasts that secrete said growth factors (vasculogenesis, angiogenesis).

Tissue mechanical stimulation



Mechanical stimulation and the Human body

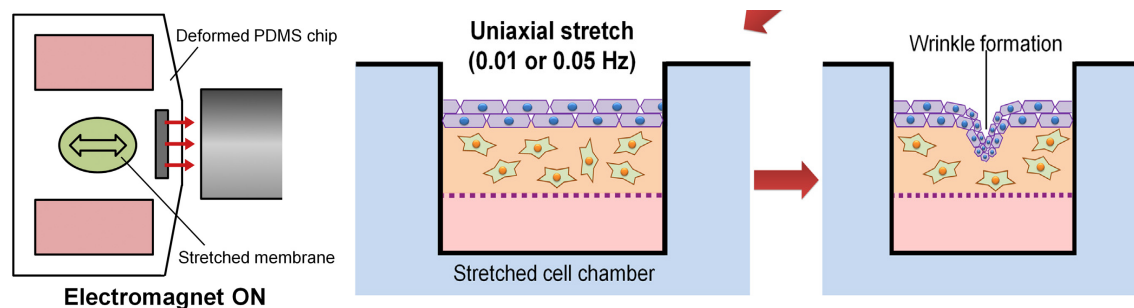
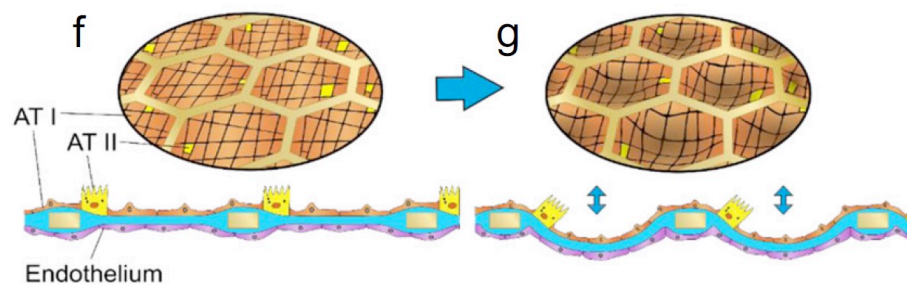
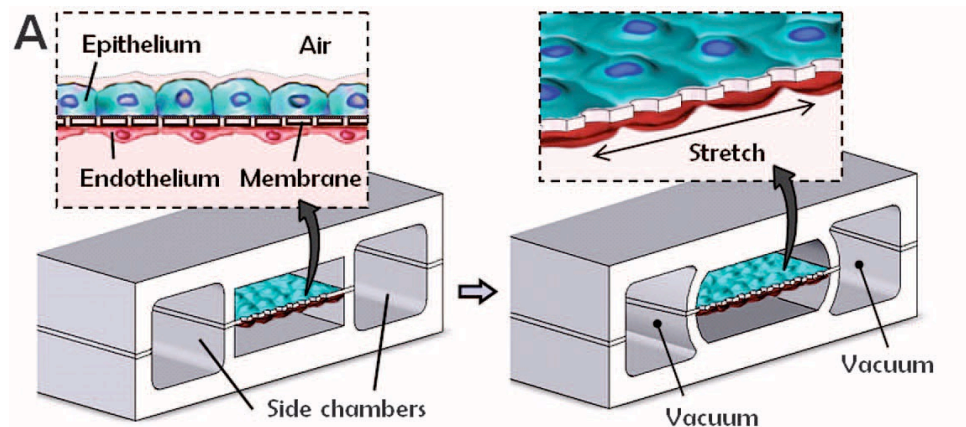
- **Variety of mechanical cues:** Stretching, compression, bulk shear, peristaltic motion, etc.
- All essential for tissue homeostasis, regulation of biological processes, inherent to physiological processes.
- Essential to include when modelling tissues and diseases.

▪ **Essential to include mechanical cues in OoC models!**

Tissue mechanical stimulation

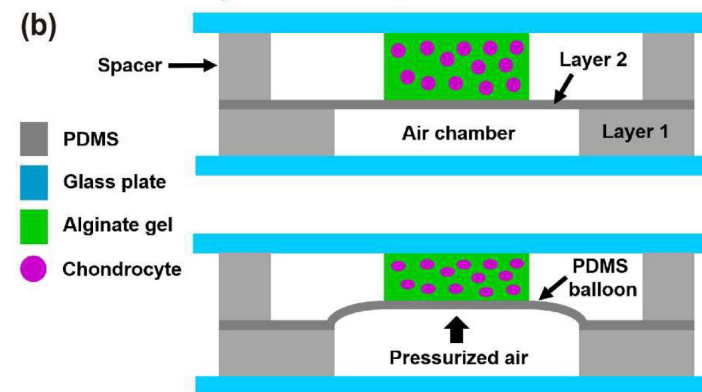
Cell stretching (lung-on-chip/skin-on-chip)

Huh et al.,
Science, 2010

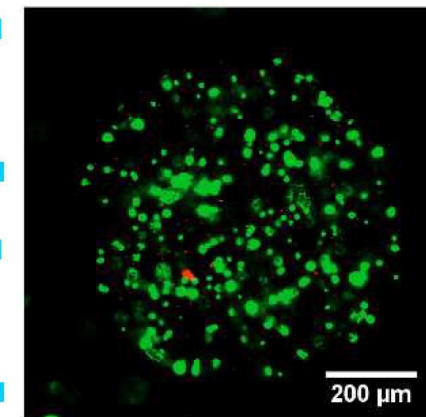


Lim et al., J. Ind. Eng. Chem., 2018.

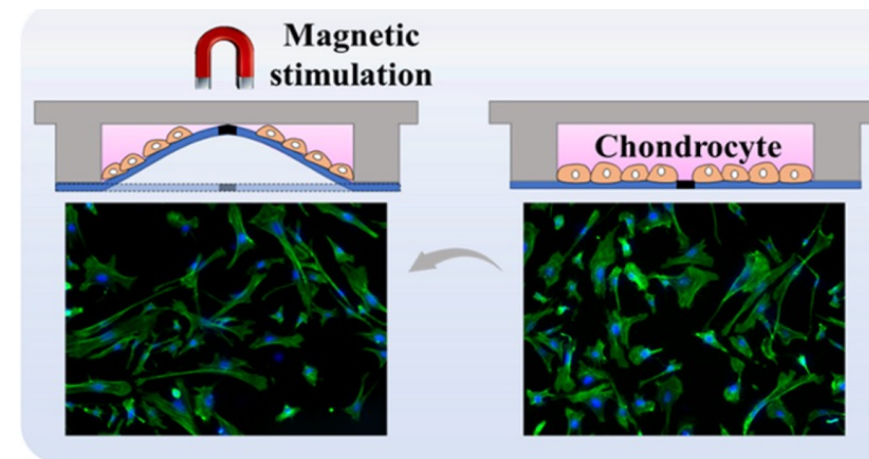
3D tissue compression



Lee et al., Lab Chip, 2018



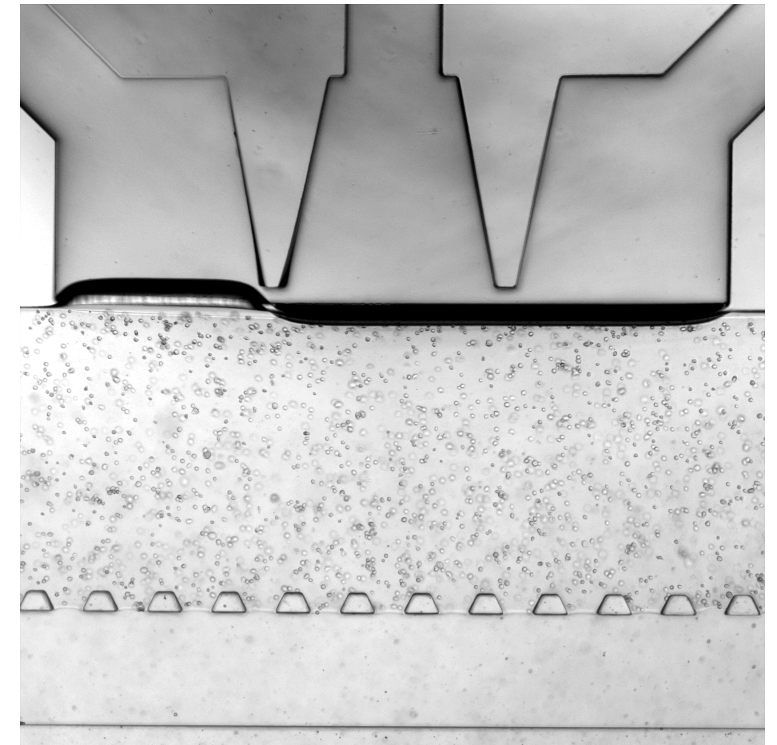
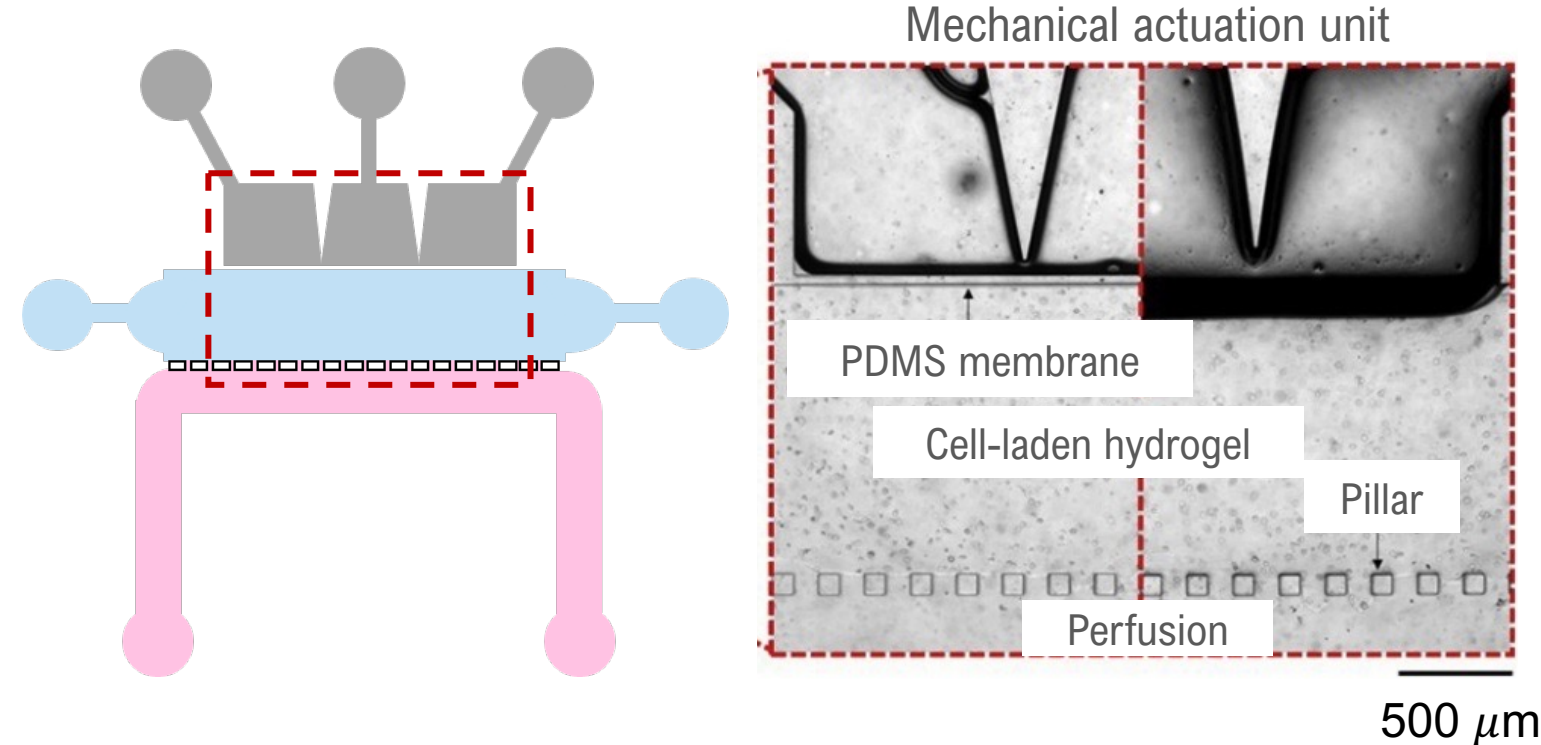
Strain application on cartilage tissue



Liu et al., Bioactive Mat., 2024

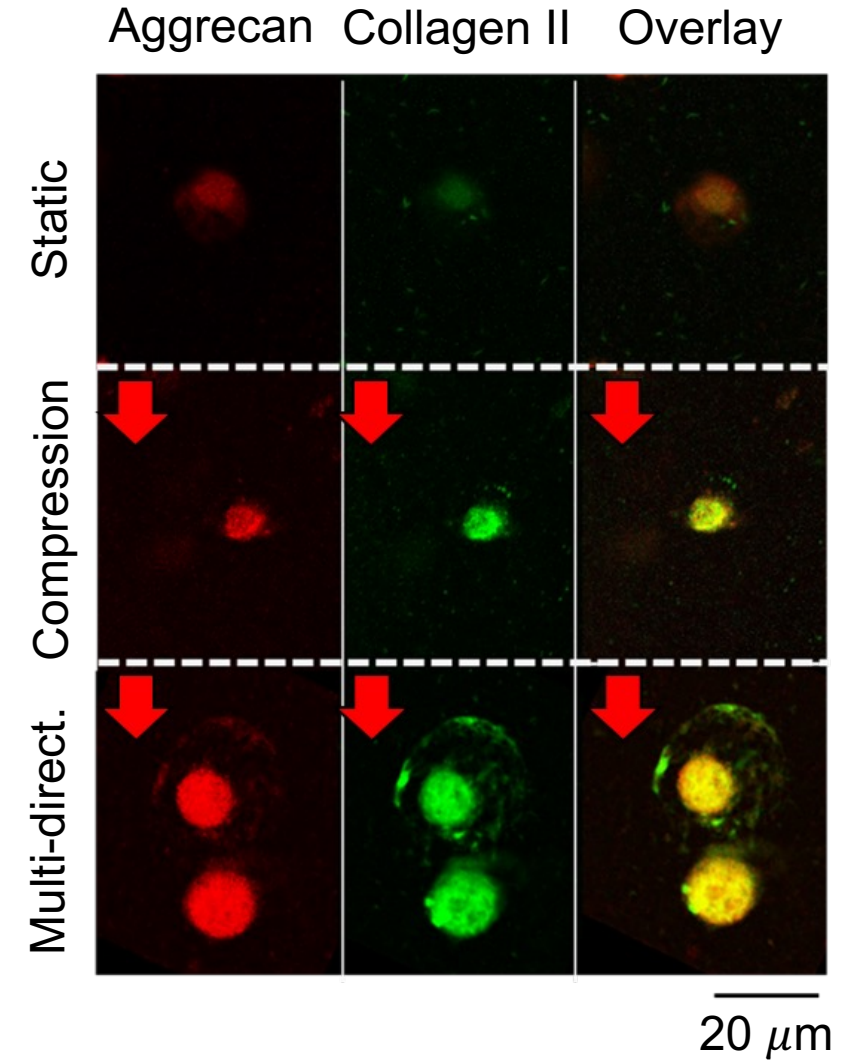
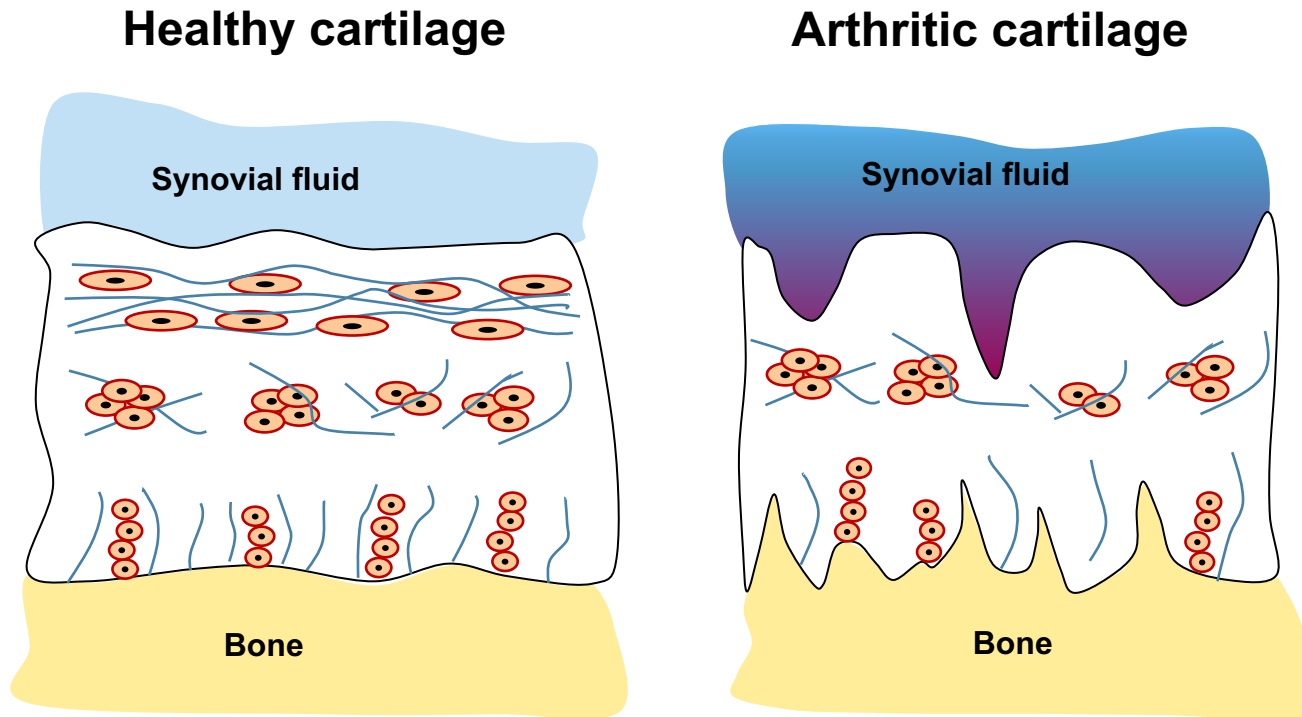
Implementing mechanical stimulation - Cartilage-on-chip

- On-chip module for the mechanical stimulation of 3D cellular models
- **Highly versatile:** possible application of compressive, shear and (possibly) stretching forces
- **Fully programmable:** duration, frequency, amplitude, negative and/or positive, etc.



Implementing mechanical stimulation - Cartilage-on-chip

- Physiological stimulation of cells
- Balance between production and degradation of the extra-cellular matrix (ECM)

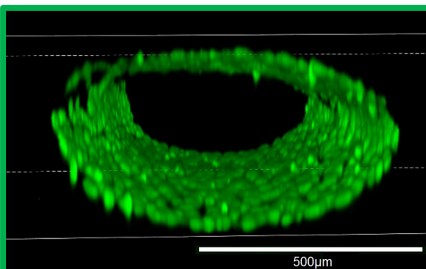
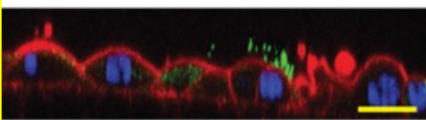
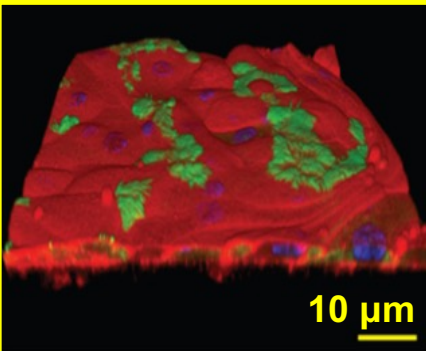
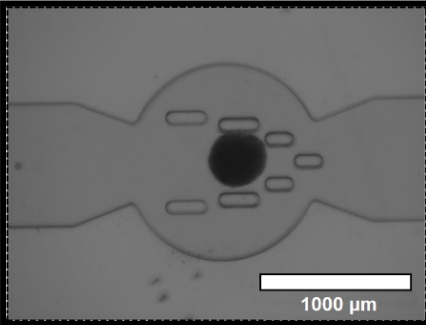
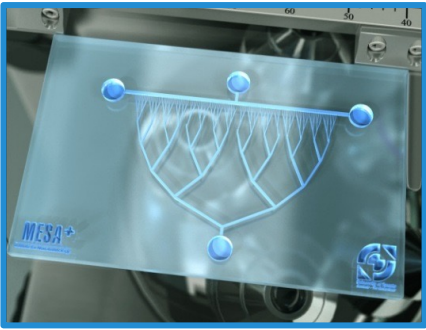


Paggi, Venzac et al., Sens. Act. B 2020;
Paggi et al., Lab Chip, 2022

- **Impact on ECM production:** Re-creation of the cell microenvironment in terms of ECM.

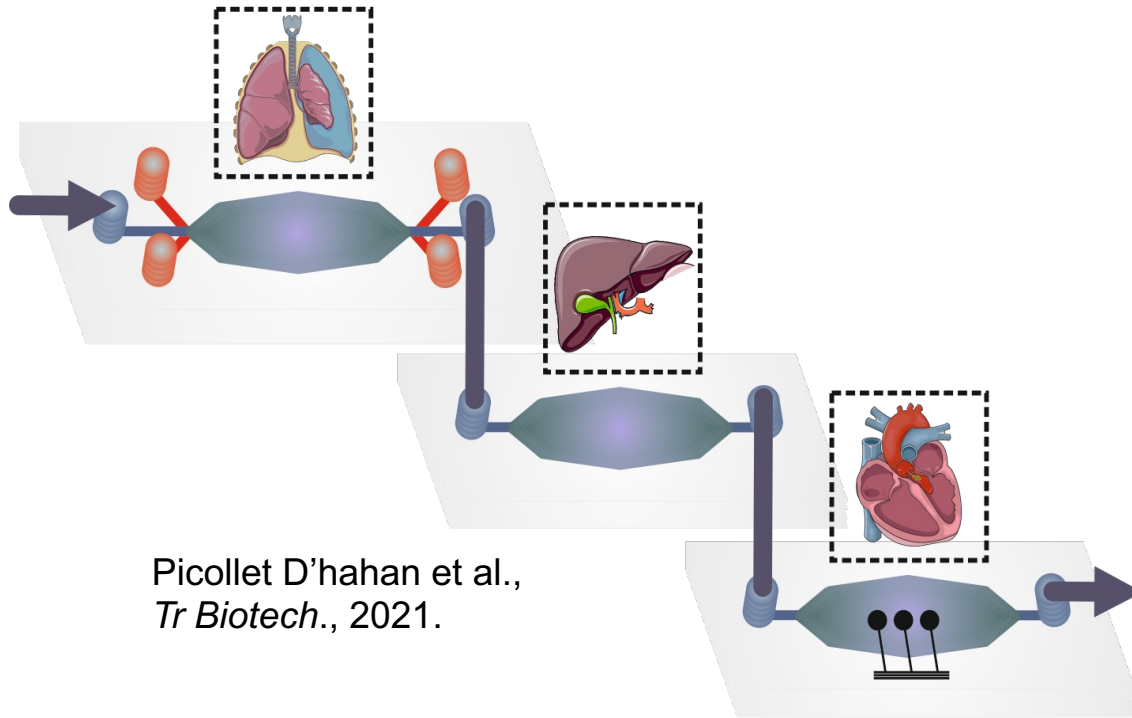
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Organ-on-chip models – A critical analysis

Promises - Powerful & sophisticated *in vitro* models



- Organ architecture; control on the cell microenvironment; patient-specific cells
- Dynamic conditions: continuous, pulsatile, direct or no direct flow
- Microfluidics: parallelization/automation/small volumes; high-throughput screening

⇒ **Excellent alternatives to animal experimentation**

Organ-on-chip models – A critical analysis

Additional points of attention

▪ Material

Mostly use of PDMS (polydimethylsiloxane); easy fabrication, elastomeric.

BUT: hydrophobic, porous, biocompatible (?), thermal sensitivity, molecular ad- and absorption, oligomer release, etc.

Need for more robust materials for routine and commercial applications

▪ Cells

Often use of commercially available cell lines or primary cells in some cases.

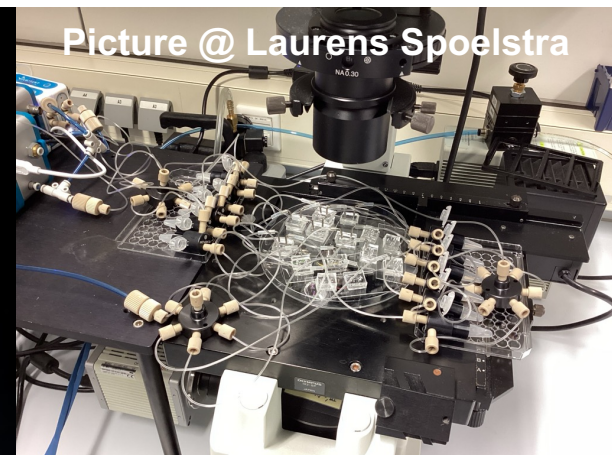
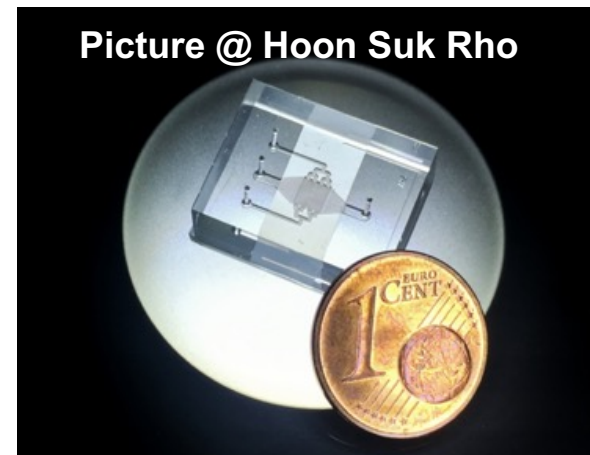
Need for more development to derive relevant cells (iPSCs) or use of ex vivo tissues

▪ Interface

Need for user-friendliness & robustness

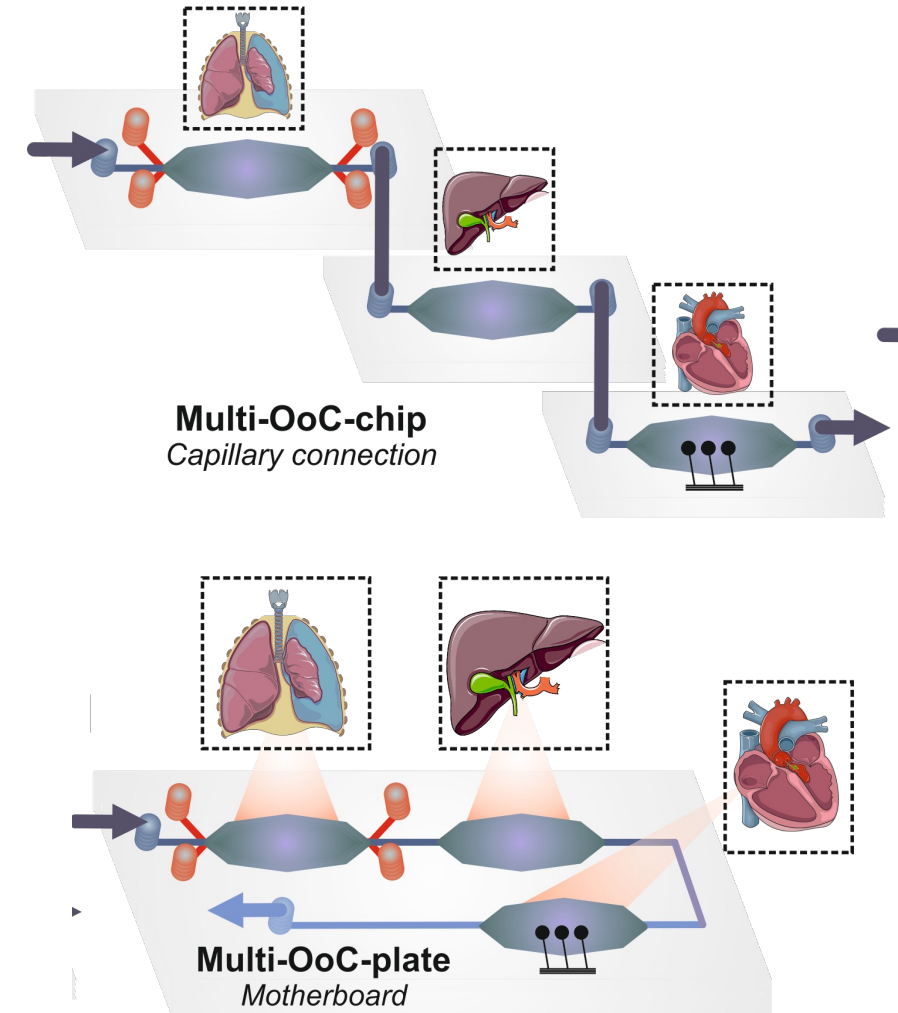
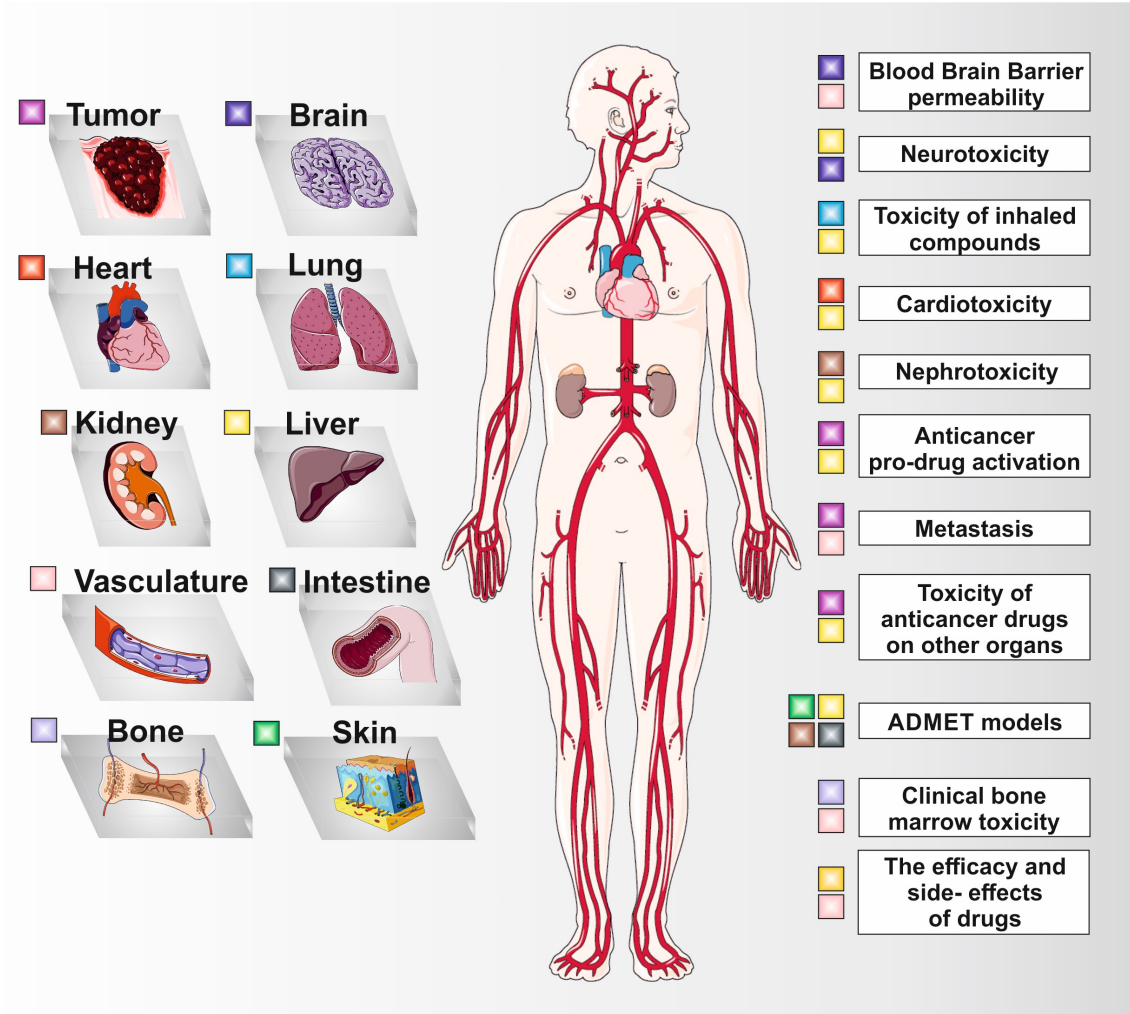
▪ Readout

Need for real-time / in situ / continuous monitoring vs. Endpoint analysis (after sample destruction)



Organ-on-chip models – The future?

Multi-OoC approach: Systemic and cross-organ communication



Conclusion

- OoCs: miniaturized and hybrid models of organs, combining living matter (cells, tissues, etc.) with microfabricated structures and possibly an ECM-like hydrogel
- Promising models to bridge the gap between the human *in vivo* situation and currently used models (*in vitro* / *in vivo* – animals) and to reduce animal experimentation
- Different approaches to realize OoC models:
 - Which ingredients are important? (cells, microenvironment, stimulation, tissue architecture, etc.)
- Remaining challenges in the OoC field
- Towards a body-on-a-chip: Multi-OoC models to include the systemic dimension and inter-organ communication

Introduction to Organ-on-Chip (OoC) models

Séverine Le Gac

**Applied Microfluidics for BioEngineering Research
University of Twente, The Netherlands**

s.legac@utwente.nl

