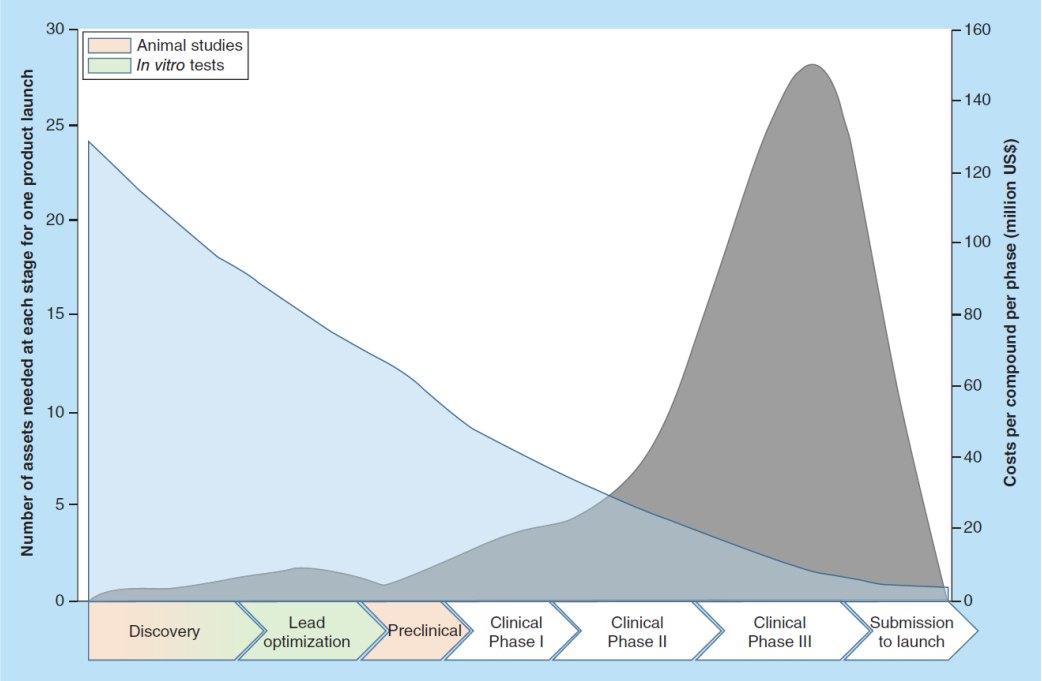
UiO : Institute of Basic Medical Sciences
University of Oslo

26.10.2020, Mathias Busek

Organ-on-Chip Technology – A short overview



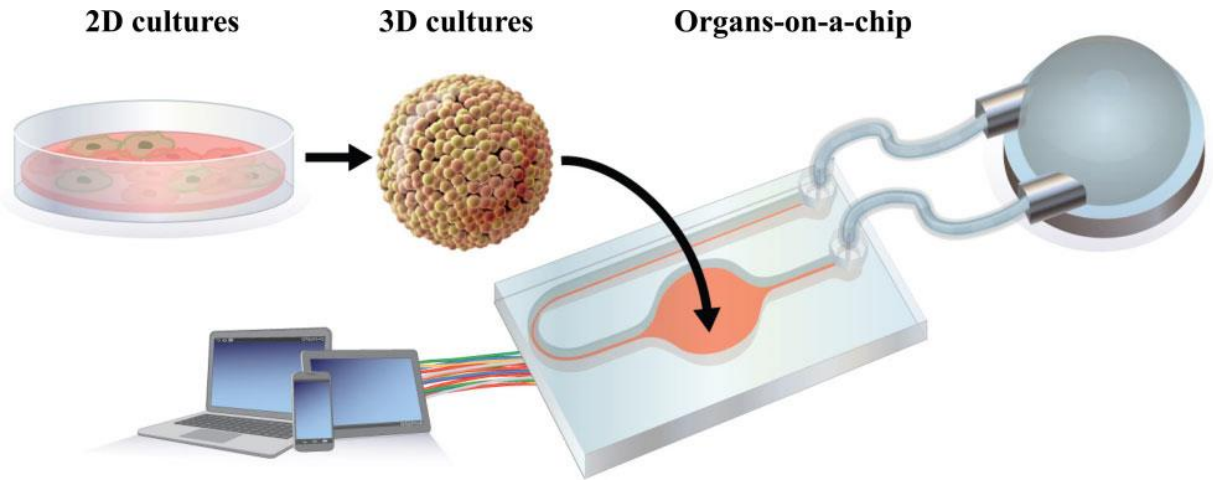
[1] E.-V. Dehne et. al.: “The ascendence of microphysiological systems to solve the drug testing dilemma”

- 90% of tested drugs fail during pharmaceutical screening process!
- Results from animal testing cannot be transferred to human exposure as it is.
- Bringing a drug to market :
  - ➢ Costs > 1 Billion $
  - ➢ 12 years with several clinical trials

OoCs are 3D microfluidic cell culture chips which simulates the activities, mechanisms, physiological response of entire organs.

*Useful to study **single organ** toxicity or diseases.*

From 2D cultures to Organs-on-a-Chip (OoC)

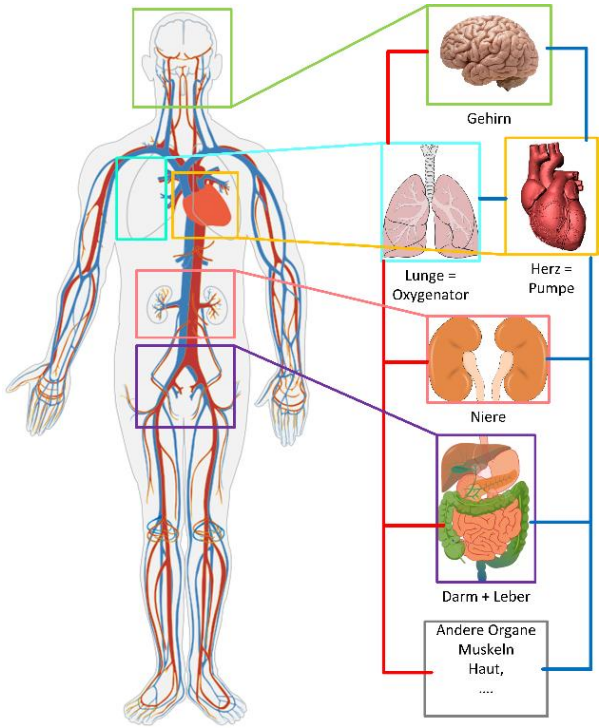


[2] D. Bovard et. al.: “Organs-on-a-chip: A new paradigm for toxicological assessment and preclinical drug development”

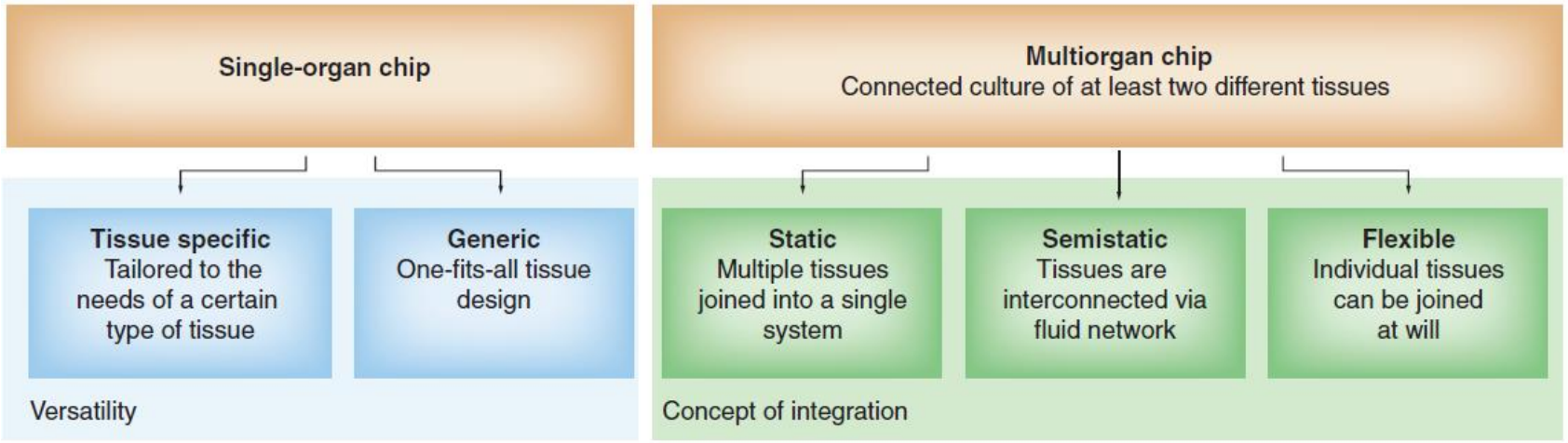
HoCs are 3D microfluidic cell culture chips which combines several OoC in one closed microfluidic circuit to rebuilt a complete body.

*Useful to study **systemic** toxicity and diseases.*

From OoCs to Humans-on-a-Chip (HoC)

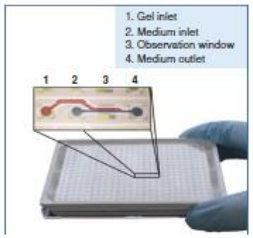


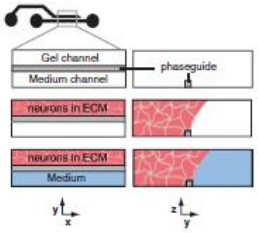
Types of OoCs



[3] J. Rogal et. al.: “Integration concepts for multi-organ chips: how to maintain flexibility?!”

Generic OOCs: Organoplates





MIMETAS
the organ-on-a-chip company

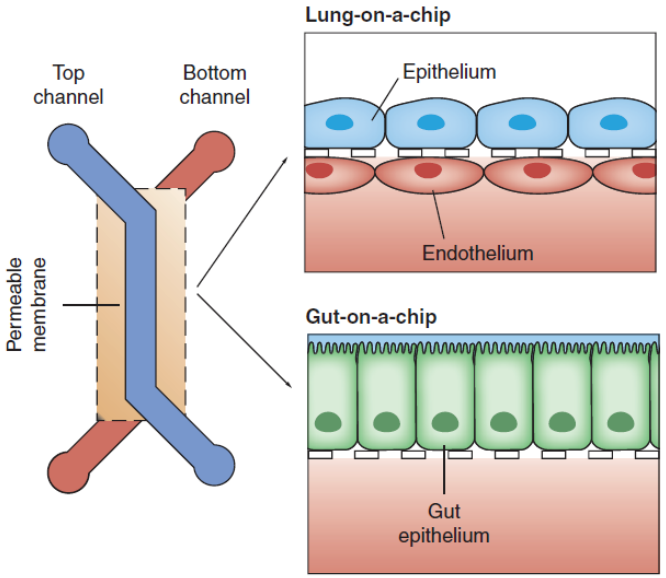
[1]

insphero

[1]

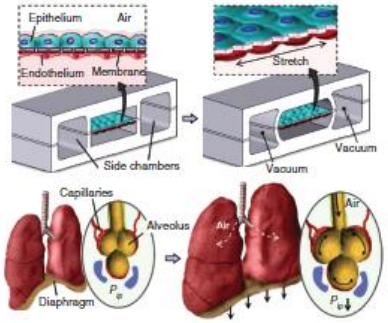
- 96-Well Plates
- Connecting channels at the bottom
- Perfusion via rocking the plate
- Co-Cultivation of different cell types in hydrogels
- No specifically adapted design

Generic OOCs: Emulate-Approach



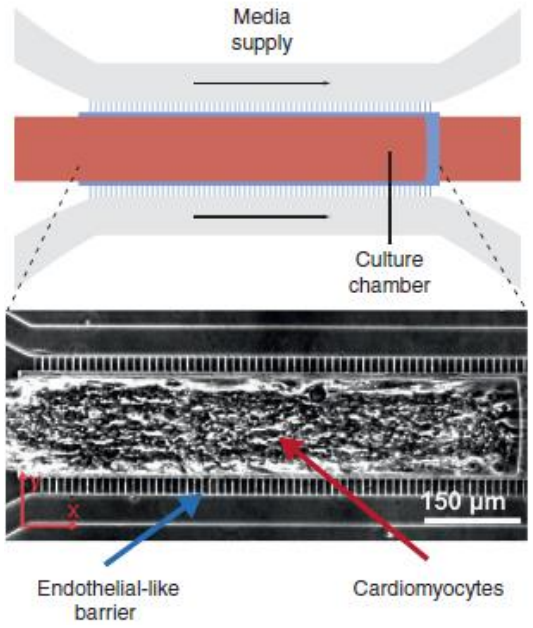
[3]

- Channels separated by permeable membrane
- Organs with mass transport function like gut, lung, kidney can be remodeled by one layout
- Flow-through system
- Membrane stretchable to mimic breathing



[1]

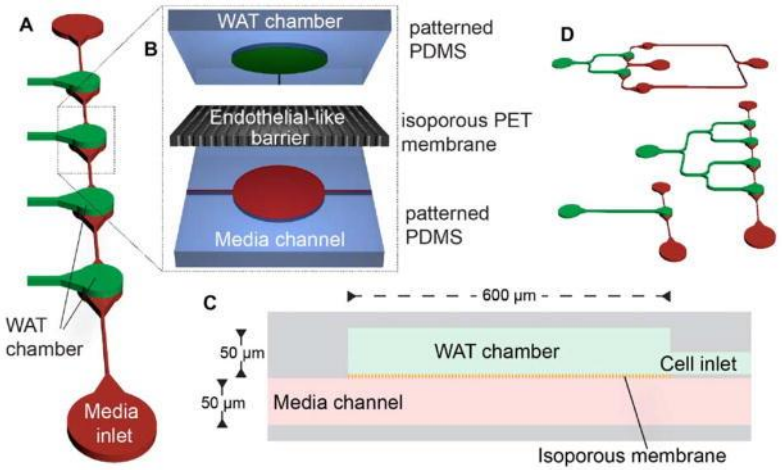
Tissue-specific OOC: Heart-on-a-Chip from UC Berkeley



[3]

- Heart diseases one of the main causes of death in Europe (WHO)
- In-vitro drug screening model still missing
- iPS-derived Cardiomyocytes (Patient specific)
- Fenestrations to prevent direct flow
- Perimysial collagen-fiber spacing (100–200 µm)

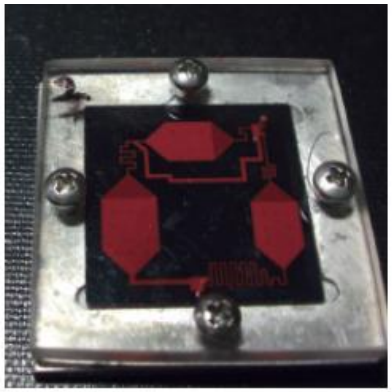
Tissue-specific OOC: Adipose tissue Chip by Fraunhofer IGB, Stuttgart



[5] P. Loskill et al. “WAT-on-a-chip: a physiologically relevant microfluidic system incorporating white adipose tissue”

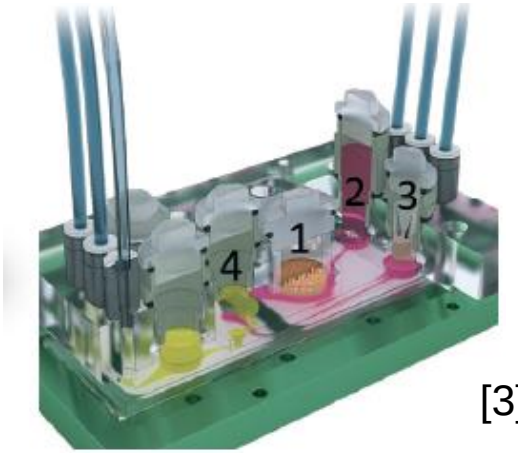
- White adipose tissue (WAT) risk factor for metabolic disorders like obesity or diabetes and highly involved in pharmacokinetics
- Up to now, only a few research on this topic
- Porous membrane as endothelial-like barrier
- Channels made by soft-lithography

Multi-Organ-Chips



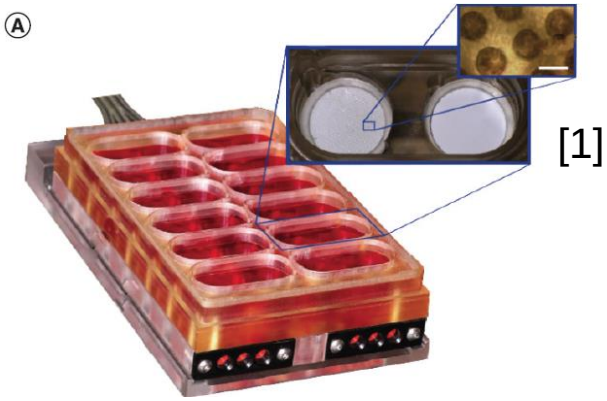
[3]

Shuler et. al. Body-on-a-Chip for PBPK models



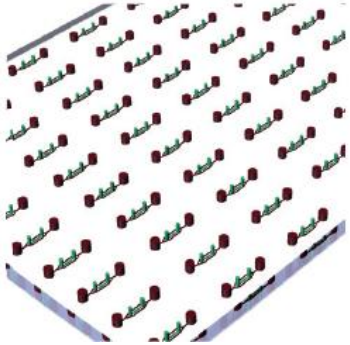
[3]

TissUse MOC with integrated micro pump



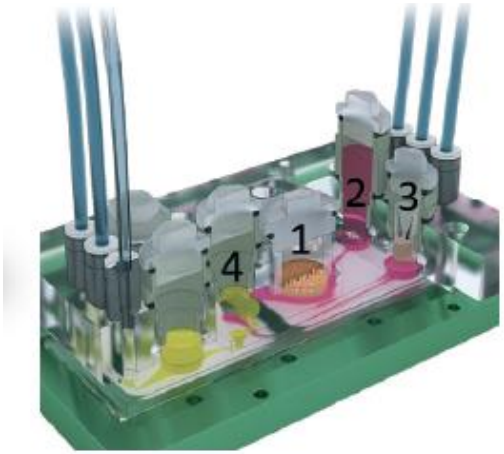
[1]

FHG IGB approach with flexibly connected single OOCs



[3]

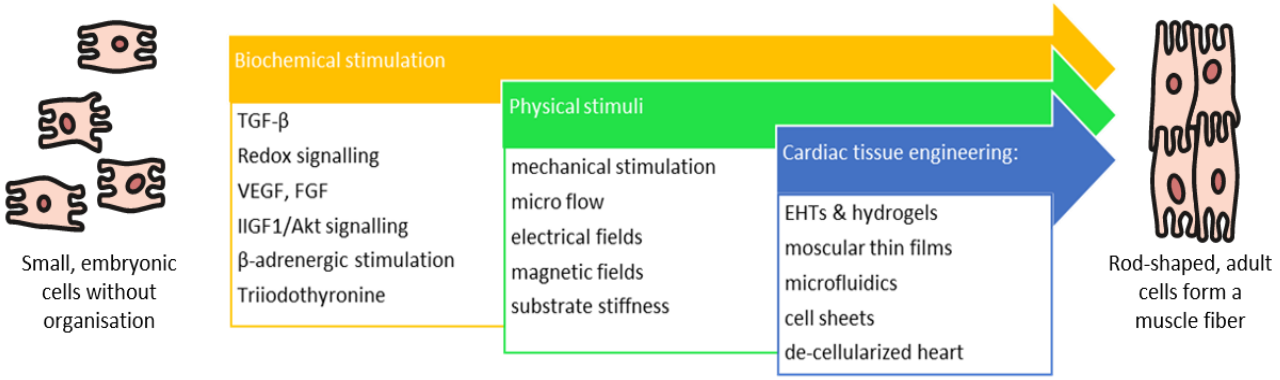
Multi-Organ-Chips: TissUse 2/4-Organ systems



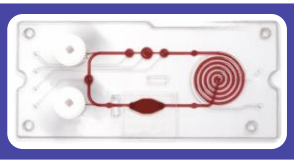
[3]

- Organoid models in 96-Transwell insert
- Static pre-cultivation
- Separated by membrane from direct flow
- co-cultivation of skin, liver, kidney, pancreas, …
- ADMET-Testing shown on different substances

Maturation problem of iPS-derived cells

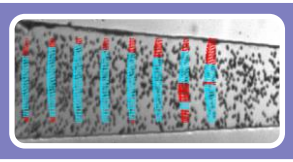


- Problem: Insufficient maturation of iPS- derived cardiomyocytes
- Can the maturation state be increased when different stimuli like micro flow are applied?

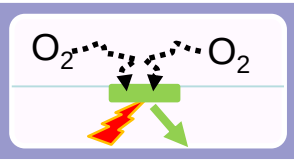


Microfluidic OoC with integrated micro pumps, valves and oxygenator

Controlling unit for pumps, valves and oxygen



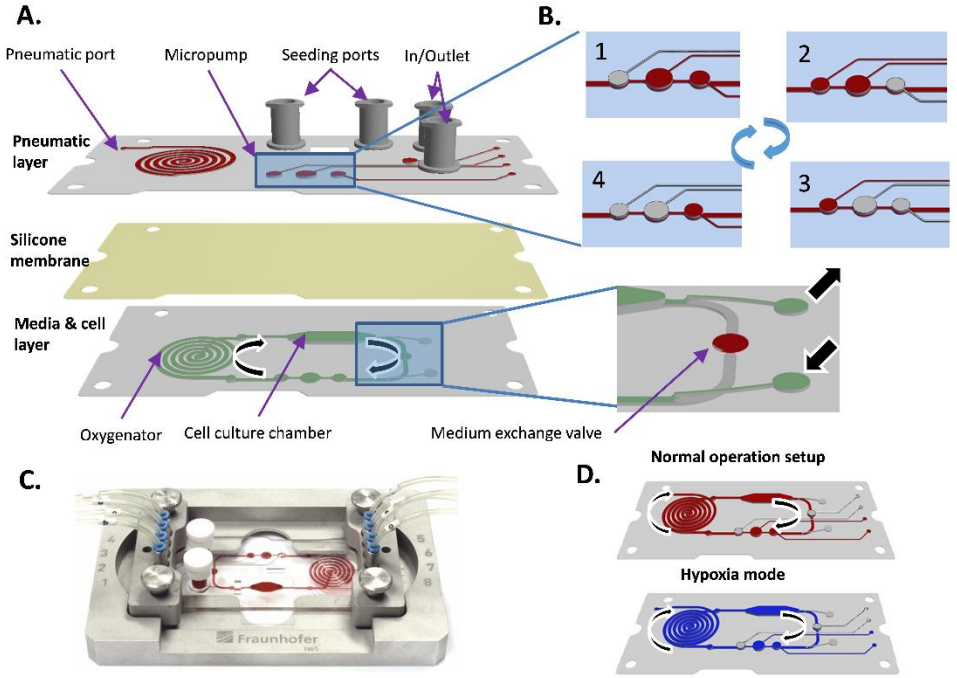
Flow measurement: Particle-Image-Velocimetry (µPIV)



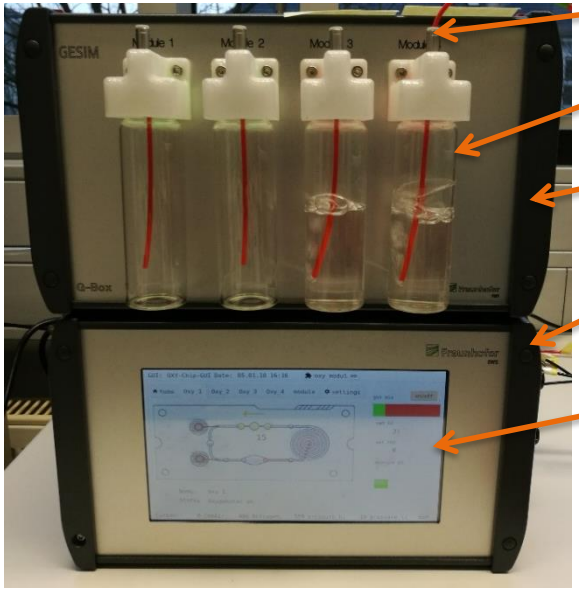
Oxygen sensing: Fluorescence quenching

Microfluidics with integrated pump and oxygen exchanger

- Pneumatic and fluidic part: laser-cut Polycarbonate (PC) foils (250 µm)
  - Bonded thermally (hot press)
- Actuators/oxygenator: flexible, gas-permeable silicone membrane (200 µm)
  - Bonded chemically (APTES)
- Holder providing pneumatic interface

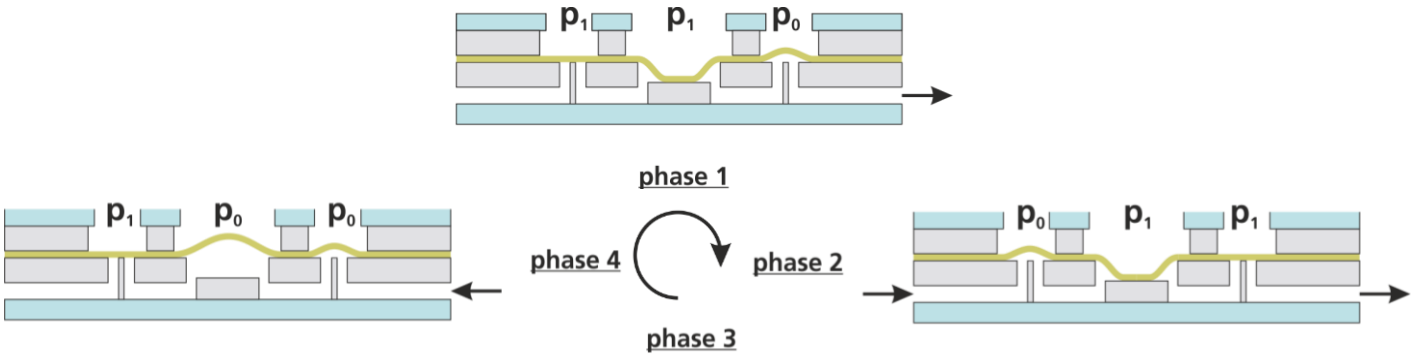


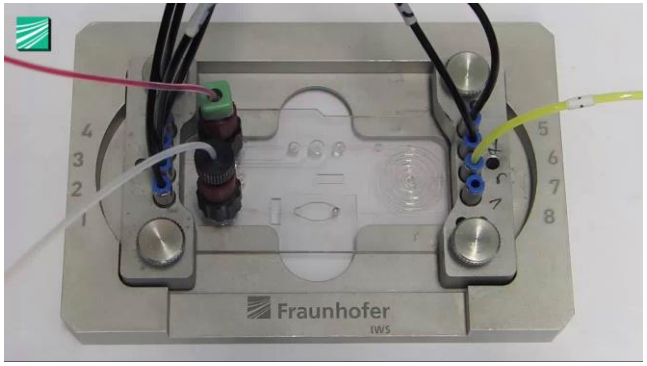
Controlling unit for pumps and oxygen exchanger



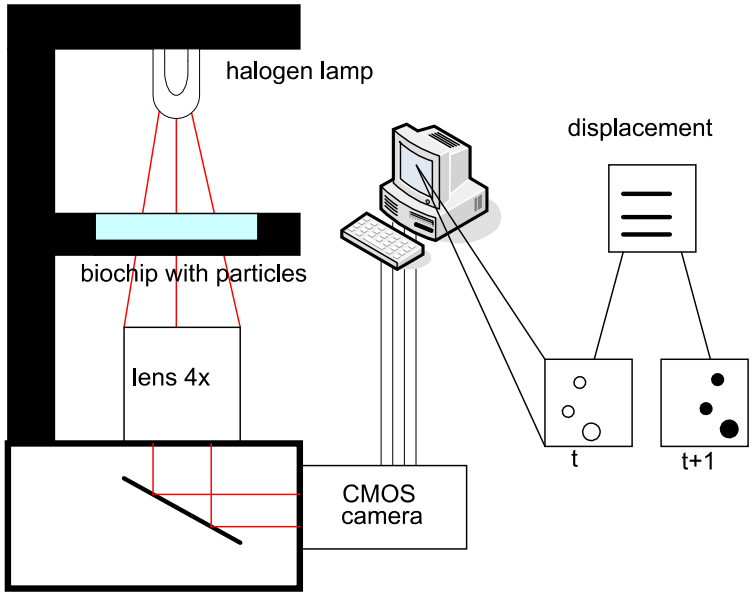
- Drives pumps and valves
- Embedded system with GUI
- Pulsed mixing of process gas for oxygenator (O_2, N_2, CO_2)
- Humidification of the gases
- Programmable

Peristaltic fluid transport



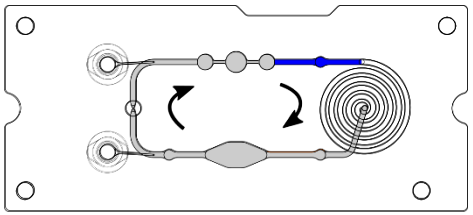


Flow measurement principle (µPIV)

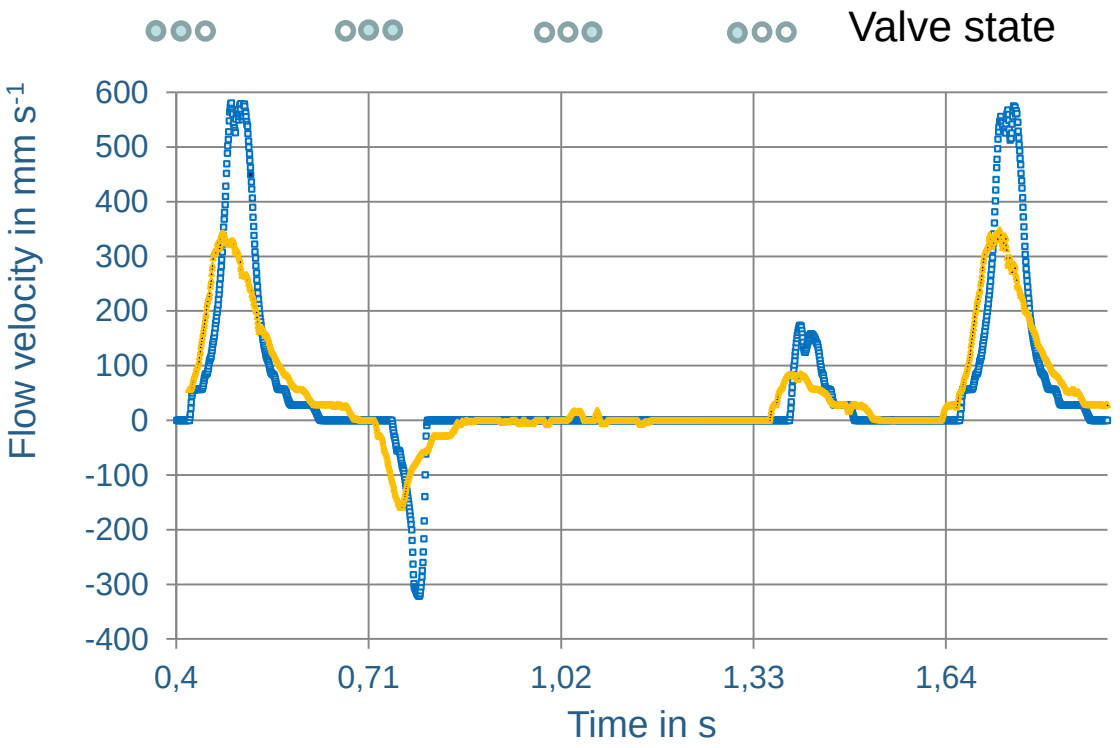


- Tracer particles in the flow: PS beads with 10 µm size
- Cross-correlation algorithm to calculate particle displacement between frames
- Only used particles flowing in the center: max. velocity

Flow characteristics

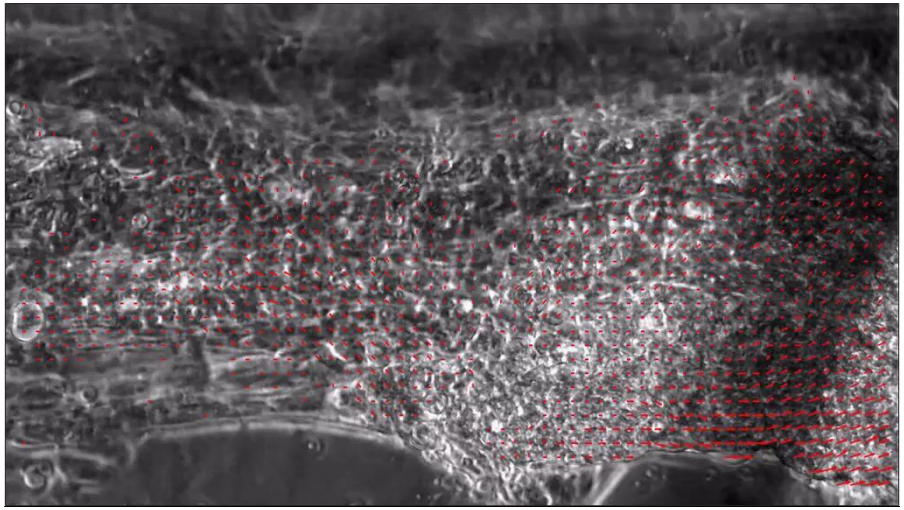


- Flow velocity dampened by 50 % due to flexible walls
- Shear forces reduced behind oxygenator in tissue chamber



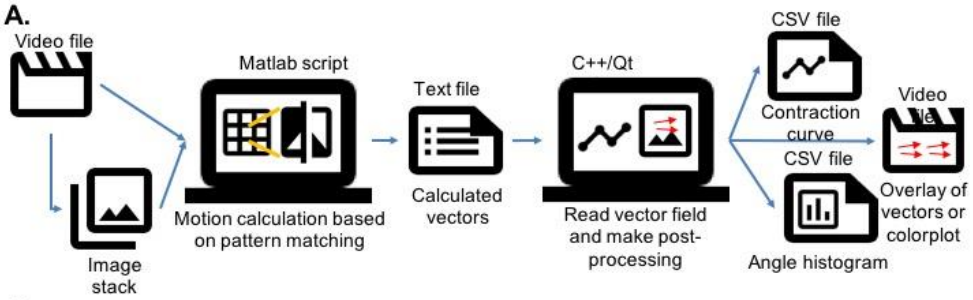
Flow characteristics

- Cells adhere at chamber bottom and start beating after several days
- Different tools applied to check maturation state:
  - Video-based analysis
  - Morphology check
  - Gene expression analysis
- Is the motion better aligned, can a higher contraction speed be detected?

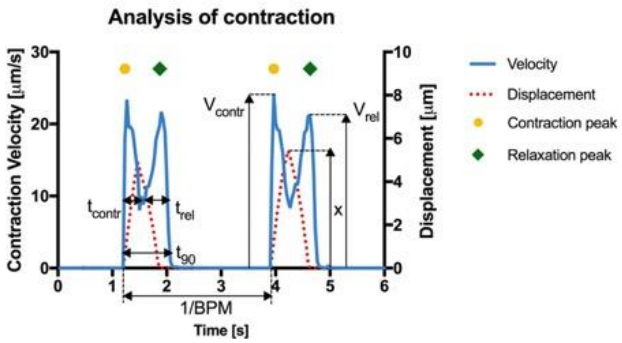


Video analysis of beating cells

- Bright field videos of 20s with beating motion of several cells.
- Calculate motion vectors of succeeding frames via Optical-flow method.
- From vector field of motion contraction relaxation speed displacement and spatial distribution can be obtained.
- Physiological parameters: BPM, amplitude, …



B.

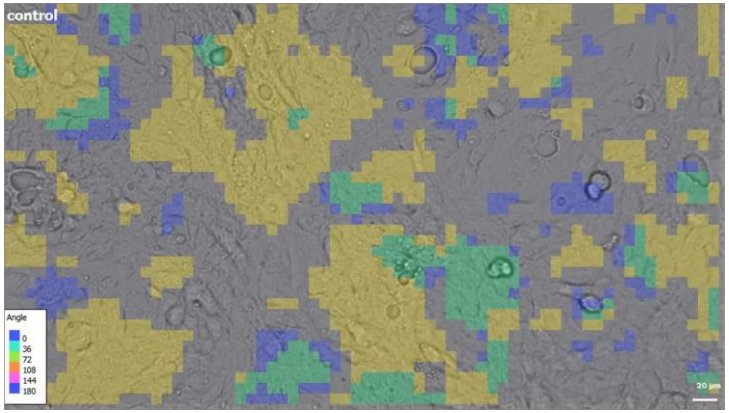


name	description	unit
BPM	Beating rate	BPM
t_{90}	Contraction time at 90% of relaxation	s
t_{contr}	Duration of contraction phase	s
t_{rel}	Duration of relaxation phase	S
v_{contr}	Contraction amplitude	µm s^{-1}
v_{rel}	Relaxation amplitude	µm s^{-1}
x	Displacement	µm

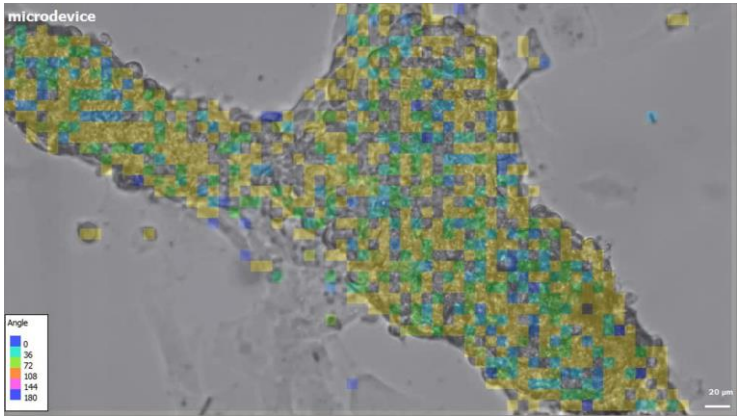
Video analysis result: Beating direction

Contraction amplitudes, displacement and orientation increased in OoC culture compared to static control

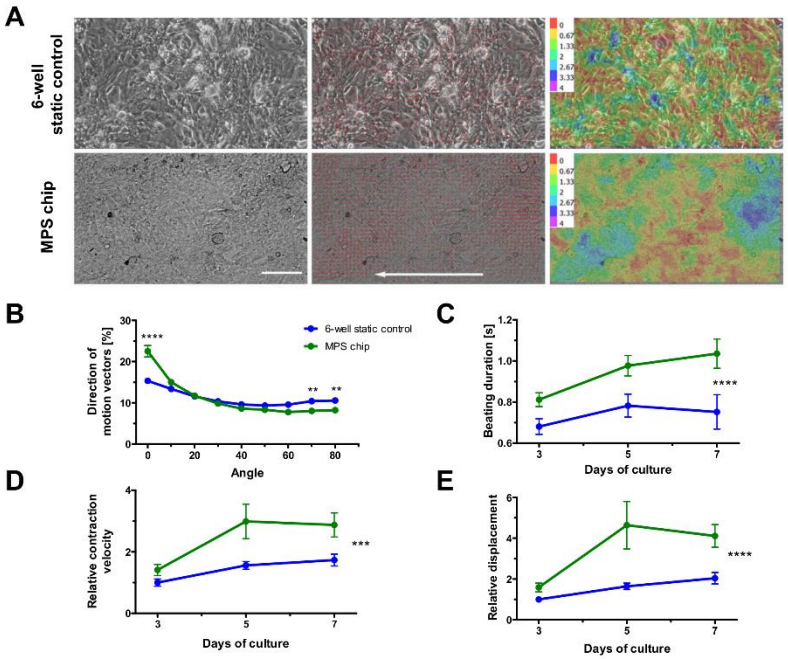
Static control

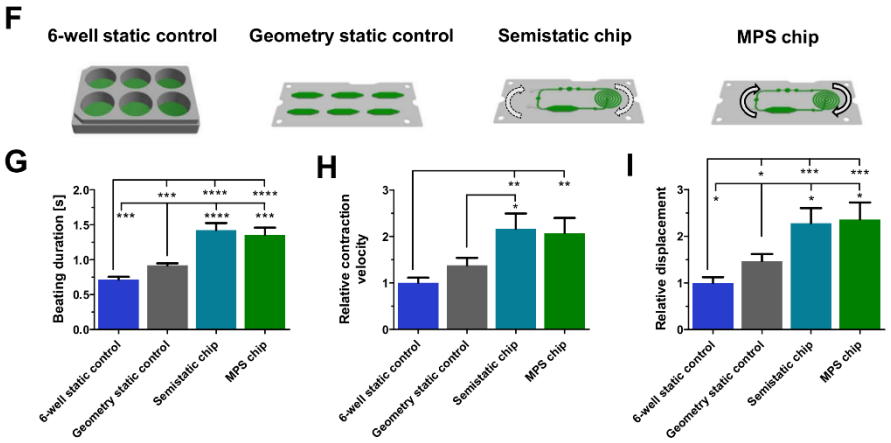


MPS culture



Video analysis result: Summary

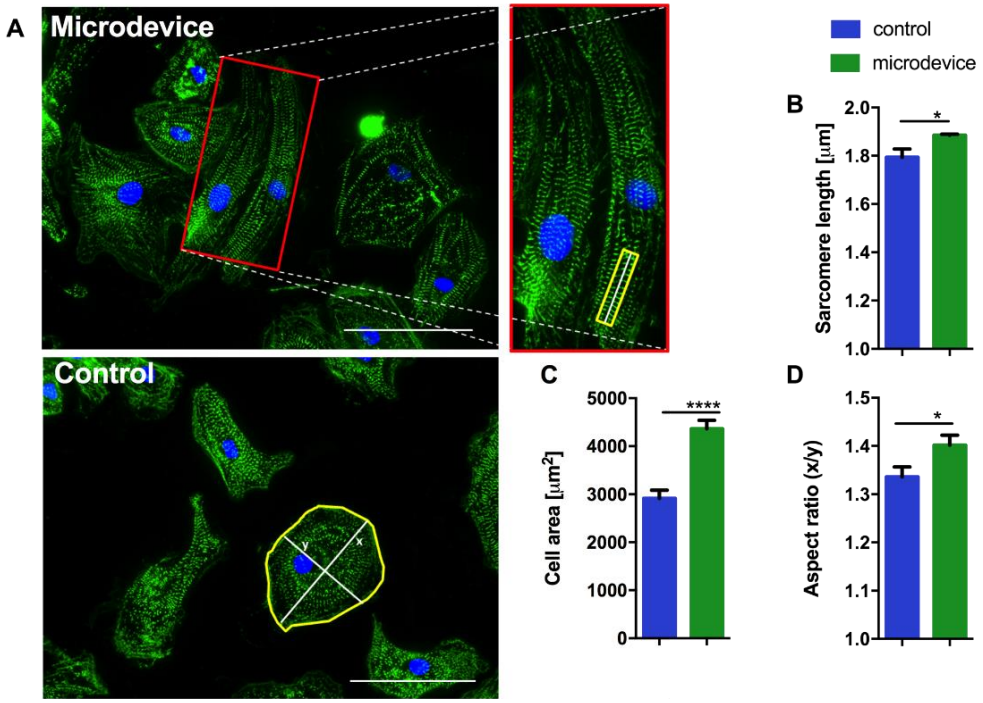




A: Brightfield images and vector/video overlays
B: Angle distribution MPS vs static
C, D, E: Beating characteristics MPS vs static
F: Different controls used
G, H, I: Beating characteristics of controls

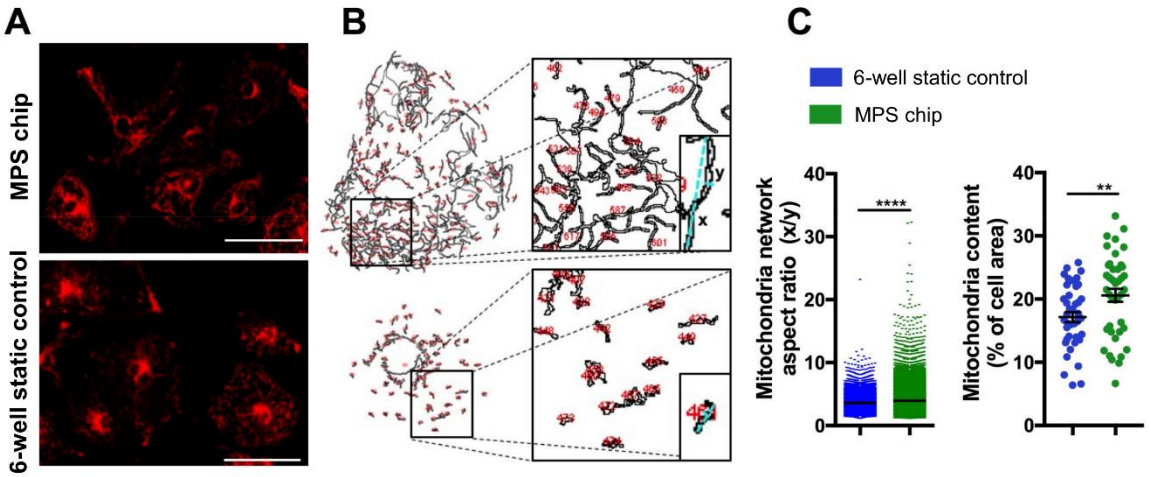
Morpholical changes: Increased sarcomere length

- Sarcomeres are active protein structures in muscle cells
- Increased length indicates better maturation of the cells
- Sarcomeres stained (green) and length measured via image analysis
- More elongated cell shape and increased cell area in OoC



Morpholical changes: Denser mitochondria network

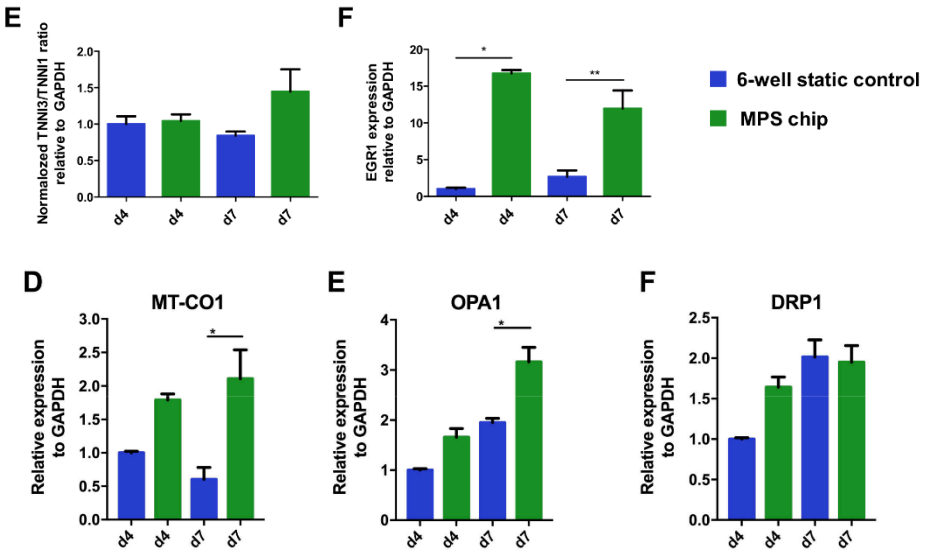
- Mitochondria network responsible for energy transport in cells
- Mitochondria's stained and there average length is calculated via image analysis (aspect ratio)



Network is much denser in MPS culture which means that energy is better distributed and cells can produce higher forces

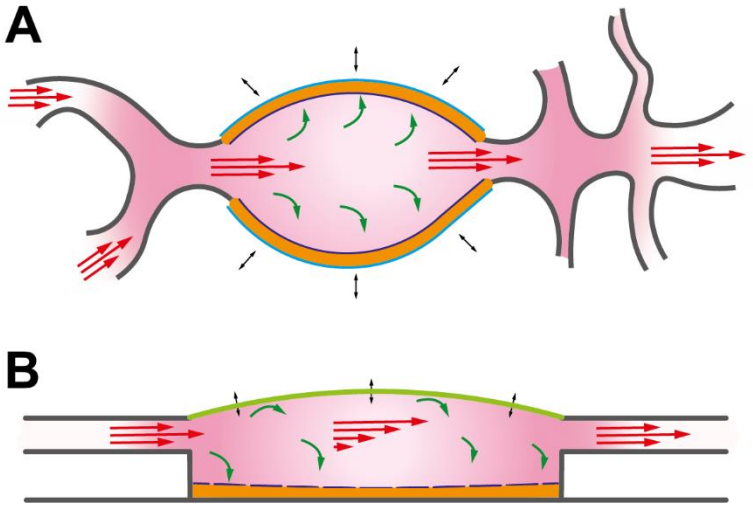
Gene expression changes

- TNNI1 marker associated with immature CMs downregulated while TNNI3 a maturation marker upregulated
- Shear forces leads to higher EGR expression in OoCs
- Mitochondrial marker genes upregulated after 7 days of perfused culture revealing a higher mitochondrial activity in OoCs

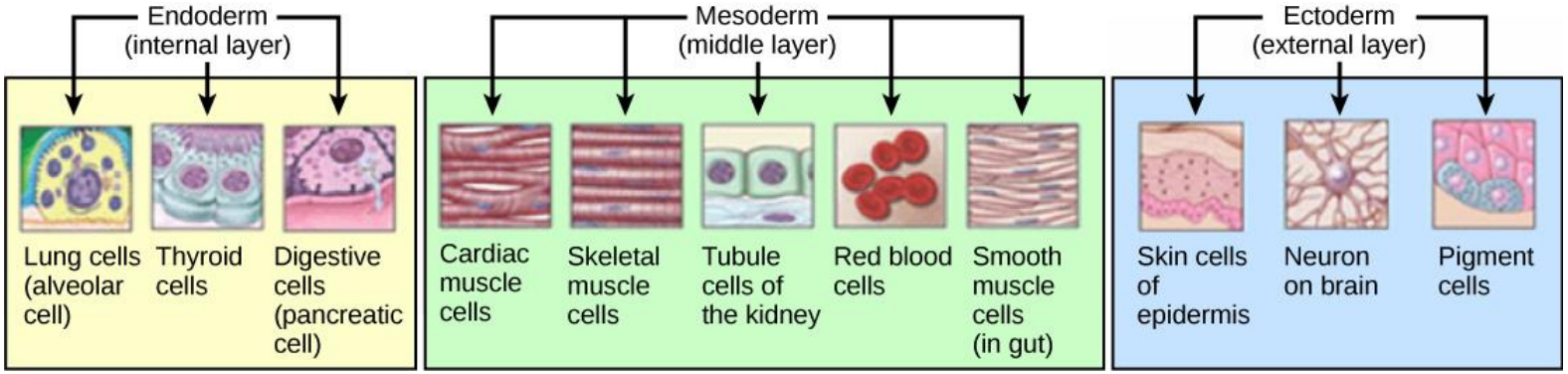


Structural maturation of iPS-CMs

- Cyclic hemodynamic force stimulation seems to drive structural maturation of iPS derived CMs
- Pressure rates are within physiological limits (10 – 20 mmHg) while shear forces remain low (no cell damage)
- OoC model close to myotube formation in the early embryo development

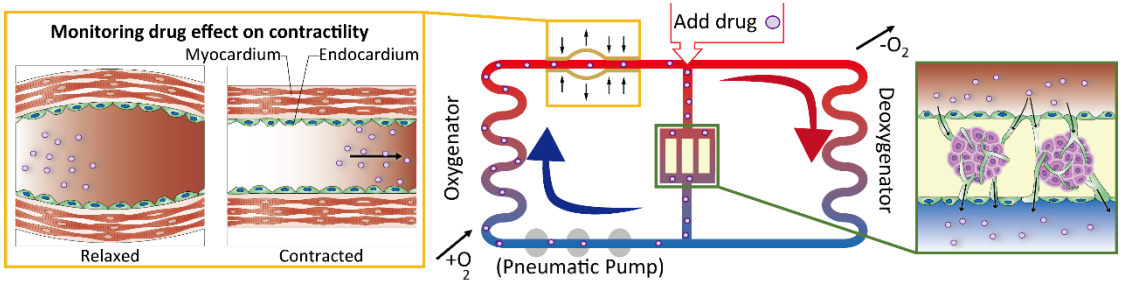


Our approach: Organogenesis-on-chip

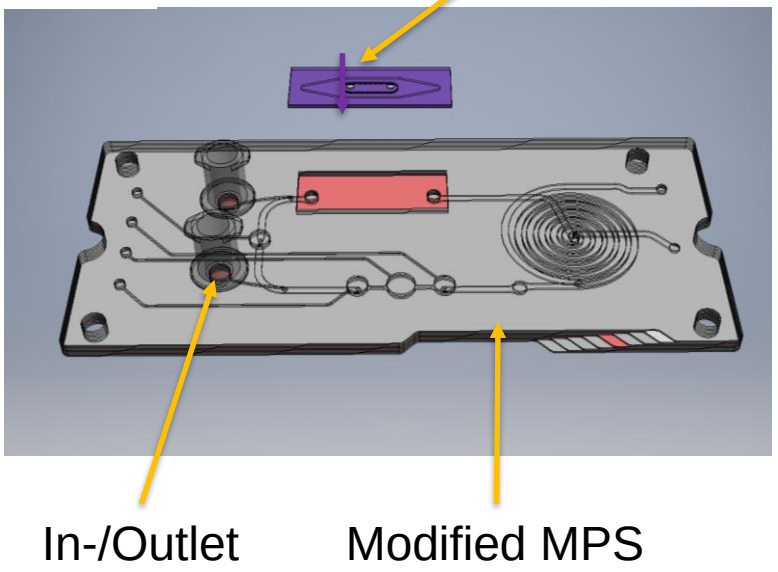


- By using iPS-derived cells we want to differentiate all needed tissue
- Provide embryonic micro environment on-chip to show organogenesis and cell differentiation/maturation
- Oxygen gradient for venous and arterial side of tissue

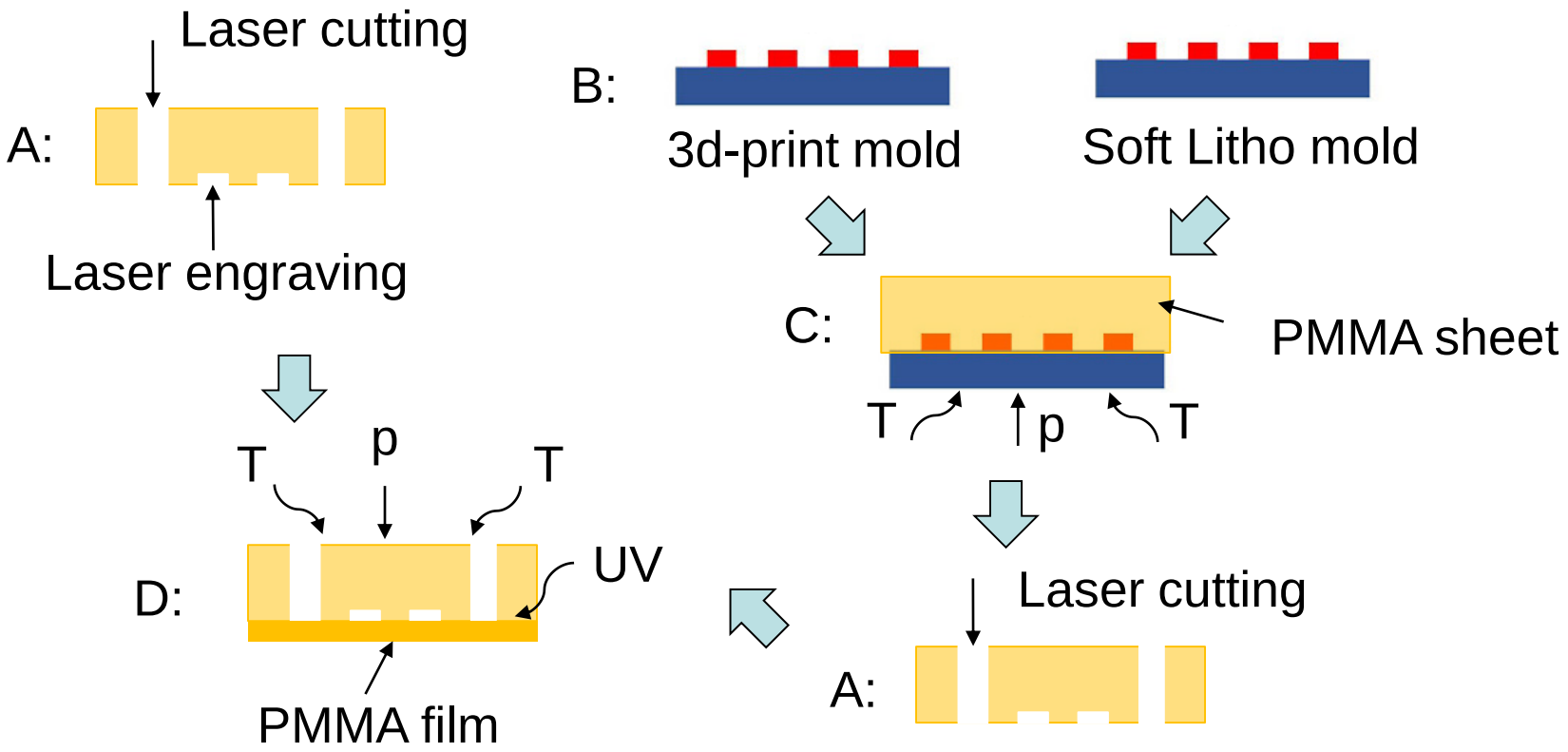
Coupling a tissue-specific module to a the microfluidic support system



- Vascularized tissue model should be coupled to MPS with media recirculation and O2 control
- MPS with micro pumps and oxygen exchanger
- Later use «pumping heart on-chip» to vascularize tissue and

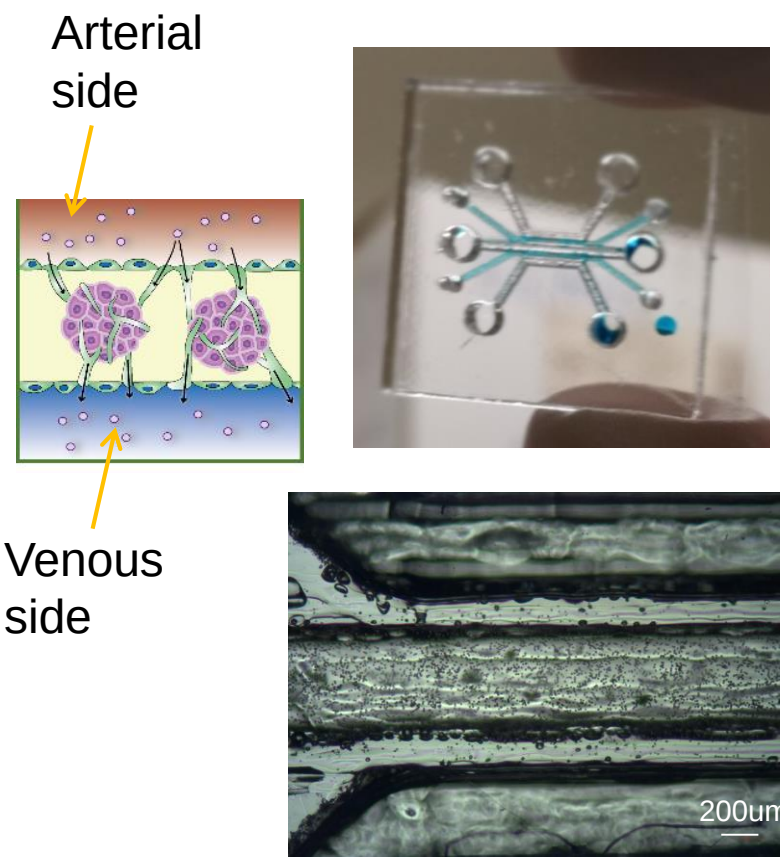


Fabrication technologies for tissue chip using PMMA



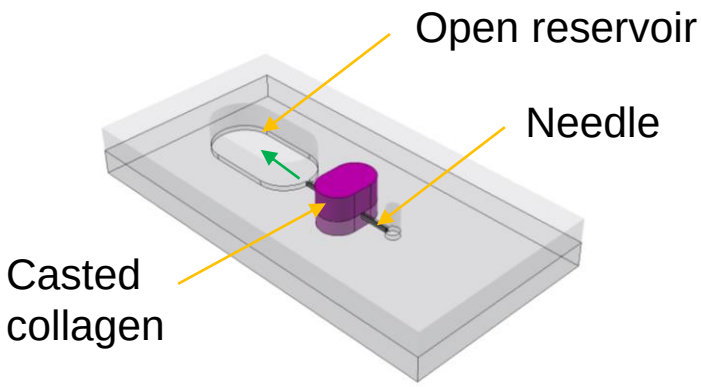
Vascularization-on-chip

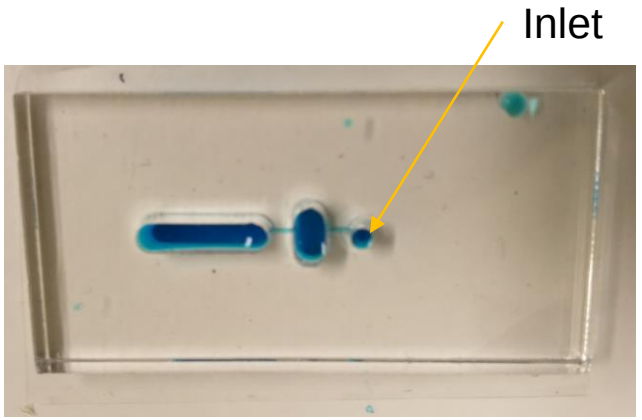
- Small organs (organoids) must be connected to the blood vessels
- Formed by endothelial cell sprouting
- Barrier of ECM needed
- Approach 1: Geltrex trapped in dead channels
- Later: cell seeded



Vascularization-on-chip - 2

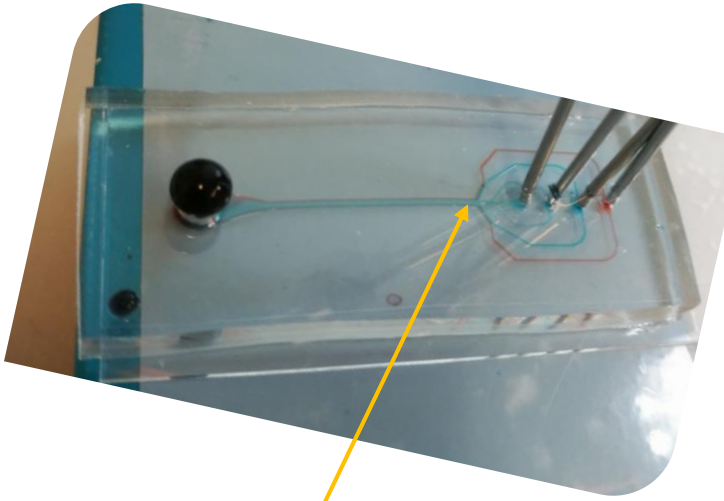
1. Insert needle in small connection channel (300x300 um)
2. Cast collagen in central open cavity
3. Let collagen polymerize
4. Remove needle at open reservoir
5. Seed and perfuse the now open channel from the inlet

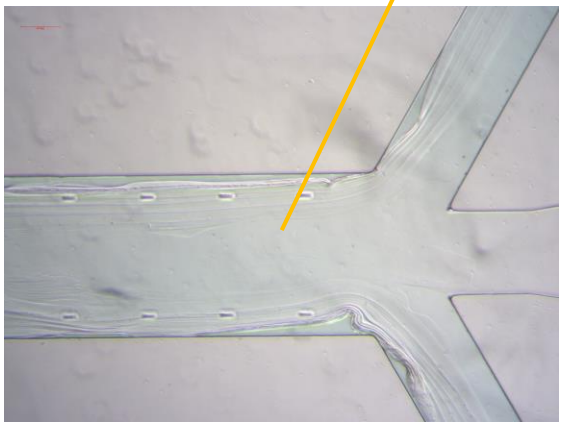




Organ-fibre-on-chip

- Basic principle: Laminar flows in micro channels are not mixing
- Fluids containing cells, ECM etc. can be “stacked” to make multilayer (hollow) structures “on-chip”
- Polymerization with Ca2+ ions, UV light or local heating/cooling
- Embed “organ-fiber” on holding structures to perfuse it





- ✓ OoCs are an emerging field in biomedical research
- ✓ Most systems are technically simple and mimic in-vivo conditions only partially
- ✓ Multilayer-manufacturing allows for complex systems with integrated actuators and sensors with controlling system and evaluation tools
- ✓ Tissue specific OoCs like the Heart-on-a-Chip system are useful for specific studies
- ✓ At HTH we want to show organogenesis-on-chip by coupling vascularized tissue modules with a microfluidic support system to provide needed gradients and tissue specific micro environment

Thank you for your attention