A multi-organ-chip platform for long-term maintenance and substance testing of human tissue co-culture

EUSAAT 2013

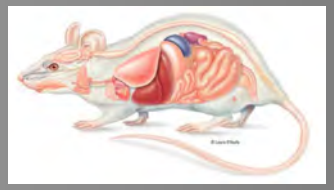
Materne

Repeated dose systemic toxicity testing

90-day: OECD 408^{o}, 411^{d}, 413^{i}

Daily observation and sampling:
- hematology (blood)
- clinical biochemistry (blood)
- urine analysis

daily exposure



~100 rodents per substance

Death
Organ weight
Necropsy:
liver, brain, heart
kidney, spleen
ovary, testes, uterus
thyroid, adrenals
epididymis others

- toxicity, dose-response relationship

28-day:
OECD 407^{o}, 410^{d}, 412^{i}, 419
~40 rodents /substance

28d — 90d — 12 mo

OECD 453

2012

A roadmap for the development of alternative (non-animal) methods for systemic toxicity testing

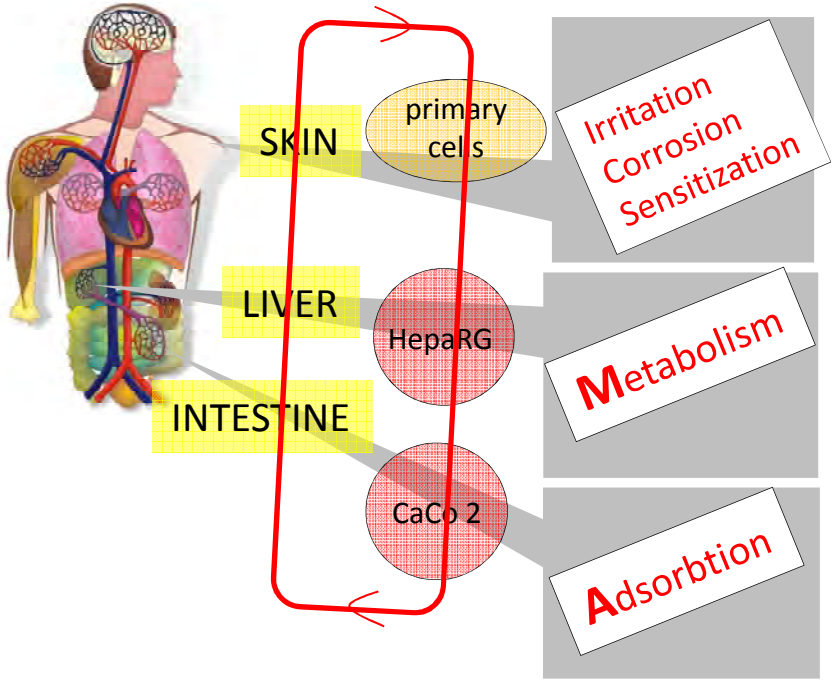
Basketter et al, ALTEX 29, 1/12, pp 1-91

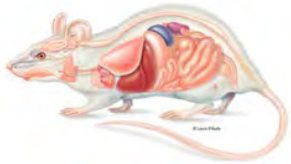
→ integrated testing strategy (ITS)

TISSUSE Emulating Human Biology

Integrated testing strategies

human *in silico* + human *in vitro* + animal *in vivo*





Roadmap request:
„more physiological culture conditions such as homeostasis, oxygen supply, cell density…“

Molecular initiation → subcellular → cellular → organ → organism

Pathway of Toxicity (PoT)

Mode of Action (MoA)

Adverse Outcome Pathway (AOP)

TISSUSE Emulating Human Biology

Solving the substance testing dilemma

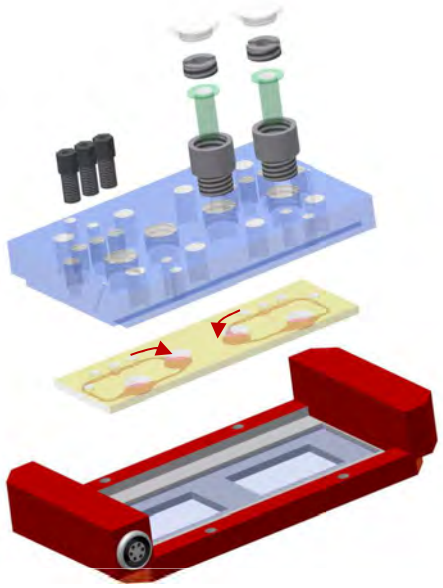
“human on a chip”
human AND systemic

animal models
systemic but NOT human

static 2D & 3D
human cell culture
human but NOT systemic

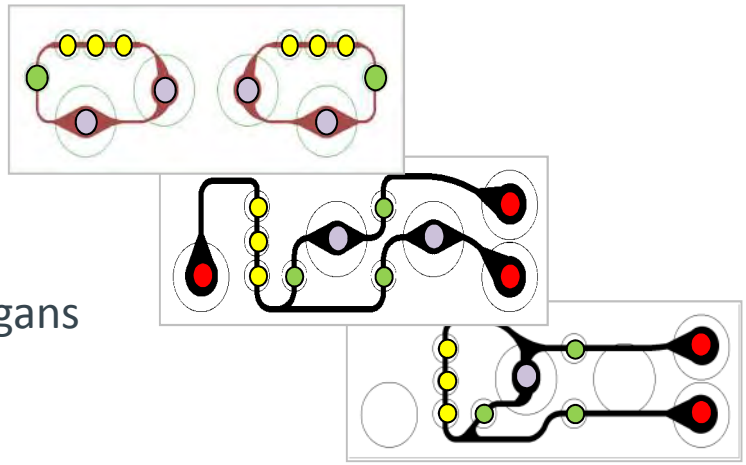
TISSUSE
Emulating Human Biology

The Multi-Organ-Chip (MOC) Technology



Features:

- Chip format of a standard microscopic slide
- On-chip micro-pump and natural tissue to fluid ratio
- Variable physiological shear stresses applicable
- Tissue cultures 100,000-fold smaller than original organs
- Rapid prototyping of any relevant chip design
- Compatible with life tissue imaging



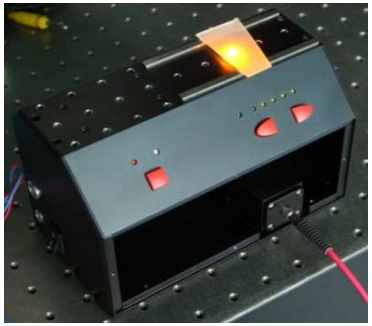
TISSUSE
Emulating Human Biology

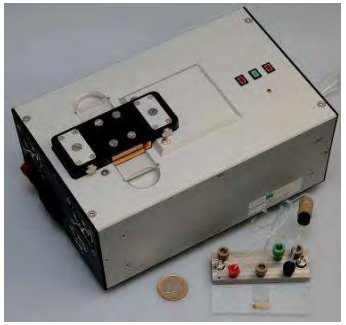
Sensors / In-process-controls

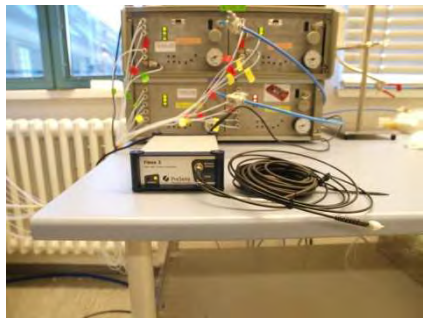
parameter / approach	flow velocity	organ viability	organ functionality	pH & pO_2	t°
principle	particle imaging velocimetry	fluorescence spectroscopy	surface plasmon resonance for secreted proteins	fluorescence lifetime	PT1000 temperature detector
features	non invasive different spots biological particles	cell tracker live imaging double staining possible	multiple proteins (46 per micro sensor 10 mm x 0.8 mm)	fibre coupled external calibration	long-term robustness

Frank Sonntag

Fraunhofer IWS

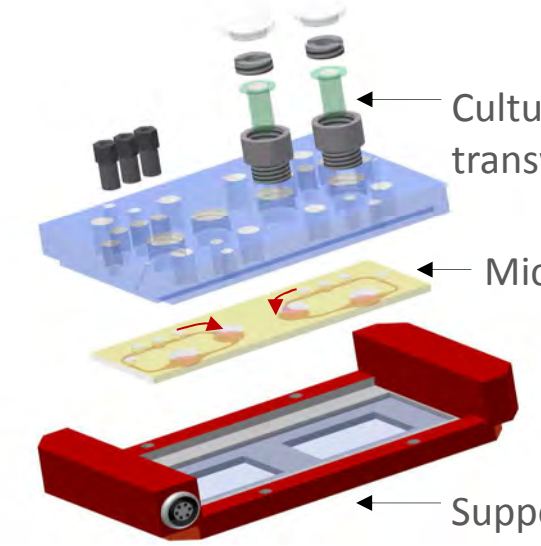






TISSUSE Emulating Human Biology

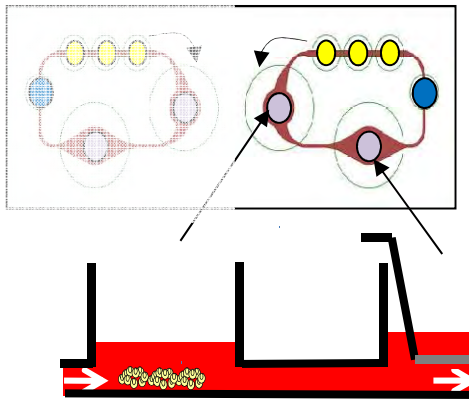
The “Two-Tissue-Culture Chip”



Culture inserts e.g. transwells

Microscopic slide format

Support with temperature control



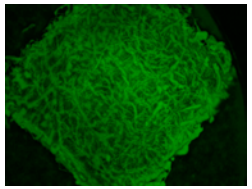
equivalent to 10 liver lobuli

cardiac, neuronal , Immune tissue bone marrow skin, intestine kidney, cancer …..

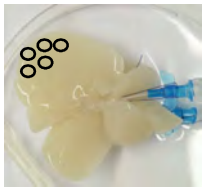
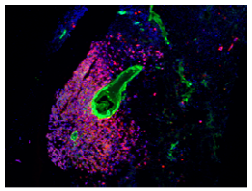
Tissue \ Duration	Short-term (<48h)	Long-term (<28d)	Homeostasis (90d, 1y…)
liver	✓	✓	in progress
skin	✓	✓	in progress
vasculature	✓	✓	in progress
neurons	✓	✓	in progress
intestine	✓	in progress	in progress
kidney	✓	in progress	in progress

TISSUSE Emulating Human Biology

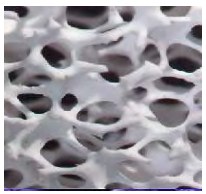
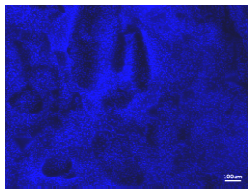
Generation of liver equivalents I

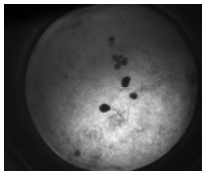
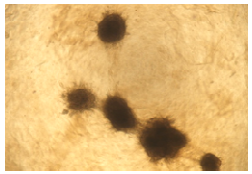
Integration of **biodegradable non-woven fabric** seeded with cells

Integration of **decellularized rat liver graft** re-seeded with cells

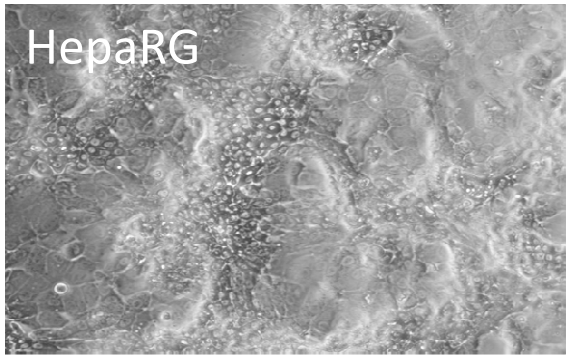
Integration of **porous ceramic** seeded with cells

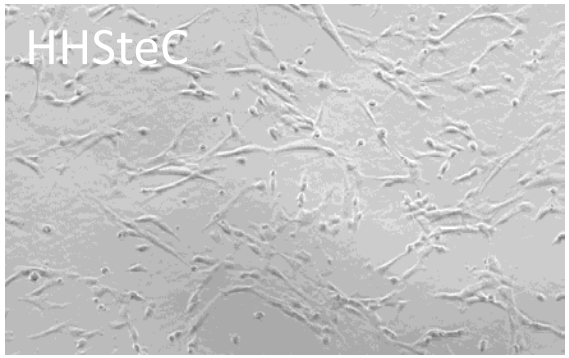



Integration of **collagen I and fibronectin hydrogels** seeded with cells or aggregates

Integration of **aggregates** produced from co-cultures

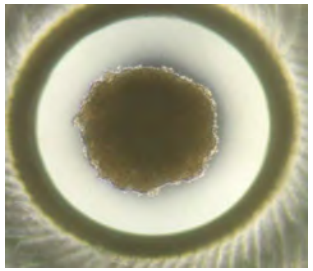
Generation of liver equivalents II

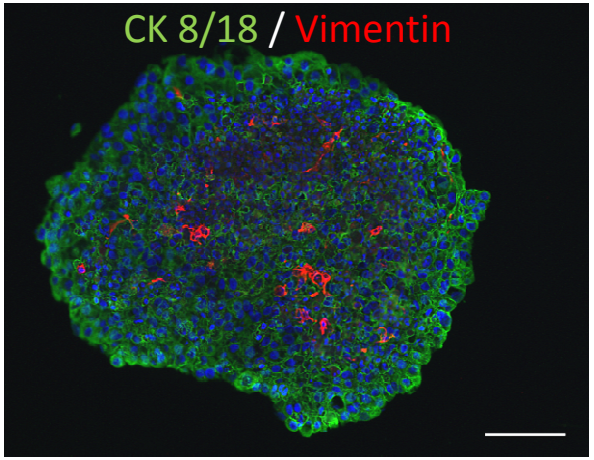




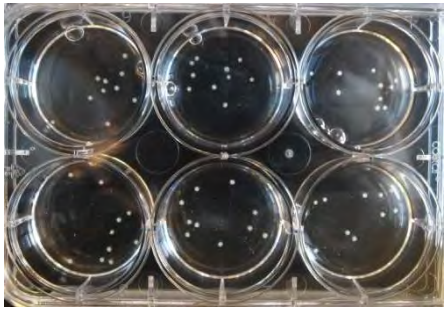
+

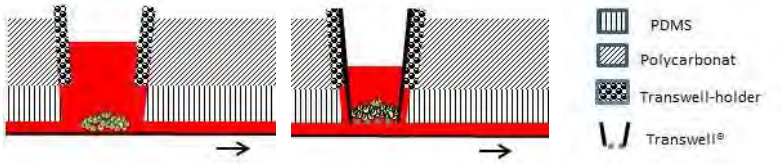
4.8 x 10^4 HepaRG and
2 x 10^3 HHSteC





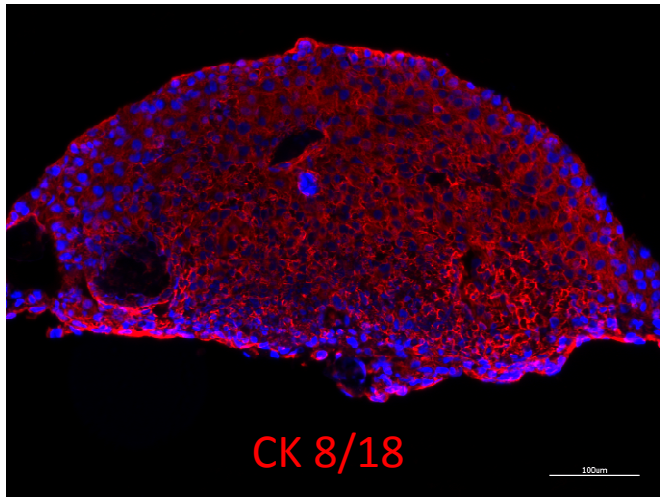
20 Aggregates ≈ 10^6 cells ≈ 1/100 000 liver mass

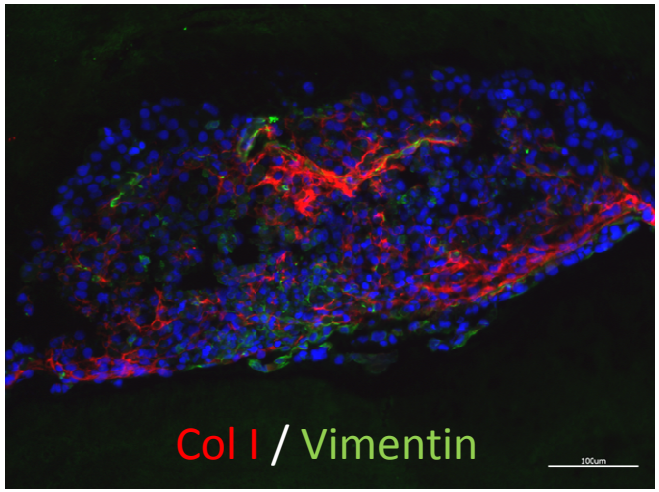


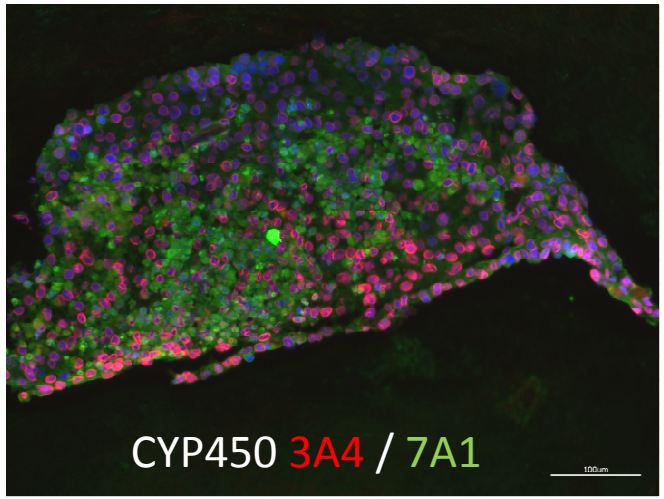


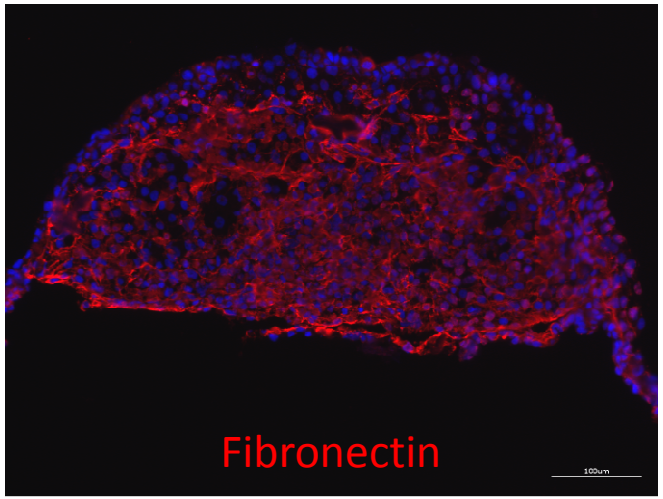
TISSUSE
Emulating Human Biology

Liver aggregate culture in the chip over 14 days

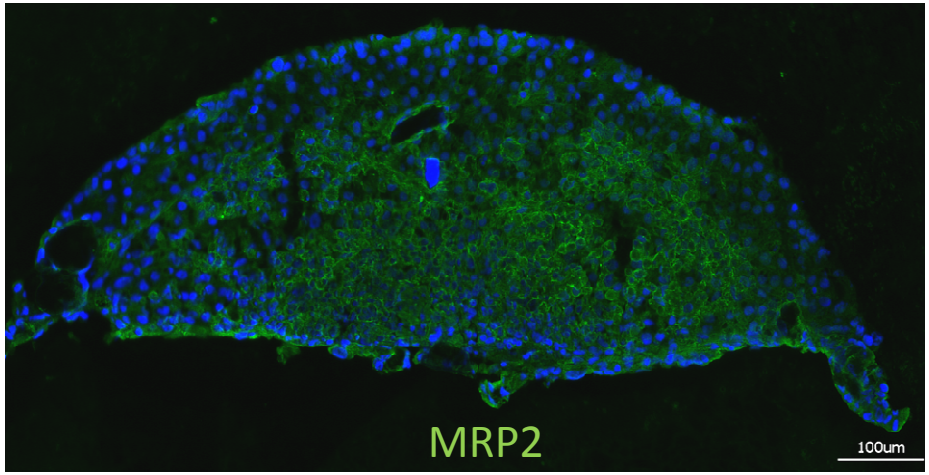


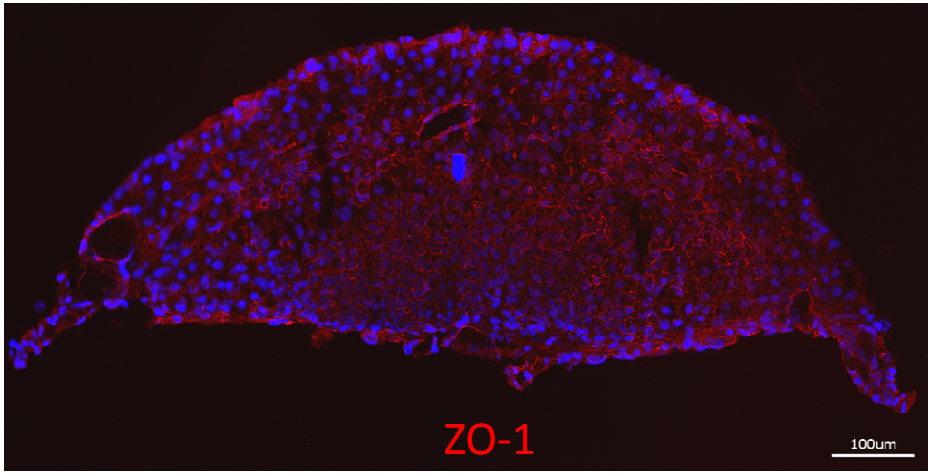


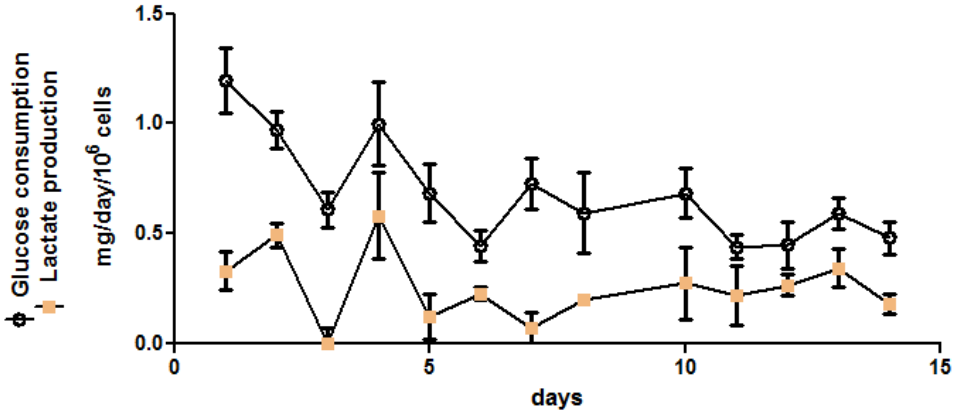


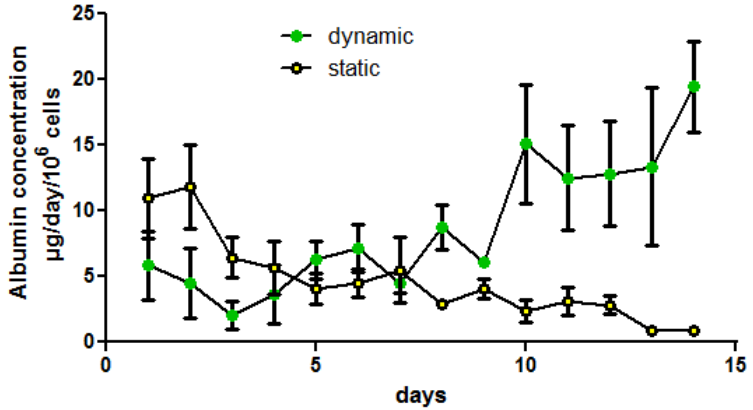


Liver aggregate culture in the chip over 14 days









Co-culture of skin and liver equivalents in the chip

3D tissue preparation and chip loading

HepaRG cells + stellate cells
(24 : 1)

↓

24h — aggregate formation in AggreWell plates

↓

3 days — low-attachment-plate culture

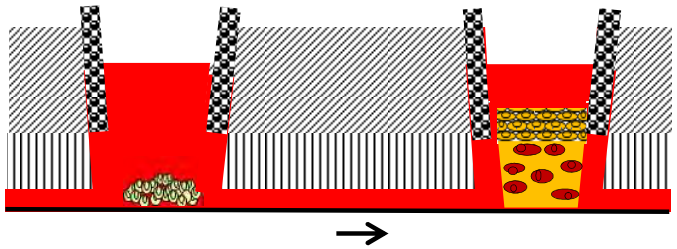
Foreskin
donor recruitment

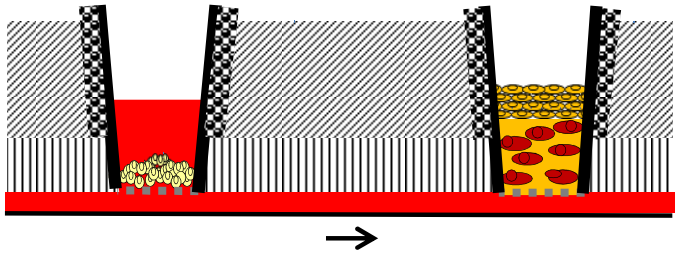
↓

tissue excision and transport to lab

↓

Preparation of sterile punch biopsies





PDMS
Polycarbonat
Transwell-holder
Transwell

TISSUSE Emulating Human Biology

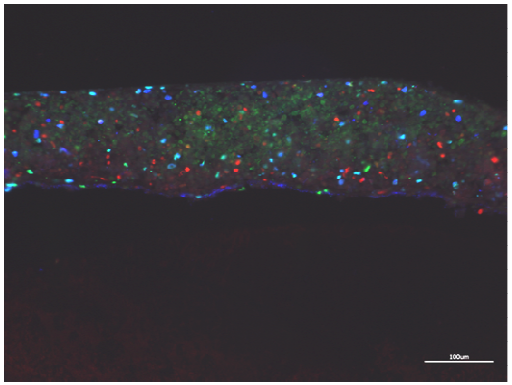
Performance of Transwell® multi-tissue cultures over 28 days

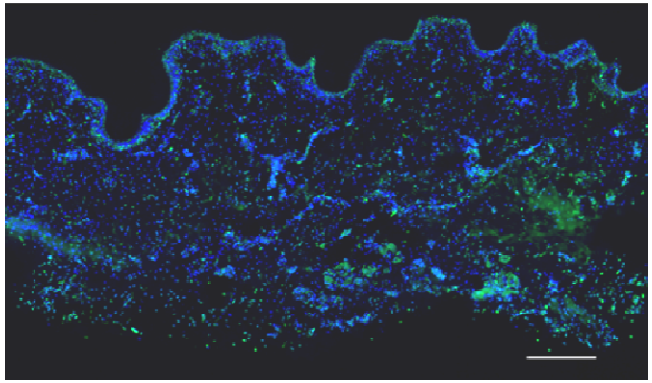
Liver

Apoptosis
Proliferation

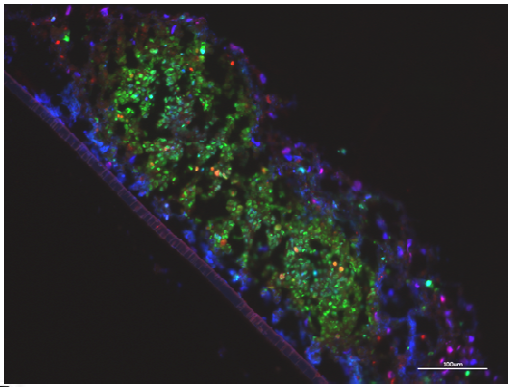
Skin

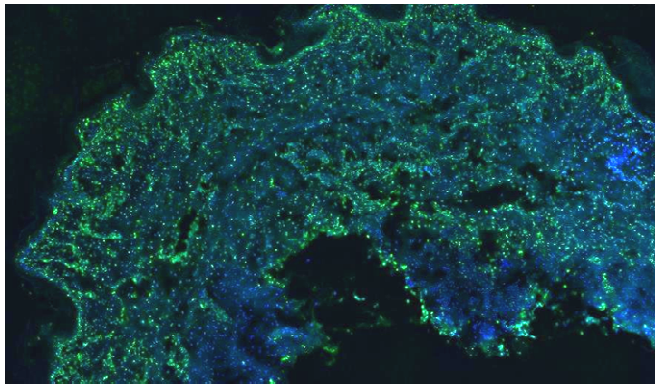
Dynamic culture in MOC

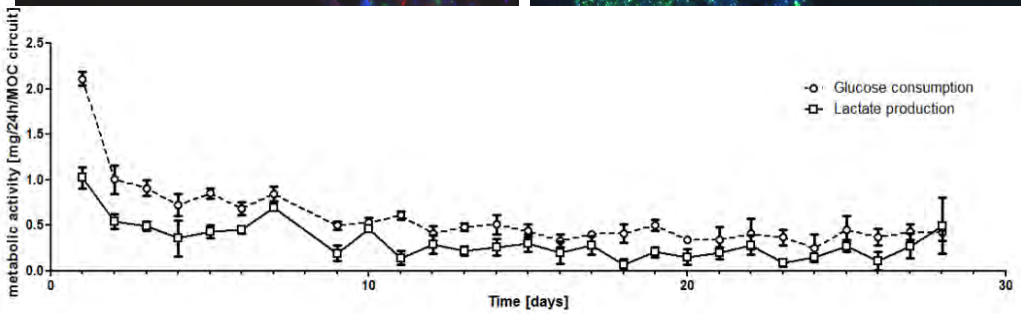




Static culture



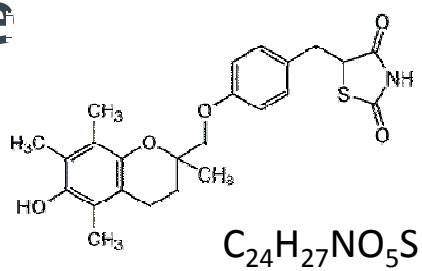




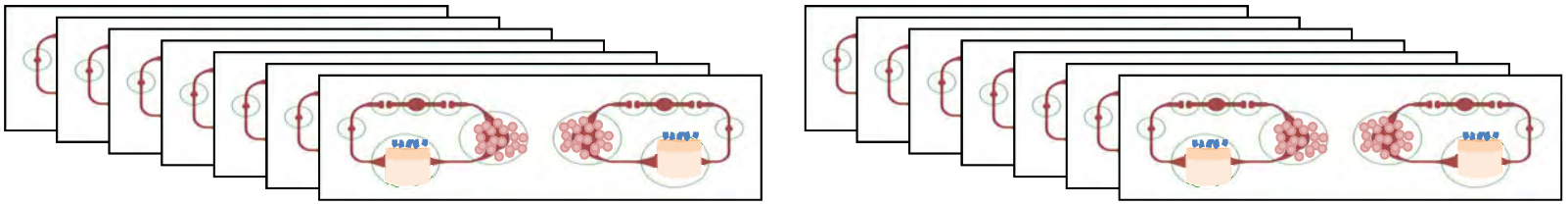
TISSUSE
Emulating Human Biology

First Tox studies using Troglitazone

Trade name: Rezulin, Rizulin, Romazin, Sensulin



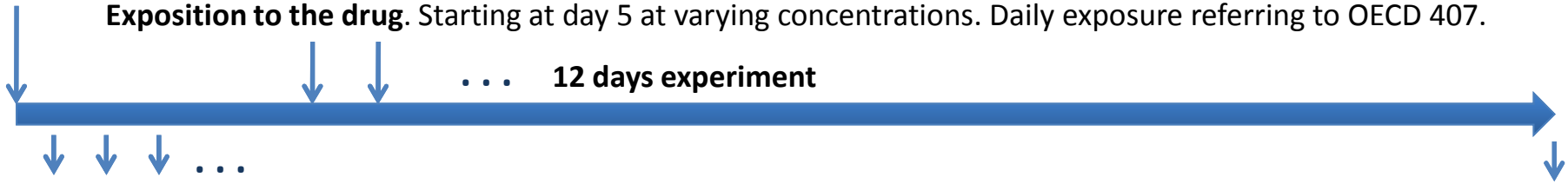
$C_{24}H_{27}NO_5S$



14 chips comprising **28 circuits** and **20 static controls.**

Inocculation of the chips on day 0

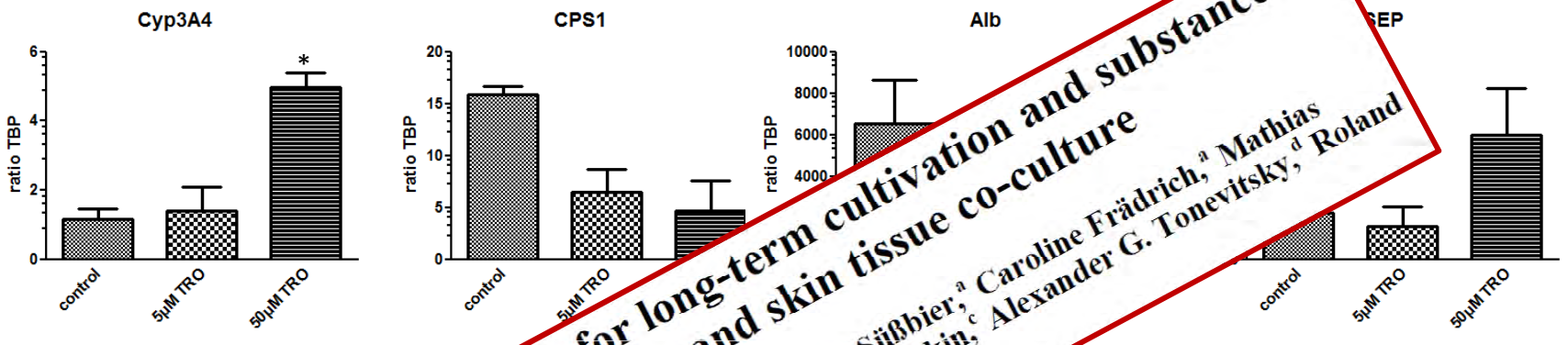
Exposition to the drug. Starting at day 5 at varying concentrations. Daily exposure referring to OECD 407.



Daily media exchange of 250µl.
The supernatants are checked for glucose, lactate, pH, albumin and LDH

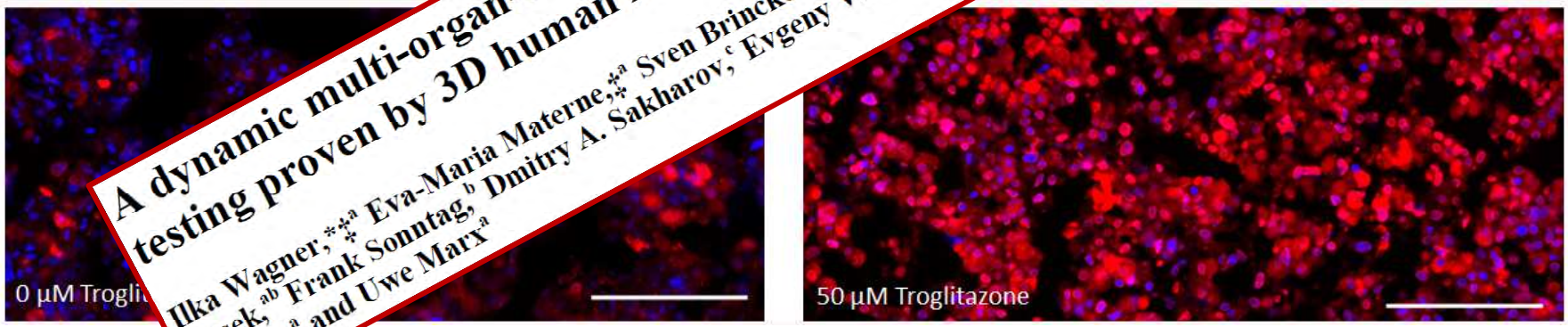
Endpoint analysis
by IHC and RT-PCR

7-day tissue performance at exposure to troglitazone



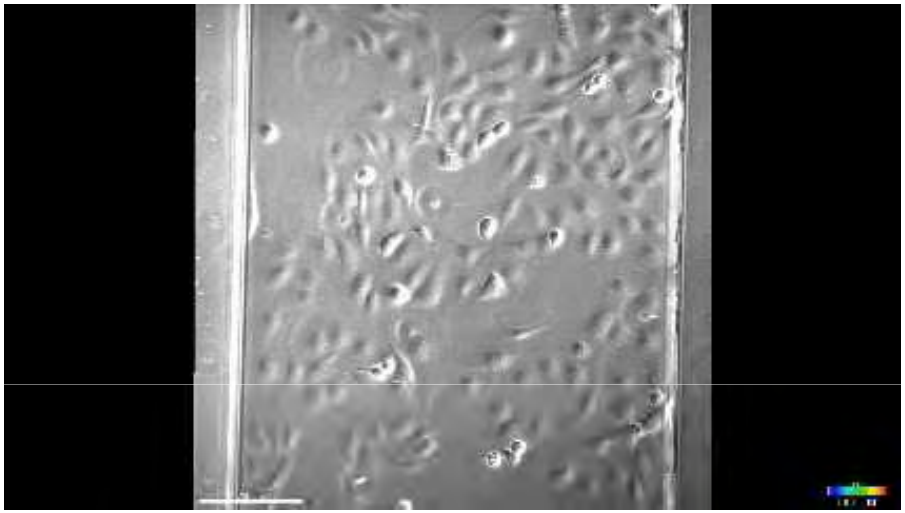
A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture

Ilka Wagner,[*a] Eva-Maria Materne,[*a] Sven Brincker,[a] Ute Süßbier,[a] Caroline Frädrich,[a] Mathias Busek,[ab] Frank Sonntag,[b] Dmitry A. Sakharov,[c] Evgeny V. Trushkin,[c] Alexander G. Tonevitsky,[d] Roland Lauster[a] and Uwe Marx[a]



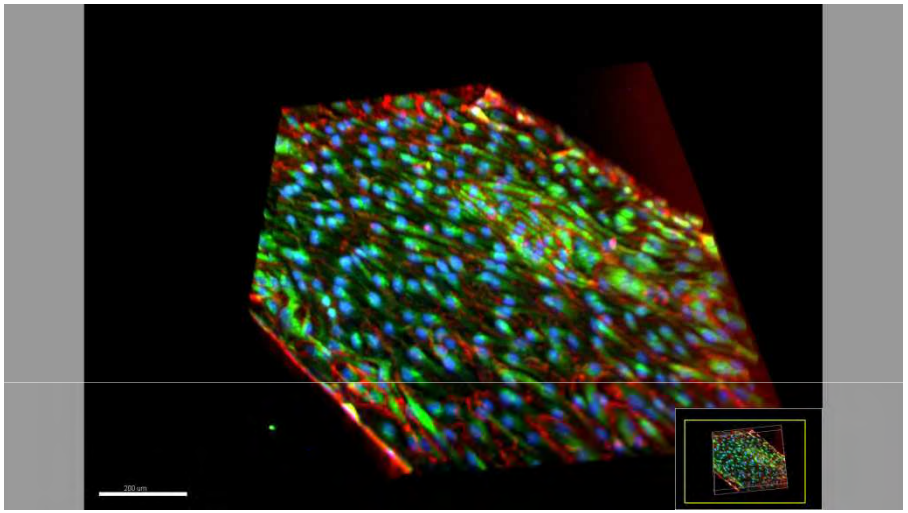
Establishment of stable microvascular circuits

Live-cell imaging



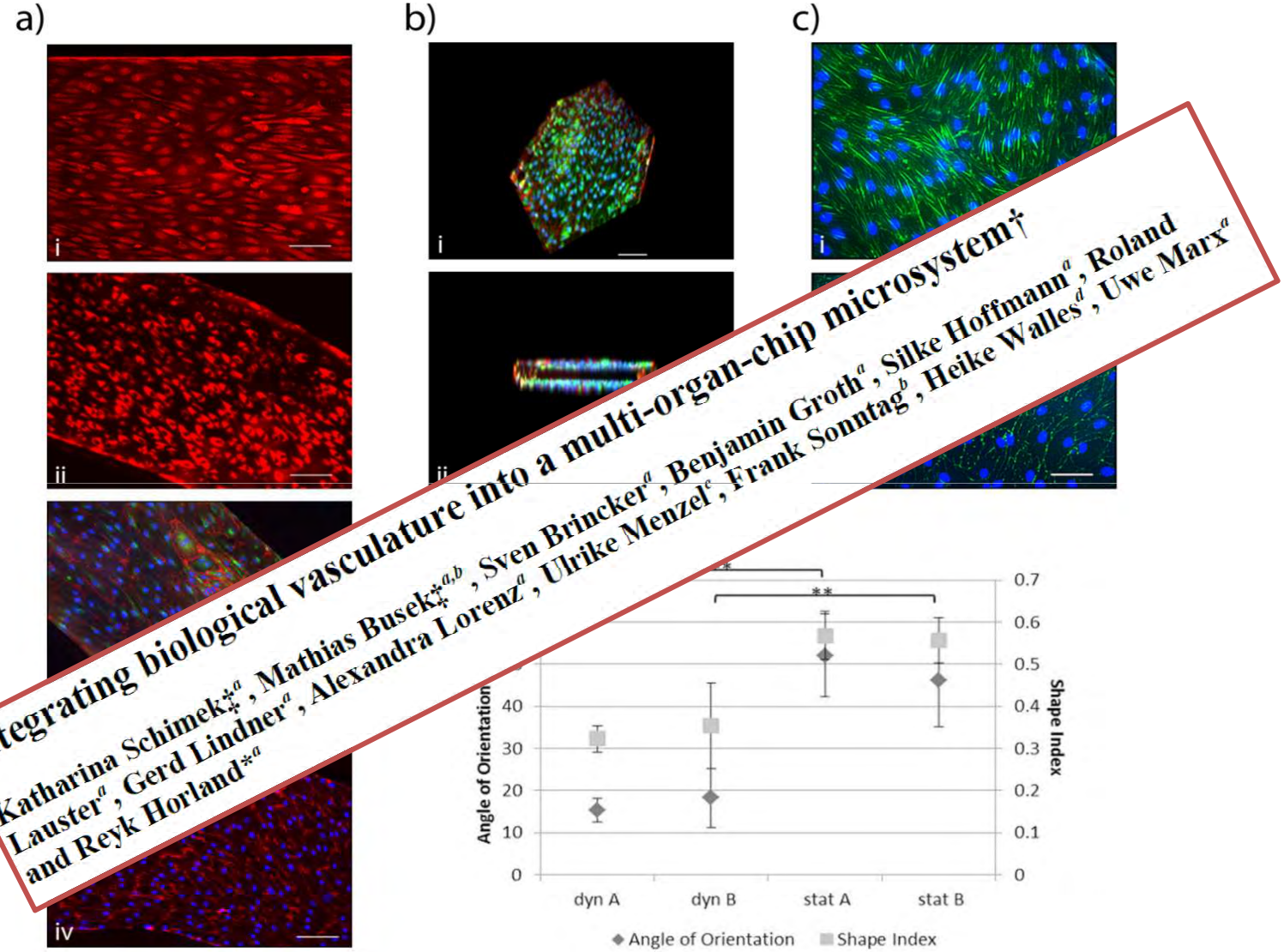
Human microvascular endothelial cells
(48h - time lapse; scale bar: 200µm)

Endpoint control

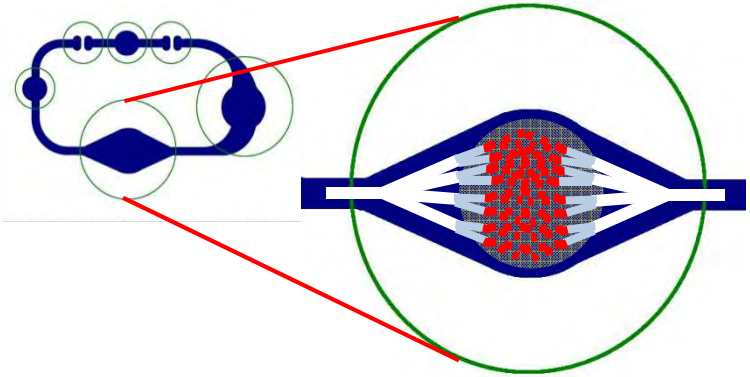


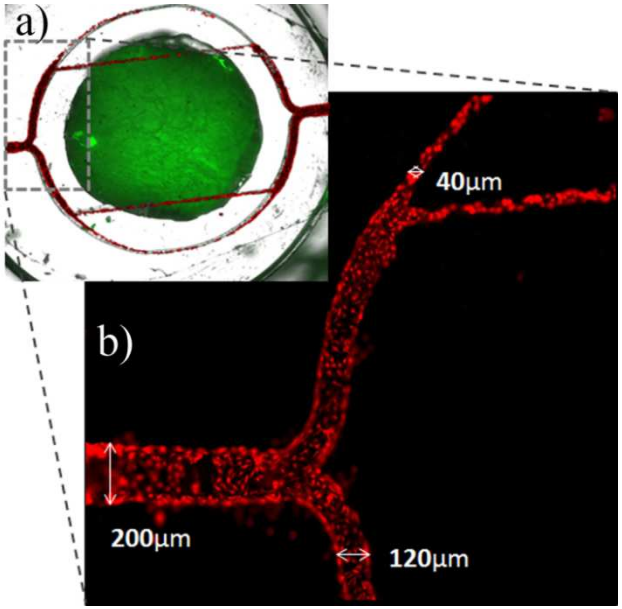
Human microvascular endothelial cells
cultured for 3 days under constant shear stress
(von Willebrand-Faktor: green; CD31: red;
Nuclei: blue; scale bar: 200µm)

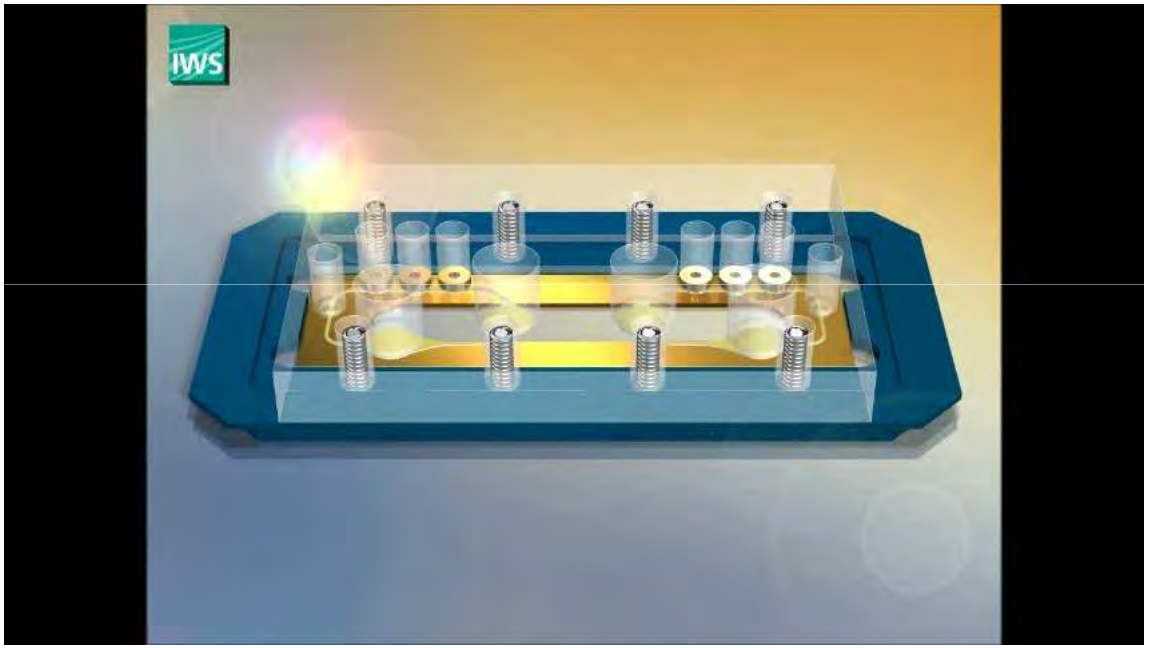
Importance of vascular transport systems



Creating a capillary bed for tissue supply







TISSUSE
Emulating Human Biology

Thank you for your attention!

Ilka Wagner, Eva-Maria Materne, Lutz Kloke, Chris Drewell, Katharina Schimek, Tobias Hasenberg, Anja Ramme, Silke Hoffmann, Gerd Lindner Juliane Hübner, Alexandra Lorenz, Caroline Frädrich, Annika Jaenicke, Agnes Schumacher, Luzie Reiners-Schramm, Jennifer Binder, Shirin Fatehi, Mark Rosowski, Beren Atac, Marielle Königsmark, Sandro Wagner, Karolina Tykwinska, Özlem Vural, Julia Bräunig, Annina Wanzek, Benjamin Groth, Corinna Magauer, Jadwiga Graczyk, Manuela Peters, Alexander Thomas, Roland Lauster, Uwe Marx