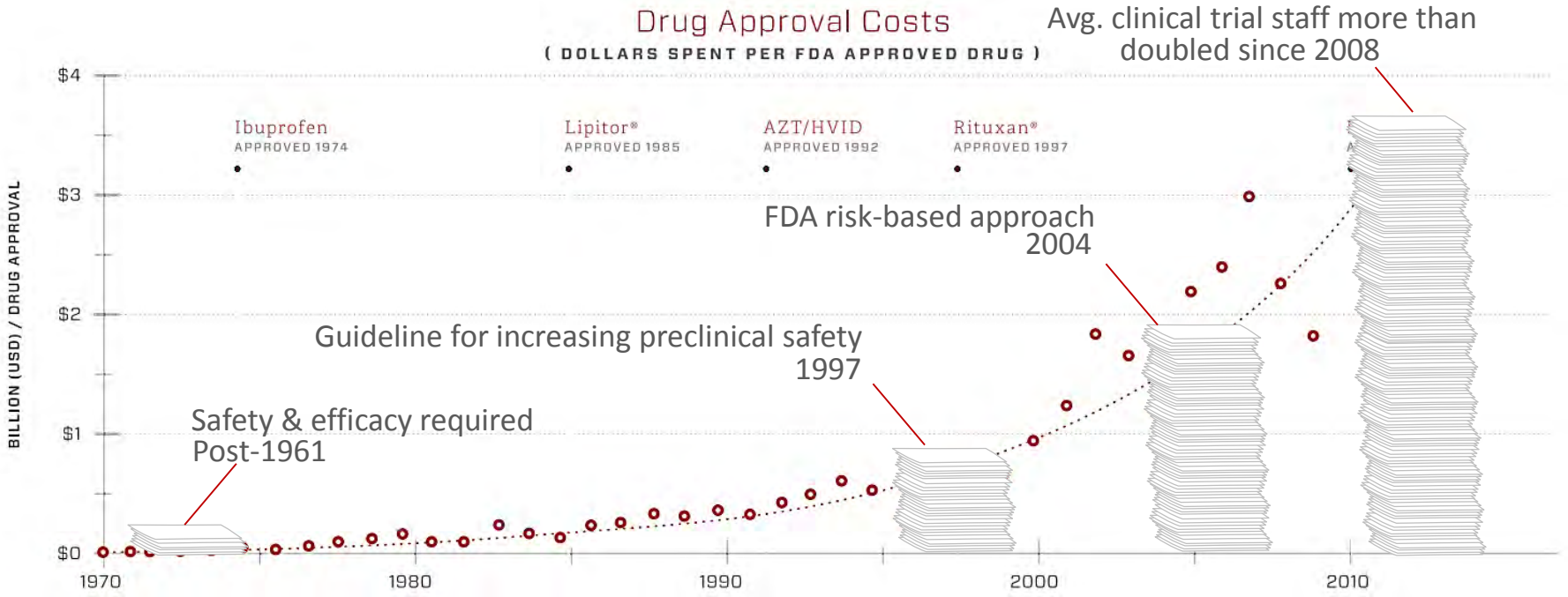
TISSUSE
Emulating Human Biology

Human-on-a-Chip: a paradigm shift in substance testing!?

A “medieval” approach to drug development



Source: Cutting Edge Information *Clinical Trial Staffing Levels Skyrocket (2011)*
Founders Fund *What Happened to the Future?*

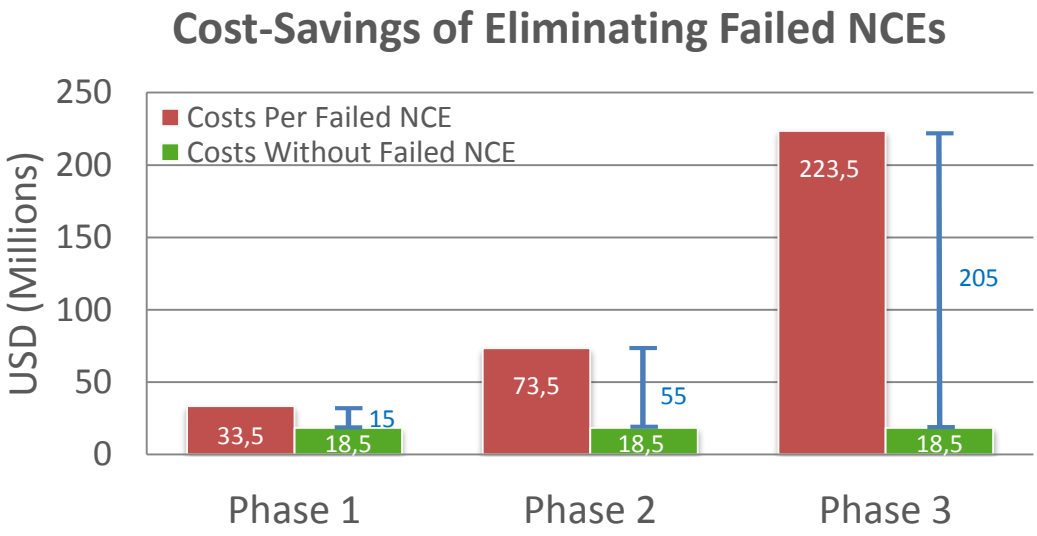
The Pharma Innovation Gap

Problem: **Drugs pass preclinical, but fail in clinical development**

Innovation Need:

Of 100 New Chemical Entities (NCEs) progressed from preclinical to clinical development :

- *46 fail due to toxicity*
- *35 fail due to lack of efficacy*



S. M. Paul et Al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nature Reviews Drug Discovery.* 2010. (9), 205-214.

Predictive power of preclinical testing

Technische Universität Berlin

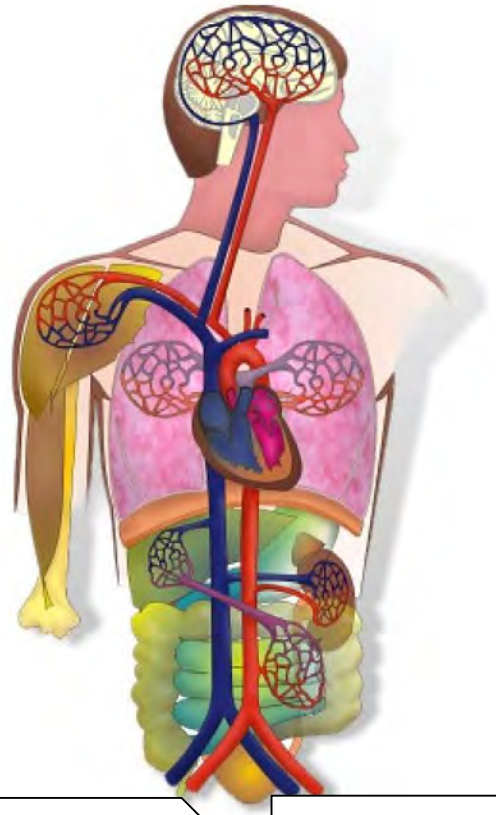
DRUG SUBSTANCES

laboratory animals

systemic but not human

human
3D- cell culture

human but not systemic



preclinics

safety
~60 volunteers/substance

efficacy
~ 3000 patients/substance

Market

US$1,4Billion., 12 years

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Microfluidic-based homeostasis *in vitro*

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number of "organs"	microscale culture systems	
	homotopic	heterotopic
single	endothelial cells: Young et al. 2010 myoblasts: Gu et al. 2004 hepatocytes: Powers et al. 2002, Leclerc et al. 2004, Ho et al. 2006, Lee et al. 2007, Toh et al. 2007 and 2009, Carraro et al. 2008, Park et al. 2008, Goral et al. 2010 neurons: Rhee et al. 2005 mammary epithelial cells: Grafton et al 2011 adipose cells: Nakayama et al. 2008 embryo cells: Hung et al. 2005, Chung et al. 2005, Villa-Diaz et al. 2009, Smith et al. 2012	lung alveola: Huh et al. 2010 liver lobulus: Kane et al. 2006, Hwa et al. 2007, Khetani et al. 2008 small artery: Günther et al. 2010 intestinal villus: Ootani et al. 2010, Sato et al. 2009, Sung et al. 2011, Lahar et al. 2011, Yu et al. 2012 nervous system: Park et al. 2009 bone-marrow units: Cui et al. 2007
multiple	bone-marrow+liver+tumour: Viravaidya et al 2004, Tabosian et al 2009, Sung et al 2009 and 2010, Mahler et al 2009 and 2012 lung+liver+kidney+adipose: Zhang et al. 2009 intestine+liver+tumour: Imura et al. 2010	

ATLA 2012, 40, 235-257 Marx et al: `Human-on-a-chip' developments: A translational cutting edge alternative to systemic safety assessment and efficiency evaluation of substances in laboratory animals and man?

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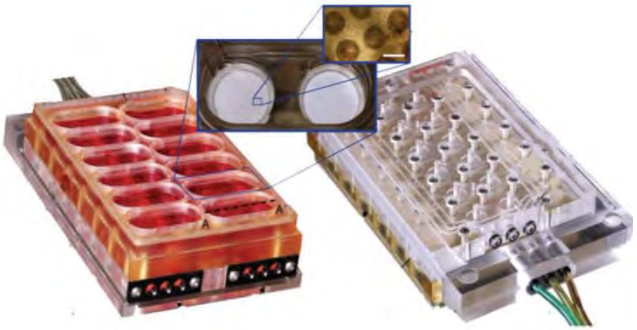
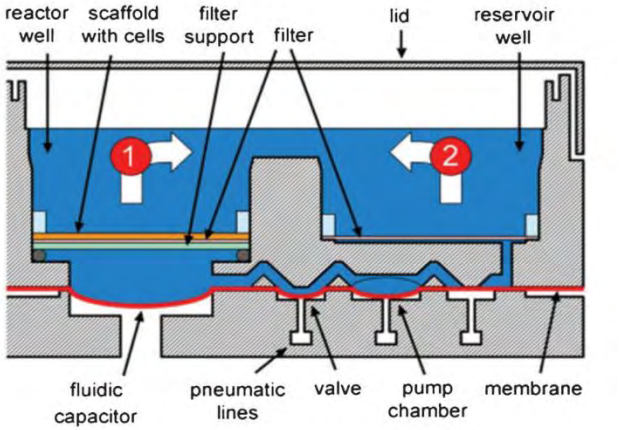
Perfused liver models

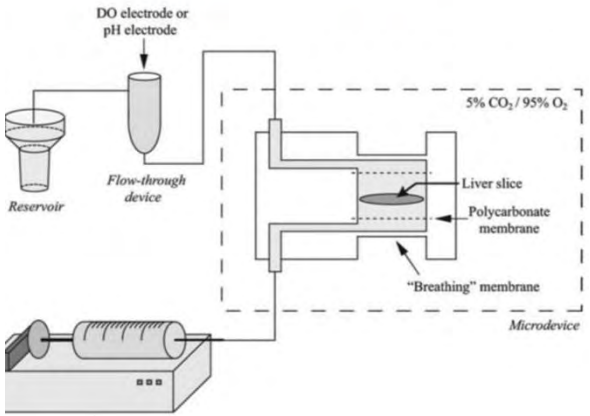
LiverChip™





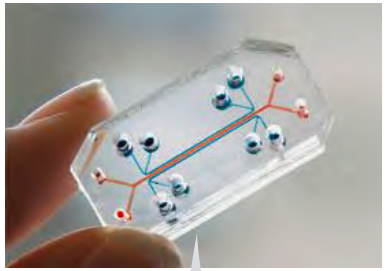
Domansky et al., Lab Chip, 2010, 10, 51-8

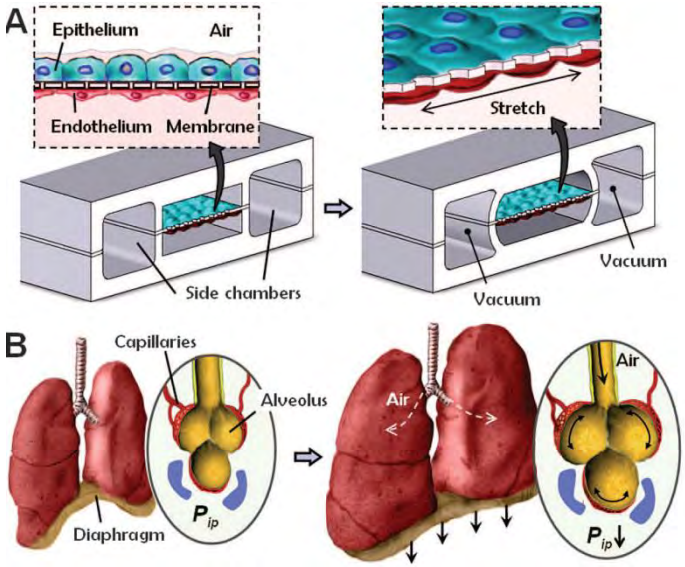
BioChip for liver slices



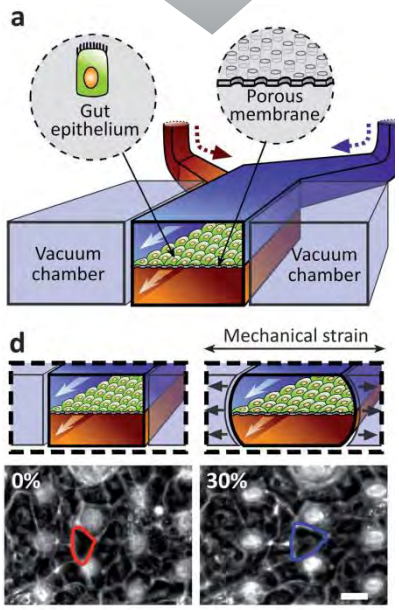
van Midwoud et al., Biotechnol. Bioeng., 2010, 105, 184-94

The Wyss Institute – Organs-on-a-Chip

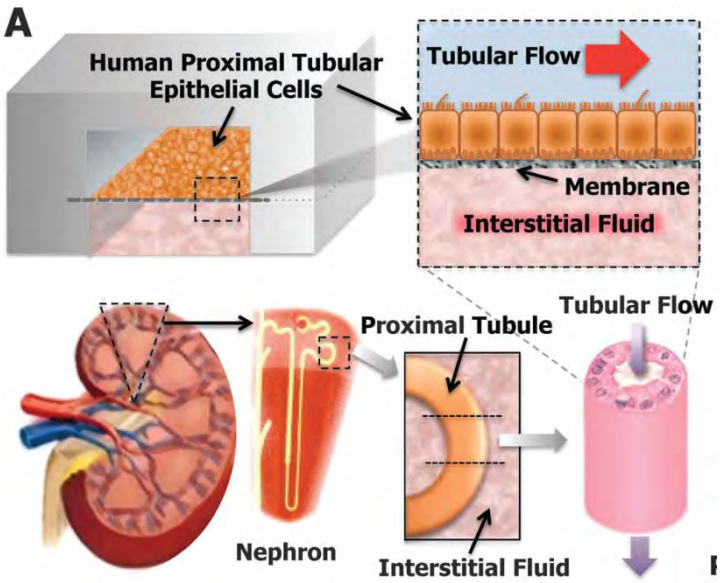




Huh et al., Science, 2010, 328, 1662

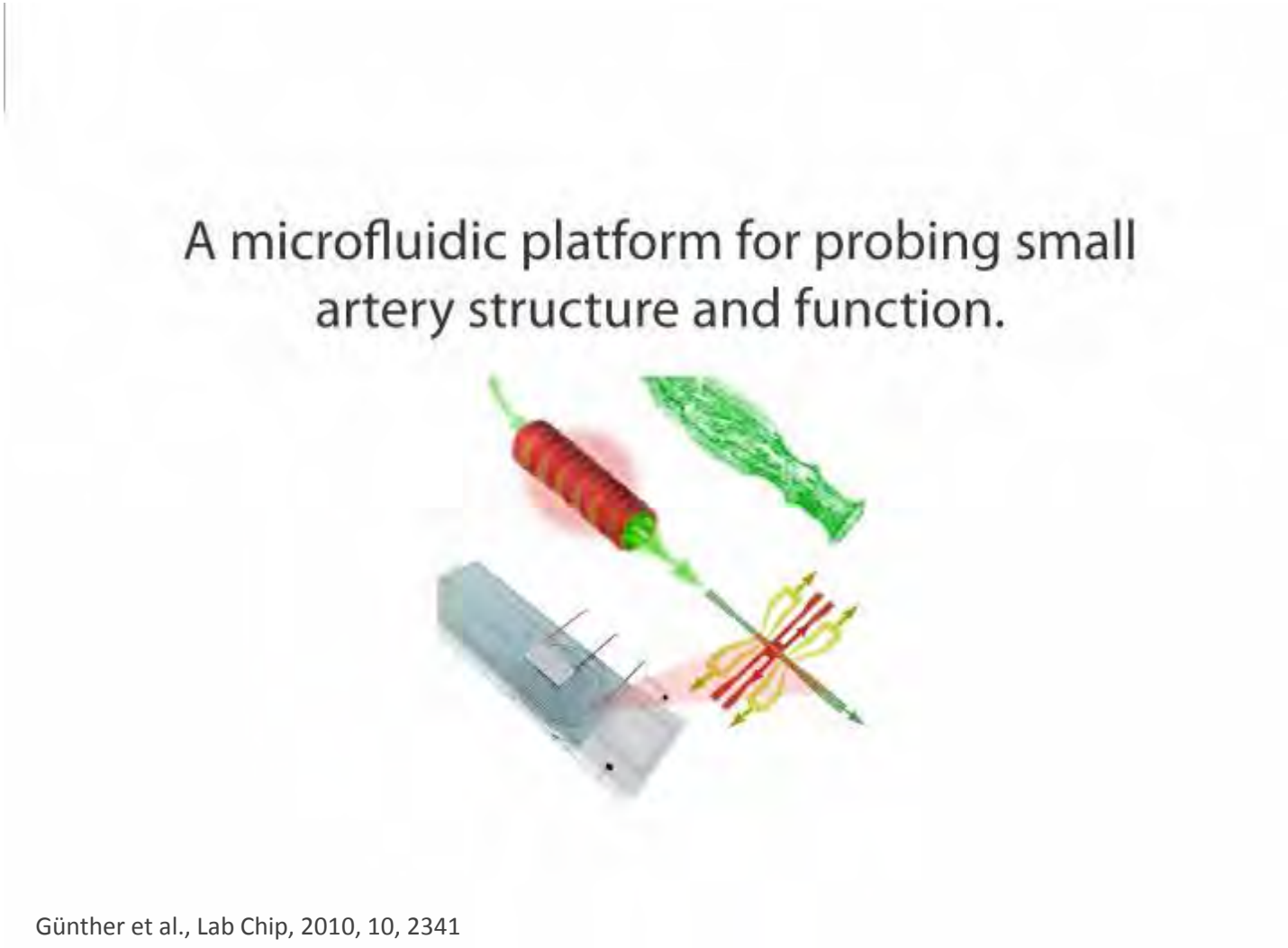


Kim et al., Lab Chip, 2012, 328, 1662



Jang et al., Integr. Biol., 2013, 5, 119

Artery-on-a-Chip



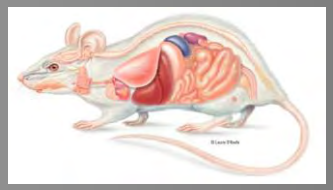
Günther et al., Lab Chip, 2010, 10, 2341

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

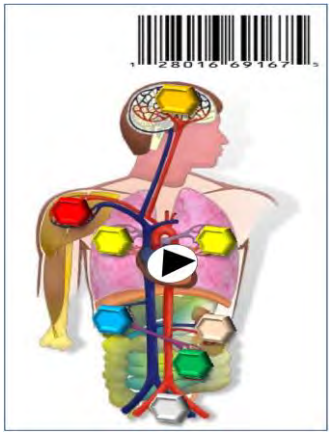
2012

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

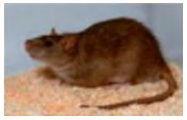
Basketter et al, ALTEX 29, 1/12, pp 1-91

→ **Integrated Testing Strategy (ITS)**

Solving the Substance Testing Dilemma



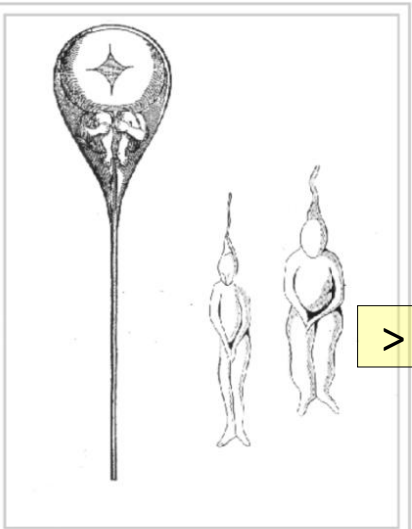
“Human-on-a-Chip”
human AND systemic




animal models
systemic but NOT human

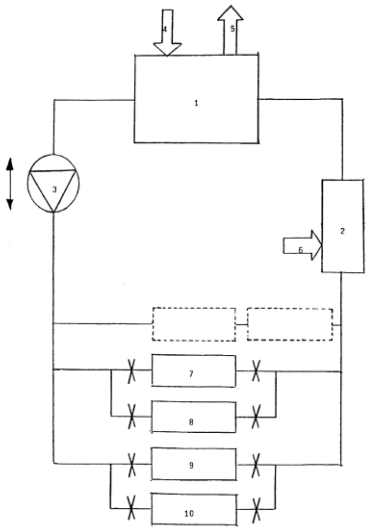
static 2D & 3D
human cell culture
human but NOT systemic

A historic imagination turned into a vision!

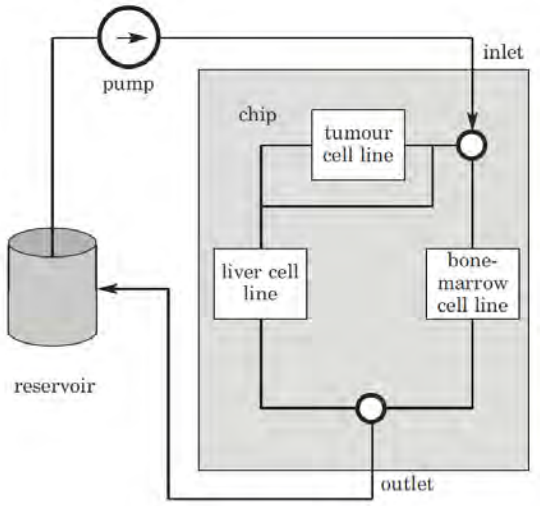


Homunculi in sperm as drawn by N. Hartsoecker in 1695

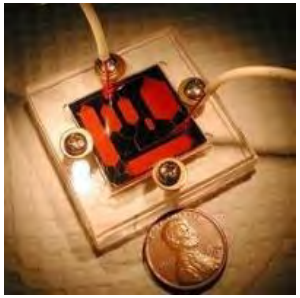
> 300 Years

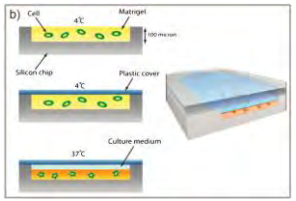


Marx et al, **1992**
V&V zur zeitgleichen Kultivierung unterschiedlicher Säugerzellen
EP0584170B1



µCCA
Micro cell culture analog





Michael Shuler et al, **2004**
„Human-on-a-chip“
Biotech. Progress 20, 590-597

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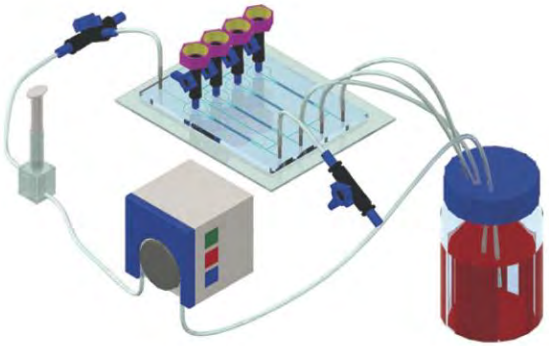
„Human-on-a-chip“ – historical sketch

Michael Shuler et al,
Cornell University

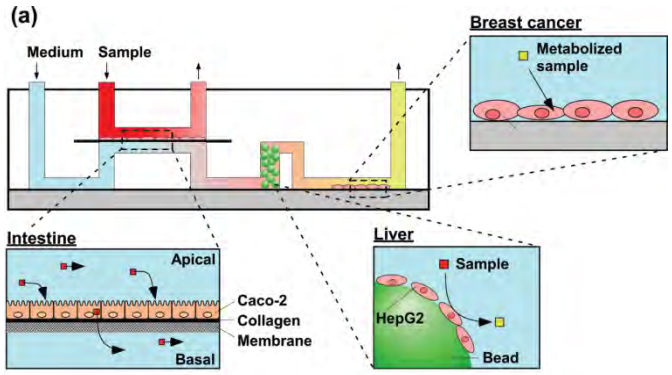
Hanry Yu et al,
Singapore – US-MIT Alliance

Kiichi Sato et al,
University of Tokyo

Uwe Marx et al,
Technische Universität Berlin



Zhang et al., Lab Chip, 2009, 9, 3185



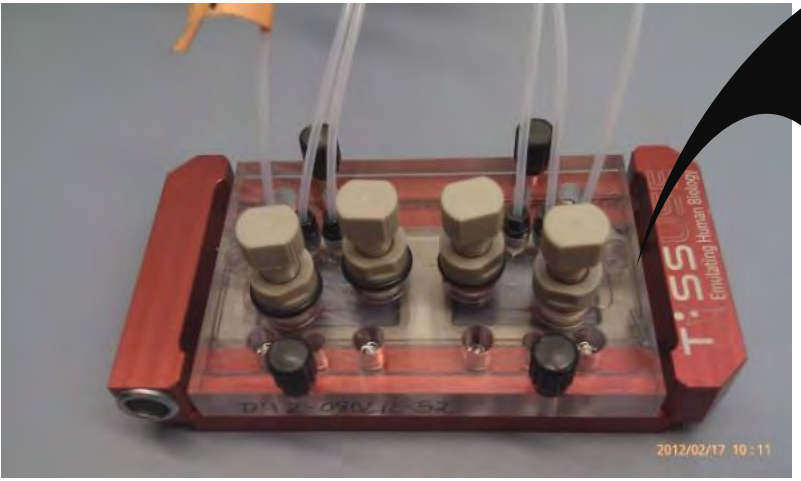
Imura et al., Anal. Chem., 2010, 82, 8

Nature March 31st 2011: Vol 471 pp. 661-665 M. Baker: Technology feature: A living system on a chip

ATLA 2012, 40, 235-257 Marx et al: `Human-on-a-chip' developments: A translational cutting edge alternative to systemic safety assessment and efficiency evaluation of substances in laboratory animals and man?

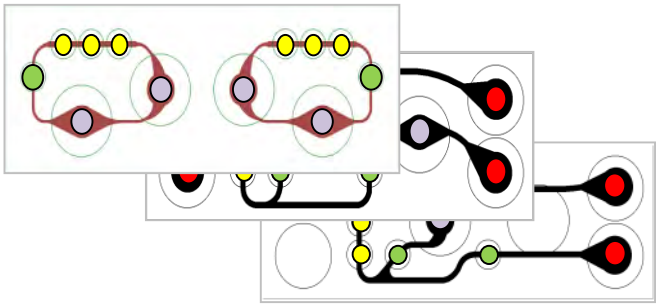
The Multi-Organ-Chip (MOC) Technology

Technische Universität Berlin



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 - Norecopa -



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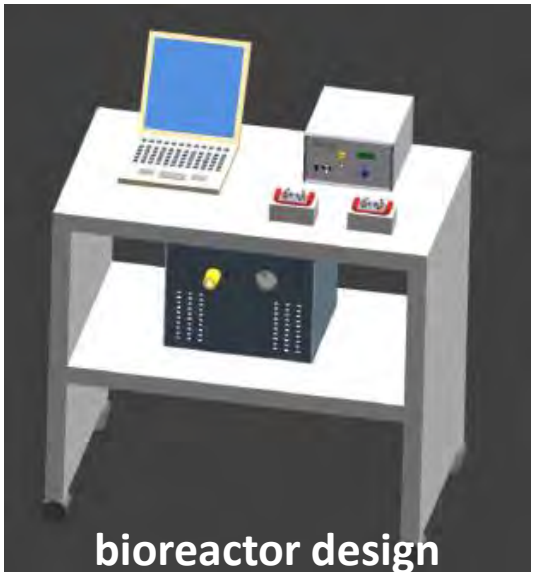
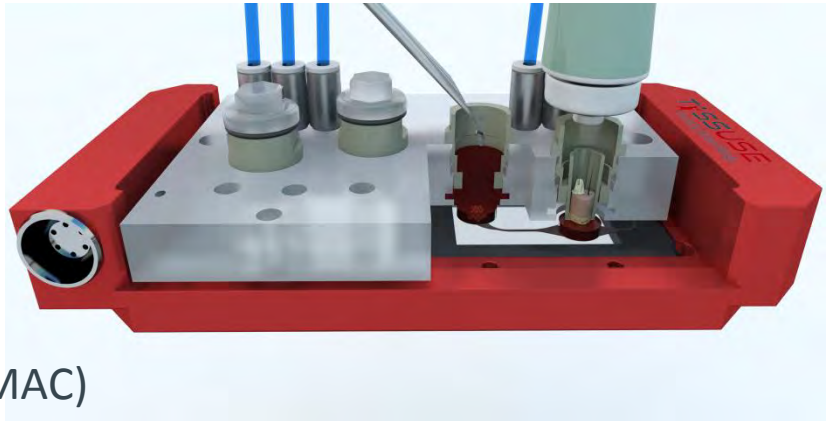
Benchtop bioreactor


bioreactor design


bioreactor in operation

Features:

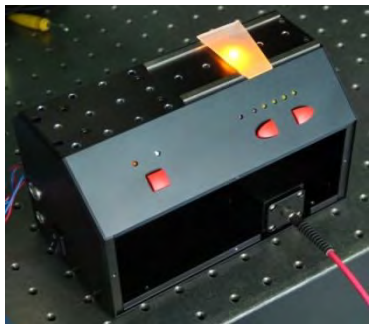
- Controlling up to 24 pneumatic actors
- Up to 4 chips per system
- Adjustable temperature and fluid flow
- Software control (e.g. WINDOWS, LINUX, MAC)
- Telemonitoring

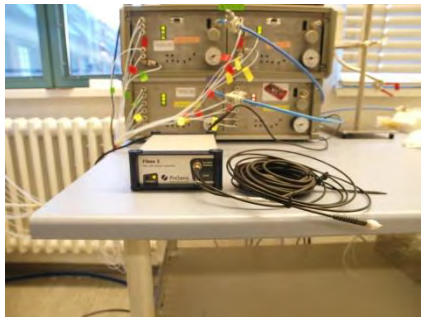


Sensors / In-process-controls

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

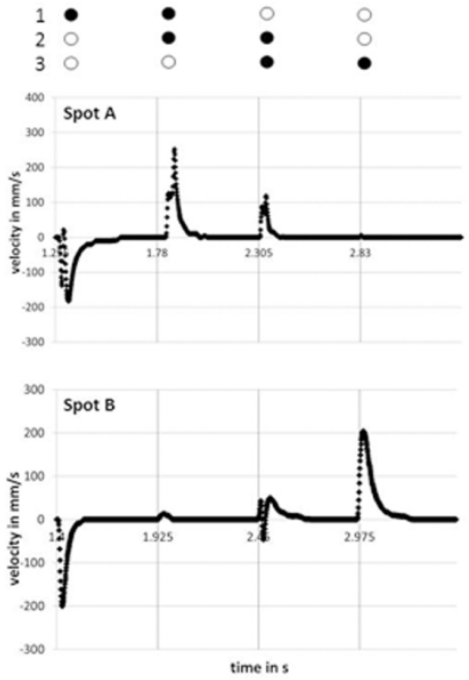




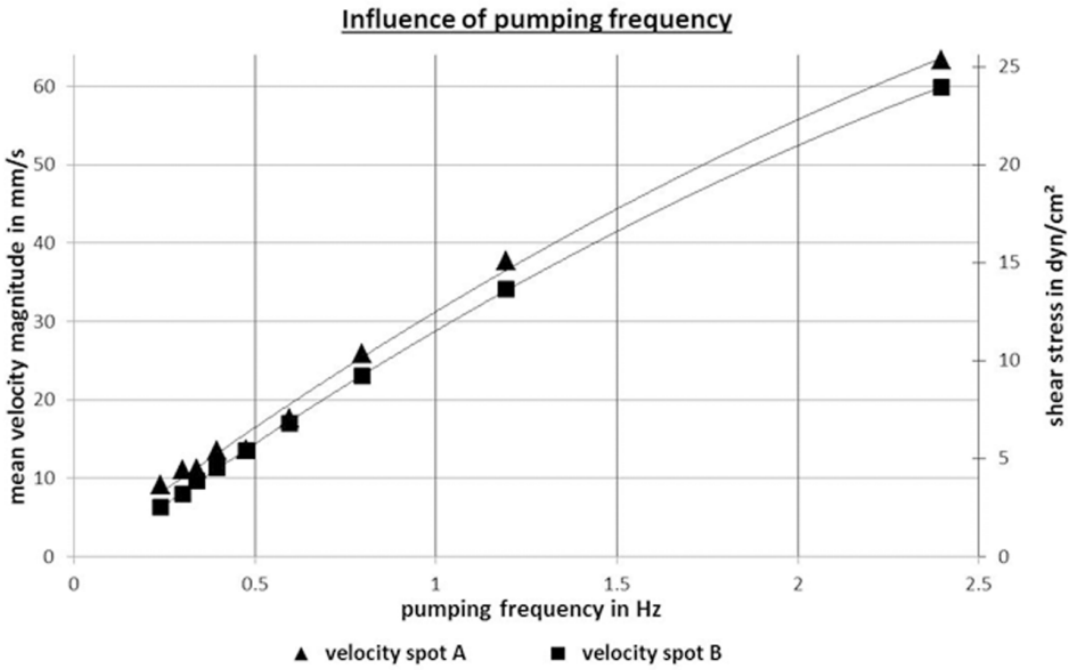
Evaluation of fluid dynamics

Particle imaging velocimetry

a)



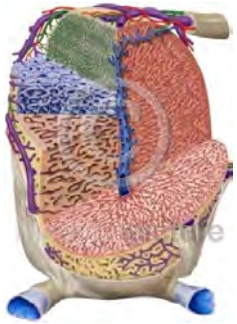
b)

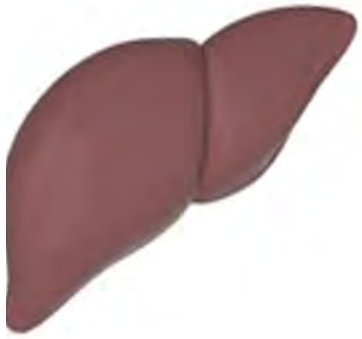


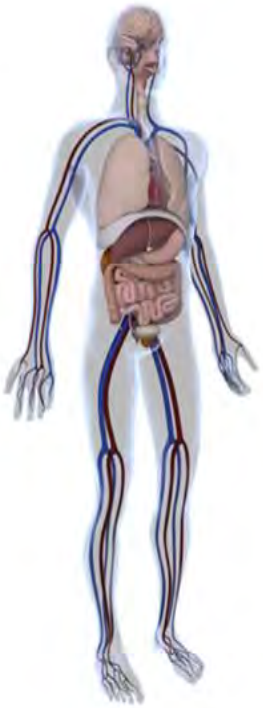
Schimek et al., Lab Chip 2013

Smallest possible scale of organs

- a single liver lobule is of 1,3 µl in scale
- 1 million liver lobules constitute a human liver







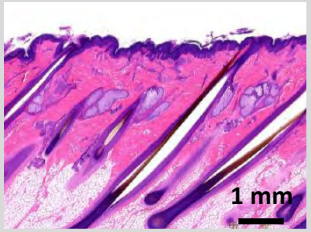
Molecule Cell Organoid Organ Individual

Ten liver lobules – the basis for a 1/100.000 "human-on-a-chip"

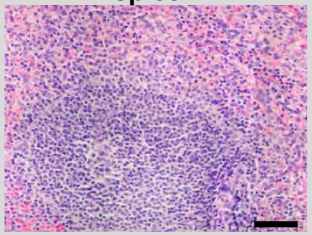
Human organoids

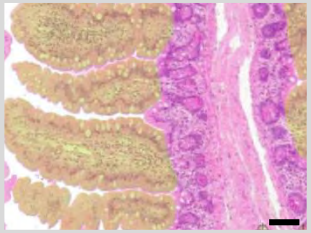
Technische Universität Berlin

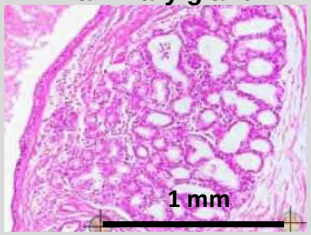
High turnover
High regenerative potential

skin


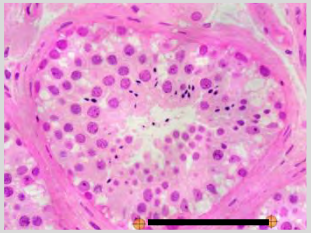
with appendices

spleen

white and red pulpa

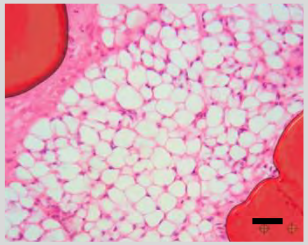
intestine

villus

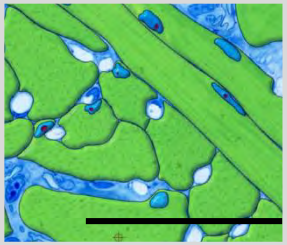
mammary gland


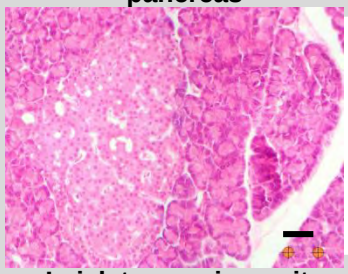
epithelium

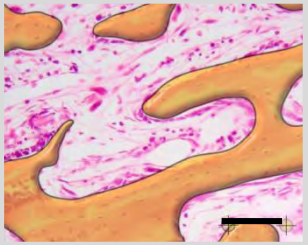
testes

follicle

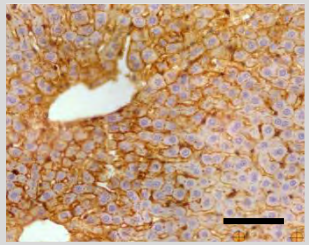
Low turnover
High regenerative potential

adipose tissue

cluster

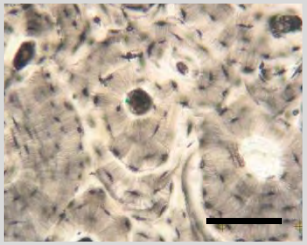
skeletal muscle

fibre segment

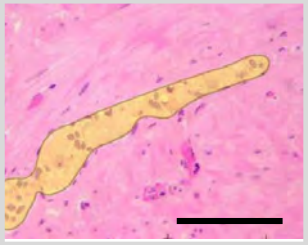
pancreas

L. islet, exocrine units

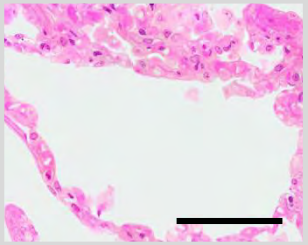
bone-marrow

unit

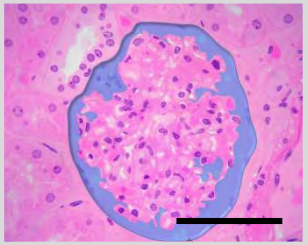
liver

lobulus

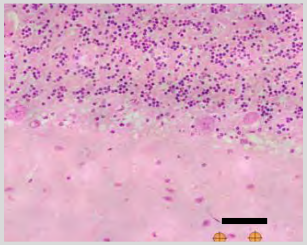
Low turnover
Low regenerative potential

bone

osteone

ENS

plexus

lung

alveola

kidney

nephron

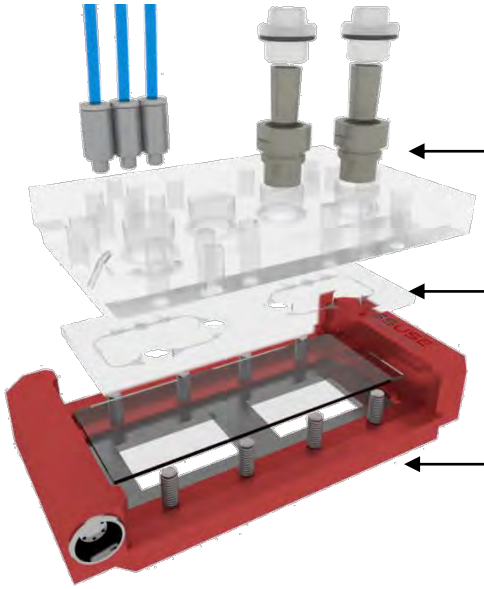
brain

cerebellar cortex

Bars: 100 µm

Marx et al., Altern Lab Anim. 2012 Oct;40(5):235-57

TISSUSE Emulating Human Biology

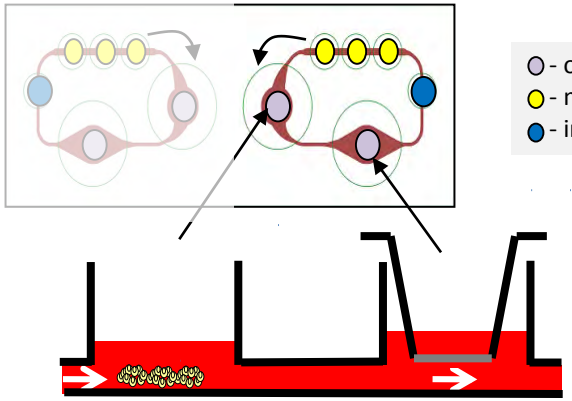
The “Two-Tissue Culture Chip”



Culture inserts e.g. Transwell®

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

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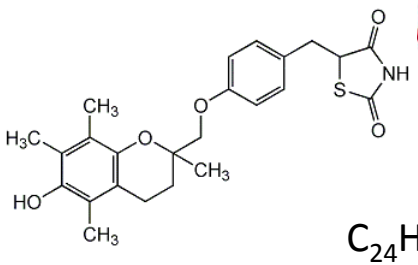
Tox study using Troglitazone

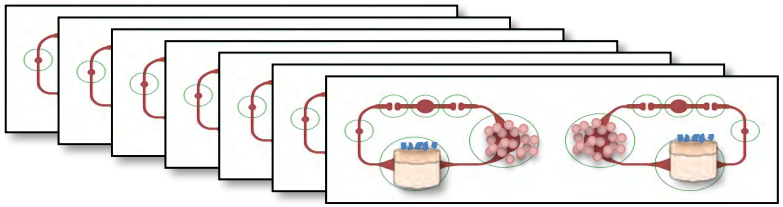


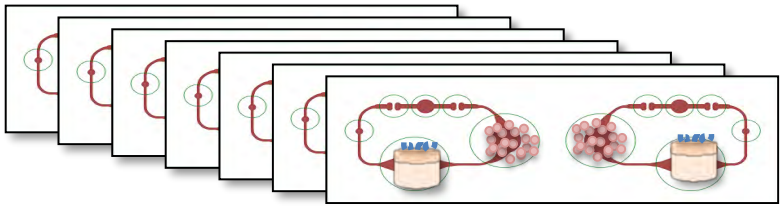
$C_{24}H_{27}NO_5S$

Trade name: Rezulin, Rizulin, Romazin, Sensulin
Developed by: Daiichi Sankyo Co (Japan)
Manucactured by: Parke-Davis (1997 approved by FDA)

Indication: Troglitazone is an antidiabetic and anti-inflammatory drug, prescribed for patients with diabetes mellitus type 2

Contraindication: Idiosyncratic reaction leading to liver failure





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

Inocculation of the chips on day 0

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

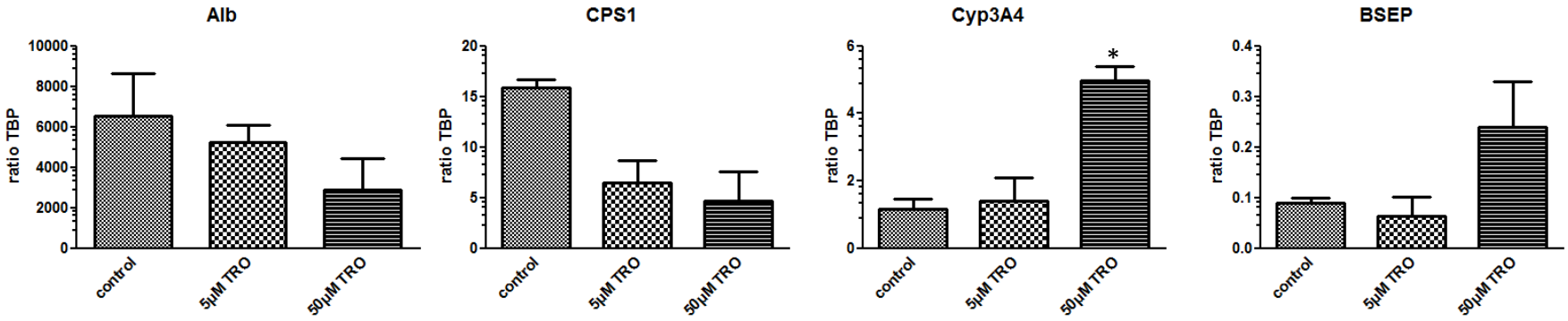
. . . **7-14 days experiment**

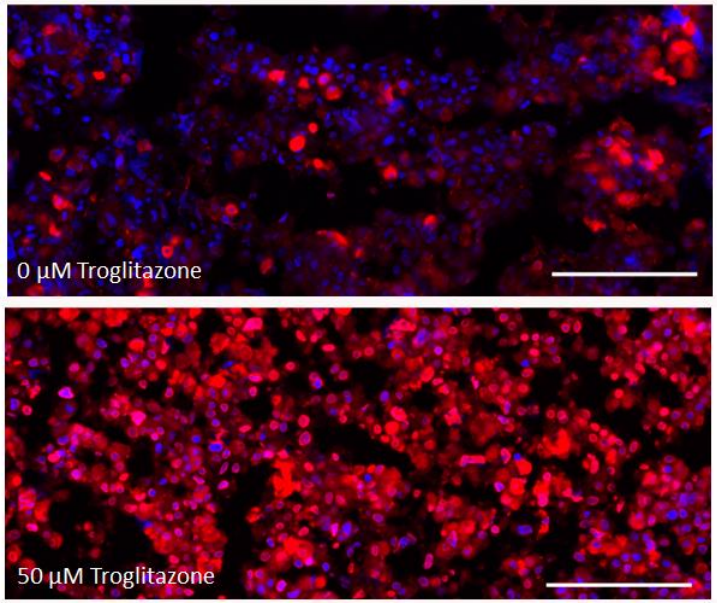
. . .

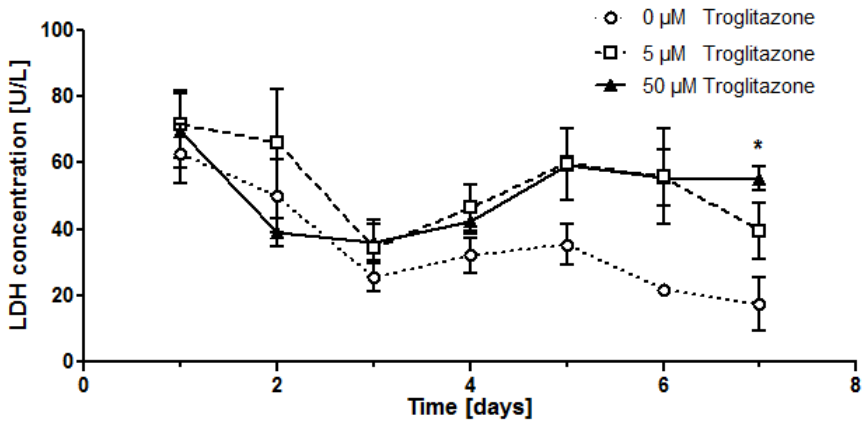
Daily media exchange of 250µl.
The supernatants are checked for glucose, lactate, pH, albumin and LDH

Endpoint analysis
by IHC and RT-PCR

Sensitivity to Troglitazone (7 day exposure)

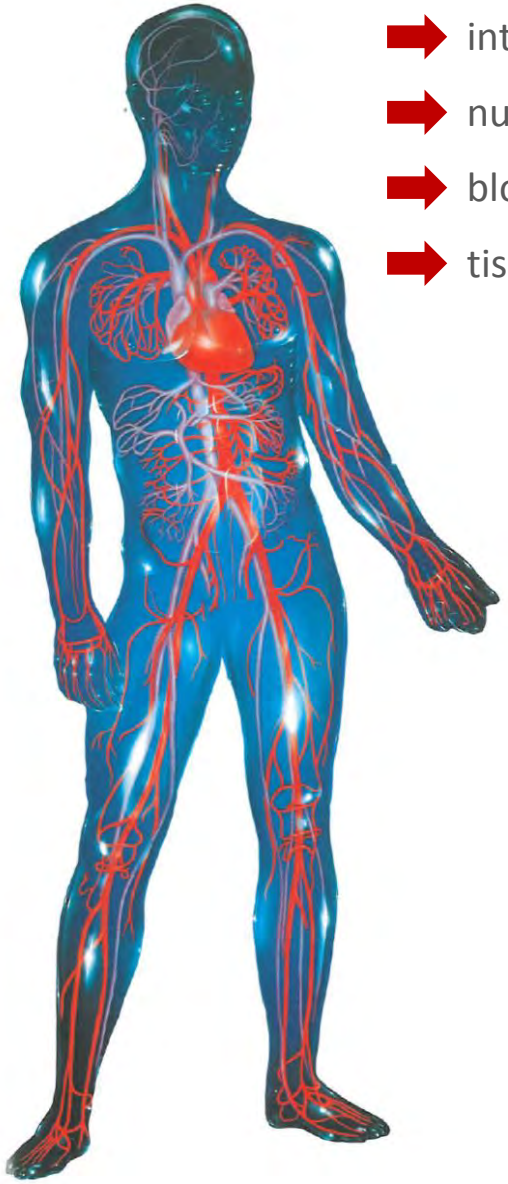




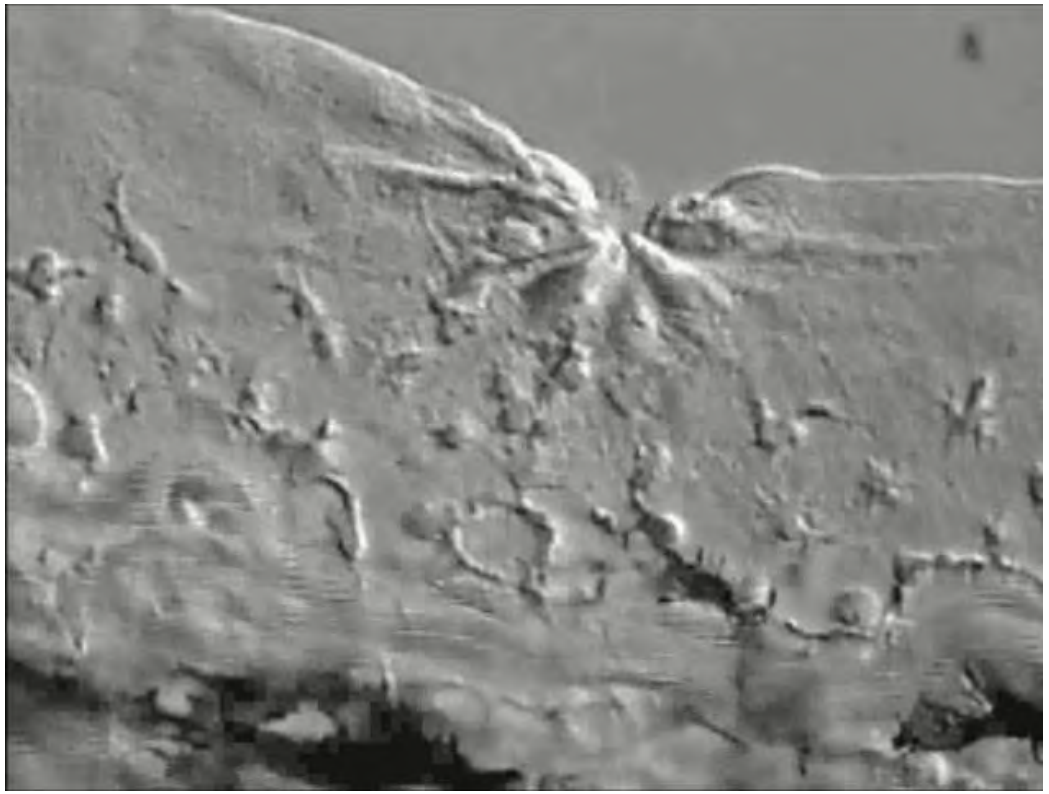


Wagner et al., Lab Chip 2013

The crucial role of dynamic blood circulation



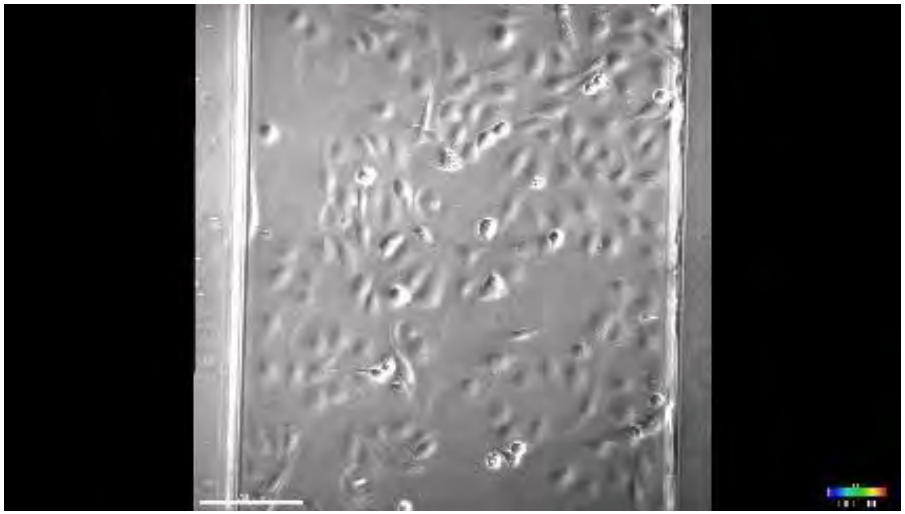
- interconnection of organs to create an **organism**
- nutrient and oxygen transport through **blood plasma and red blood cells**
- blood-tissue barrier and neo angiogenesis through **endothelial cells**
- tissue repair and immune response through **white blood cells**



Establishment of stable microvascular circuits

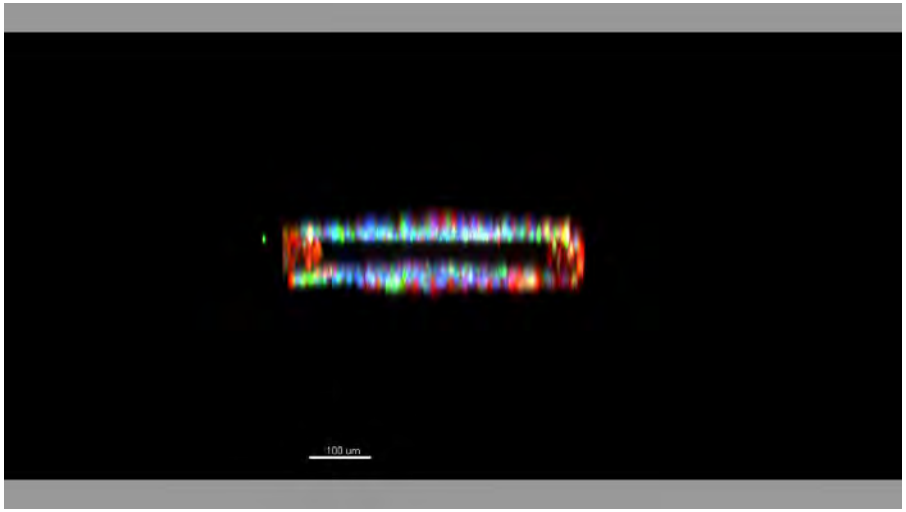
Technische Universität Berlin

Live-cell imaging



Human microvascular endothelial cells
(66h - 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)

Schimek et al., Lab Chip 2013

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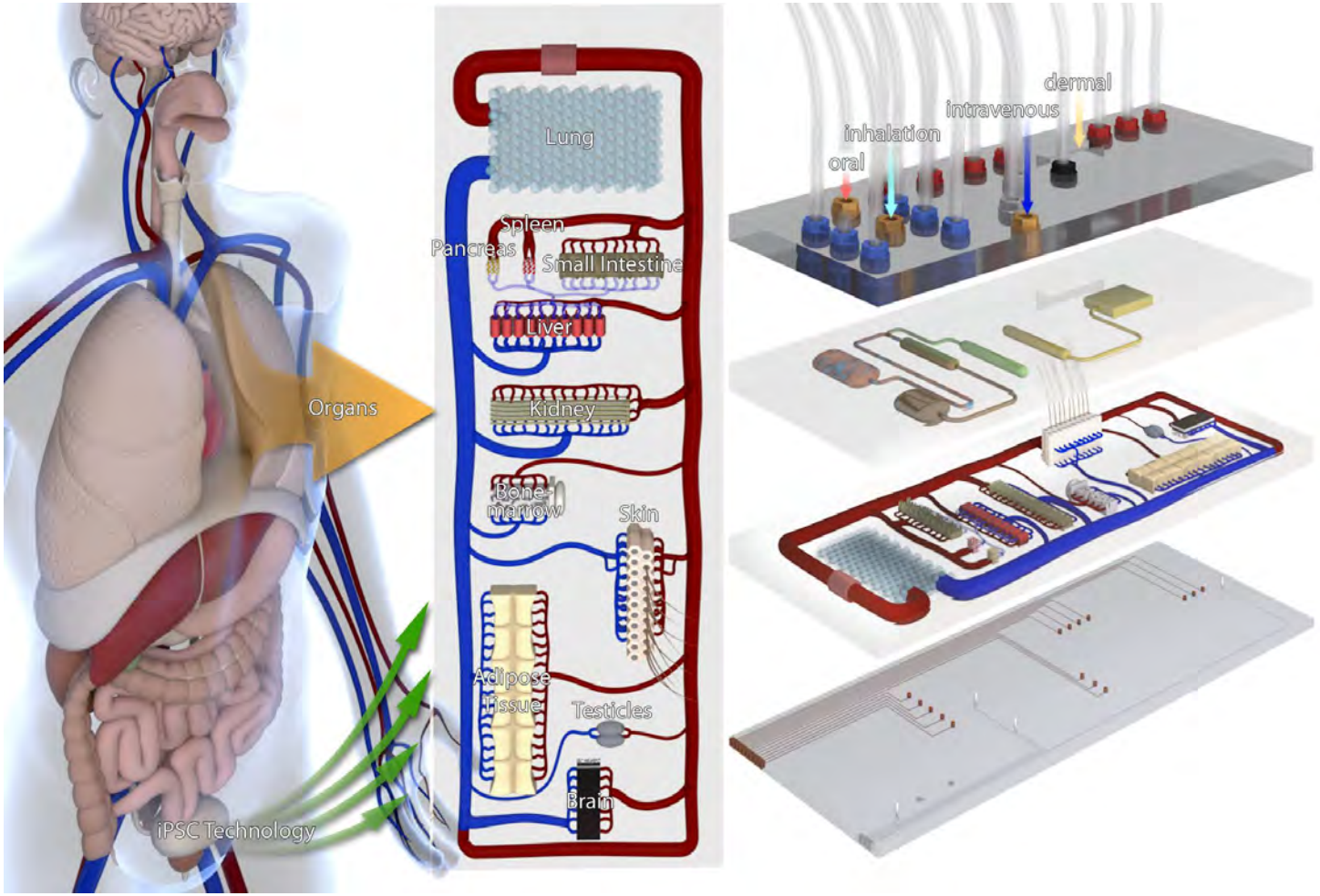
Next generation Multi-Organ-Chip



Marx et al., Altern Lab Anim. 2012 Oct;40(5):235-57

US Initiative “Human-on-chip”

US-Initiative adopted the “human-on-a-chip” strategy 2012 in a unique way

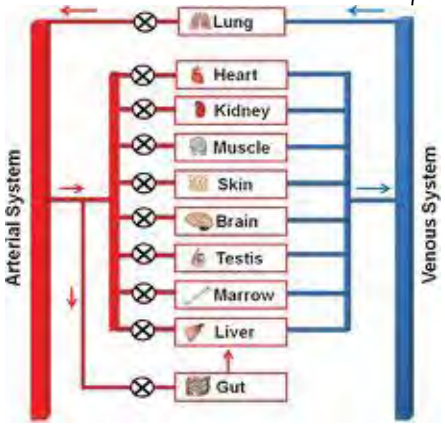
July 1st 2012 ---------- June 30th 2017

DARPA
Defence Advanced
Research Projects Agency

US$ 69Mio/5 Years

NIH/NCATS
National Center for Advancing
Translational Sciences

US$ 64Mio/5 Years



FDA

17 NIH groups and …

Linda Griffith
MIT

Donald Ingber
Wyss Institute
(Harvard MS)

Three players – NIH, FDA, DARPA

Source: The Burrill Report. http://www.burrillreport.com/article-nih_and_darpa_fund_development_of_organ_on_a_chip_systems.html

Adopted from Suzanne Fitzpatrick, FDA 27.10.2012

Thank you for your attention!

Ilka Wagner, Eva-Maria Materne, Lutz Kloke, Chris Drewell, Katharina Schimek, Tobias Hasenberg, 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, Manuela Peters, Alexander Thomas, Roland Lauster, Uwe Marx