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

A “medieval” approach to drug development

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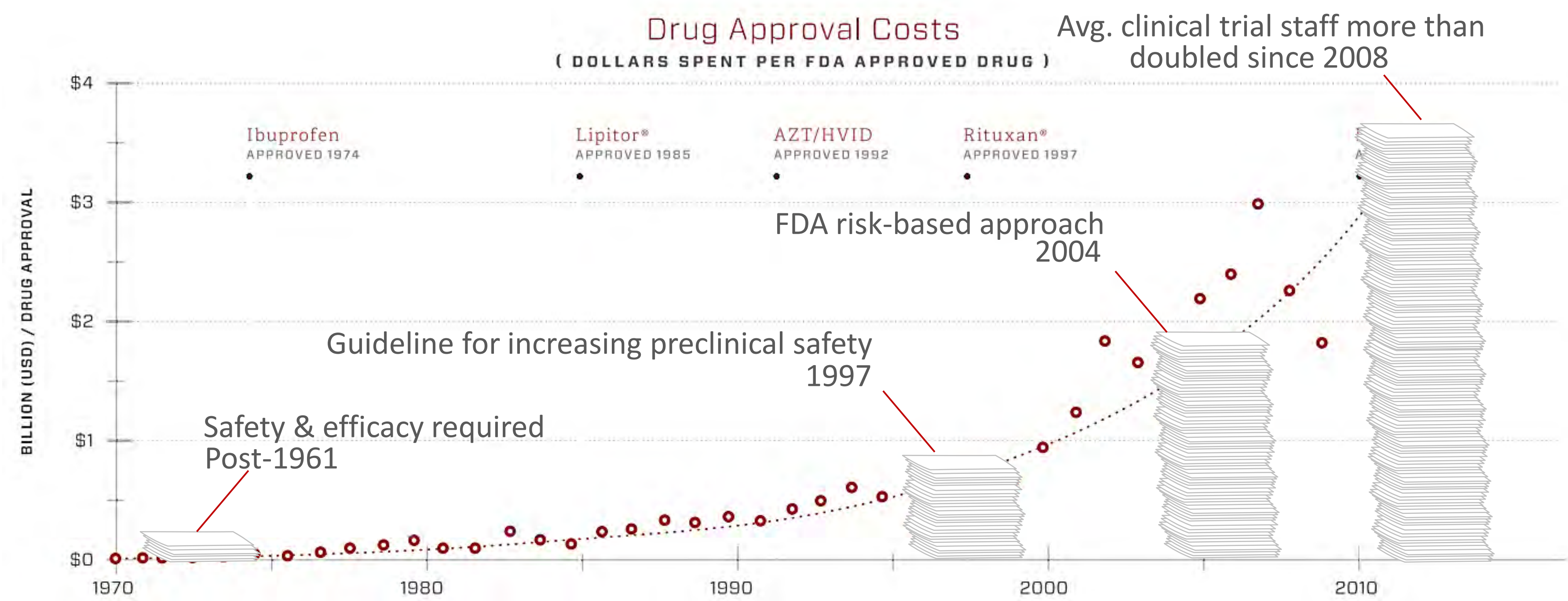

Source: Cutting Edge Information *Clinical Trial Staffing Levels Skyrocket (2011)*
Founders Fund *What Happened to the Future?*

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The Pharma Innovation Gap

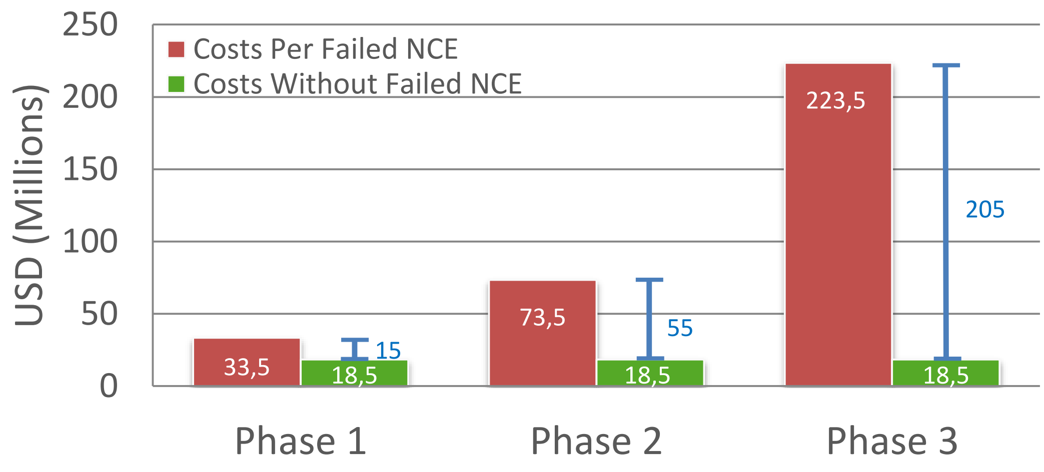
Problem: **Drugs pass preclinical, but fail in clinical development**

Innovation Need:

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

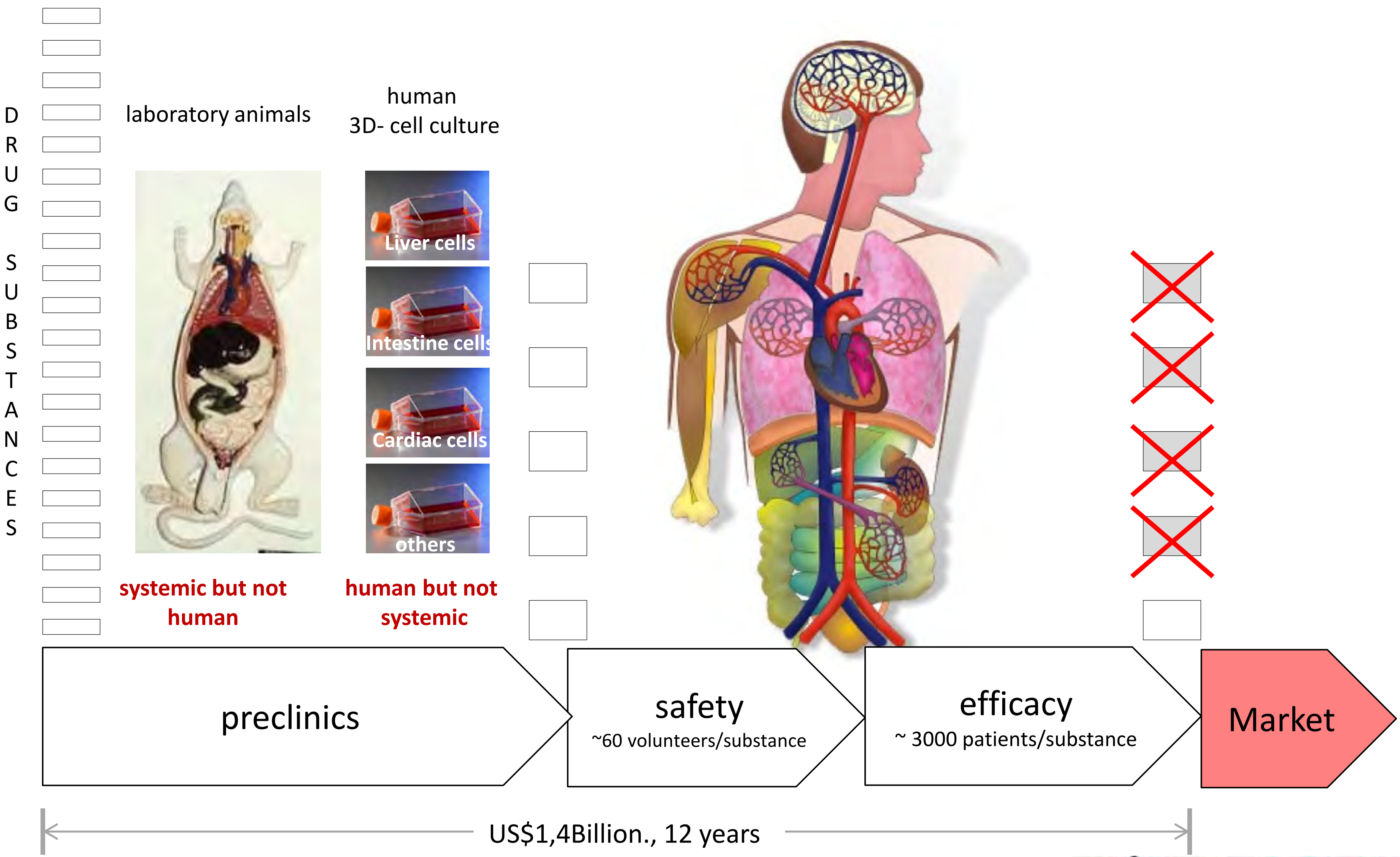
- *46 fail due to toxicity*
- *35 fail due to lack of efficacy*

Cost-Savings of Eliminating Failed NCEs



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



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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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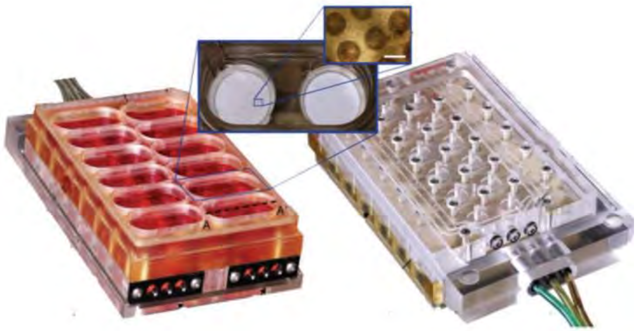
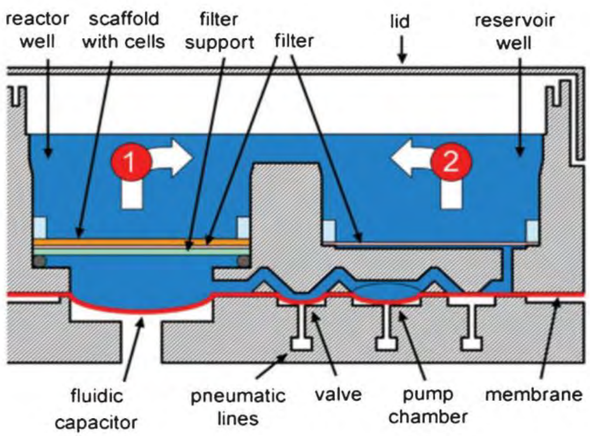
Perfused liver models

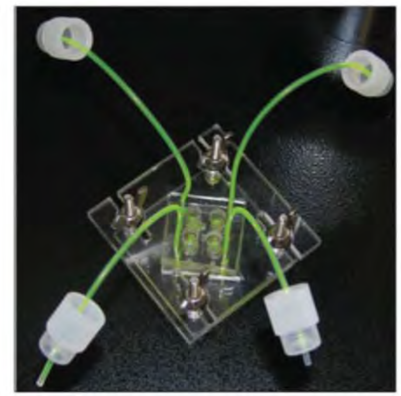
LiverChip™

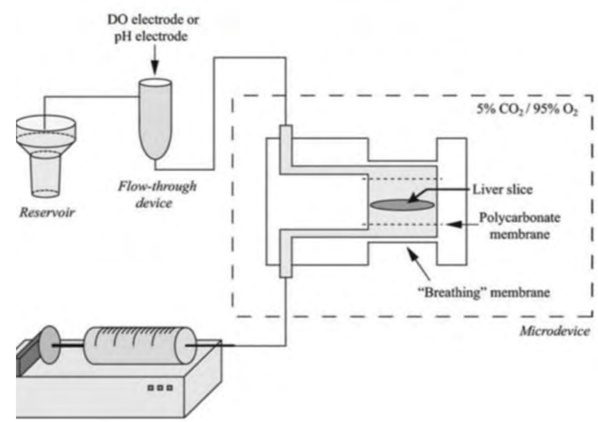




Domansky et al., Lab Chip, 2010, 10, 51-8

BioChip for liver slices





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

The Wyss Institute – Organs-on-a-Chip

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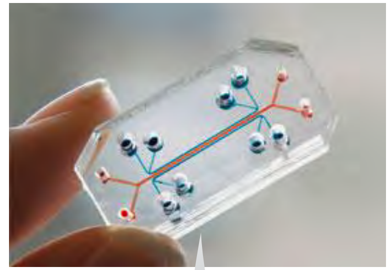
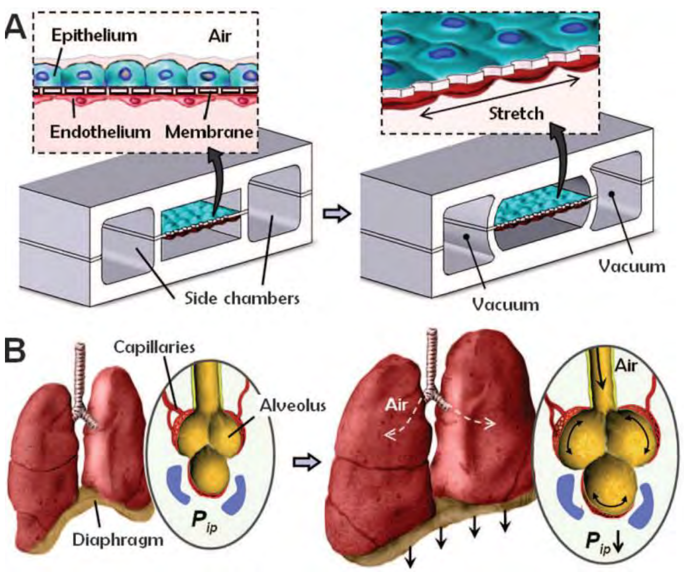
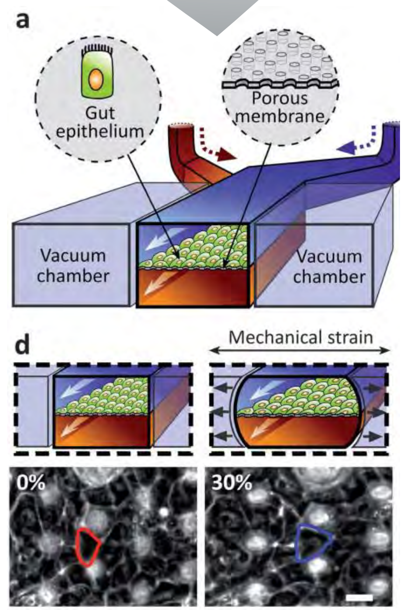
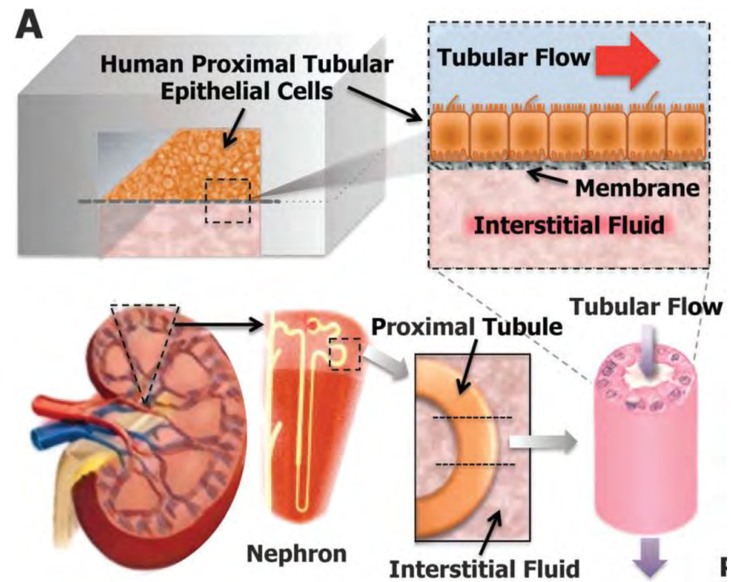




Huh et al., Science, 2010, 328, 1662



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



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

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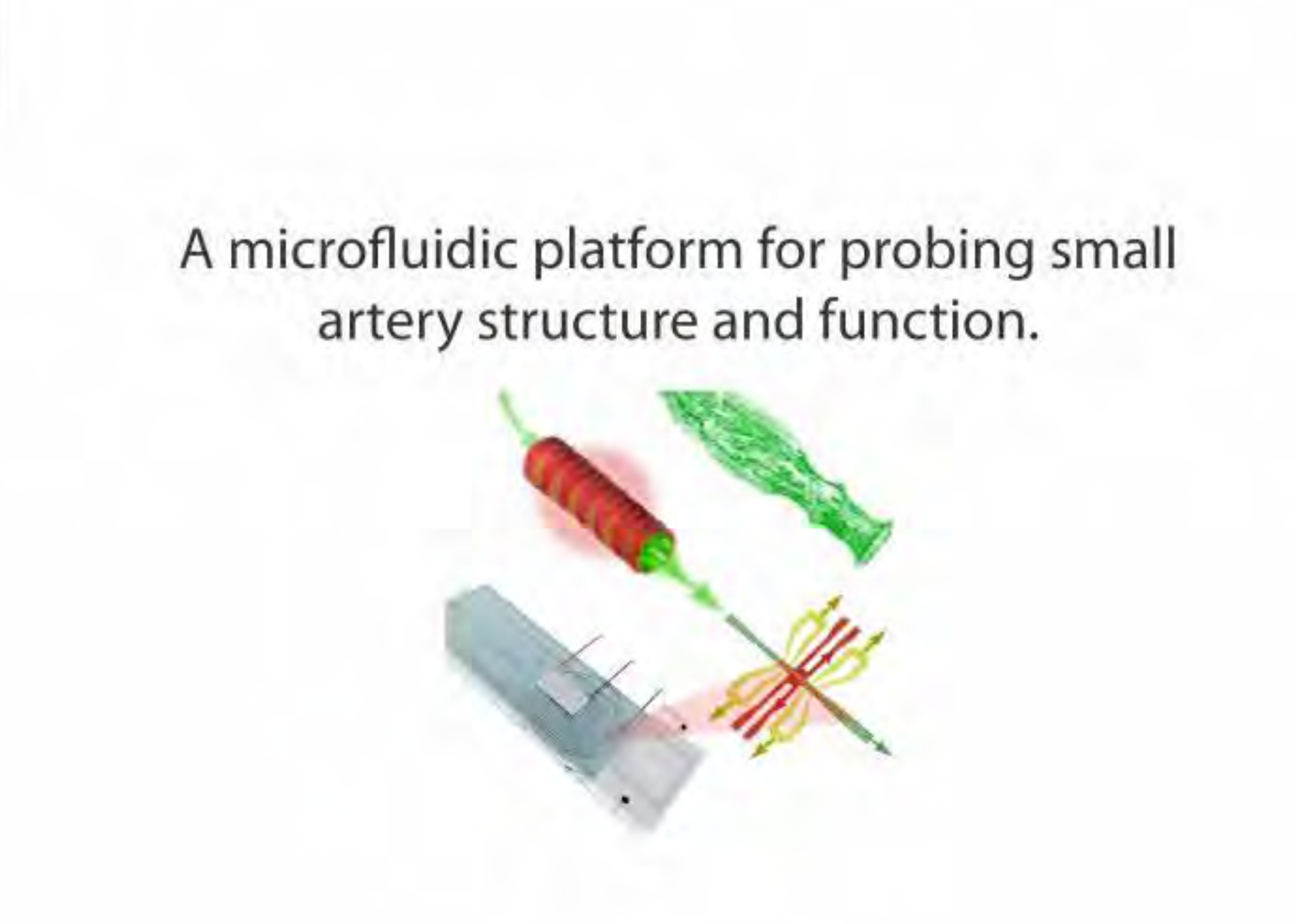
Artery-on-a-Chip



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

Repeated dose systemic toxicity testing

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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)**

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Solving the Substance Testing Dilemma

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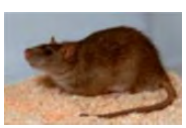
“Human-on-a-Chip”

human AND systemic



animal models

systemic but NOT human

static 2D & 3D

human cell culture

human but NOT systemic

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A historic imagination turned into a vision!

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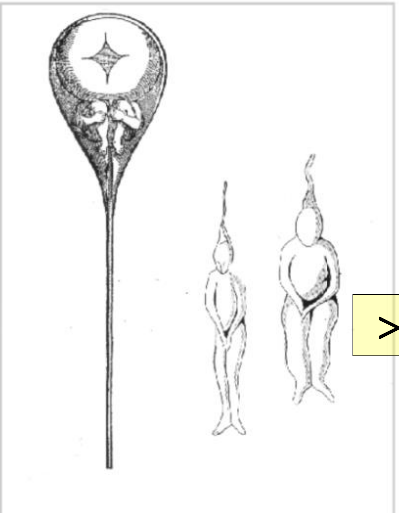
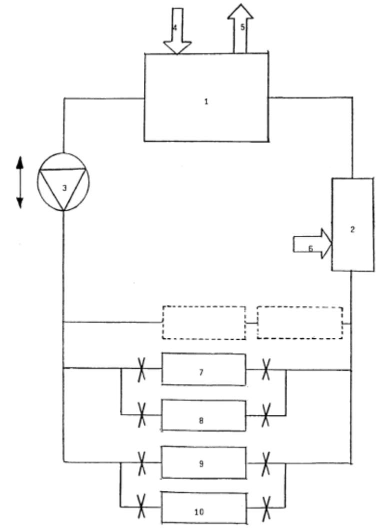
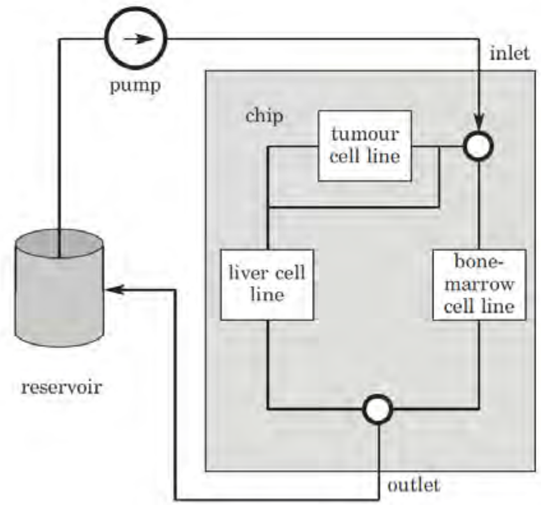


Homunculi in sperm as drawn by N. Hartsoecker in 1695

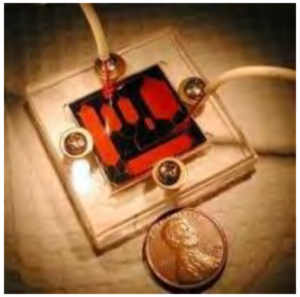
> 300 Years

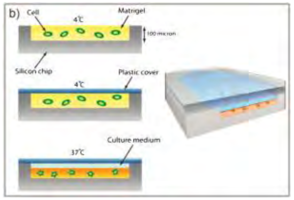


Marx et al, **1992**
V&V zur zeitgleichen Kultivierung unterschiedlicher Säugerzellen
EP0584170B1



µCCA
Micro cell culture analog





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

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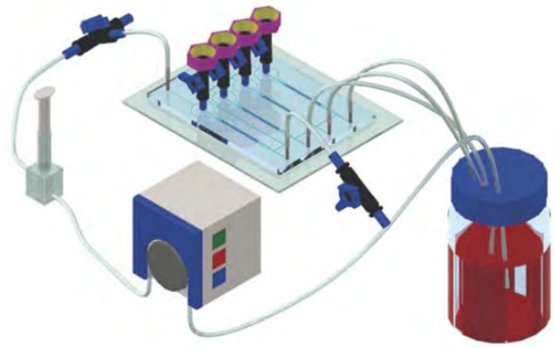
„Human-on-a-chip“ – historical sketch

Michael Shuler et al,
Cornell University

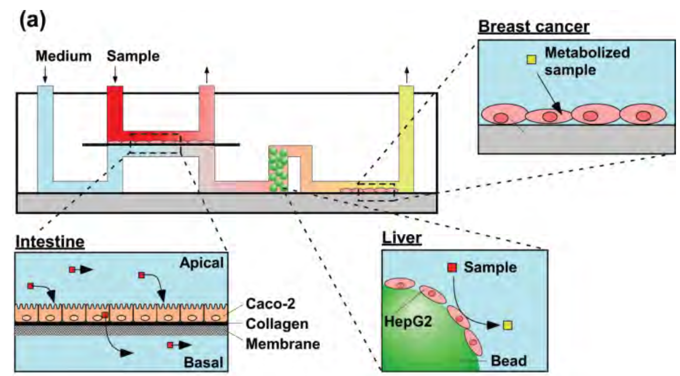
Hanry Yu et al,
Singapore – US-MIT Alliance

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

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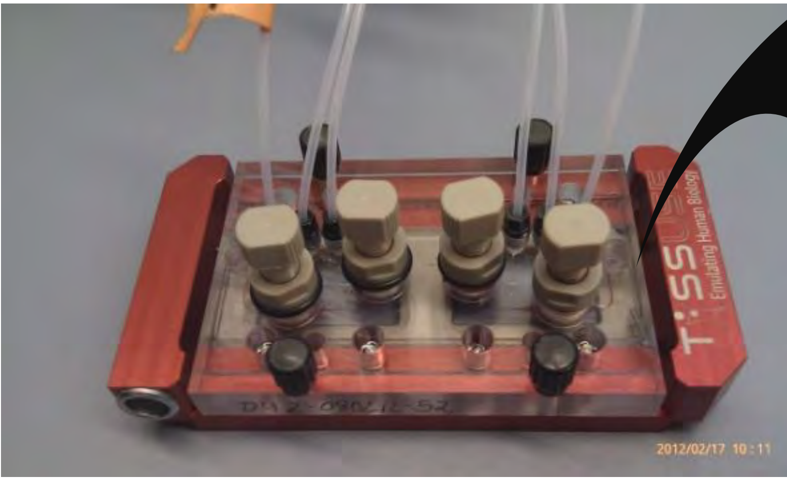
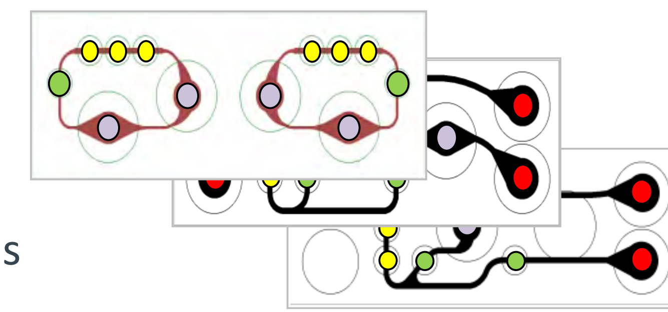


Features:

- 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



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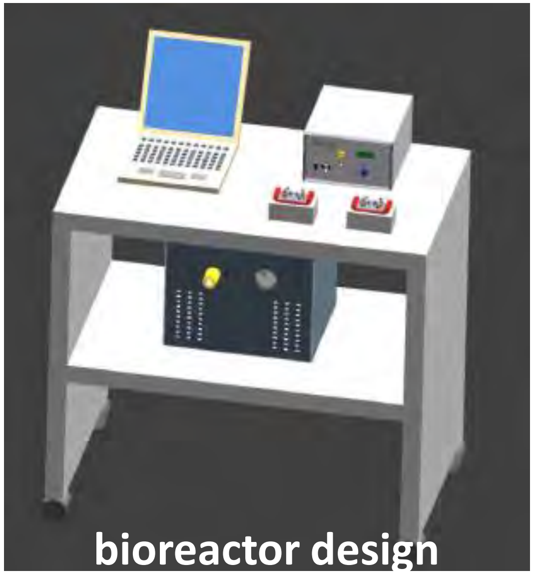
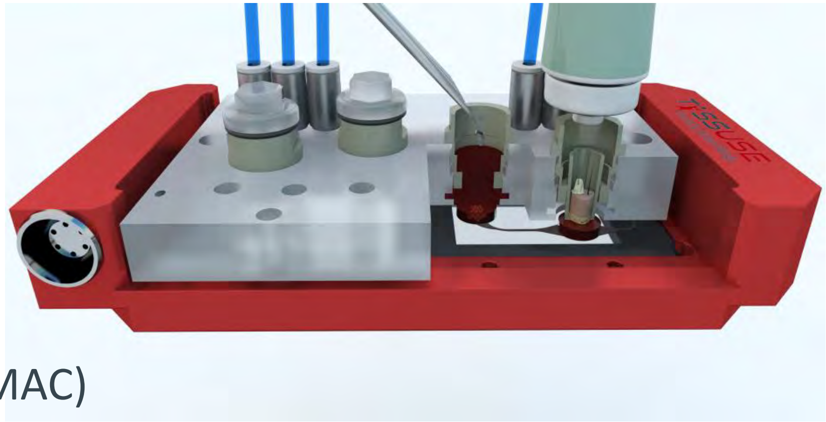
Benchtop bioreactor



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

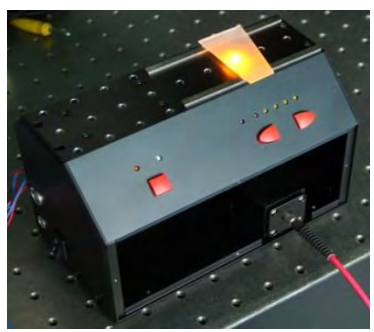


Sensors / In-process-controls

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

Fraunhofer IWS



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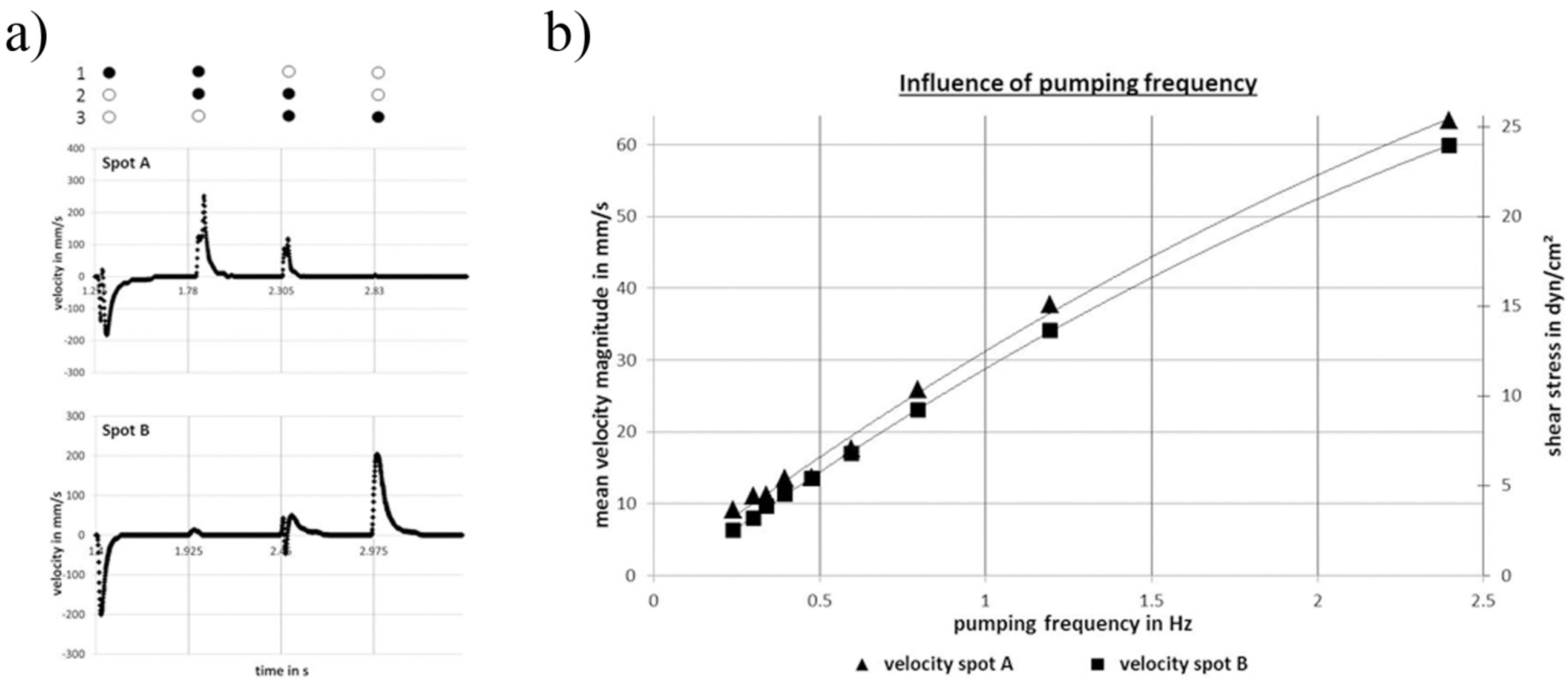
Evaluation of fluid dynamics

Particle imaging velocimetry



Schimek et al., Lab Chip 2013

Smallest possible scale of organs

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

Molecule

Cell

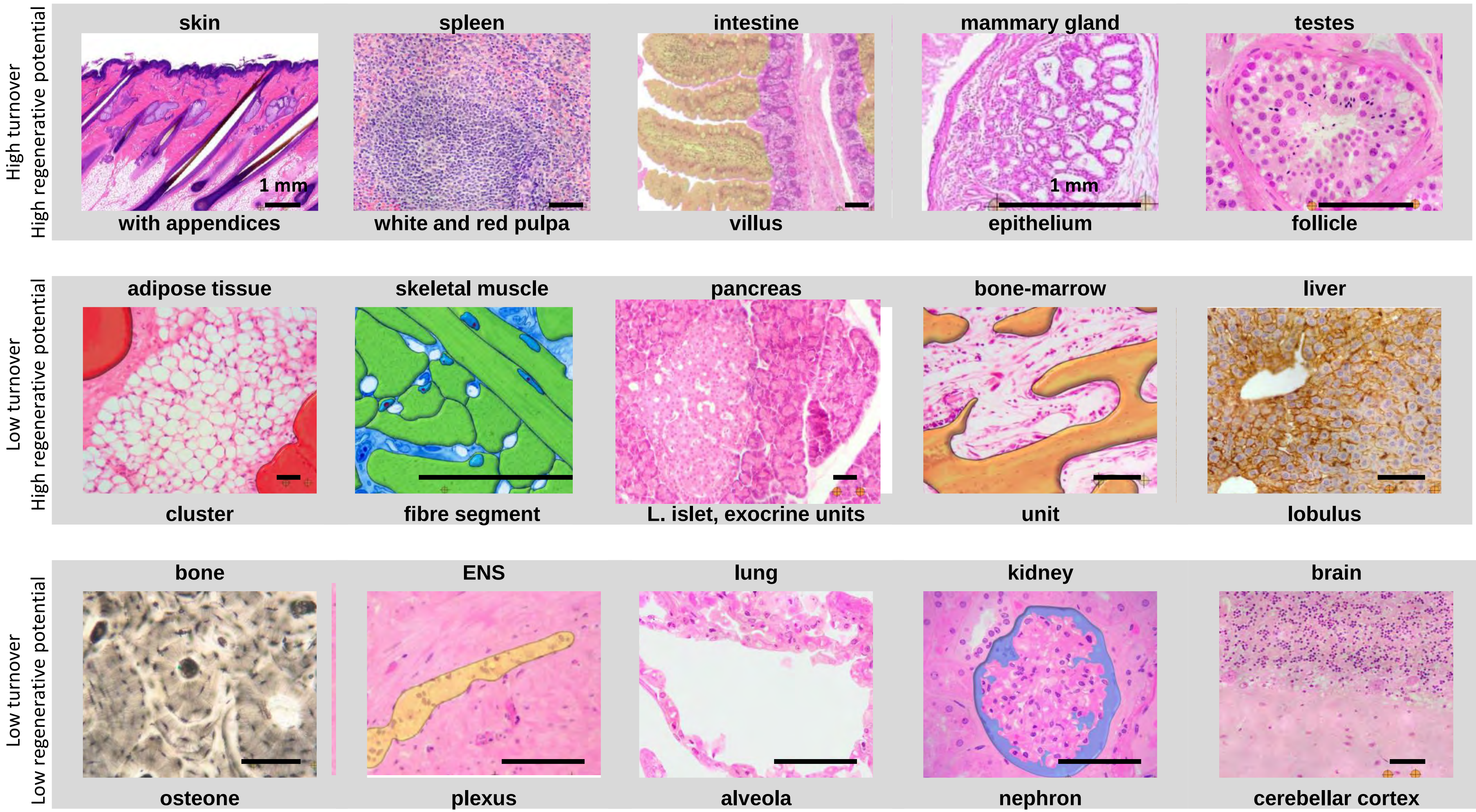
Organoid

Organ

Individual

<u>Ten liver lobules</u> – the basis for a $^{1}/_{100.000}$ "human-on-a-chip"

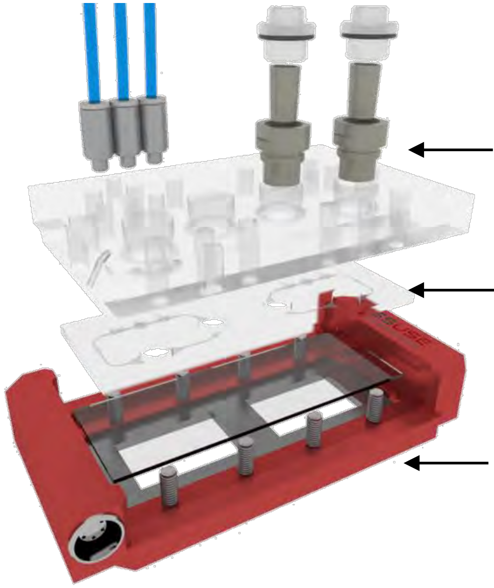
Human organoids

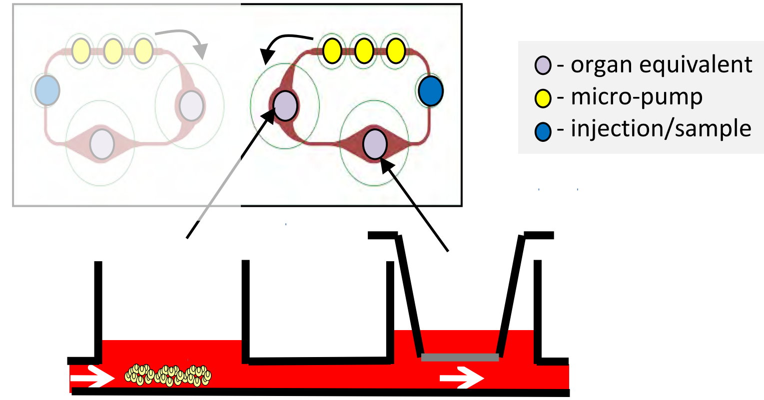


Bars: 100 µm

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

The "Two-Tissue Culture Chip"





equivalent to 10 liver lobuli

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

Duration / Tissue	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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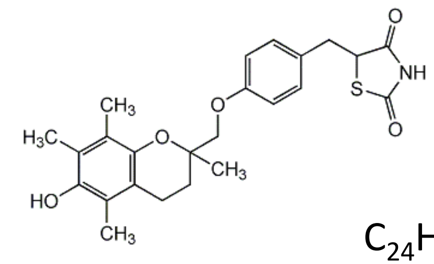
Tox study using Troglitazone

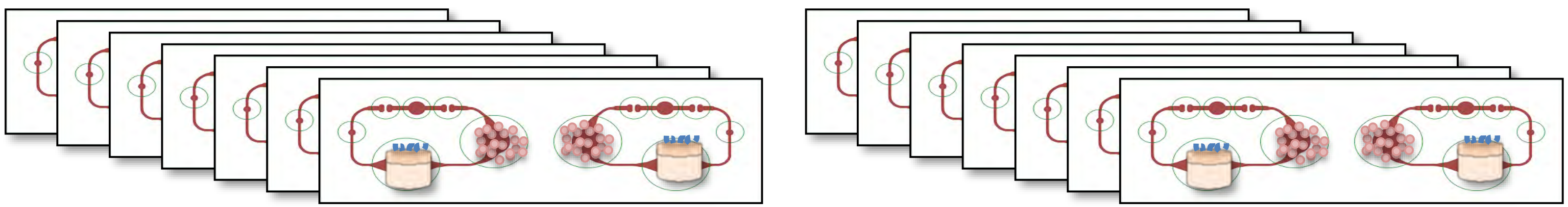
Trade name: Rezulin, Rizulin, Romazin, Sensulin
Developed by: Daiichi Sankyo Co (Japan)
Manucactured by: Parke-Davis (1997 approved by FDA)



$C_{24}H_{27}NO_5S$

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

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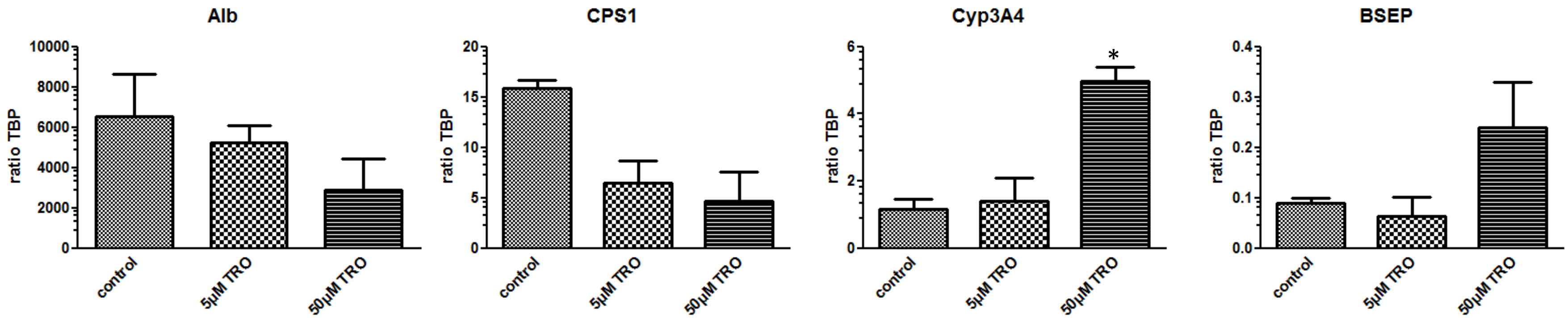
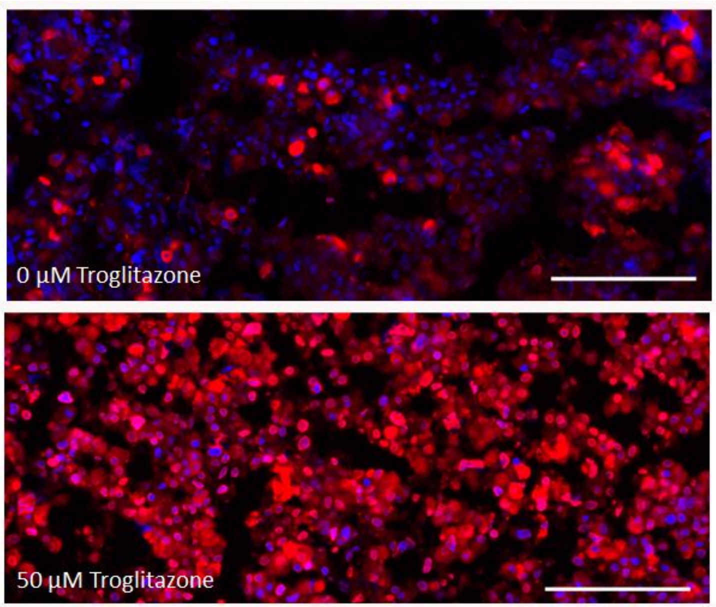
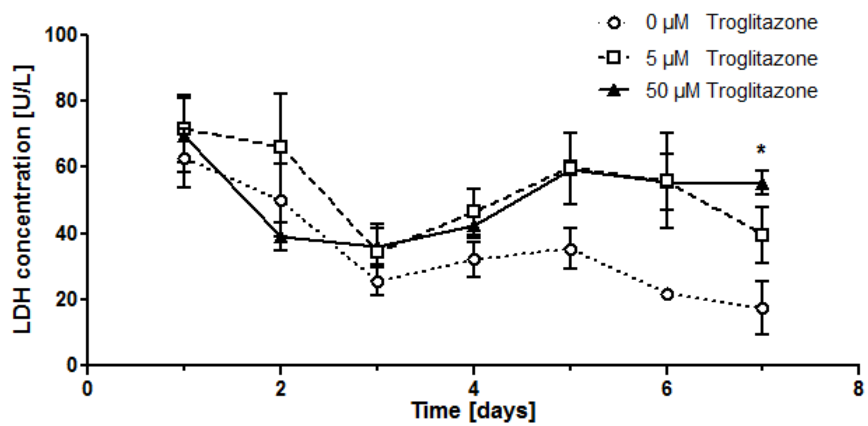
Sensitivity to Troglitazone (7 day exposure)







Wagner et al., Lab Chip 2013

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The crucial role of dynamic blood circulation

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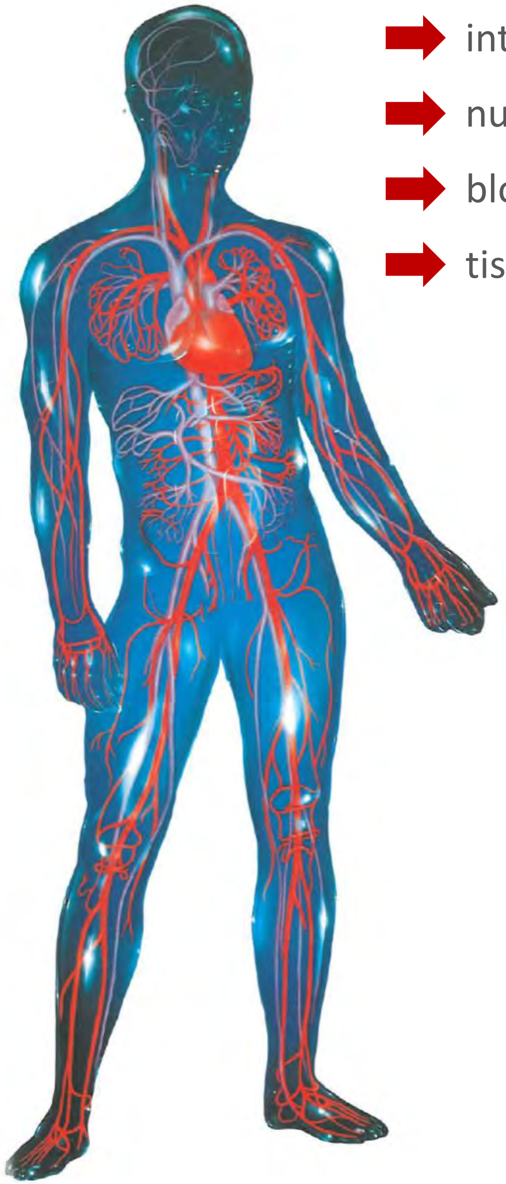
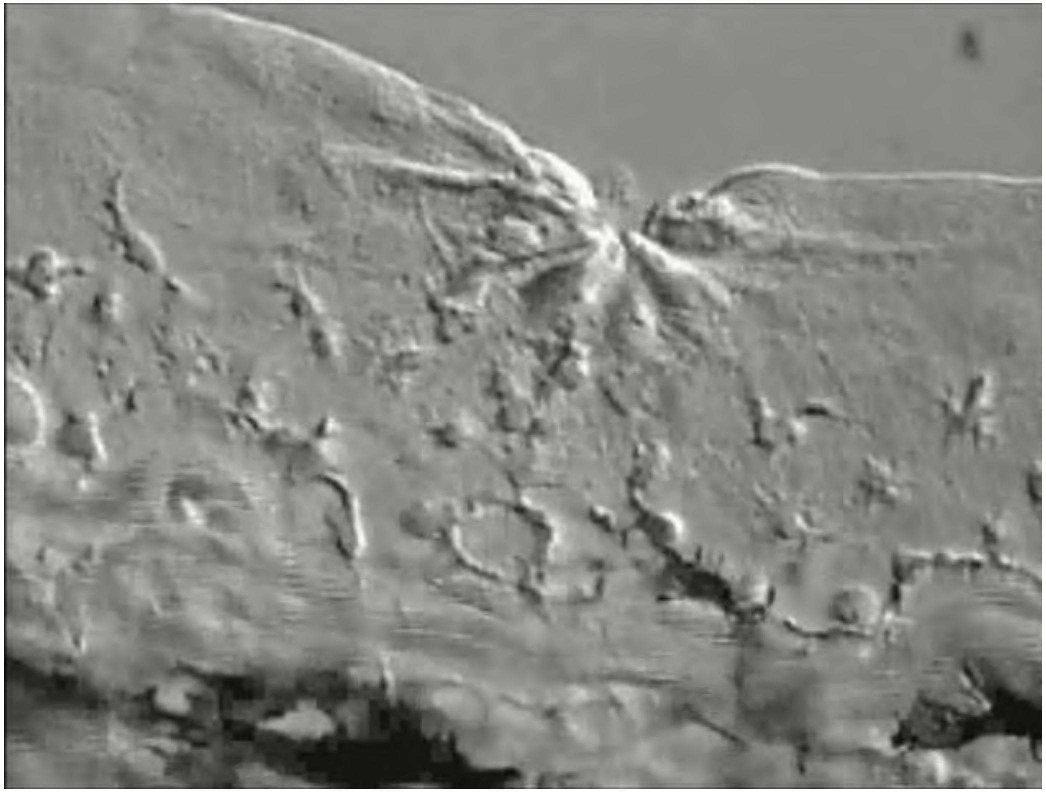


- interconnection of organs to create an **organism**
- nutrient and oxygen transport through **blood plasma and red blood cells**
- blood-tissue barrier and neo angiogenesis through **endothelial cells**
- tissue repair and immune response through **white blood cells**



- Norecopa -

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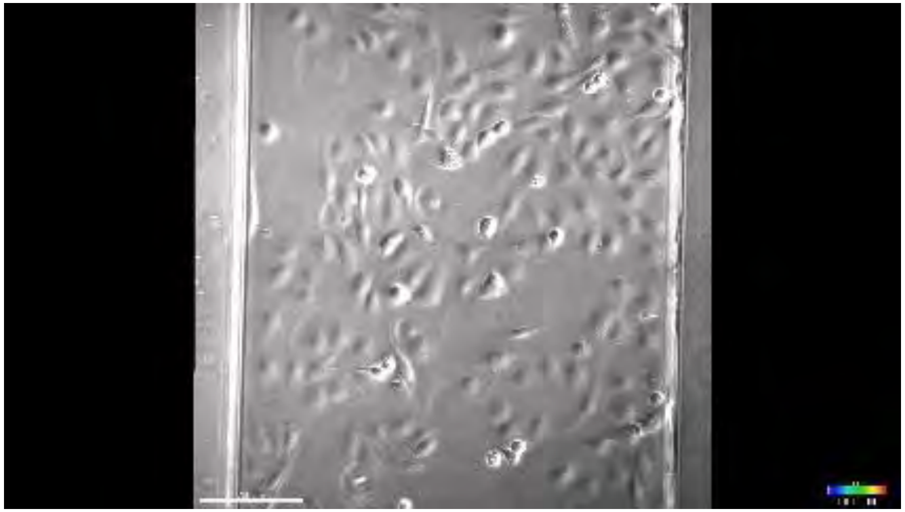
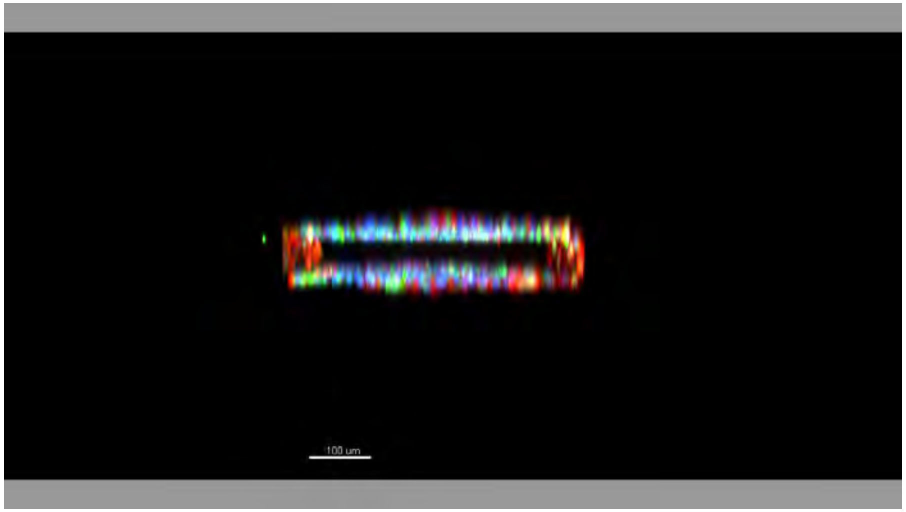
Establishment of stable microvascular circuits

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

Next generation Multi-Organ-Chip

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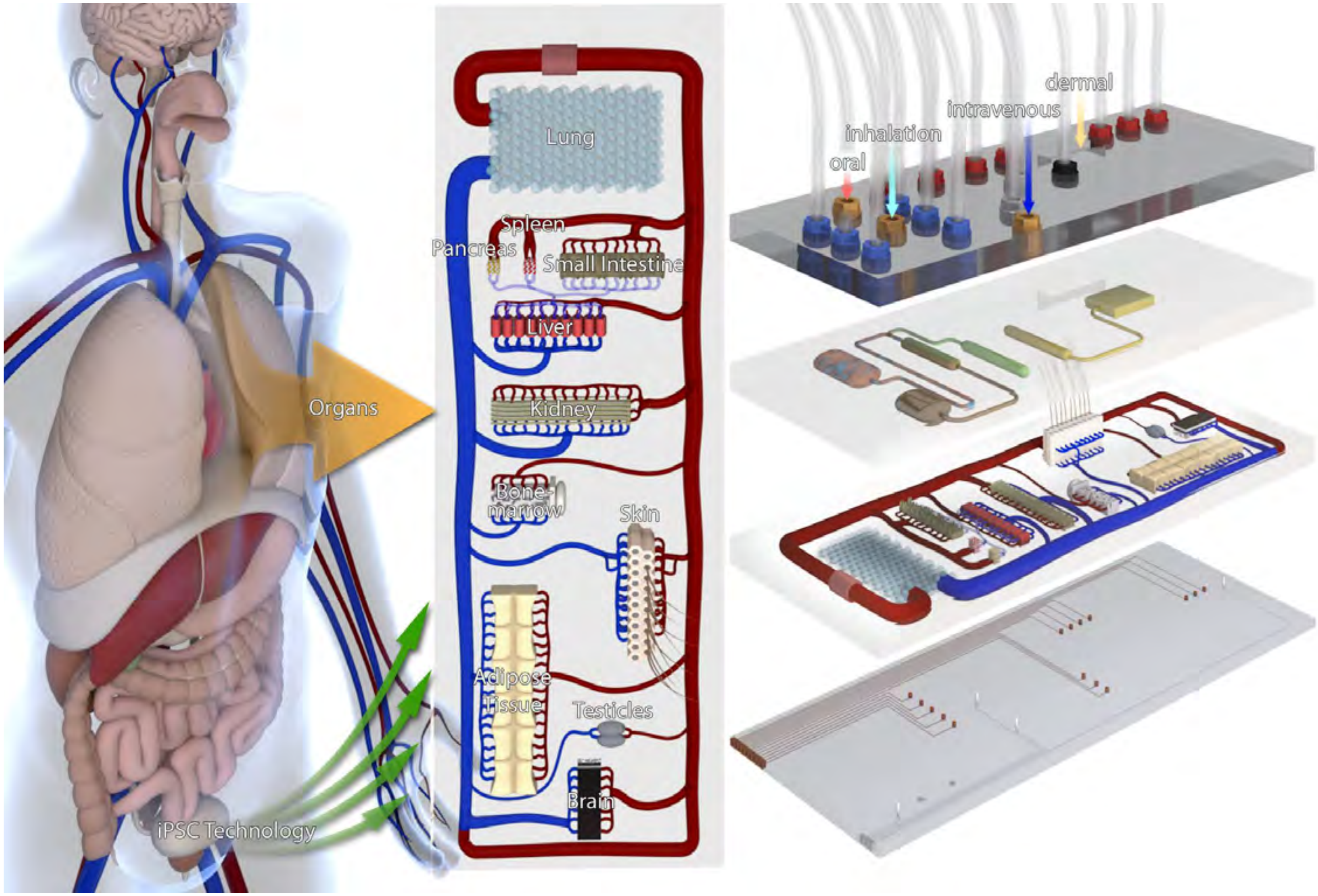

Marx et al., Altern Lab Anim. 2012 Oct;40(5):235-57

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US Initiative “Human-on-chip”

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

FDA

July 1st 2012 → June 30th 2017

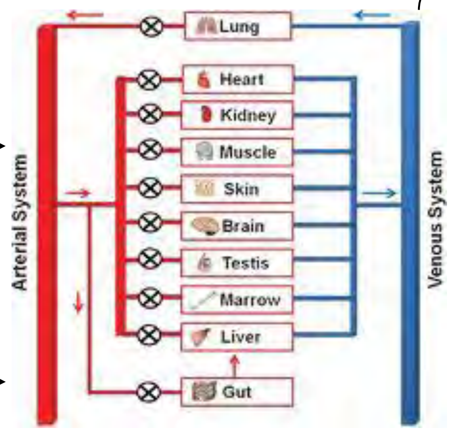
17 NIH groups and …

DARPA
Defence Advanced Research Projects Agency

US$ 69Mio/5 Years

NIH/NCATS
National Center for Advancing Translational Sciences

US$ 64Mio/5 Years



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