

# Animal-free testing and humans-on-a-chip: How far have we come?

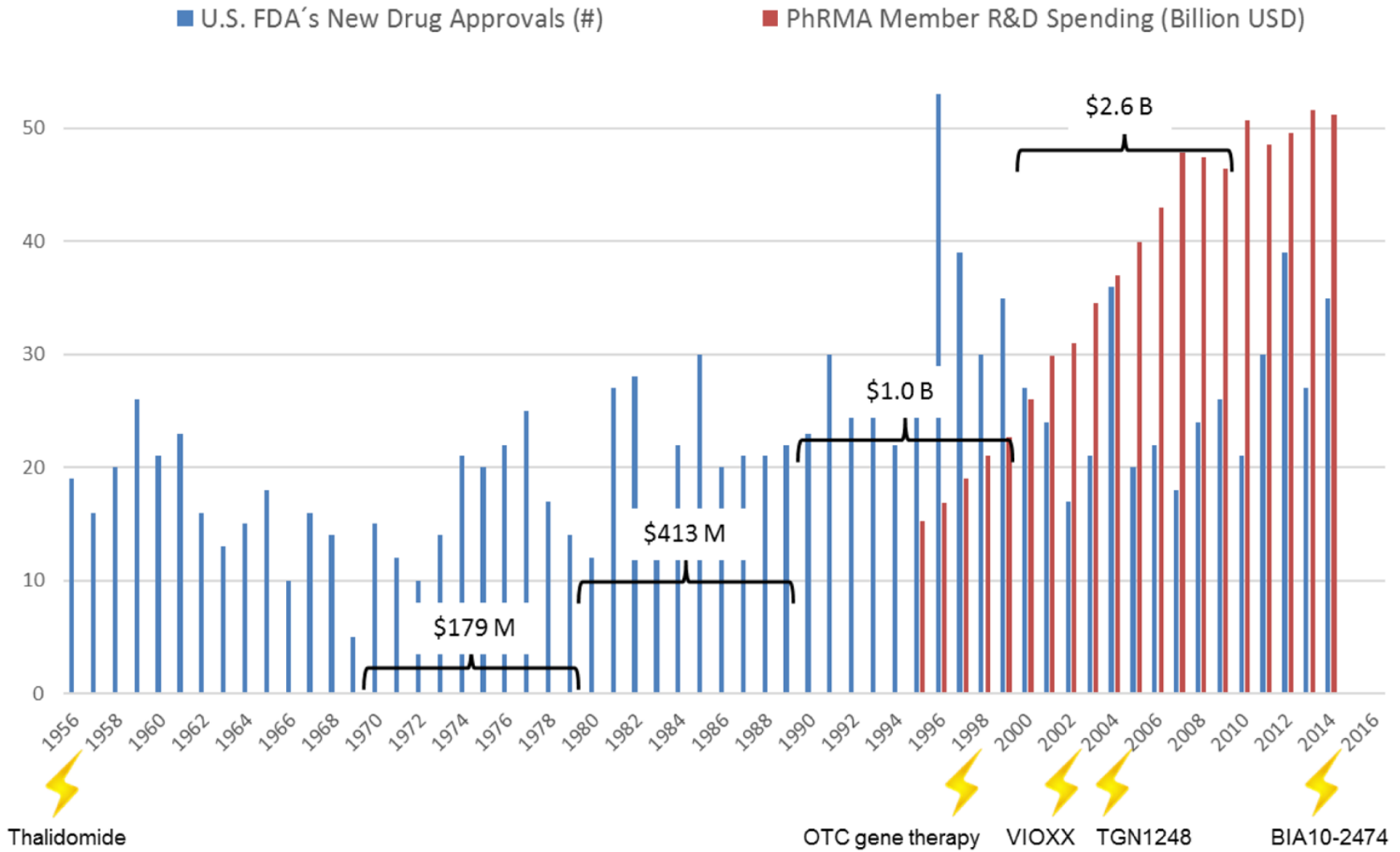
## OUTLINE

- Solving the drug development dilemma
- Microphysiological systems – a historical sketch
- Status quo of the Multi-Organ Chip platform

Leopold Koenig  
R&D scientist

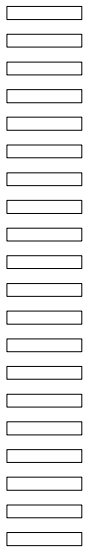
Leopold Koenig – NORECOPA – 10.10.2017

# The R&D-Dilemma

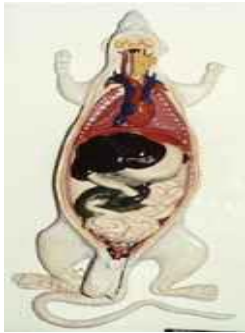


# Drug development today

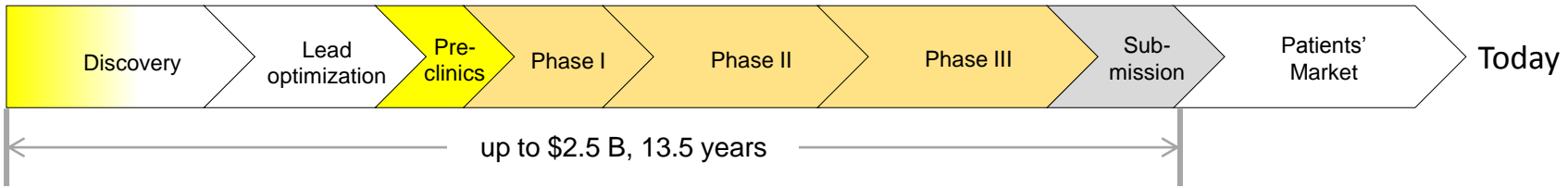
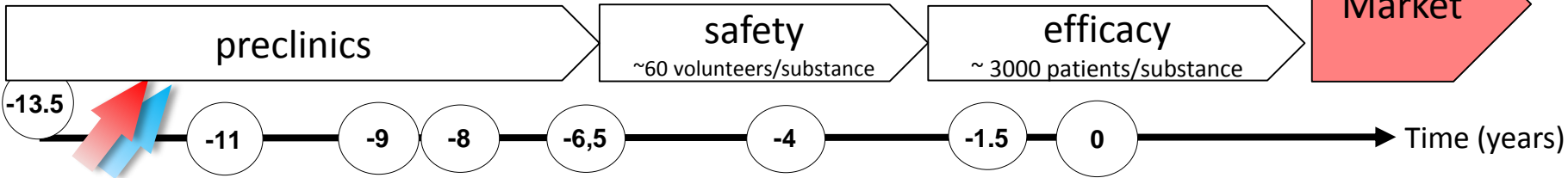
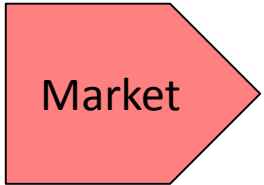
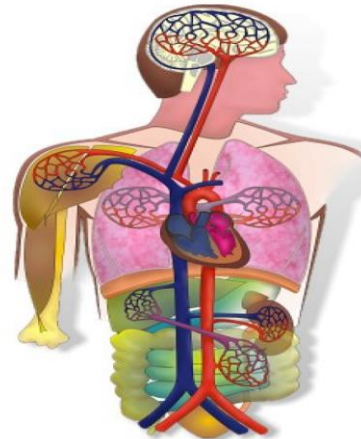
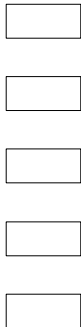
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laboratory animals



human  
2D & 3D-  
cell culture



laboratory animal studies -      *in vitro* testing -      clinical trials -

# The drug development dilemma



Animal models

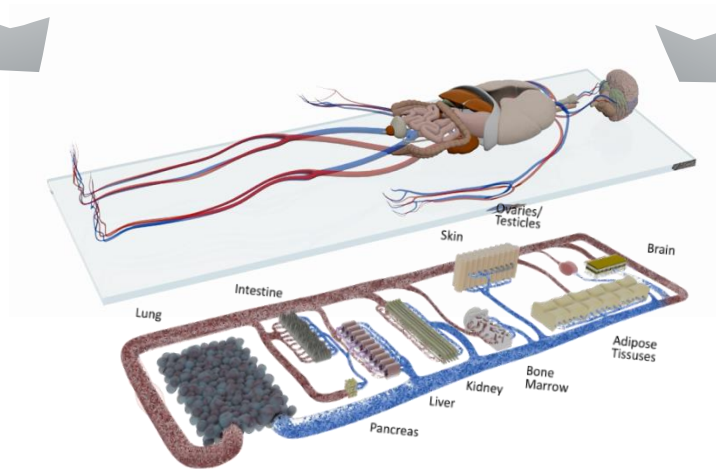
systemic but not human



static 2D & 3D

human cell culture

human but not systemic



**“Human-on-a-Chip”**  
**human and systemic**

# Definition

**“Human-on-a-chip” platforms** aim to establish microfluidic **microphysiological systems**, emulating human biology at smallest biologically acceptable scale

**micro:** at smallest biologically acceptable scale

- testing at relevant throughput
- minimum use of human tissue
- affordable assay economy

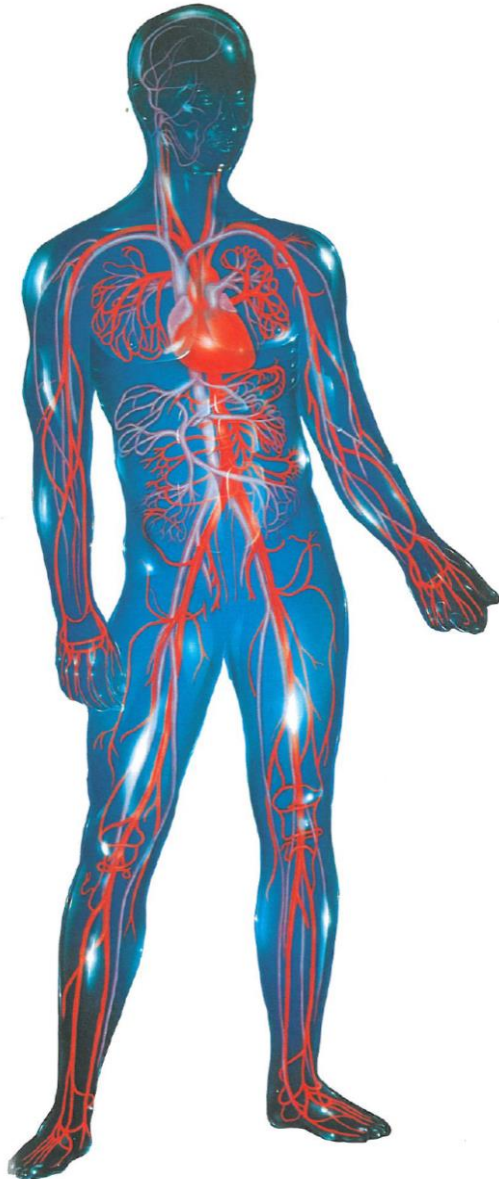
**physiological:** truly emulating human biology

- human organ architecture
- healthy long term homeostasis
- damage, disease, repair, regeneration

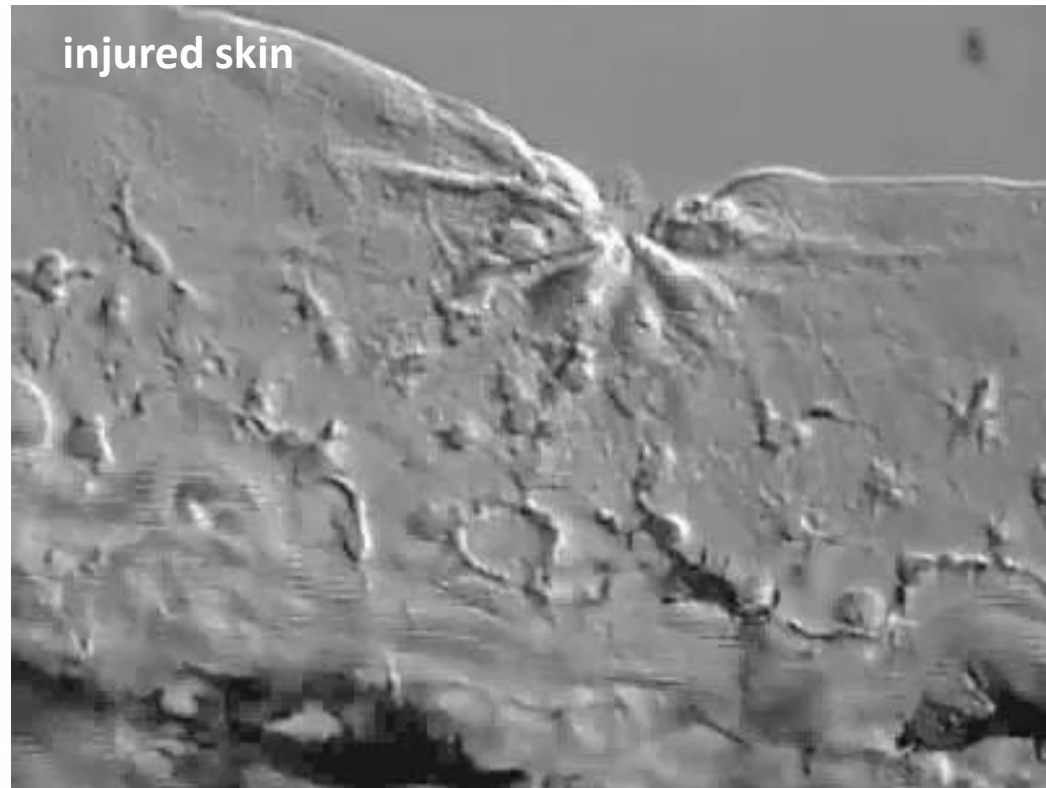
**systems:** devices supporting human-like organ maintenance

- temperature, humidity, pH, O<sub>2</sub>-supply
- mechanical and electrical coupling
- optical imaging

# Why set tissues under flow?



- continuous efficient **nutrition**
- generation of stable **oxygen** and **protein gradients**
- organ **crosstalk** and **immune surveillance**

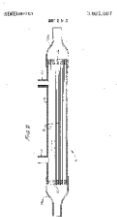


# Historical sketch – dynamic cell culture

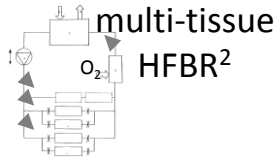
dynamic

1972      1991      2002      2004      2010      2012      2013      2016/17

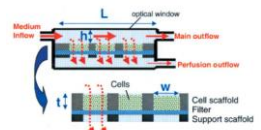
multi-organ engineering



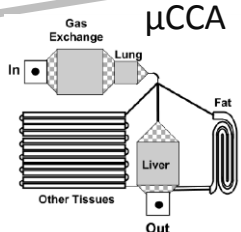
HFBR<sup>1</sup>



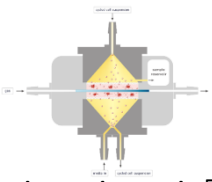
multi-tissue HFBR<sup>2</sup>



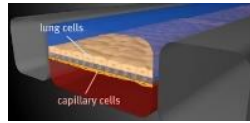
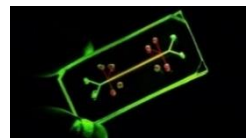
single-tissue MPS<sup>3</sup>



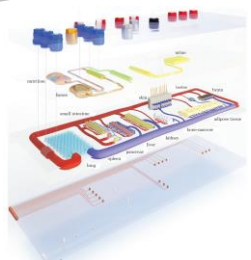
multi-tissue MPS<sup>4</sup>



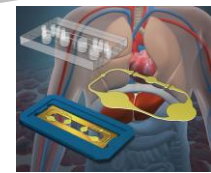
lymph node<sup>5</sup>



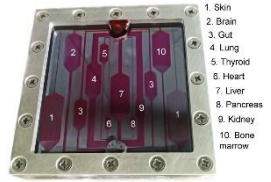
lung-on-a-chip<sup>6</sup>



on-chip organism<sup>7</sup>



3D skin-liver chip<sup>8</sup>



14-compartment MPS<sup>9</sup>



“EVATAR”<sup>10</sup>



MIT  
Wyss  
institute

MPS programme

17 NIH groups  
NCATS/FDA/DARPA  
(~ \$140 Mio)

single-organ engineering

<sup>1</sup> Patent, Knazek et al., US 3821087 A (1972)  
<sup>2</sup> Patent, Marx et al., WO1992020780 A1 (1991)  
<sup>3</sup> Powers et al., Biotech Bioeng (2002); <sup>4</sup>Shuler et al., Biotech Progress (2004)  
<sup>5</sup> Patent, Marx et al., WO2004101773 A1 (2004)  
<sup>6</sup>Huh et al., Science (2010); <sup>7</sup>Marx et al., ATLA and EP2712918 A1 (2012); <sup>8</sup>Wagner et al., LabChip (2013)  
<sup>9</sup>Miller et al., Biotech Bioeng (2016); <sup>10</sup>Xiao et al., Nat Com (2017)

# Organ on a chip systems : International landscape

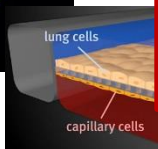
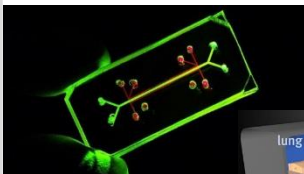
## “single-organ-chips”

2002, Powers et al.  
 2004, Gu et al., Leclerc et al.  
 2005, Chung et al., Rhee et al.  
 2005, Hung et al.  
 2006, Ho et al., Kane et al.  
 2007, Cui et al., Hwa et al.  
 2007, Lee et al., Toh et al.  
 2008, Carraro et al., Park et al.  
 2008, Nakayama et al., Khetani et al.  
 2009, Sato et al., Park et al.  
 2010, Goral et al., Günther et al.  
 2010, Ootani et al., Young et al.

2010, Huh et al. Science



Lung-on-a-chip  
 Donald Ingber  
 Wyss Institute  
 Harvard



## “multi-organ-chips”

- human cell lines instead of primary cells
- lack of 3D environment
- less than 10.000 cells per compartment
- non-physiological tissue-to-fluid ratio
- short-term cultures (24h-72h)



Yong Yu group  
 Singapore – US-MIT  
 Science

et al. Lab Chip 9  
 Issues of:



Sato group  
 University

et al. Anal Chem 82  
 Issues of:

+ tumour

2009, Sung et al., Ivanier et al.

see: 2011, Baker Nature 471, A living system on a chip.



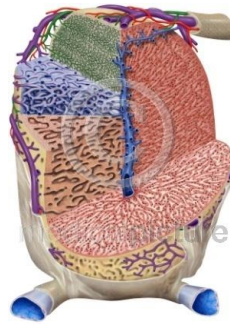
# Smallest biologically acceptable scale!?



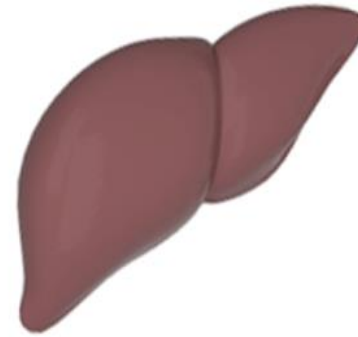
Molecule



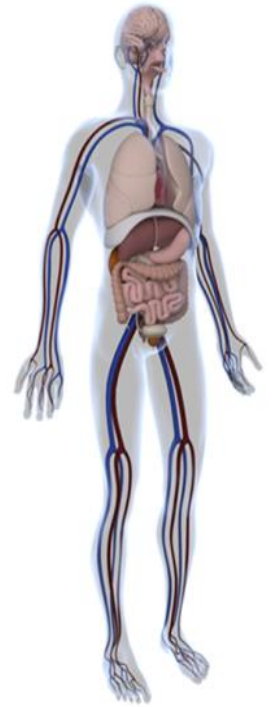
Cell



Organoid



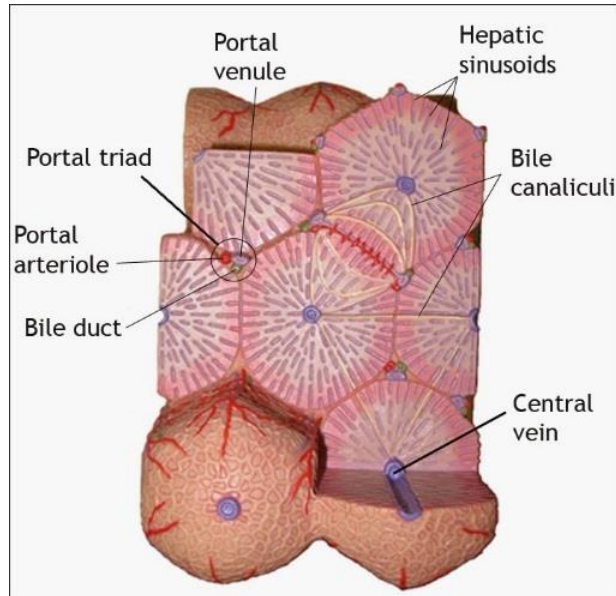
Organ



Individual

# Working hypothesis and starting point

- Organs are built up by multiple, identical, functionally self-reliant structural organoids
- Organoids are evolutionarily conserved and subject to genetically encoded self-assembly



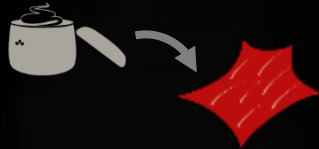
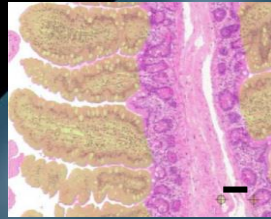
- **1 million** liver lobules constitute a human liver
- a single liver lobule is of **1,3  $\mu$ l** in scale

Ten liver lobules – the basis for a  $1/100.000$  liver model

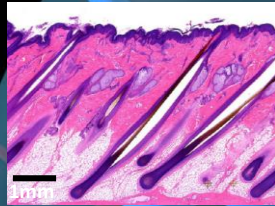
# Downscaling a human body: How small can we go?



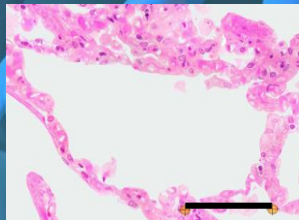
70 small intestine villi



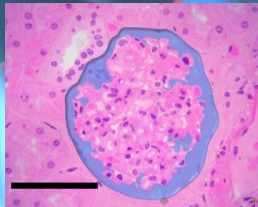
18 mm<sup>2</sup> skin segment



3000 lung alveoli



20 kidney nephrons

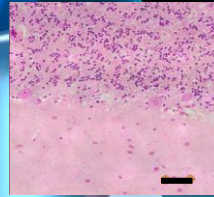


or

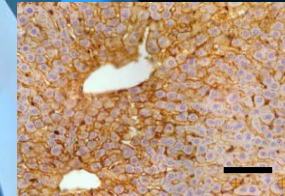


0.1 mg of  
ovary follicles

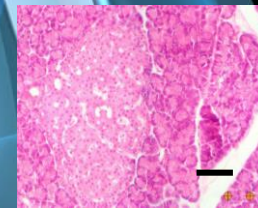
0.4 mg of testes  
seminiferous tubules



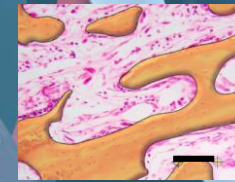
4 mm<sup>2</sup> brain cerebral cortex



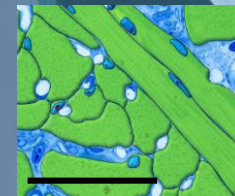
10 liver lobuli



5 pancreatic islets



20mg of  
bone marrow units



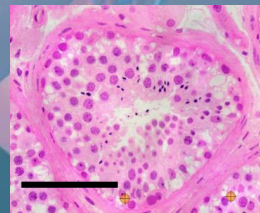
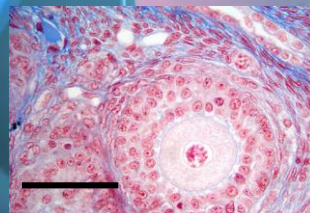
300 mg of skeletal  
muscle fiber segments



125 mg of adipose  
tissue clusters

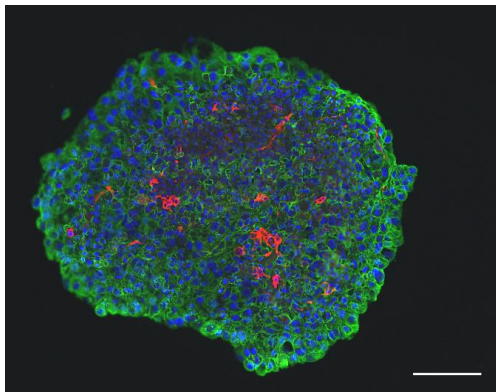
**Scaling factor: 1/100.000**

Marx et al. (2012)  
ATLA 40, 235-257

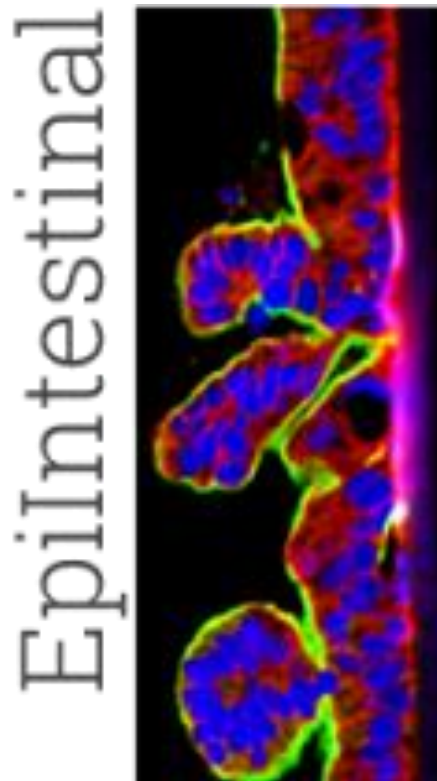


# Human cell supply for MPS

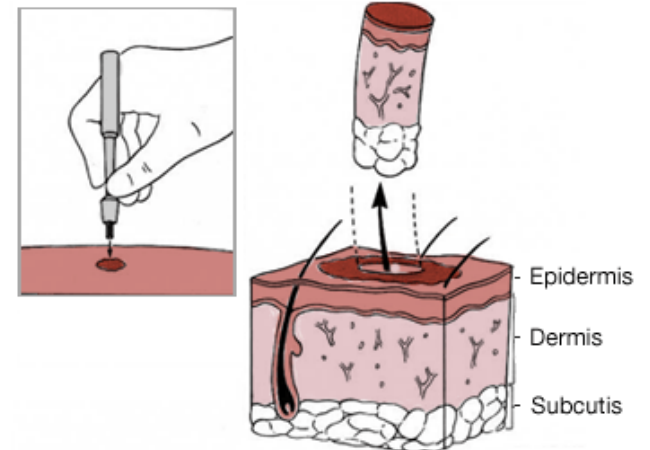
Cell lines



Primary Cells



Tissue Explants



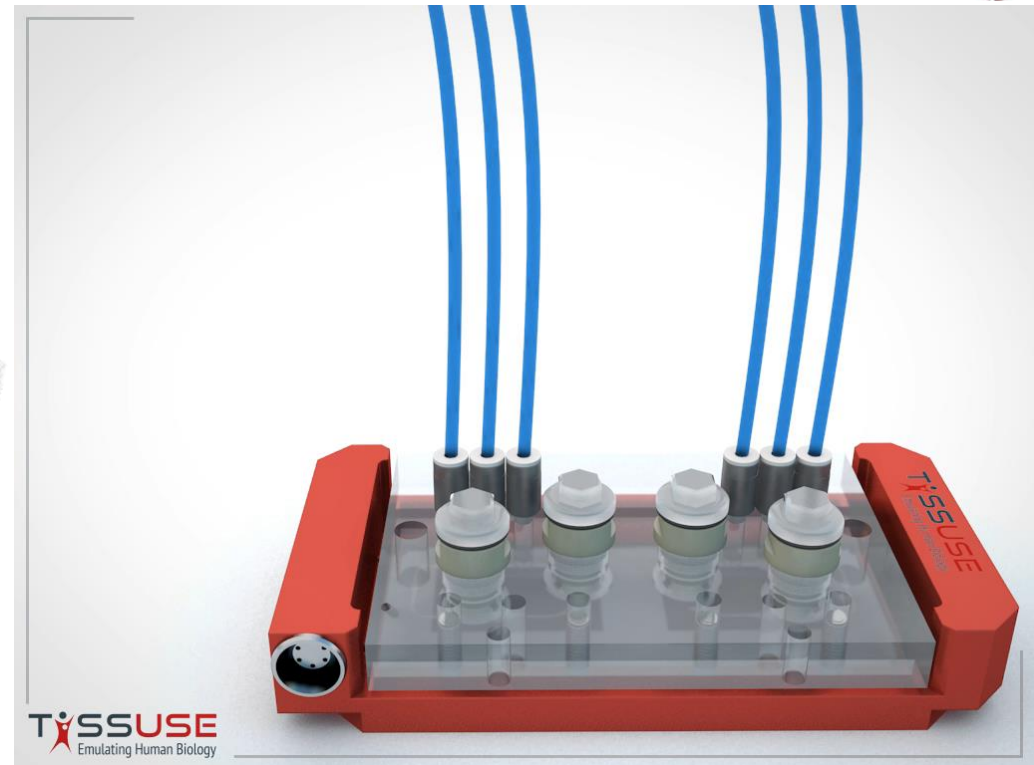
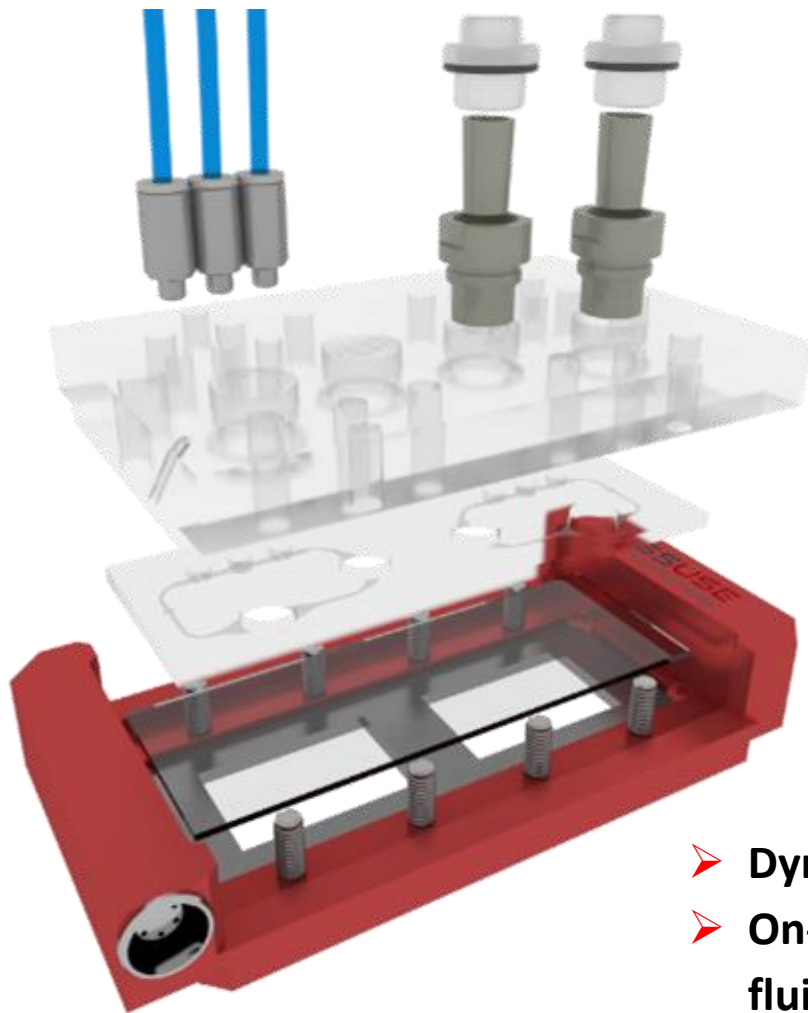
# The Multi-Organ-Chip (MOC) Technology



## Features:

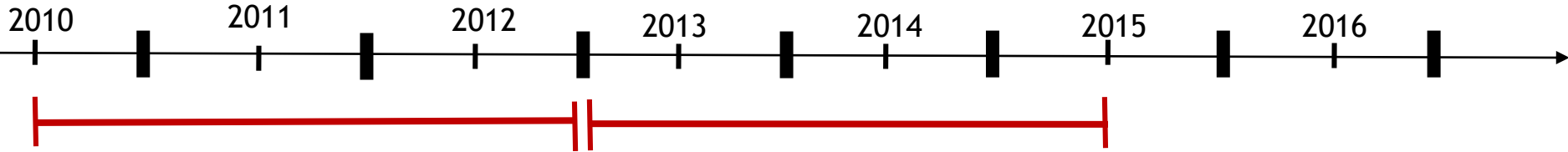
- Area of a standard microscopic slide
- On-chip micro-pump enabling pulsatile flow
- Suitable for primary cells, 3D tissues and cell lines
- Compatible with live tissue imaging

# The 2-organ-chip at a glance



- **Dynamic system allows tissue-tissue communication**
- **On-chip micro-pump allows for near to physiological fluid-to-tissue ratio**
- **Long term cultivation of cells, tissues and biopsies possible**

# Multi-Organ-Chip platform development



### 2-Organ-Chip

- Liver**
  - prime metabolic organ
  - blood protein supplier
- Skin**
  - target for cosmetics
  - dermal administration

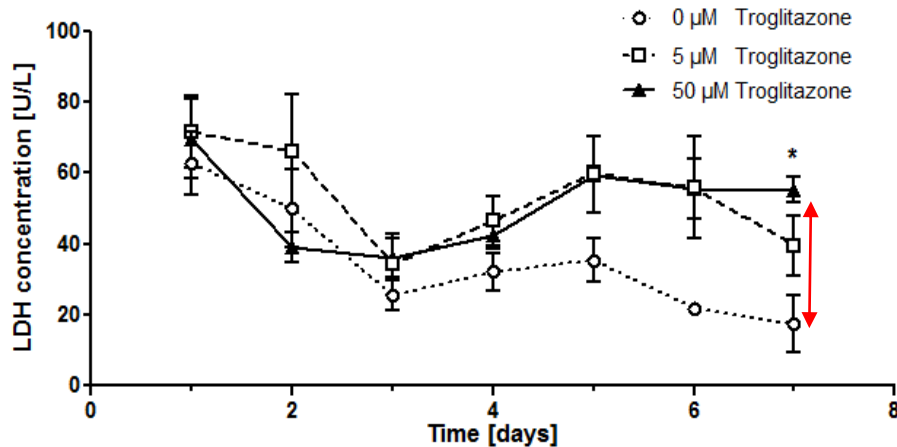
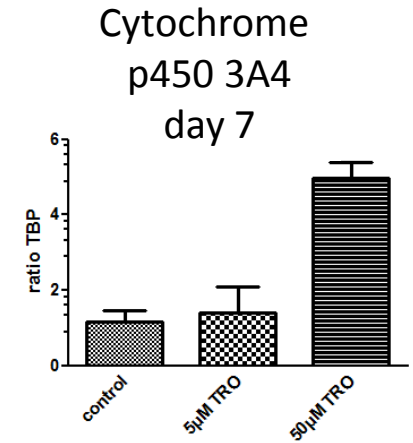
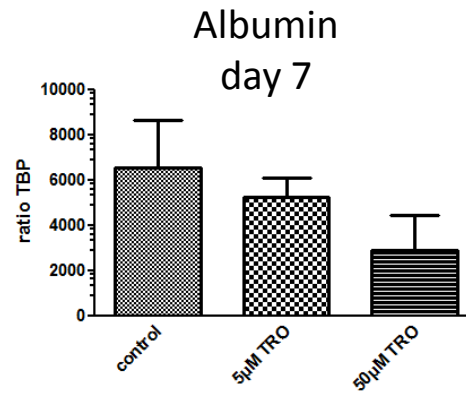
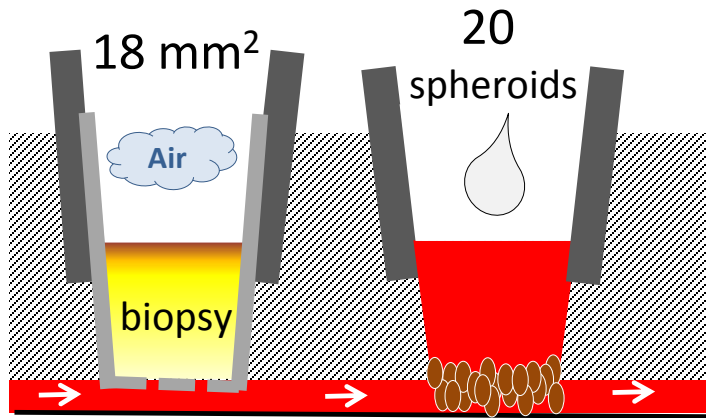
### 4-Organ-Chip

- Liver**
- Skin**
- Intestine**
  - target for food additives
  - oral administration
- Kidney**
  - urine removal
  - toxicity target

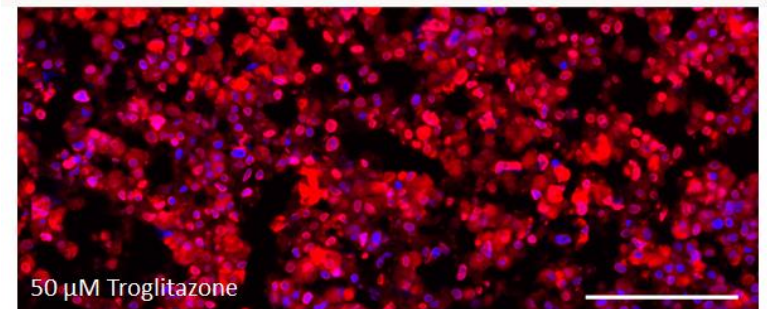
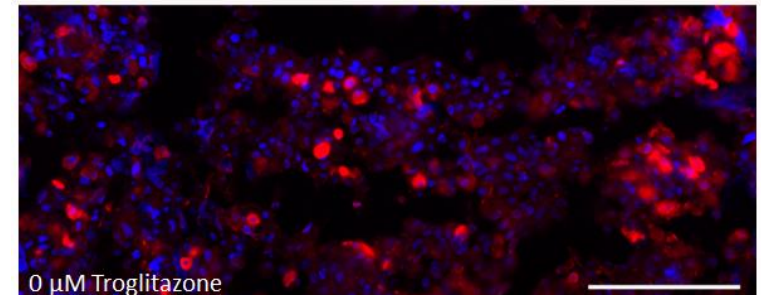
### Human-on-a-Chip

→ 2018

# Skin-liver chip: proof of concept



Wagner et al. (2013) Lab Chip

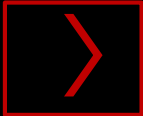
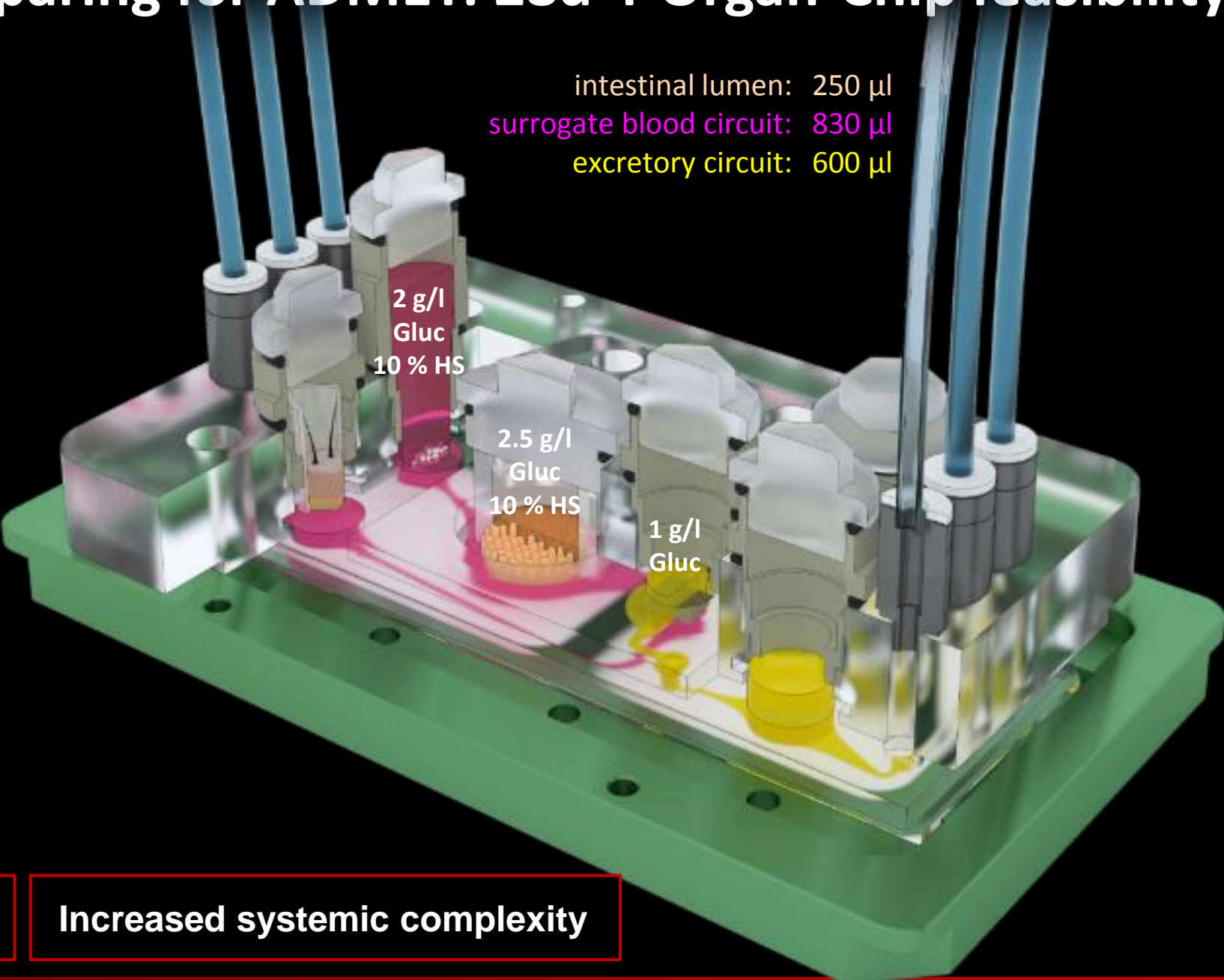


**28-d homeostasis and dose-dependent toxicity**



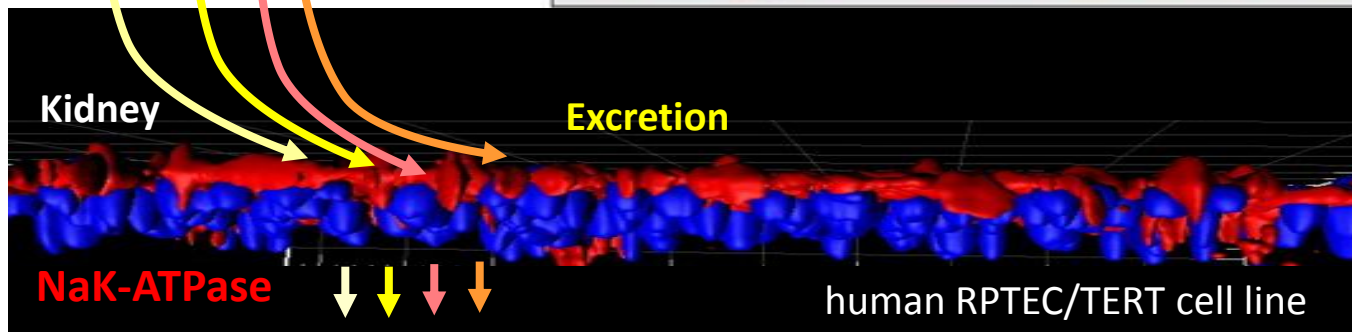
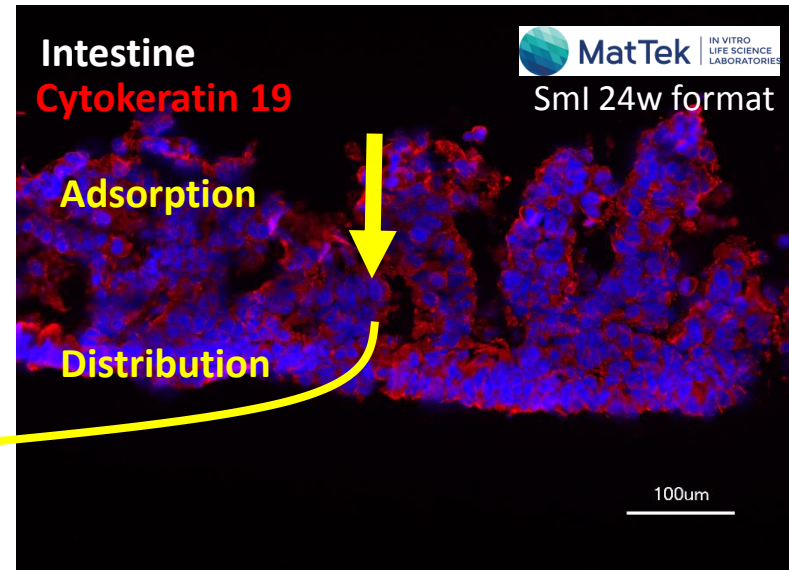
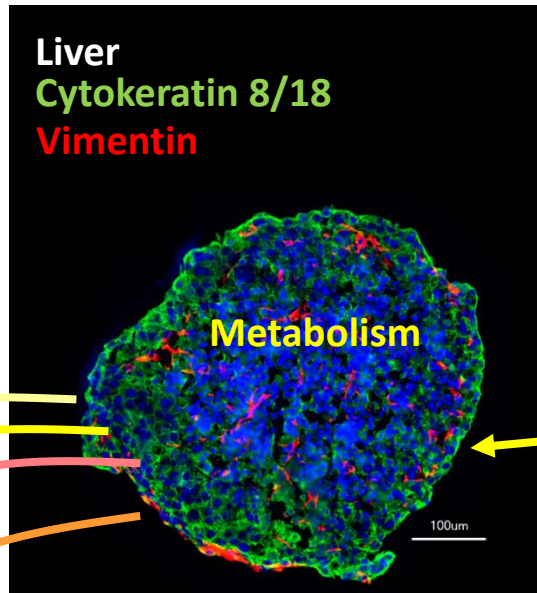
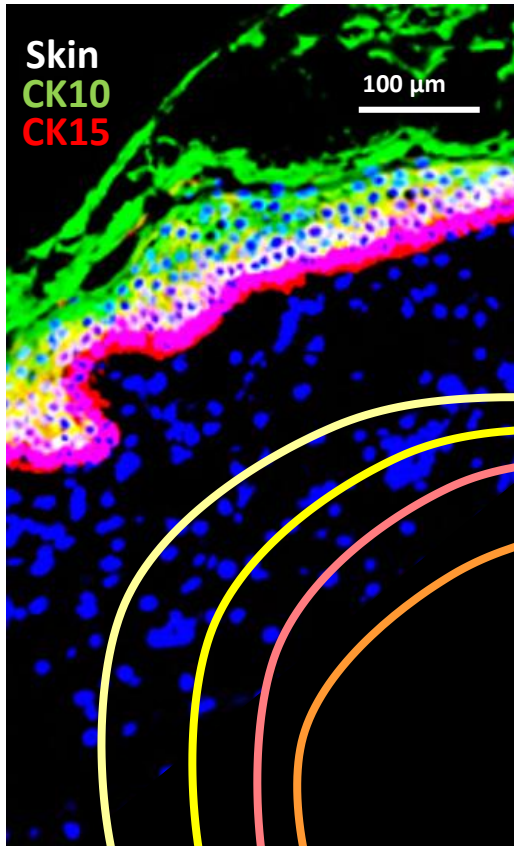
# Preparing for ADMET: 28d 4-Organ-Chip feasibility

intestinal lumen: 250  $\mu$ l  
surrogate blood circuit: 830  $\mu$ l  
excretory circuit: 600  $\mu$ l



Increased systemic complexity

# The 4-Organ-Chip: Intestine – Liver – Skin - Kidney

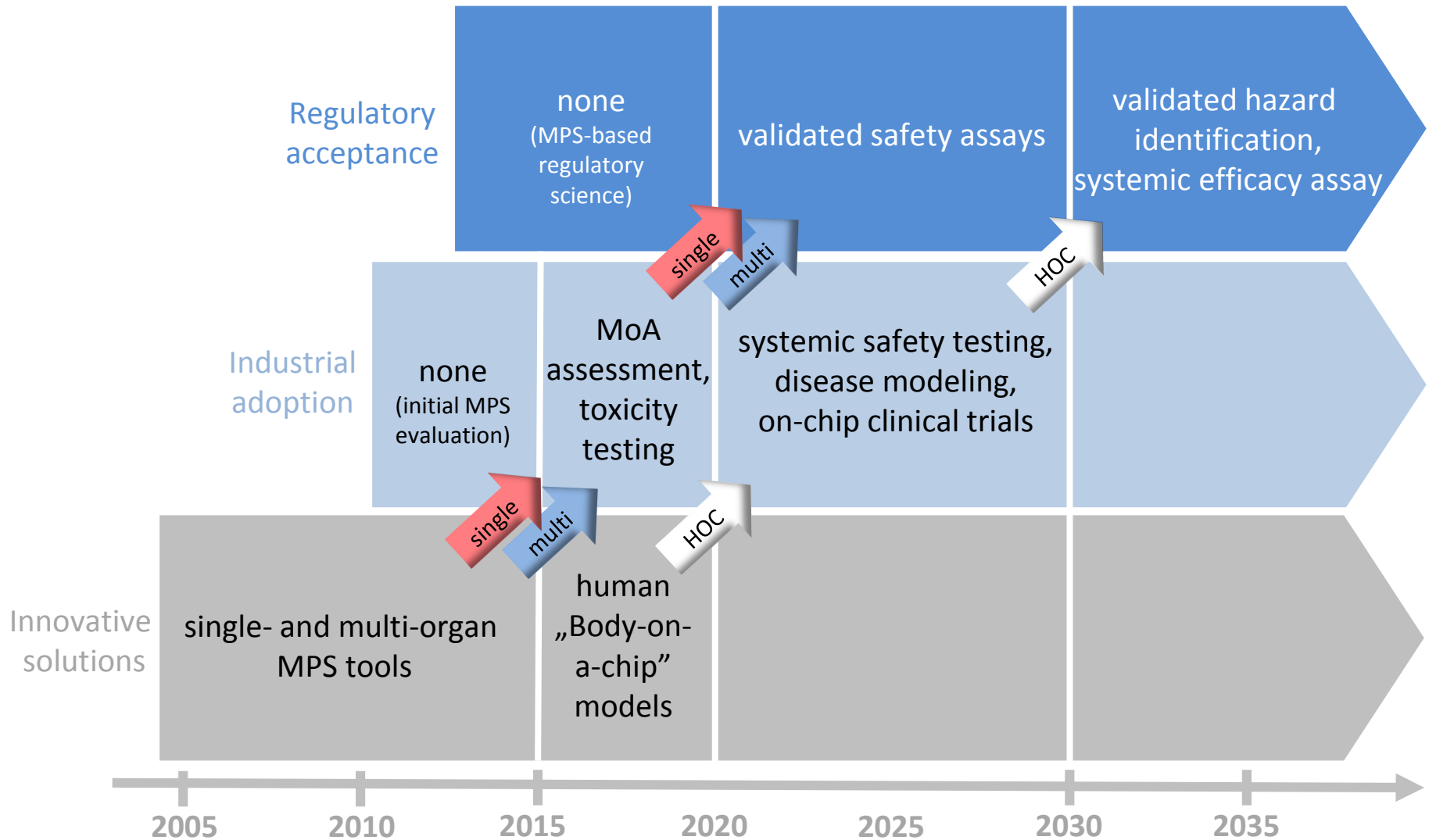


## ADMET-Chip

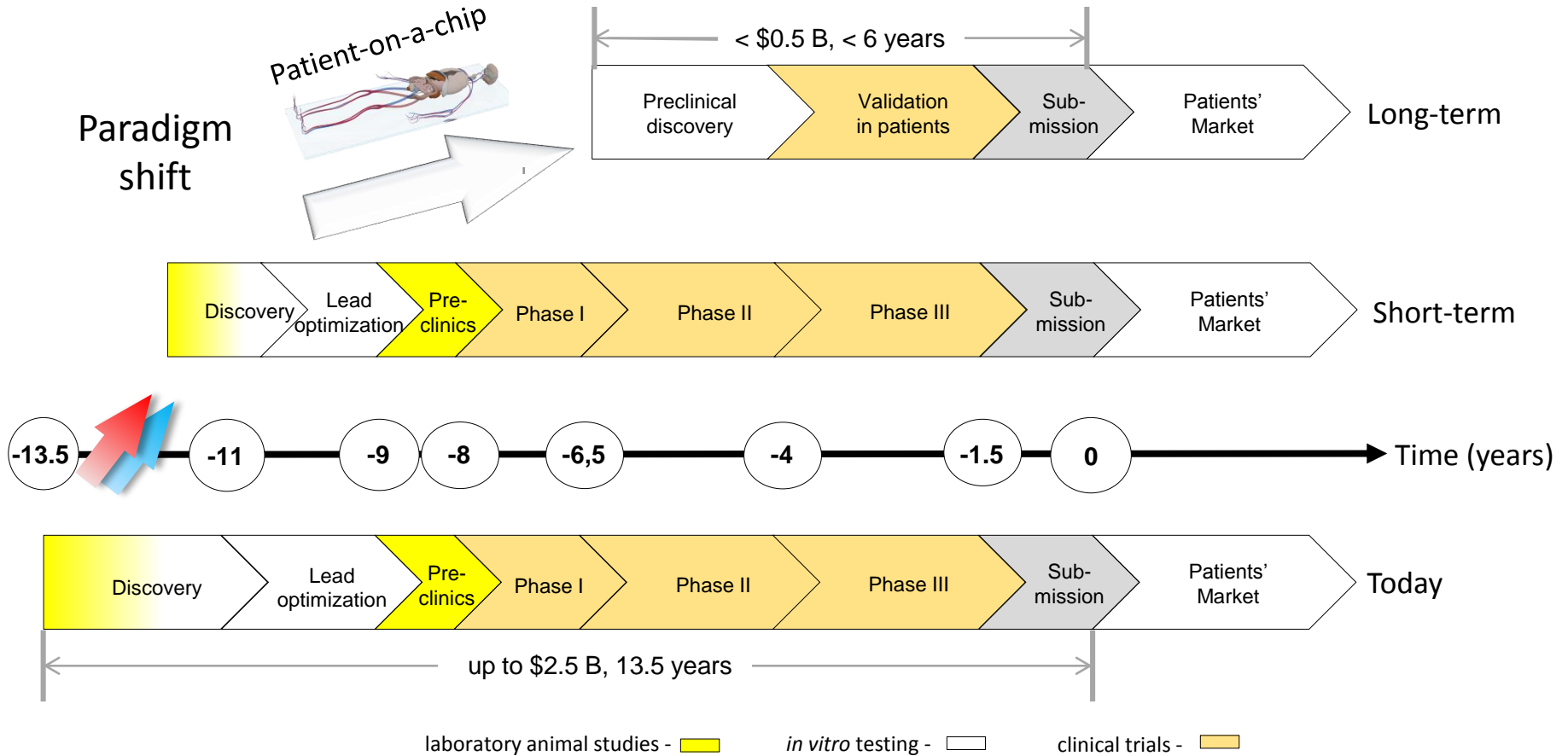
Maschmeyer et al., *Lab Chip*.  
2015, 15(12):2688-99



# A roadmap towards MPS-based decision-making

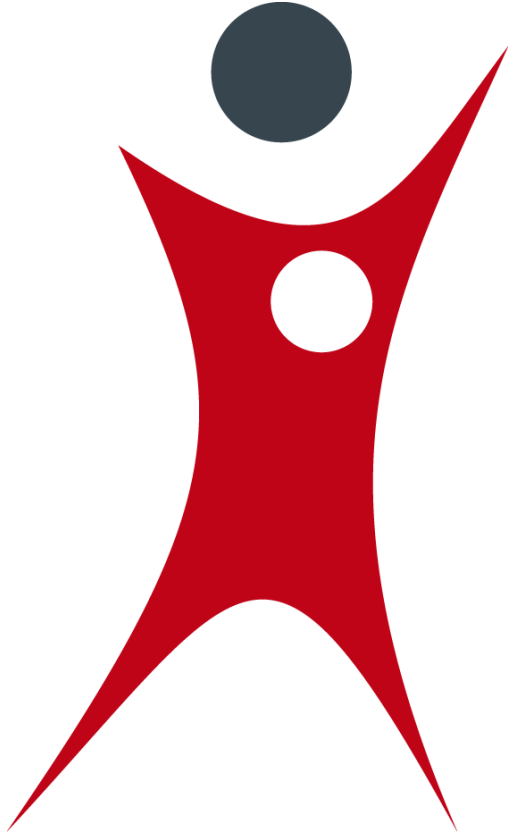


# The paradigm shift



Marx et al., ALTEX 2016 May 15. doi: 10.14573/altex.1603161.

# Thank you for your attention



[leopold.koenig@tissuse.com](mailto:leopold.koenig@tissuse.com)

[www.tissuse.com](http://www.tissuse.com)