A circulation system for long-term maintenance of multiple human tissues or organoids on a chip

Uwe Marx & Reyk Horland

Berlin University of Technology
Institute of Biotechnology
Department of Medical Biotechnology
Germany
Objective

animal models
systemic but NOT human

“human-on-a-chip”
human AND systemic

static and dynamic
3D human cell culture
human but NOT systemic

Organismal long-term homeostasis
for safety assessment and efficacy evaluation of drug candidates in vitro

acute systemic testing – hours and days
chronic systemic testing – months and years
(e.g. OECD guidelines: 1 year, 90d, 28d)

definition of exposure time
short-term – hours and days
mid-term – weeks
long-term – months and years
<table>
<thead>
<tr>
<th>number of “organs”</th>
<th>microscale culture systems</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>single</td>
<td><strong>homotopic</strong></td>
<td><strong>heterotopic</strong></td>
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<td></td>
<td>endothelial cells: Young et al. 2010</td>
<td>lung alveola: Huh et al. 2010</td>
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<td>myoblasts: Gu et al. 2004</td>
<td>liver lobulus: Kane et al. 2006, Hwa et al. 2007, Khetani et al. 2008</td>
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<td></td>
<td>adipose cells: Nakayama et al. 2008</td>
<td>bone-marrow units: Cui et al. 2007</td>
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<tr>
<td></td>
<td>lung+liver+kidney+adipose: Zhang et al. 2009</td>
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<tr>
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<td>intestine+liver+tumour: Imura et al. 2010</td>
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# Microfluidic-based human homeostasis in vitro

## Number of “organs”

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<td>hepatocytes: Powers et al. 2002, Leclerc et al. 2004</td>
<td>Hwa et al. 2007,</td>
</tr>
<tr>
<td>Ho et al. 2006, Lee et al. 2007</td>
<td>Khetani et al. 2008</td>
</tr>
<tr>
<td>neurons: Rhee et al. 2005</td>
<td>intestinal villus: Ootani et al. 2010,</td>
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<tr>
<td>mammary epithelial cells: Young et al. 2010</td>
<td>Sato et al. 2009,</td>
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<tr>
<td>adipose cells: Young et al. 2010</td>
<td>Sung et al. 2011,</td>
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<tr>
<td>embryo cells: Young et al. 2010</td>
<td>Lahar et al. 2011,</td>
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<td></td>
<td>Yu et al. 2012</td>
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## Multiple

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**Short-term exposure (max. 7d)**

2012 Marx et al. ATLA In press
Lessons learned at miniscale

human bone-marrow, thymus, PBMC’s, leukemia (5ml tissue per cell culture space)

human artificial lymph node (0.5ml CCS)


Lessons learned at miniscale

human bone-marrow, thymus, PBMC’s, leukemia
(5ml tissue per cell culture space)

1) mid-term homeostasis (weeks) can be secured using primary tissues

2) proper media perfusion, oxygen supply and debris removal (by macrophages) is essential

3) gradual loss of functionality and viability over months can not be prevented

human artificial lymph node (0,5ml CCS)


24.09.2012
Lorentz workshop Marx & Horland
1) a roadmap towards µl-scale organ engineering *in vitro* has been proposed

2) Organoids (functionally self reliant structural units of each organ) should be emulated *in vitro* at numbers, relevant to the respective research target

3) Micro-bioreactor design, process development and human cell supply remain the major challenges
Human organoids

<table>
<thead>
<tr>
<th>Tissue</th>
<th>High turnover</th>
<th>High regenerative potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>30 days</td>
<td>follicular bulge SCN</td>
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<tr>
<td></td>
<td></td>
<td>Kloepper et al. 2008</td>
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<tr>
<td>Skin with appendices</td>
<td>20 days</td>
<td>White and red pulpa</td>
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<tr>
<td>Intestine</td>
<td>1 day</td>
<td>Villus</td>
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<td>Mammary gland</td>
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<td>Epithelium</td>
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<tr>
<td>Testes</td>
<td></td>
<td>Follicle</td>
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<tr>
<td>Adipose tissue</td>
<td>8.5 years</td>
<td>Cluster</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>15 years</td>
<td>Fibre segment</td>
</tr>
<tr>
<td>Pancreas</td>
<td>20 years</td>
<td>L. islet, exocrine units</td>
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<tr>
<td>Bone-marrow</td>
<td></td>
<td>Unit</td>
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<tr>
<td>Liver</td>
<td></td>
<td>Oval SCN</td>
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<td></td>
<td></td>
<td>Sigal et al. 1992</td>
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<td></td>
<td></td>
<td>Oh et al. 2002</td>
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<tr>
<td>Bone</td>
<td>10 years</td>
<td>Osteone</td>
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<tr>
<td>ENS</td>
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<td>Plexus</td>
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<tr>
<td>Lung</td>
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<td>Bronchio-alveolar SCN</td>
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<tr>
<td>Kidney</td>
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<td>Nephron</td>
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<tr>
<td>Brain</td>
<td></td>
<td>Cerebellar cortex</td>
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<td></td>
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<td>Lifelong</td>
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Bars: 100 µm

Histoimages were kindly provided by www.wesapiens.org

2012 Marx et al. ATLA In press
Vasculature - the missing piece in the puzzle

BioVaSc®-based 5ml liver cultures

1. Albumin synthesis achieves homeostasis & correlates to metabolism
2. Metabolism of dextometorphan (cough suppressant) to dextorphan-glucuronidid (Phase II metabolite) is stable during the entire experiment.

No signs of loss of function for over 3 months!

BioVaSc®- technology (vascularized liver, trachea, intestine ...)

Walles et al. 2005
Mertsching et al. 2005
Walles et al. 2009
Schanz et al. 2010

Angiogenesis in vitro
Sudo et al. 2009
Young et al. 2010
Rivron et al. 2012

2012 Marx et al. ATLA In press
Our concept – the $\frac{1}{100,000}$ “human-on-a-chip”

2009/2010 Grant acquisition activities

7 European partners

- budget: €10Mio over 3 years
- 10 organ equivalents per chip

2 German partners

- budget: €3Mio over 3 years
- a liver and a skin equivalent per chip

- on-chip micro-pump
- microfluidic circulation
- standard microscopic slide base area
- accessible for live tissue imaging

FP7 Health 2010
Alternatives to ...

BMBF GO-BIO
Go-BIO Initiative ..

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Flexible microfluidic chip design

multi-“organ“-chip

- organ equivalent
- micro-pump
- injection/sample
- valve
- reservoir

single „organ“ circulation

single „organ“ perfusion

culture inserts

„skin“

„liver“
Laboratory bioreactor manufactured

- controlling 12 pneumatic actors
- two chips per system
- software control (e.g. WINDOWS, LINUX, MAC)
- telemonitoring
<table>
<thead>
<tr>
<th>parameter</th>
<th>flow velocity</th>
<th>organ viability</th>
<th>organ functionality</th>
<th>pH &amp; pO₂</th>
<th>t°</th>
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<tbody>
<tr>
<td>approach</td>
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<td>principle</td>
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<td>particle imaging velocimetry</td>
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<td>fluorescence spectroscopy</td>
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<tr>
<td>surface plasmon resonance for secreted proteins</td>
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<td>fluorescence lifetime</td>
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<td>PT1000 temperature detector</td>
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<td>features</td>
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<td>non invasive different spots biological particles</td>
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<td>cell tracker live imaging double staining possible</td>
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<td>multiple proteins (46 per micro sensor 10 mm x 0.8 mm)</td>
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<td>fibre coupled external calibration</td>
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<td>long-term robustness</td>
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Frank Sonntag

Fraunhofer IWS
28-day survival with poor functionality

3D tissue preparation and chip loading

HepaRG cells + stellate cells (12 : 1)

24h aggregate formation in AggreWell plates

4 days low-attachment-plate culture

Foreskin donor recruitment

4 days tissue excision and transport to lab

Preparation of sterile punch biopsies

tissue volume: ~30µl
channel volume: ~10µl
total volume: 300µl

IMMUNOHISTOSTAINING:
- CYP3A4
- CYP7A1

D28

Lorentz workshop Marx & Horland

IMMUNOHISTOSTAINING:
- Tenascin
- Col IV
How to add the missing peace?

- immune system
  (macrophages, leucocytes)
- stromal bed
  (fibroblasts + ECM)
- stem cell niche
- biological vasculature?
- Pulsatile fluid flow
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**
Establishing on-chip vasculature

GFP-transduced human micro-vascular endothelial cell line (HMEC-1 cell line); flow < 6 dyn/cm² → no orientation of cell line according to flow
Seeding of primary HDMEC

Human dermal microvascular endothelial cells (HDMEC), static attachment, dynamic flow after 3 hours

Day 0
Day 4
Day 15
Day 25
Day 40
Alexa594-ac-LDL

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Seeding of primary HDMEC
Two-photon microscopy time-lapse image
Seeding of primary HDMEC
in-chip characterization – endpoint control

intracellular von Willebrand factor / cell-cell adhesion CD31 / cell nuclei Hoechst33342
Determination of fluid flow velocity:
Particle Image Velocimetry (PIV) of erythrocytes

Hematocrit (HCT): 0.01

Particle Image Velocimetry

Two-photon imaging of erythrocytes
Creating a capillary bed for tissue supply
MOC-based liver tissue culture

- Dise space
- bile segregation
- stellate cell function
- adequate flow rates
- zonal albumin secretion
- drug metabolism (CYP3A4)
- bile synthesis (CYP7A1)
- ...

HepaRG cell aggregates mixed with primary stellate cells

Seed primary hepatocytes into acellularized liver matrix

Designed Ormocer® photo resist inserts in place

Drug metabolism and bile synthesis

Cell culture compartment

Cyp3A4/Collagen IV

Tunel / Ki-67

~400 hepatocytes

500 µm

50 µm

pO2

24.09.2012

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BMBF: GO-BIO
uwe.marx@tu-berlin.de
reyk.horland@tu-berlin.de