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

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

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

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

- Toxicity, dose-response relationship
- ~100 rodents per substance

28-day:

- OECD 407\textsuperscript{o}, 410\textsuperscript{d}, 412\textsuperscript{i}, 419
- ~40 rodents/substance
- Toxicity, dose-response relationship
- ~100 rodents per substance

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)

OECD 453

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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)
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
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
## Sensors / In-process-controls

<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>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Approach</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Principle</strong></td>
<td>particle imaging velocimetry</td>
<td>fluorescence spectroscopy</td>
<td>surface plasmon resonance for secreted proteins</td>
<td>fluorescence lifetime</td>
<td>PT1000 temperature detector</td>
</tr>
<tr>
<td><strong>Features</strong></td>
<td>non invasive different spots biological particles</td>
<td>cell tracker live imaging double staining possible</td>
<td>multiple proteins (46 per micro sensor 10 mm x 0.8 mm)</td>
<td>fibre coupled external calibration</td>
<td>long-term robustness</td>
</tr>
</tbody>
</table>

**Frank Sonntag**

**Fraunhofer IWS**

**EMUSAAT 2013 Materne**
The “Two-Tissue-Culture Chip”

Culture inserts e.g. transwells

Microscopic slide format

Support with temperature control

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Duration</th>
<th>Short-term (&lt;48h)</th>
<th>Long-term (&lt;28d)</th>
<th>Homeostasis (90d, 1y...)</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>in progress</td>
</tr>
<tr>
<td>skin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>in progress</td>
</tr>
<tr>
<td>vasculature</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>in progress</td>
</tr>
<tr>
<td>neurons</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>in progress</td>
</tr>
<tr>
<td>intestine</td>
<td>✓</td>
<td>in progress</td>
<td>in progress</td>
<td>in progress</td>
</tr>
<tr>
<td>kidney</td>
<td>✓</td>
<td>in progress</td>
<td>in progress</td>
<td>in progress</td>
</tr>
</tbody>
</table>

equivalent to 10 liver lobuli

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

Duration

- Short-term (<48h)
- Long-term (<28d)
- Homeostasis (90d, 1y...)

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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

HepaRG + HHSteC

4.8 $\times 10^4$ HepaRG and 2 $\times 10^3$ HHSteC

20 Aggregates $\approx 10^6$ cells $\approx 1/100$ 000 liver mass
Liver aggregate culture in the chip over 14 days

CK 8/18

Col I / Vimentin

CYP450 3A4 / 7A1

Fibronectin
Liver aggregate culture in the chip over 14 days

MRP2  ZO-1

Glucose consumption and lactate production over 14 days.

Albumin concentration over 14 days, dynamic vs. static conditions.
Co-culture of skin and liver equivalents in the chip

3D tissue preparation and chip loading

HepaRG cells + stellate cells (24 : 1)
aggregate formation in AggreWell plates
low-attachment-plate culture

24h

Foreskin donor recruitment
tissue excision and transport to lab
Preparation of sterile punch biopsies

3 days

PDMS
Polycarbonat
Transwell-holder
Transwell

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Performance of Transwell® multi-tissue cultures over 28 days

Dynamic culture in MOC

Static culture

Liver  Apoptosis  Proliferation  Skin

Dynamic culture in MOC

Static culture

Emulating Human Biology
First Tox studies using Troglitazone

**Trade name:** Rezulin, Rizulin, Romazin, Sensulin

![Chemical structure of Troglitazone](image)

\[ \text{C}_{24}\text{H}_{27}\text{NO}_{5}\text{S} \]

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

### Inoculation of the chips on day 0

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

[Diagram showing the 14 chips and their arrangement]

- **12 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
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, Eva-Maria Materne, Sven Brincker, Ute Sößbier, Caroline Frädrich, Mathias Busek, Frank Sonntag, Dmitry A. Sakharov, Evgeny V. Trushkin, Alexander G. Tönevesky, Roland Laustey, and Uwe Marx

0 μM Troglitazone

50 μM Troglitazone
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

Integrating biological vasculature into a multi-organ-chip microsystem

Katharina Schmees, Markus Bieske, Steff Brueck, Benjamin Gretsch, Silke Holtermann, Roland Lauscher, Gerd Lindner, Alexandra Lenz, Uwe Marx, and Ralf Holst.
Creating a capillary bed for tissue supply
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

Institut für Mikrotechnik Mainz