

# Design and prototyping of a novel “Organs-on-a-chip”-bioreactor system for substance testing

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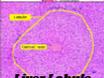
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## Abstract

There has quite plainly been an embarrassing gap in adequate measures to predict the interaction of consumer-relevant synthetic or natural substances with the human body in its typical environment and with its individual genotypic specificity prior to human exposure. Dynamic miniaturized human multi-micro-organ systems are postulated to be a solution for this bottleneck. We have designed and prototyped an “organs-on-a-chip” (OOC) platform technology for the dynamic long-term culture of different human sub-organoids. First chip prototypes were manufactured by means of micro-system technologies and tested with regard to microstructure accuracy, liquid handling performance and resistance to sterilisation procedures.

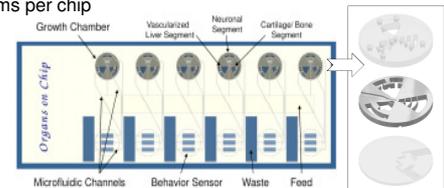
## Design Principles and Criteria

Fundamental paradigms of *in vivo* behaviour of human organs can be translated into rational design principles for dynamic multi-micro-organ bioreactors

	<ul style="list-style-type: none"> <li>organs consist of identical functionally self-reliant sub-organoid units and stem cell niches</li> <li>interconnected through vasculature</li> <li>monitored by various biological sensors of the body</li> </ul>	<ul style="list-style-type: none"> <li>Miniaturize culture systems to sub-organoid size and parallelize bioreactors in standard formats</li> <li>aim for common vasculature</li> <li>add non-invasive sensing tools</li> </ul> <p><b>Device</b></p>
	<p>Specific microstructures provided by an extra-cellular matrix and a local dynamic microenvironment are crucial prerequisites of re-organization and maintenance of sub-organoids <i>in vivo</i></p>	<p>Emulate the extra-cellular matrix backbone of the original tissue at a minimum scale, allowing for the re-organization of at least one organ specific sub-organoid per dedicated culture space</p> <p><b>Architecture</b></p>
	<p>Organ homeostasis is ensured by the systemic supply and removal of soluble factors. Regeneration at sites of damage relies on local recruitment of precursor cells from proximate adult stem cell niches</p>	<p>Provide a dynamic process microenvironment, which ensures re-organisation, maintenance, exposure and regeneration of sub-organoids over long culture periods</p> <p><b>Process</b></p>

## Design of Multi-Bioreactor-Chips

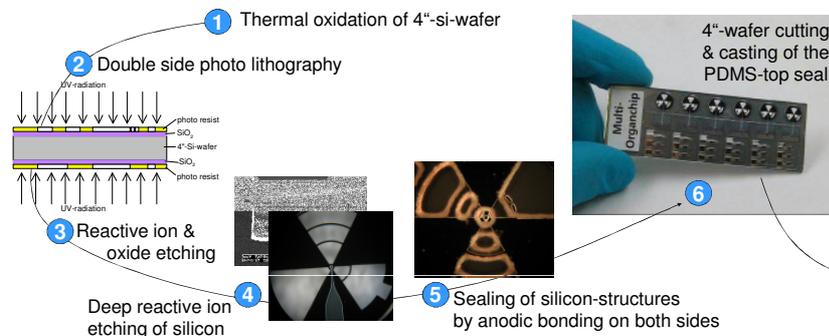
- standard microscope slide format
- six identical micro-bioreactor systems per chip
- common central medium reservoir
- live tissue imaging
- each micro-bioreactor consists of :
  - three organ growth segments
  - a stem cell niche cavity
  - three behavior sensors
  - three waste reservoirs



Layer	Material	Technology	Function
1	PDMS (2000µm)	Casting	Channels and reservoirs
2	Glas (200 µm)	Lithography, Laser	Closing layer
3	Silizium (400 µm)	Lithography, Laser	Channels, organ growth segments & stem cell niche
4	Glas (200 µm)	Lithography, Laser	Sensors and heater

## Manufacture of Chip-Prototypes

... is a six step procedure:



## Supply Unit Prototype

- adapted to workplates of inverse & upright microscopes, easy to handle
- data exchange with PC
- battery-supplied operation for 2 hours
- automatic charge in docking station
- limit value monitoring and alerting
- data acquisition and logging for at least 14 days (e.g. temperature)
- actuator control (e.g. heater) / closing loop control



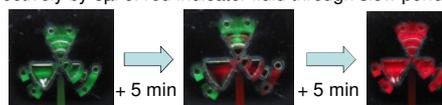
## Performance Evaluation of Multi-Bioreactor-Chips

### Accuracy of Microstructures

- lithographic and laser-based manufacturing techniques are limited to channel widths > 30µm at channel depths > 200 µm (side-wall-angles, undercut, roughness based bonding problems)
- channel geometries of widths < 10µm eliminate fluiddynamic liquid movement in the micro-bioreactors due to surface tension (capillary-driven stop valves)

### Liquid Handling

Manual fill and flush procedure in place: 12µl of green indicator fluid were filled into a growth chamber with three tissue culture segments and were replaced effectively by 8µl of red indicator fluid through slow perfusion



### Resistance to Sterilisation

- Easy to sterilise
- Multiple cycles are acceptable
- QC measures for the thin film system are mandatory to prevent dissolution of electrodes, caused by thermal tensions

## Summary

We were able to translate an “Organs-on-a-chip” bioreactor design into a chip prototype, containing six identical micro-bioreactors, with three different tissue culture segments each. In addition feed and waste reservoirs for long term culture could be successfully integrated into the PDMS layer of the chip prototype. The chips are chemically resistant and sterilizable through autoclaving. The applied manufacturing technology is perfectly suited to produce microstructures of a size above 30µm. Also of very small dimensions, the chip arrangement supports fast and easy fill and flush procedures.

## Outlook

Next steps for the evaluation of the technology are cell and tissue culture experiments, to evaluate biocompatibility of the multi-bioreactor-chips. Mid term it is envisioned, to generate and maintain human sub-organoids of various tissues, such as liver lobuli or skin segments over a period of at least 14 days within the multi-bioreactor-chips. In a further step, generated sub-organoids are planned to be exposed to important substances at relevant regimens.