Multi-Organ-Chip Developments:

Towards a Paradigm Shift In Drug Development

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Abstract

Present *in vitro* and animal tests for drug development do not reliable predict the human outcomes of tested drugs or substances because they are failing to emulate the organ complexity of the human body, leading to high attrition rates in clinical studies. For example, absorption, distribution, metabolism and excretion (ADME) are key determinants of efficacy and safety for therapeutic candidates. However, these systemic responses of applied substances are ignored in most *in vitro* tests. Here we present a universal microfluidic chip platform the size of a microscopic slide, consisting of an on-chip micro-pump and, capable to interconnect different organ equivalents.

Experimental Set Up

The micro-pump ensures stable long-term circulation of media through the tissue culture compartments at variable flow rates, adjustable to physiological mechanical stresses of the respective tissues. In order to emulate the physiological relevant *in vivo* crosstalk with the ability to perform systemic preclinical substance testing, we have developed a universal 2-Organ-Chip (2OC) for long-term culture of human organ equivalents interconnected within a common capillary microfluidic network. Several combinations of organs have been performed on this 2OC platform (e.g. a combination of liver tissues with skin, intestine, pancreatic islets or neuronal tissues).

In addition we present a new 4-Organ-Chip (4-OC) platform for ADME profiling. In this 4-Organ-Chip platform, a human primary intestinal model and a skin biopsy have been integrated on standard cell culture inserts. A fluid flow connected these barrier models with a 3D-based liver spheroid. Finally, a barrier segregating the media flow through the organs from fluids excreted by the kidney has been generated by a polymeric membrane covered by a monolayer of human proximal tubule epithelial cells.

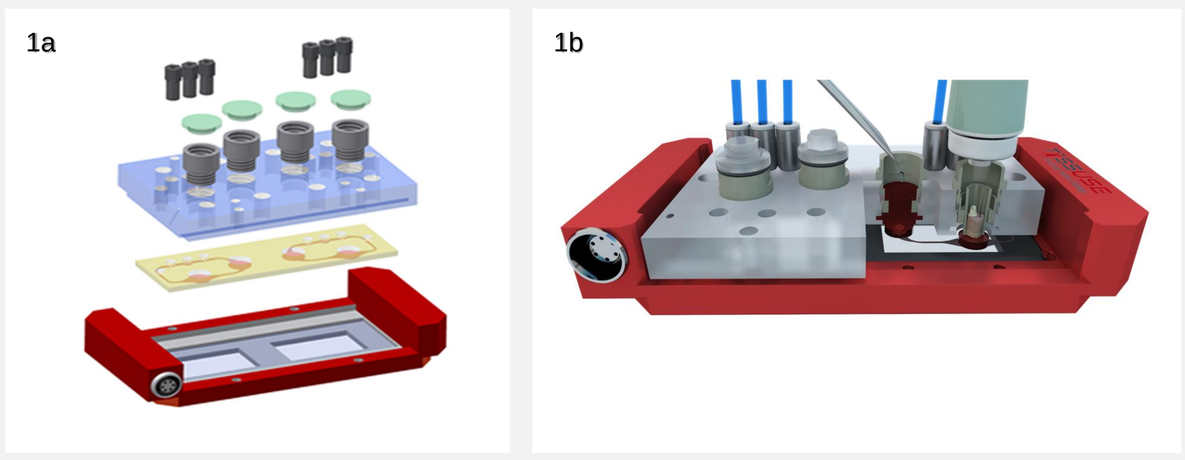

Fig. 1. The microfluidic 2-OC device at a glance. a) Exploded view of the device comprising a polycarbonate cover-plate (blue), the PDMS-glass chip accommodating two microfluidic circuits (yellow; footprint: 76 mm x 25 mm; height: 3 mm) and a heatable MOC-holder (red) **b)** 3D drawing of substance exposure, either systemic or topical/apical.

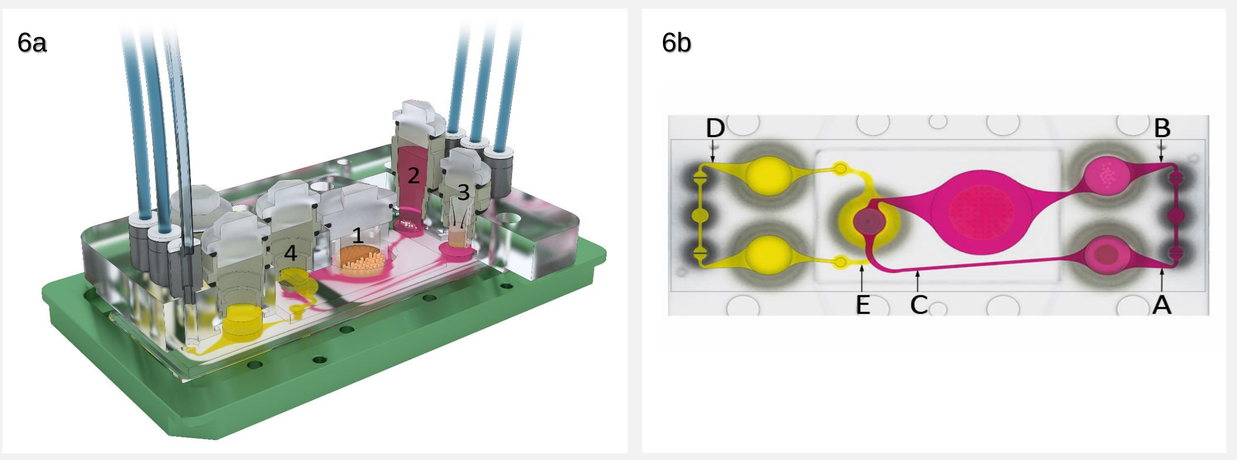

Fig. 6. The microfluidic 4-OC device at a glance. a) 3D view of the 4OC device with culture compartments for intestine (1), liver (2), skin (3), and kidney (4) tissue **b)** Top view of the four-organ-chip layout (footprint: 76 mm x 25 mm; height: 3 mm) accommodating a surrogate blood flow circuit (pink) and an excretory flow circuit (yellow).

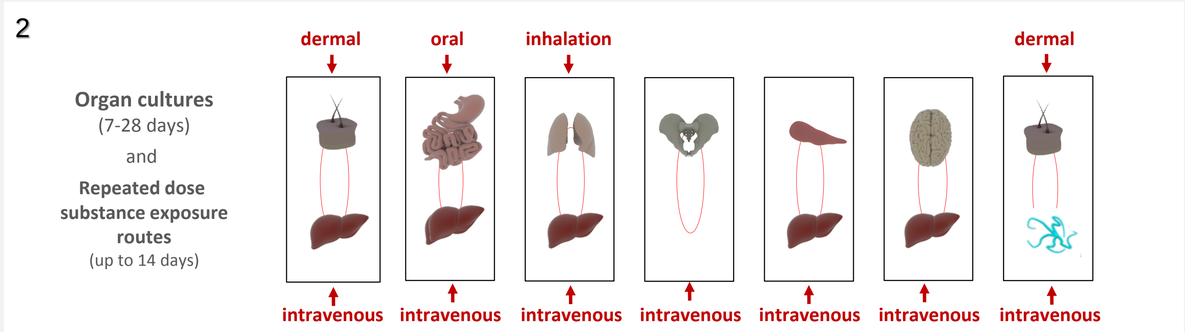

Fig. 2. Examples of co-cultures run on the 2OC. From left to right: Liver-skin, Intestine-liver, lung-liver, bone-marrow, pancreatic islets-liver, neurospheres-liver, skin and dentric cells and their possible substance exposure routes.

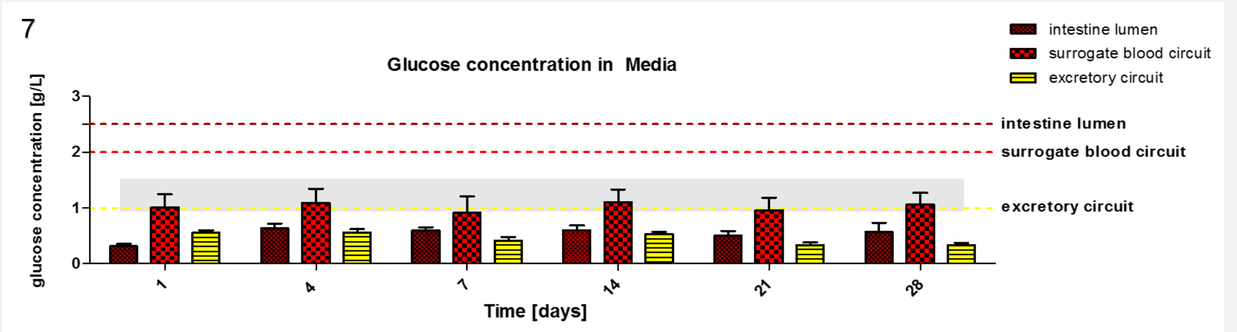

Fig. 7. Glucose balance in the three segregated media pools. Bars: Brown for media from intestine lumen, red for media from the blood circuit and yellow for media from the excretory circuit. Bars are plotted against the background of the physiological glucose concentration (grey area).

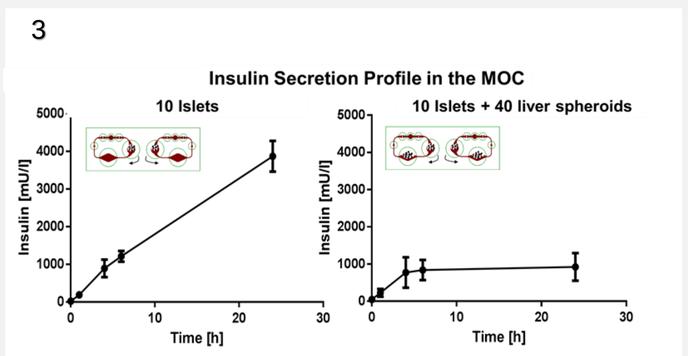

Fig 3. Insulin Secretion Profile in the 2OC. a) with pancreatic islets single culture b) in co/culture with liver spheroids.

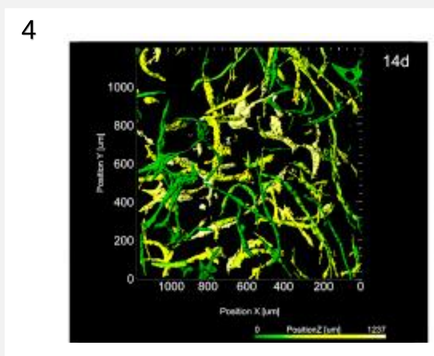

Fig 4. Formation of microvascular structures of GFP-expressing HUVECs in fibrin gel in the 2OC

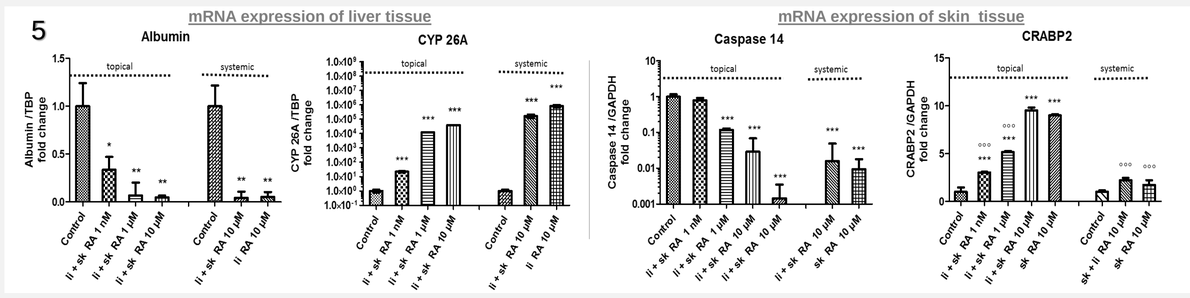

Fig 5. mRNA expression profiles of multi-tissue cultures after chronic substance exposure. mRNA expression of albumin and CYP26A in liver tissue and caspase14 and CRABP2 in epidermis tissue after retinoic acid exposure. Values are mean ± S.E.M. Asterisks (*,**,***) indicate statistically significant differences between control and treatment groups and Degrees (°,°°,°°°) between samples after topical application of highest concentrations and other treatment groups (p≤0.05,p≤0.01.p≤0.001). Li-liver, sk–skin.

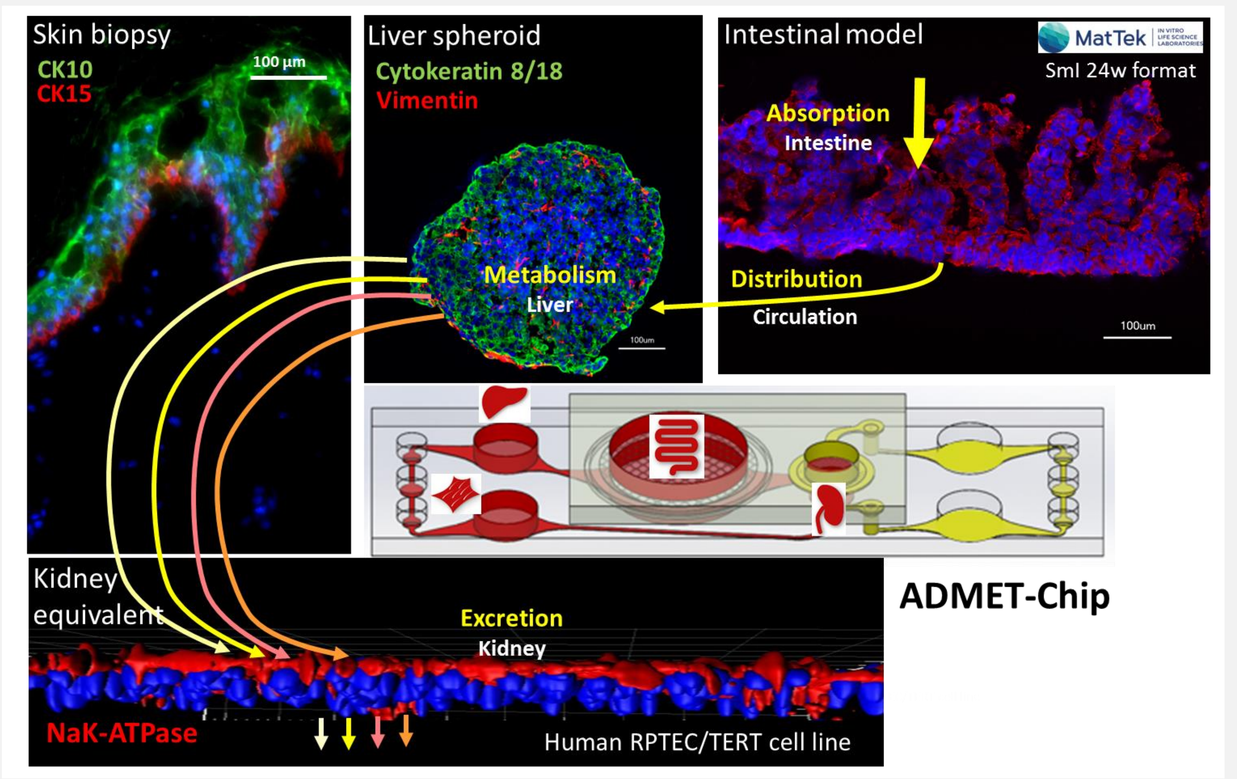

Fig. 8. Performance of human tissues in the 4OC after 28 days of co-culture. Staining of a) for Ctk 19 (red) expression in small intestinal epithelial tissue, b) Ctk 8/18 (green) Vimentin (red) expression in liver aggregates, c) epidermal markers Ctk 10 (green) and Ctk 15 (red) in skin biopsies, d) transporter NaK-ATPase (red) in proximal tubule epithelial monolayer. (a - d) Nuclei were stained with DAPI (blue).

Results

It could be shown, that our Multi-Organ-Chip is universally applicable to co-culture different organ models over a culture period of up to 28 days. Tissue engineering data and assay performance data for repeated dose substance exposures through topical, apical and systemic administration routes will be presented. 4-OC results showed steady homeostasis during the complete culture period. Hence, a unique Multi-Organ-Chip platform was developed, enabling the testing of effects of substances on a set of miniaturized human organs.

Outlook

Based on the developments of recent years on ‘organs-on-a-chip’ we are working on a further improvement of our 2OC and 4OC with several more organ combinations. Next to this, we are developing a „body-on-a-chip“, which combines a fluid flow with at least 10 different organ units. A prototype can be seen in figure 9.

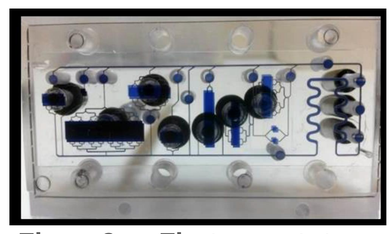

Fig. 9. First prototype towards a human body-on-a-chip

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