Substance exposure to skin and liver co-cultures in a Multi-Organ-Chip

Ilka Wagner[1], Juliane Hübner, Alexandra Lorenz[1], Reyk Horland[1],
Thamée Lade[2], Andreas Schepky[2], Roland Lauster[1], Jochen Kühnl[2], Uwe Marx[1,3]

[1]Technische Universität Berlin, Germany, [2]Fraunhofer IWS, Dresden, Germany
[2] Front End Innovation, Dept. Toxicology, Beiersdorf AG, Hamburg, Germany
[3]TissUse GmbH, Markgrafenstraße 18, 15528 Spreenhagen, Germany

Abstract

Current *in vitro* tests fail to emulate the systemic response of applied substances. In this study, a dynamically-perfused Multi-Organ-Chip (MOC) system was used to integrate the culture of liver and skin equivalents in one system. An on-chip micro-pump enabled metabolic transport and added physiological shear stress. Liver and skin equivalents were viable for 10 days and showed expression of tight junctions and specific transporters. Caffeine, retinoic acid and betamethasone-21-valerate were applied daily for 7 days to investigate the effect of compounds known to be metabolised by skin and liver. The effects of topical application onto the epidermis were compared to the effects of direct substance application to the medium to analyse the influence of skin penetration and metabolism. Liver and skin equivalents were analysed for the expression of metabolising enzymes, transporters, differentiation markers and for viability. Results showed constitutive and inducible phase I and II enzyme expression on protein and mRNA level, according to the substances applied. Hence, the Multi-Organ-Chip is a promising *in vitro* approach for systemic and topical dosage of drugs and cosmetics in a combined culture of liver and skin.

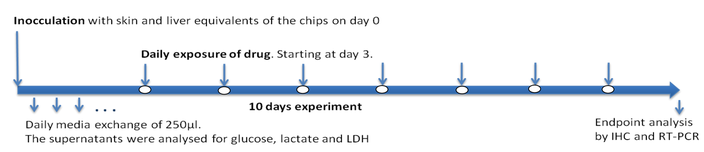
Experimental Set Up

Tissue loading

Liver microtissues (aggregates of HepaRG+human hepatic stellate cells) and MatTek epidermis models were loaded onto the Multi-Organ-Chip: submerged in media (liver equivalent) and air/liquid interfaced (skin) with a total volume of 460 µl (Fig 1D). The cultures were allowed 3 days of adaption to shear stress and the shared medium, before substances were added.

Multi-tissue exposure to substances

Caffeine (5 µM, 250 µM and 500 µM), retinoic acid (1 nM. 1 µM and 10µM) and betamethasone-21-valerate (2, 4 and 6 mg/14,6L) were dissolved in DMSO and were applied daily either topically or systemic (only highest concentration) to the co-culture. Caffeine is a OECD standard compound for epidermal permeation and is metabolised by the liver. Betamethasone-21-valerate has been shown to be degraded to betamethasone when applied topical to skin models, while retinoic acid affects gene. transcription and modulates a wide variety of biological processes like cell proliferation and differentiation, including apoptosis



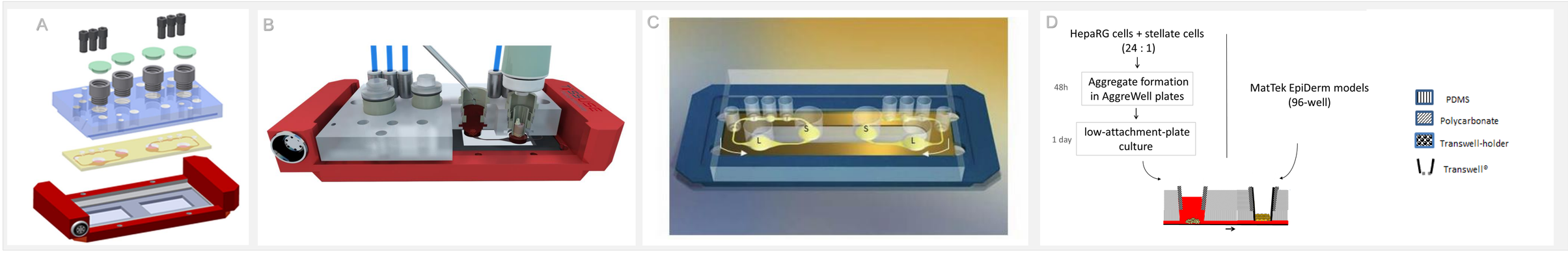


Fig. 1. The microfluidic MOC device at a glance. (A) Exploded view of the device comprising a polycarbonate cover-plate (blue), the PDMS-glass chip accommodating two microfluidic circuits (yellow; footprint: 76 mm x 25 mm; height: 3 mm) and a heatable MOC-holder (red). **(B)** 3D drawing of substance exposure, either systemic or topical. **(C)** 3D drawing of an assembled MOC. Arrows indicate fluid flow direction of each circuit. S – skin culture compartments, L –liver culture compartments **(D)** Schematic sections through the tissue culture compartments supporting skin cultures in Transwells® and submersed cultures in the fluid flow.

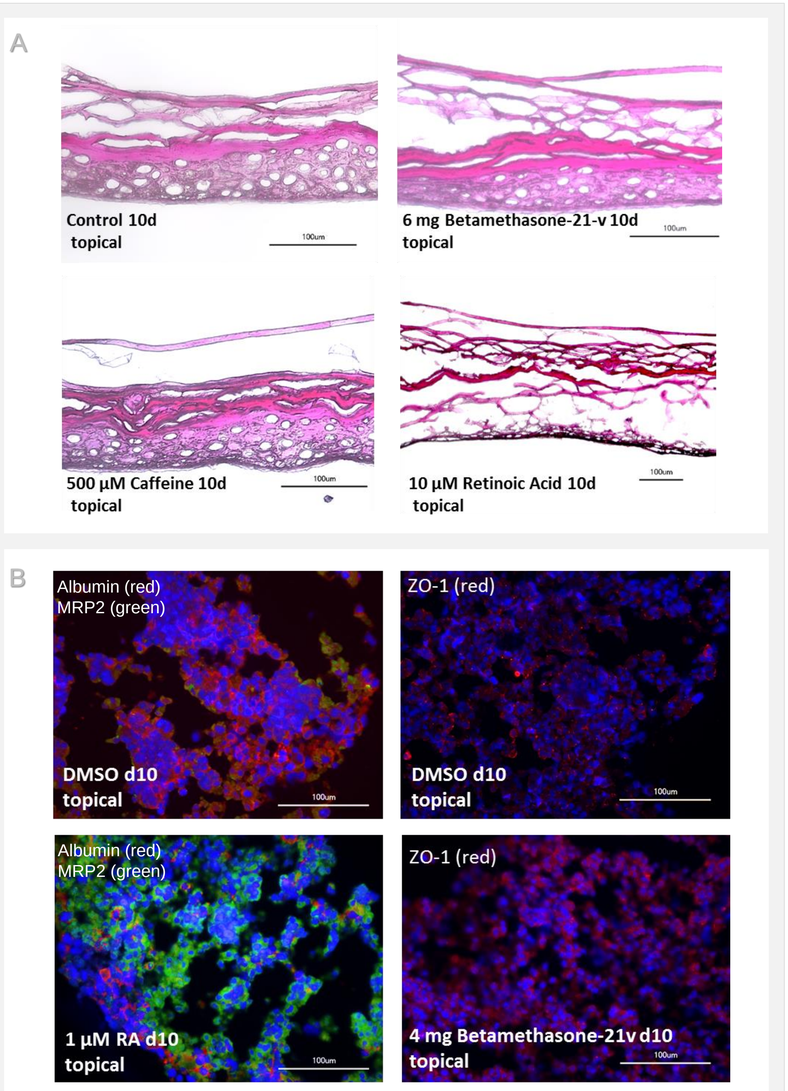


Fig. 2) Staining of multi-tissue culture after 7 days of chronic substance application in the MOC. (A) H/E staining of epidermis models after topical application of highest concentrations. **(B)** Immunofluorescence staining of liver tissue after chronic application of retinoic acid and control (left, albumin, red and MRP2, green) and betamethasone-21-valerate and control (right, ZO-1, red)

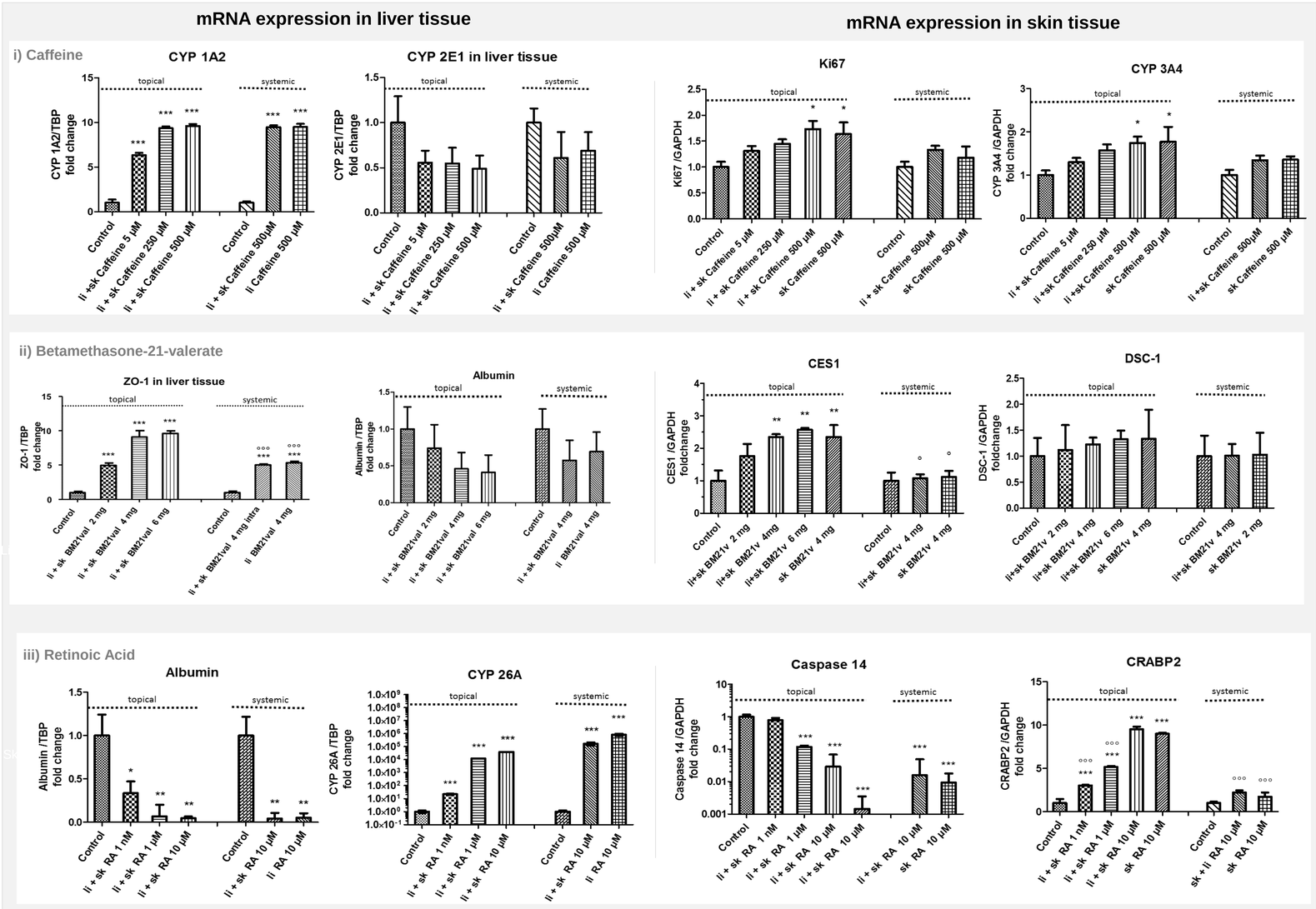


Fig. 3) mRNA expression profiles of multi-tissue cultures after chronic substance exposure (i) mRNA expression of CYP1A2 and CYP 2E1 in liver tissue and Ki67 and CYP 3A4 in epidermis tissue after caffeine exposure **(ii)** mRNA expression of ZO-1 and albumin in liver tissue and CES1 and DSC-1 in epidermis tissue after betamethasone-21-valerate exposure **(iii)** mRNA expression of albumin and CYP 26A in liver tissue and caspase 14 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 ($p \leq 0.05$, $p \leq 0.01$. $p \leq 0.001$). Li- liver, sk – skin.

Results

Substance exposure to co-cultures showed no distinct differences in histology of epidermis in betamethasone-21-valerate and caffeine treated cultures compared to control but a destructed epidermis after chronic 10 µM retinoic acid treatment. Albumin expression was downregulated in liver tissues treated with retinoic acid, seen in immunofluorescence staining (Fig. 2B) and mRNA expression (Fig 3iii). mRNA expression showed significant inductions and reductions of relevant genes after exposure with all three substances. Significant differences could be seen in epidermal mRNA expression between topical and systemic application in several tissues.

Summary

A unique chip-based tissue culture platform has been developed enabling the testing of effects of cosmetics and drugs on a set of miniaturized human organs. This “human-on-a-chip” platform is designed to generate reproducible, high-quality *in vitro* data predictive of substance safety in humans. The tested substances penetrated the epidermis or were metabolised in the epidermis before they were pumped to the liver and activated the corresponding CYPs. Hence, the Multi-Organ-Chip is a promising *in vitro* approach for systemic and topical dosage of cosmetics and drugs in a combined culture of liver and skin.

Technische Universität Berlin • Department of Biotechnology • D-13355 Berlin • Germany • ilka.wagner@tu-berlin.de • www.medbt.tu-berlin.de