Human Multi-Organ-Chip co-culture approach of bronchial airway and liver models for substance exposure studies

Stefan Frentzel[1], Katharina Schimek[2], Karsta Luettich[1], David Bovard[1], Isabel Rütschle[2], Laura Boden[2], Felix Rambo[2], Annika Winter[2], Uwe Marx[2] and Julia Hoeng[1]

[1]Philip Morris Products S.A. (part of Philip Morris International group of companies), Quai Jeanrenaud 3, 2000 Neuchâtel, Switzerland
[2]TissUse GmbH, Oudenarder Str. 16, 13347 Berlin, Germany

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Introduction

At present, the predictability of cell culture based test methods to assess the effects of substances on the human body is limited, as they are failing to emulate organ complexity and cross-talk. Biology inspired microphysiological systems such as TissUse's Multi-Organ-Chip (MOC) platform provide preclinical insight into absorption, distribution, metabolism and toxicity of substances on a systemic level using human tissues. In order to be able to elucidate the toxicity of inhaled compounds, we adjusted the MOC design for the optimized co-cultivation of a human liver equivalent based on the HepaRG™ cell line, combined with human stellate cells and a bronchial equivalent based on the MucilAir™ model. Compared to an earlier version, the MOC was redesigned to optimize medium supply, as well as allowing better oxygenation of the organ models. Viability and homeostasis could be demonstrated for 14-days MOC co-culture.

Experimental Set Up

40 liver HepaRG™/stellate cell spheroids (each 24.000 HepaRG™ cells and 1.000 stellate cells) and one 24-well Transwell®-based MucilAir™ bronchial epithelial model have been co-cultured using the 3-Organ-Chip optimized for medium supply (3-OCplus) for 14 days. The third organ compartment was used as a reservoir for media exchange (Figure 1 A-D). A 50% media exchange rate was applied every 2nd to 3rd day during the whole culture period. All supernatants were collected and stored at 4 °C until metabolic analysis was performed (Figure 1 E).

The microfluidic 3-Organ-Chip high medium (3-OCplus) at a glance

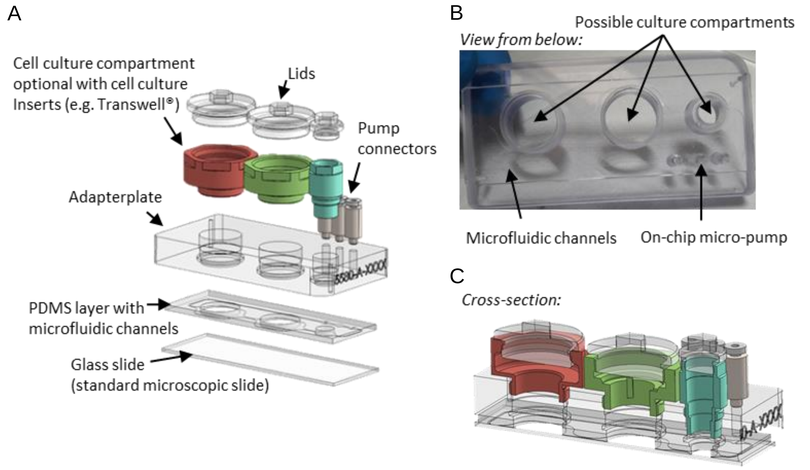

Figure 1: The Multi-Organ-Chip (MOC) platform and the experimental procedure for the chip culture at a glance. (A) Components of the 3-Organ-Chip optimized for medium supply (3-OCplus). (B) Bottom view of the 3-OCplus. (C) Cross-section of the 3-OCplus.

14 day 3-OCplus co-culture with repeated medium exchange

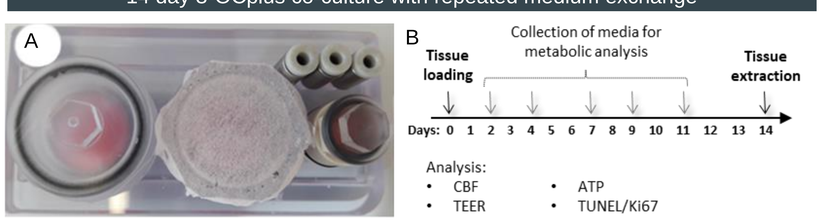

Figure 2: Experimental set-up and Timeline of chip experiment.. (A) Illustration of the 3-OCplus with breathable lid above the lung culture compartment. (B) Timeline of the 14-day experiment. Medium is exchanged every 2nd to 3rd day.

Relevant organ equivalents for liver and bronchial tissues

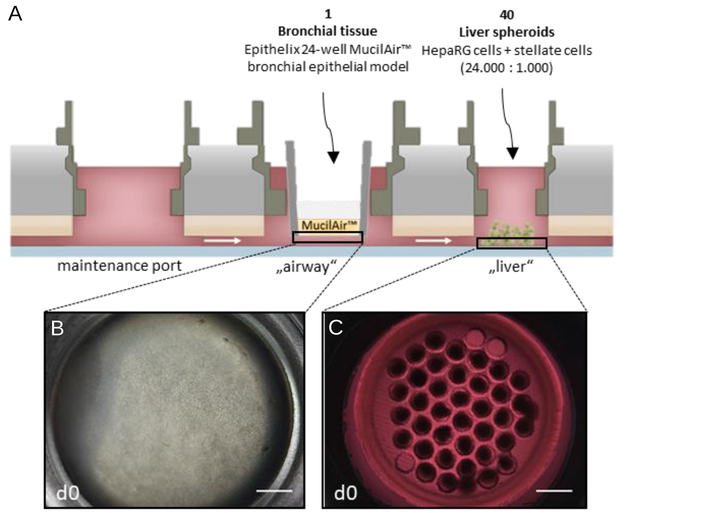

Figure 3: Relevant organ equivalents and loading scheme for 3-OCplus culture. (A) Distribution of 40 HepaRG™/stellate cell spheroids (liver model) and one bronchial epithelial (MucilAir™) model in the 3-OCplus circuit (sectional representation). Clockwise, pulsatile pumping direction so medium first passes through the maintenance compartment, then the MucilAir™ model in the middle and afterwards the liver model. (B) One bronchial epithelial model and (C) 40 liver spheroids in the respective 3-OCplus culture compartment. Scale bar: 1000 µm.

Results

Oxygenation of the organ equivalents during 3-OCplus culture

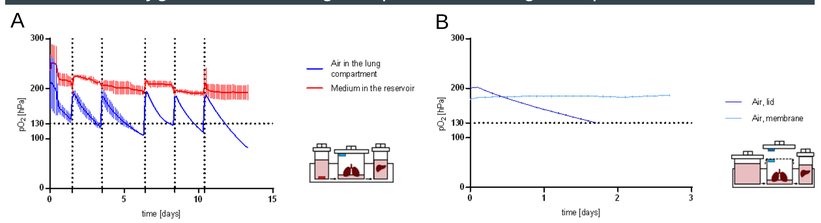

Figure 4: On-line oxygen measurement with PreSens technology in the 3-OCplus. (A) pO2 dynamics in the reservoir medium and in the lung compartment air during 3-OCplus co-cultivation. Means and standard deviations, N = 2. pO2 fell below 130 hPa in the lung compartment, two days after every media exchange (dotted vertical lines). (B) pO2 dynamics in the lung compartment. Comparison of a gas-permeable membrane and a standard lid as a covering for the lung compartment. Means and standard deviations, N = 3.

Viability of organ equivalents

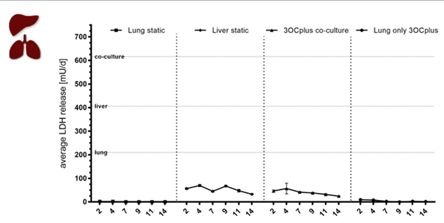

Figure 5: LDH release of the cultured tissues over 14 days. Single static cultures of bronchial MucilAir™ tissues and liver tissues are compared with lung/liver co-cultures and lung only cultures in the 3-OCplus.

Long term functionality of organ equivalents

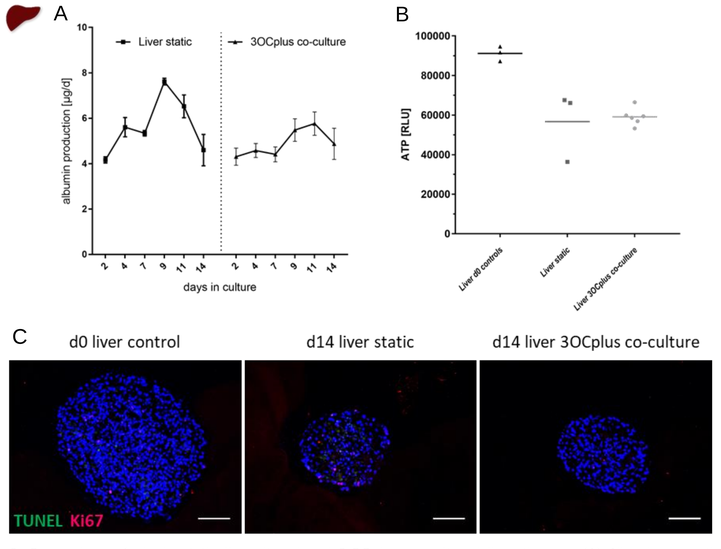

Figure 6: Comparison of single statically cultured and 3-OCplus co-cultured liver tissues (A) Albumin production was measured every 48 to 72h in media collected during 50% media exchange. Co-cultures on the right (triangle) are compared to single cultures on the left (square). (B) ATP based CelltiterGlo® 3D viability assay performed with single liver spheroids extracted from static liver and 3-OCplus co-cultures compared to day 0 controls. (C) TUNEL (green, apoptotic cells) and Ki67 (red, proliferative cells) double staining of HepaRG™/stellate cell spheroids. A low number of TUNEL positive apoptotic cells and an equal amount of Ki67 positive proliferative cells could be detected in all conditions indicating that liver tissues are still viable and metabolically active on day 14 of co-culture. Scale bar: 100µm. Means and standard deviations, N = 3-8.

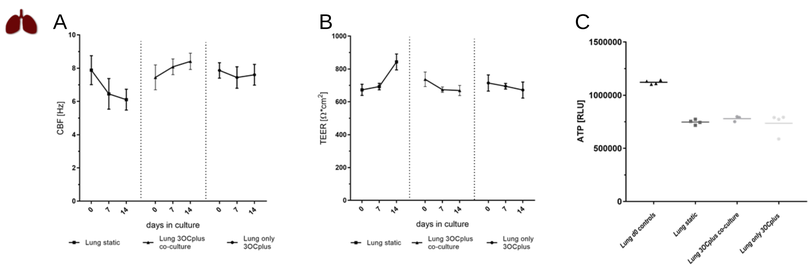

Figure 7: Comparison of single statically cultured and 3-OCplus co-cultured bronchial tissues (A) Cilia beating frequency (CBF) and (B) Transepithelial electrical resistance (TEER) measurement on day 0, 7 and 14 of culture. (C) ATP based CelltiterGlo® 3D viability assay performed with bronchial MucilAir™ tissues extracted from the cultures. 3-OCplus co-cultures are compared to single static static cultures and day 0 controls. On d14 of co-culture, lung tissues are still viable and active.
Means and standard deviations, N = 3-8.

Conclusions

TissUse's 3-OC could be successfully optimized for medium supply and better oxygenation of the organ models in long-term co-culture of human airway and liver equivalents. To this end, increased medium volume (up to 4ml) and an air-permeable lid above the MucilAir™ lung models was developed. Experiments demonstrated a stable co-culture for up to 14 days.
As the MOC platform allows for easy integration of additional organ models, more complex model systems could be implemented, e.g. endothelial cell layers to emulate vasculature.
In the future, routine use of the MOC system for evaluation of inhaled substances at repeated exposure regimens is envisioned.