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

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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 prescient 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 inhalated 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 MucAir™ 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 hemostasis 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 MucAir™ bronchial epithelial model have been co-cultured using the 3-Org-Chip optimized for medium supply (3-OCPplus) 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-Org-Chip high medium (3-OCPplus) at a glance

**Results**

**Oxygenation of the organ equivalents during 3-OCPplus culture**

**Viability of organ equivalents**

**Long term functionality of organ equivalents**

**Discussion**

TissUse’s 3-OCP 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 MucAir™ lung cell layer 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 inhalated substances at repeated exposure regimens is envisioned.

**References**

1. Frentzel, S., Schimek, K., Luetich, K., Bovardi, D., Rütschle, I., Boden, L., Rambo, F., Winter, A., Marx, U., Hoeng, J. (2021). Human Multi-Organ-Chip co-culture approach of bronchial airway and liver models for substance exposure studies. TissUse GmbH • www.tissuse.com • Oudenarder Straße 16 • 13347 Berlin, Germany • Tel. +49(0)-30-5130264-00; Email: katharina.schimek@tissuse.com

**Abstract Number/Poster Board number:** 2036 / P426