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Towards a multi-organ-chip combining human liver, pancreatic islets, skeletal muscle and kidney equivalents to study metabolic diseases

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Abstract

Present *in vitro* and in vivo 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 microfluidic chip platform the size of a microscopic slide, consisting of an on-chip micro-pump and, capable to interconnect different organ equivalents with the aim to not only study therapeutic intervention but also pathogenic factors in metabolic diseases like non-alcoholic fatty liver disease (NAFLD) and the metabolic syndrome.

Experimental Set Up

In order to emulate the physiological relevant *in vivo* crosstalk, we have developed a 2-Organ-Chip (2OC) for long-term culture of human organ equivalents. At the size of a microscopic glass slide, the 2OC consists of two spatially separated cavities which are used for the culture of three-dimensional organ models. These culture compartments are interconnected by a microfluidic circuit representing the human blood circulation. An integrated on-chip micropump circulates a nutrient solution through the microfluidic channel system. This connection of the organ models enables their cross-talk. Samples can be taken at any time during culture as the culture compartments can be easily accessed through lids above the cavities.

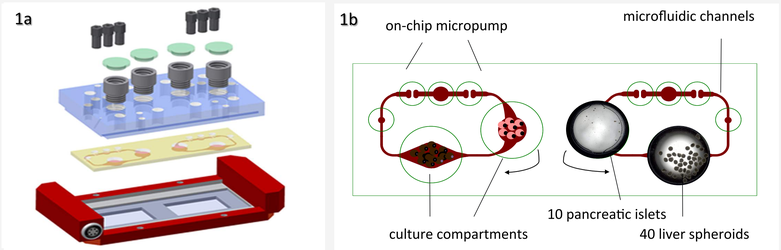

Fig. 1. The microfluidic 2-OC device at a glance. a) Exploded view of the device comprising a polycarbonate cover-plate (blue), the PDMS-glass chip accommodating two microfluidic circuits (yellow; footprint: 76 mm x 25 mm; height: 3 mm) and a heatable MOC-holder (red) **b)** Experimental Set up with 40 liver spheroids co-cultured with 10 human pancreatic islets to investigate insulin-glucose cross-talk in the 2OC.

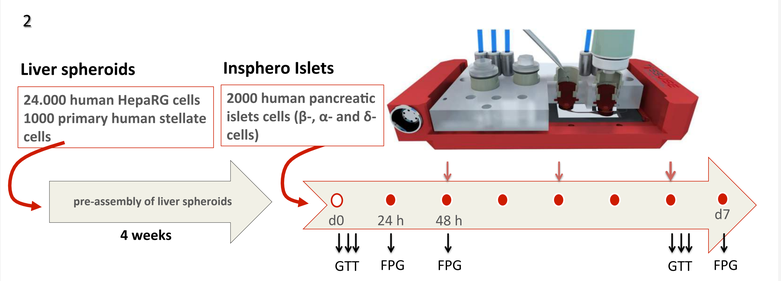

Fig. 2 Experimental design of islet-liver co-culture. On the first day of culture 40 pre-assembled liver spheroids and 10 pancreatic islets are transferred to the 2OC. They can be cultured for at least 7 days in a common medium. On the first and last day of culture a glucose tolerance test **(GTT)** is performed by sampling the medium at different time points (0h, 2h, 4h). After 24 h and 48 h the fasting plasma glucose **(FPG)** level is measured. Medium is exchanged every two days enabling a repeated substance application if desired.

Results

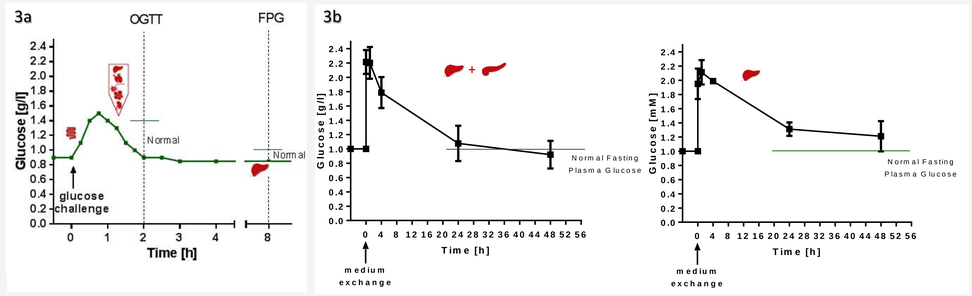

Fig. 3. The oral glucose tolerance test (OGTT) *in vivo* vs *in chipro. a)* *In vivo* blood glucose is decreased within 2 h after a glucose challenge to physiological ranges. The fasting plasma glucose (FPG) is balanced by glucose production through the liver around 1 g/l. **b)** *In chipro* glucose is decreased to physiological ranges within 24 h. A physiological fasting plasma glucose concentration can be maintained for up to 48 h. Without insulin producing pancreatic islets the liver aggregates are not able to decrease the glucose concentration to physiological ranges.

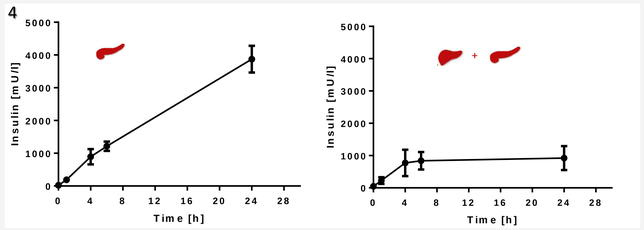

Fig. 4. Pancreatic islet performance in the 2OC. Cultivation of islets alone results in a steady increase of the insulin in the circulation while in co-culture the insulin secretion stagnates due to a faster consumption of glucose and a possible clearance of insulin by the liver aggregates.

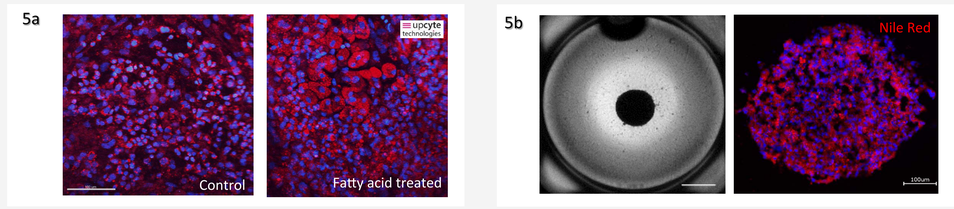

Fig 5. A steatosis model with fat loaded upcyte hepatocytes (proliferating, primary hepatocytes) to study the progression of NAFLD and the metabolic syndrome. (A) Nile Red staining of upcyte hepatocytes treated for 7 days with 0,6 mM palmitate (right images) or without palmitate (left images). Scale 100 µm (B) Steatotic liver aggregates formed with 25.000 upcyte hepatocytes that were pretreated for 7 days with palmitate.

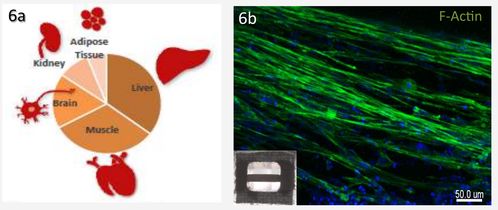

Fig 6. a) Postprandial glucose disposal rate shows that muscle is a main glucose consumer and therefore essential for physiological glucose tolerance. b) 3D skeletal muscle model made of iPSC-derived human skeletal muscle cells grown in a fibrin gel.

Outlook

In addition we are aiming for ADME profiling of drug candidates on the platform. Therefore we developed a 4-Organ-Chip (4-OC) which allows for the integration of a human primary intestinal model for drug absorbtion to a 'blood' circulation. A second urinary circulation is segregated from the blood circulation by a polymeric membrane covered with human proximal tubule epithelial cells. Through this kidney barrier excretion and reabsorption can be studied.

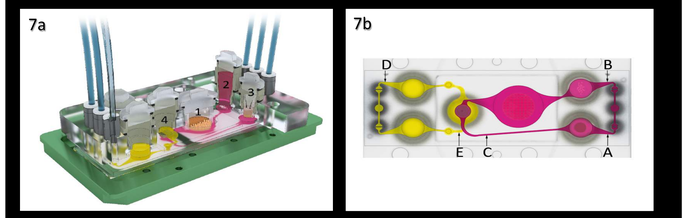

Fig. 7. The microfluidic 4-OC device at a glance. a) 3D view of the 4OC device with culture compartments for intestine (1), pancreatic islets (2), liver (3), and kidney (4) tissue **b)** Top view of the four-organ-chip layout (footprint: 76 mm x 25 mm; height: 3 mm) accommodating a surrogate blood flow circuit (pink) and an excretory flow circuit (yellow).

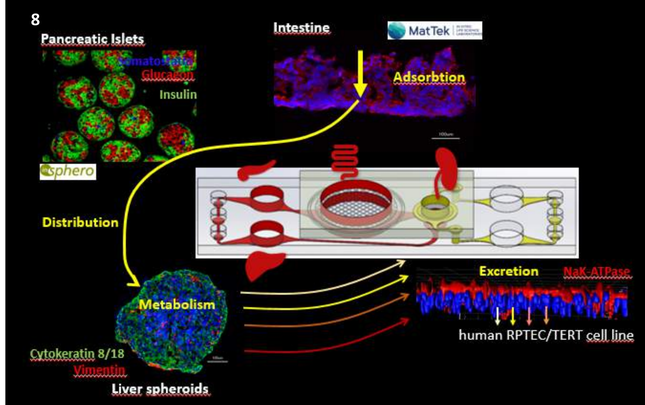

Fig. 8. A 4-Organ-Chip platform for in vitro microfluidic ADME profiling and repeated dose systemic toxicity testing of drug candidates. Oral applied drugs as well as nutrients are taken up by a preformed human intestine (Absorbtion). A peristaltic on-chip micropump ensures pulsatile flow interconnecting all 4 culture compartments and allowing for the distribution of metabolites. A 3D-based spheroid, equivalent to ten liver lobules, mimics liver function (Metabolism). A second microfluidic circulation ensures drainage of the fluid excreted through the kidney epithelial cell layer (Excretion). Organs are integrated at a size 100,000-fold smaller than their human counterpart organs to assures near to physiological fluid-to-tissue ratios.