Use of upcyte® hepatocytes as a steatosis model on a multi-organ-chip
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INTRODUCTION
Multi-Organ-Chips are platforms capable of systemically combining human organ equivalents for the study of organ cross-talk as well as chemical toxicity and drug efficacy. For liver equivalent production primary human hepatocytes (pHiH) would be the ideal cell source. However, the supply of pHiH is limited by the low and sporadic availability of human liver tissue. To address this, we have developed a technique which causes pHiH to proliferate up to 40 population doublings whilst still retaining a metabolically competent phenotype when cultured at confluence (”upcyte® hepatocytes”). Hepatic steatosis, also known as fatty liver disease (FLD), is a reversible condition wherein large vacuoles of triglyceride fat accumulate in liver cells. It is commonly associated with the metabolic syndrome, but can also be due to chemical or drug toxicity (e.g. valproic acid or tamoxifen).

The upcyte® technology
The Multi-Organ-Chip Platform

Expansion of primary hepatocytes using a defined cocktail of lentiviral vectors
We first generated a library of lentiviral vectors carrying proliferation-inducing genes, allowing primary human hepatocytes (pHiH) to bypass senescence. Resulting upcyte® hepatocytes gained the ability to proliferate for up to 40 additional population doublings without losing functional and phenotypic characteristics of mature cells. All cells exhibited expected morphology patterns and were restricted by the presence of specific growth factors, contact inhibition and anchorage dependence.

RESULTS

Formation and depletion of fat vesicles in upcyte® hepatocytes in monolayers...

and aggregates in the two-organ-chip

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Conclusions
In conclusion upcyte® hepatocytes are a suitable cell source to study the development and progression of hepatic steatosis. The utilization of 3D steatotic liver aggregates cultured in a multi-organ-chip enables the study of glucose homeostasis in a physiologically relevant scale as well as the possibility to study cross talk with organs involved in the progression of NAFLD. In future studies the control medium has to be optimized (reduced insulin and glucose concentrations, as well as lower Gentamycin concentrations) in order to avoid lipid accumulation in untreated liver aggregates (control).

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