Use of upcyte® hepatocytes as a steatosis model on a multi-organ-chip

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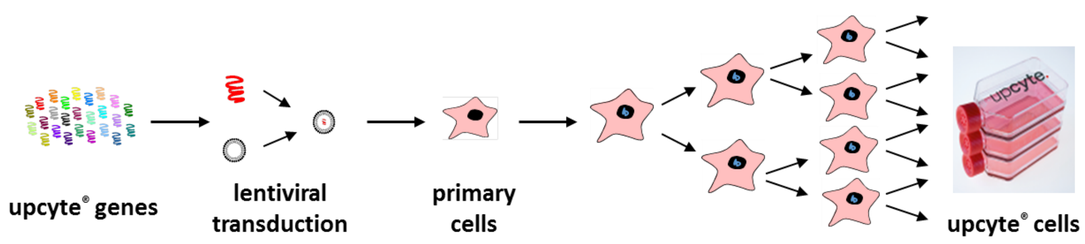
INTRODUCTION

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 (pHH) would be the ideal cell source. However, the supply of pHH is limited by the low and sporadic availability of human liver tissue. To address this, we have developed a technique which causes pHH 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).

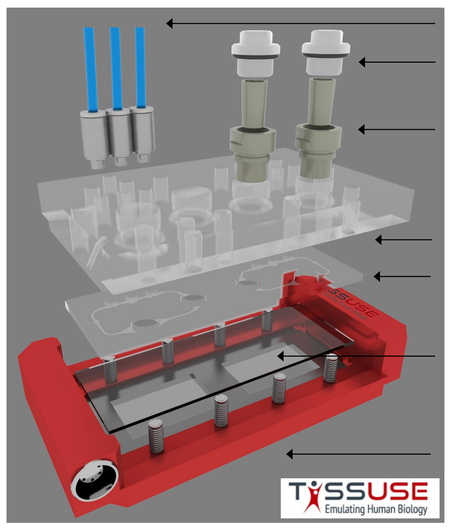
The upcyte® technology



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 (pHH) 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**.

The Multi-Organ-Chip Platform

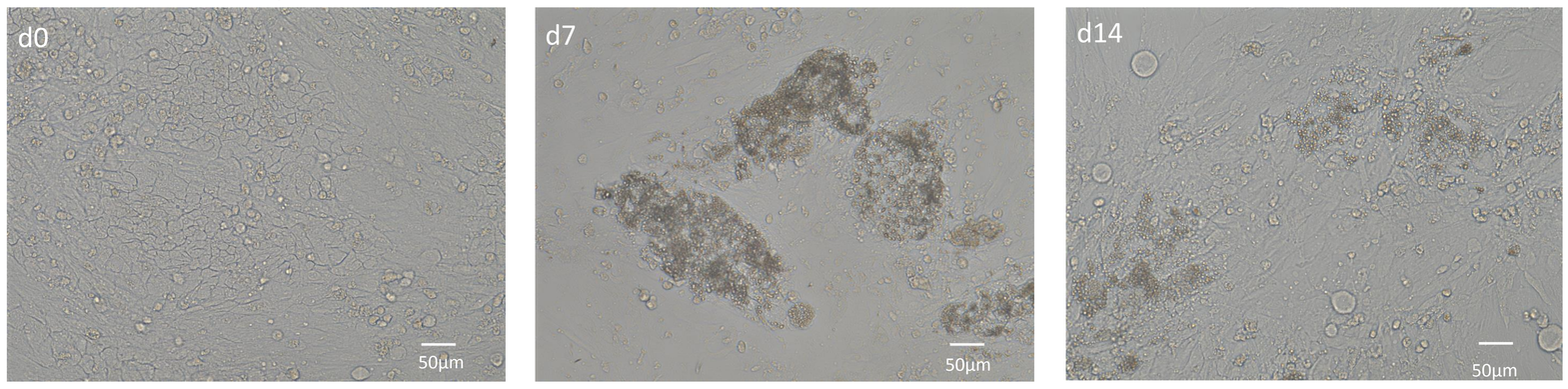


A dynamic Two-Organ-Chip has been established for the simultaneous cultivation of two different organ models in a common media perfusion circuit at a **miniaturized scale**. Cells or tissues can be applied both into the two culture spaces on standard Transwell inserts to model **biological barriers**, such as intestine epithelia, or on matrix supports to mimic the **3D environment** of parenchymal organs, such as the liver. The on-chip micro-pump and **microfluidic channels interconnect these organs** and provide lifelike behavior. This enables the direct prediction of effects of chemicals and their metabolism on near real-life models.

RESULTS

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

upcyte® hepatocytes monolayer → +7 days 0.6mM palmitate → +7 days w/o palmitate

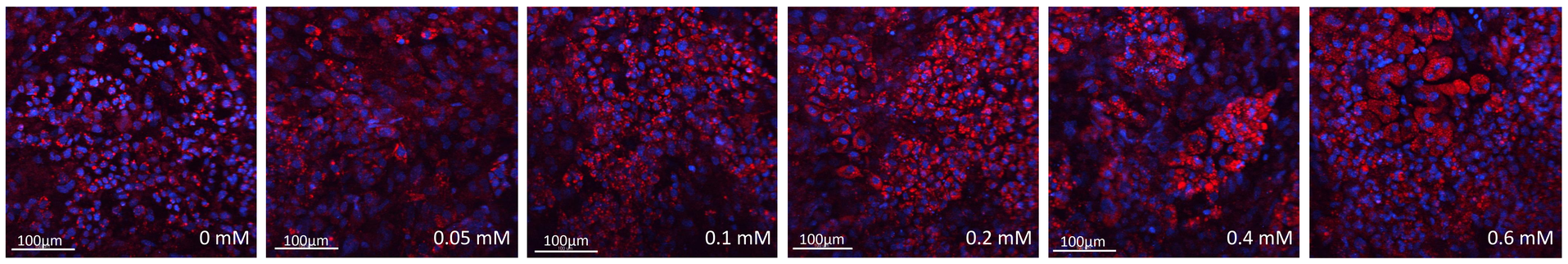


← upcyte® hepatocytes in a monolayer

upcyte® hepatocytes were cultured in a monolayer for 7 days with and treated with palmitate (0.6 mM). After 7 days fat vesicles were clearly visible throughout the culture After additional 7 days w/o palmitate, cells depleted their vesicles again.

↓& treated with different plamitate concentrations:

Cells were cultured in high (2 g/l) or low (1 g/l) glucose medium and treated with different concentrations of Palmitate (0.6 mM; 0.4 mM; 0.2 mM; 0.1 mM; 0.05 mM; 0 mM) for 7 days in a 96-well plate.



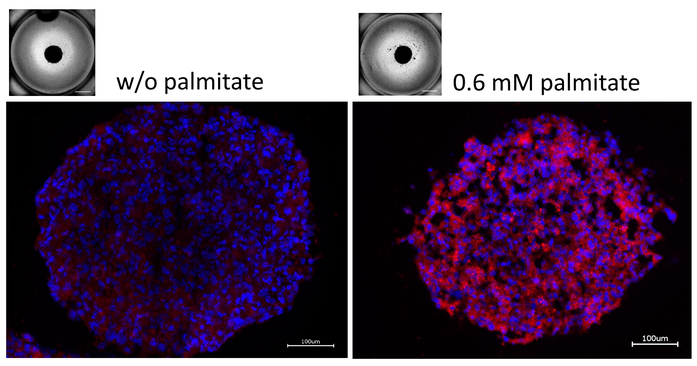
Nile Red staining:

Fat vesicles were more prominent when cells are treated with Palmitate and cultured in low glucose medium (data of high glucose medium not shown).

….and in aggregates…

Forming aggregates of palmitate pretreated cells

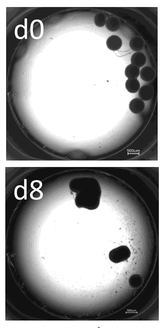
upcyte® hepatocytes und stellate cells can form aggregates , even if they are pre-treated with palmitate (7 days). 25,000 cells/well (physiological mixture of 24,000 hepatocytes to 1,000 stellate cells) were seeded into a shaking in 384-well round bottom spheroid plate for 3 days. One spheroid formed per well with a smooth surface.

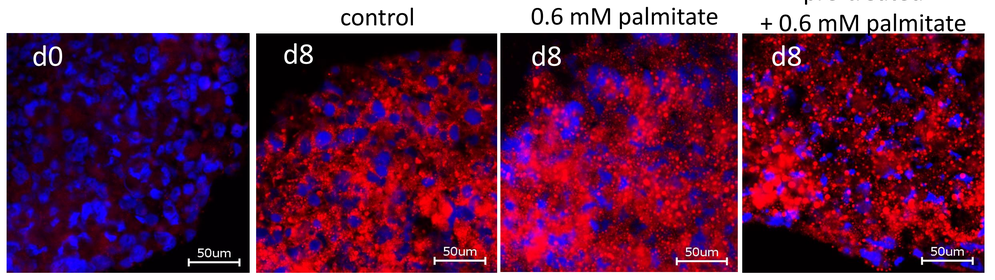


…and aggregates in the Two-Organ-Chip

Two-Organ-Chip culture of steatotic liver aggregates

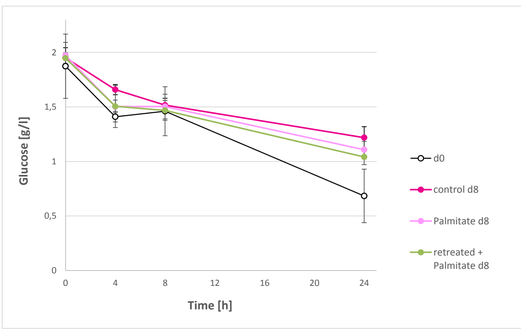
Liver aggregates formed of untreated or palmitate pre-treated upcyte® hepatocytes were cultured for 8 days in one compartment of a 2-organ-chip (25.000 cells/aggregate). High glucose Medium containing 0.6 mM palmitate was added daily to both untreated and pre-treated aggregates. Nile Red staining shows accumulation of neutral lipids after culture in all conditions including control. However lipid droplets are bigger and more pronounced in palmitate treated conditions.





↓ Insulin sensitivity

Insulin sensitivity was intended to be measured by an *in vitro* glucose tolerance test. Glucose disposal was reduced in all conditions after 8 days culture. This might indicate a reduced insulin action due to an inhibition of the insulin signaling pathway by accumulated fatty acids.



CONCLUSION

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).