Liver organoid formation using differentiated human upcyte® cells

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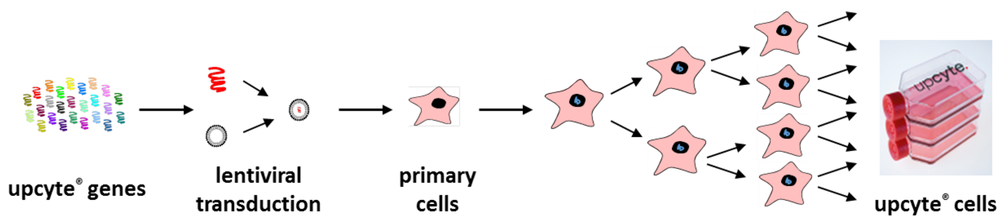
INTRODUCTION

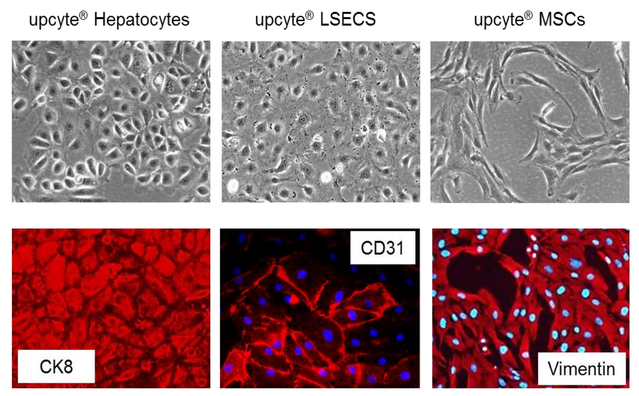
Cultures of different primary human liver cells, especially hepatocytes (pHH), are routinely used in drug development to evaluate metabolic fate, drug-drug interactions and drug toxicity. However, the supply of primary cells is limited by the low and sporadic availability of human liver tissue. To address this, we have developed a technique which causes pHH and liver sinusoidal endothelial cells (LSECs) to proliferate up to 40 population doublings whilst still retaining a metabolically competent phenotype when cultured at confluence ("upcyte cells"). 3D structures resembling embryonic liver organoids have recently been described using stem cell-derived cell types (Takebe *et al.*, 2013). For the *in vitro* generation of liver organoids (LO), we used differentiated adult human upcyte® liver cells. By co-culturing a defined mixture of cells (hepatocytes, LSECs, mesenchymal stem cells (MSCs) and later on stellate cells (HSCs)) on a layer of Matrigel, the cells self-organized to form liver organoid-like structures within 24 hours. When cultured for another 10 days in a bioreactor, these liver organoids show typical functional characteristics of liver cells including basal as well as induced CYP3A4 enzyme activity.
In conclusion, we describe the generation of 3D functional liver structures composed of primary human upcyte® cells. These liver organoids can be cultured for a prolonged period of time, and potentially represent an *ex vivo* model to study liver functions.

Generation of upcyte® hepatocytes, LSECs & MSCs

upcyte® - expanded primary cells

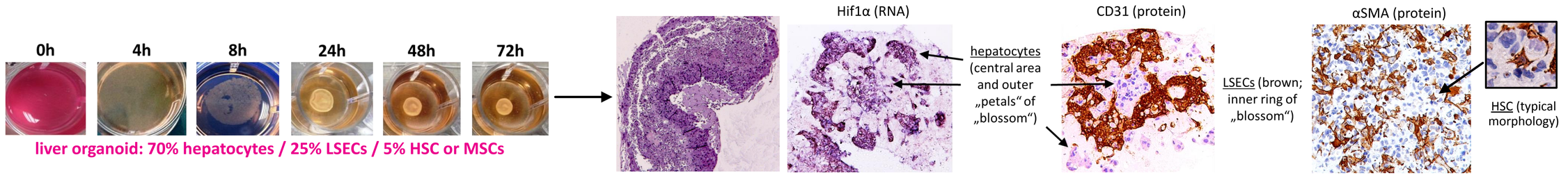
The upcyte® technique enables the generation of non-transformed proliferating cells from e.g. primary human hepatocytes while maintaining adult phenotype. upcyte® cells start to grow from primary cells after transduction with a defined cocktail of lentiviral vectors carrying proliferation inducing genes. upcyte® cells have the ability to proliferate for additional cell doublings, depending on the cell type, without losing functional and phenotypic characteristics of mature cells.





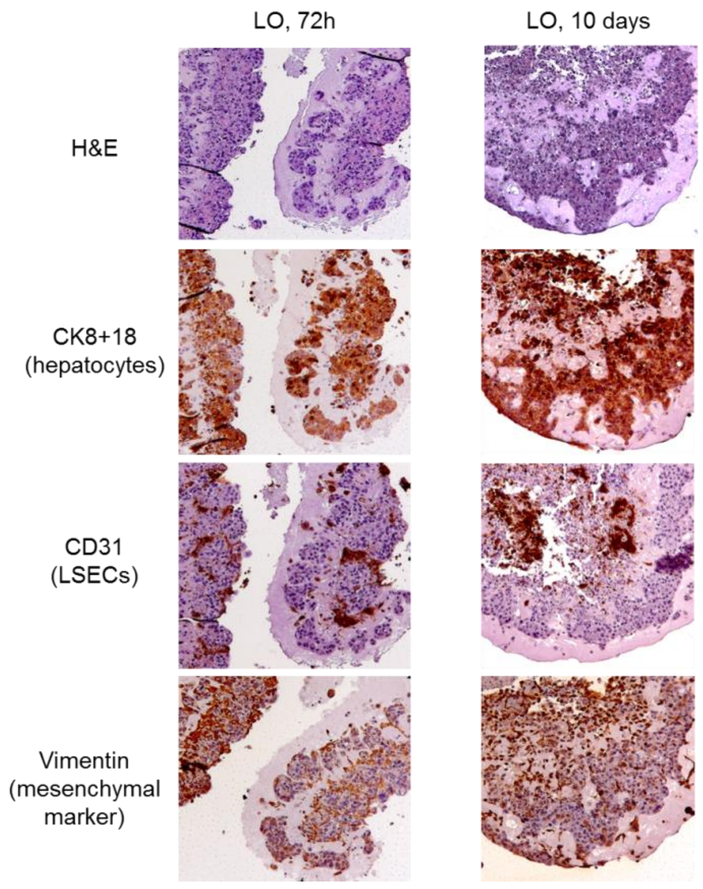
RESULTS

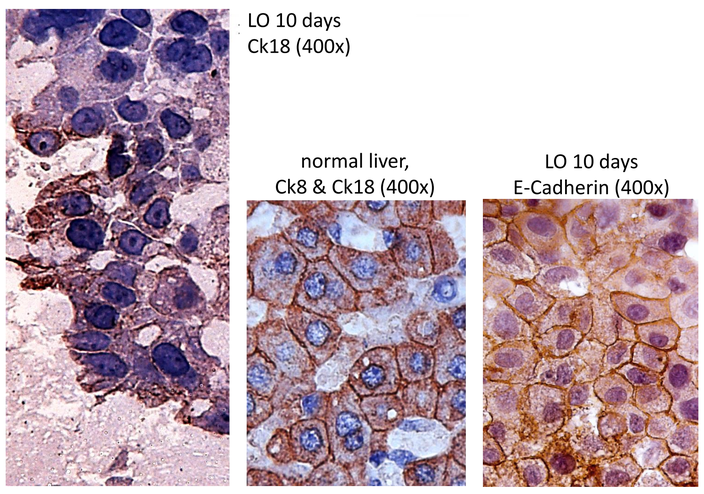
Generation of liver organoids using human upcyte® cells



Time-dependent self-assembly of liver organoids in matrigel-coated 24-well plates: Instead of just forming a randomly assembled "cell clump", an LO after 72h shows formation of structures resembling a blossom with hepatocytes located in the center and forming the outer petals (shown here by *in situ* hybridization against the hepatocyte marker, Hif1α). Liver sinusoidal endothelial cells (LSECs) tend to form an inner ring between the hepatocytic areas (demonstrated here by immunostaining against CD31, a general endothelial cell marker). HSCs adapt to their typcial, „star-formed" morphology (demonstrated here by immunostaining against α smooth muscle actin (α-SMC)).

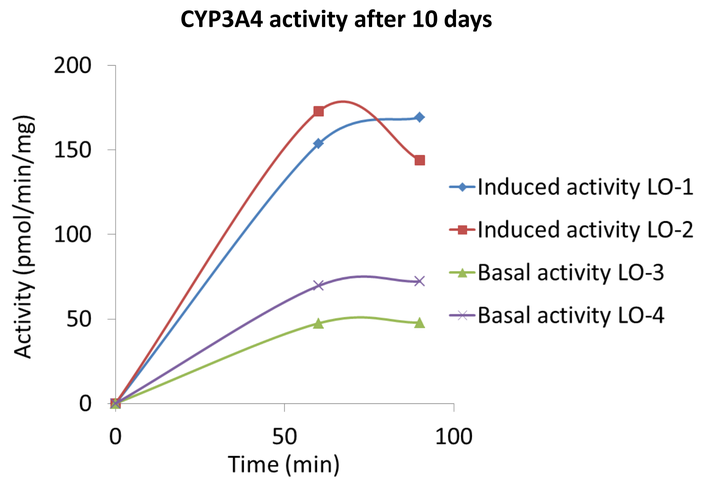
Long-term culture (>48h) in a dynamic system (Kirkstall® quasi vivo system)





↑ Ck18 and E-Cadherin immunostaining of a 10-day liver organoid showing a bar-like alignment of hepatocytes, and cellular arrangement surrounding circular openings similar to the *in vivo* liver architecture.

← Spatial distribution of the different cell types within the liver organoids. H&E- and immunostainings for cell-type markers were performed in serial sections of liver organoids (LO) after 72h and 10 days of culture. original magnification: 200x



CYP3A4 activity in 10-day liver organoids: After 7 days of culture rifampicin (20 µM) was added to LO-1 and LO-2 to induce CYP3A4 activity and substrate incubation was performed for 60 and 90 min for all LOs on day 10 of culture. CYP3A4 activity was measured after each incubation interval and was normalized to total protein content of the organoids. Even after 10 days in culture, hepatocytes have functional metabolism.

Data from 2015 Ramachandran *et al.* – PLOS one - *In vitro* generation of functional liver organoid-like structures using adult human cells

CONCLUSION

The results obtained with the process of self-assembly structures demonstrate that upcyte® hepatocytes combined with upcyte® LSECs and HSCs/MSCs can form organoids *in vitro* which show typical features of healthy human liver, e.g. polarized phenotype of the hepatocytes and CYP3A4 activity. We are currently working on integrating more liver cell types such as Kupffer cells.
The potential applications of such human structures include toxicity tests of new drugs even under long-time exposure conditions and applications as models of liver disease in basic science. For example we could incubate the organoids under hypoxic or steatotic conditions and study the cellular responses in a time- and dose-dependent manner. By using human cells the obtained results will be even more suitable than those which could be generated using animal models.