in vitro organoid model of the human liver sinusoid

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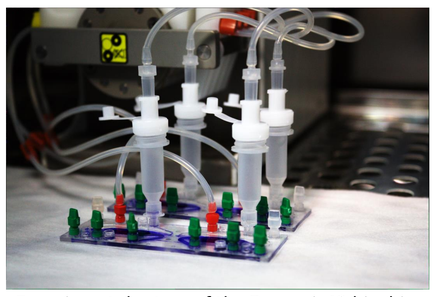
INTRODUCTION

Isolated liver cells, such as **hepatocytes**, **liver sinusoidal endothelial cells** (LSECs), **Kupffer Cells** (macrophages of the liver) and hepatic stellate cells are frequently used to study **hepatic metabolism**, **toxicity** and **diseases**. LSECs play a pivotal role in the onset and progression of chronic hepatitis and liver cancer. It is well known that the major functions of LSECs are to control the transport of molecules in blood to the hepatocytes, and to remove foreign or unwanted materials by means of scavenger receptors. In **chronic hepatitis** and **cirrhosis**, LSECs undergo transformation to a vascular type and changes into LSECs that interfere with the bi-directional exchange of molecules and therefore have deleterious effects on liver physiology. However, so far chronic conditions affecting the LSECs have not been able to be investigated *in vitro* due to the lack of primary cells in sufficient quantity.

upcyte technologies **expands isolated human LSECs** which have preserved the typical characteristics as well as the functionality of the primary cells. These cells are suitable for long-term culture enabling the study of chronic liver conditions *in vitro* and are available in large quantities.

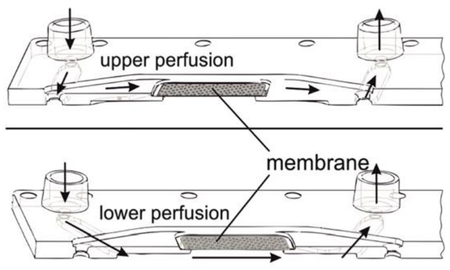
The Dynamic42 biochip is a **two-sided chip system** in which **individual perfusion of tissue constructs** is implemented in order to address cell-type specific micro-environmental needs. The here presented liver chip is composed of vascular and hepatocyte cell layers integrating non-parenchymal cells closely reflecting liver tissue architecture.



Experimental set up of the Dynamic42 biochip for upcyte® LSECs cultivation

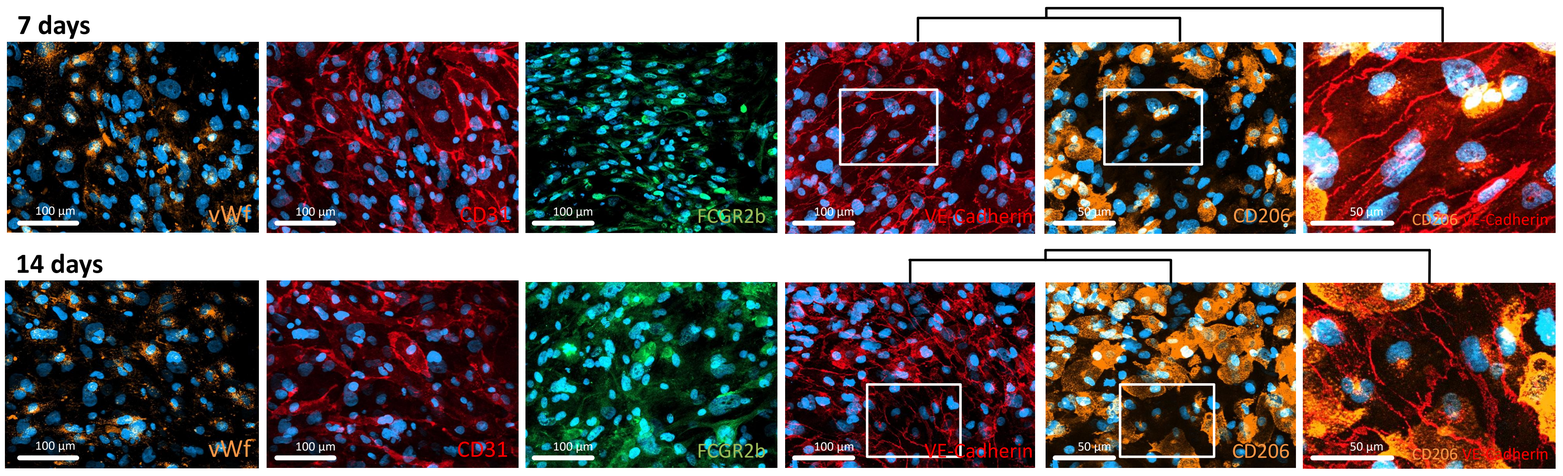
RESULTS

upcyte® LSECs in micro-physiological perfused biochip in co-culture with tissue-resident macrophages



The design of Dynamic42 biochip. (Raasch et al, 2015)

The biochip used in this study was generated by injection molding with cyclic olefin polymers with welded membranes of **polyethylene terephthalate**. As for the design, the biochip contains two cavities both of which have an integrated membrane serving as cell culture area. This enables cells to be cultured under independent perfusion from either apical or basal side through upward directed (upper perfusion) and downward directed (lower perfusion) channels. **upcyte® LSECs** were seeded together with human **monocyte-derived macrophages** onto collagen IV coated membranes of the upper channel. On the other hand, **upcyte® hepatocytes** were seeded on the other side of the membrane into the lower channel and cultured in a hanging fashion.

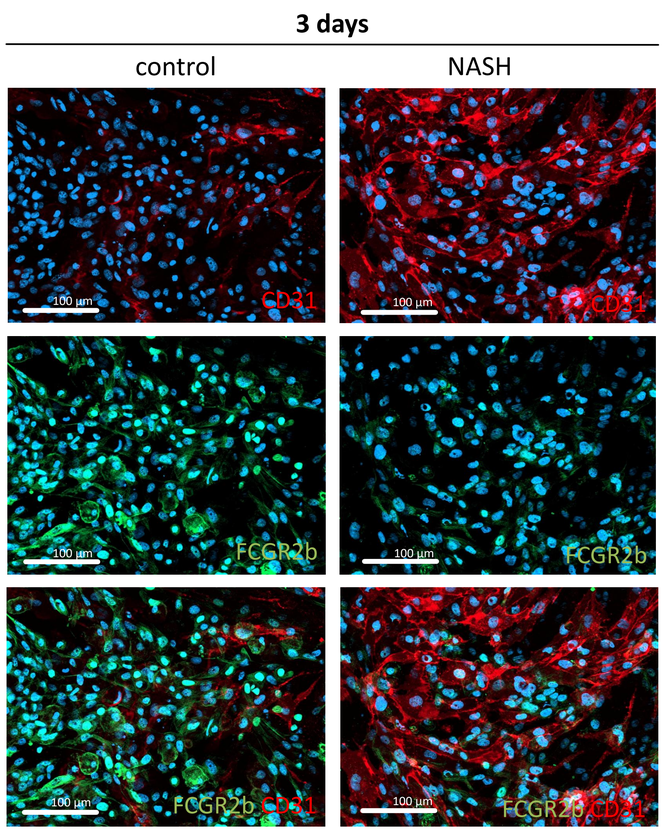


upcyte LSECs in co-culture with tissue-resident macrophages in the Dynamic42 biochip.

upcyte® LSECs have been tested in micro-physiological perfused biochip culture with a shear rate of 0.7 dyn/cm² for 7 and 14 days in co-culture with tissue-resident macrophages. Typical LSEC marker proteins such as **von Willebrand factor** (vWf), **platelet endothelial cell adhesion molecule** (CD31), **low-affinity gamma immunoglobulin Fc region receptor IIb** (FCGR2B - CD32b), **vascular endothelial cadherin** (VE-Cadherin) and **mannose receptor** (CD206) could be confirmed by immunofluorescence staining.

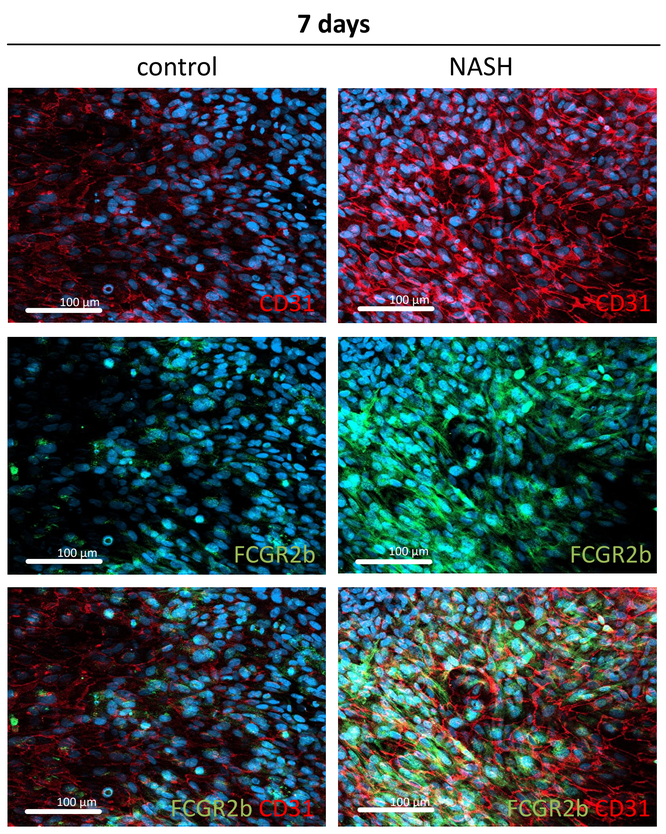
FCGR2B contributes to **receptor-mediated endocytosis and small immune complex clearance** while mannose receptors are largely responsible for the **removal of circulating lysosomal enzymes via endocytosis**. These data show that upcyte® LSECs could be stably cultured and were found to keep all the characteristic markers over the course of 14 days.

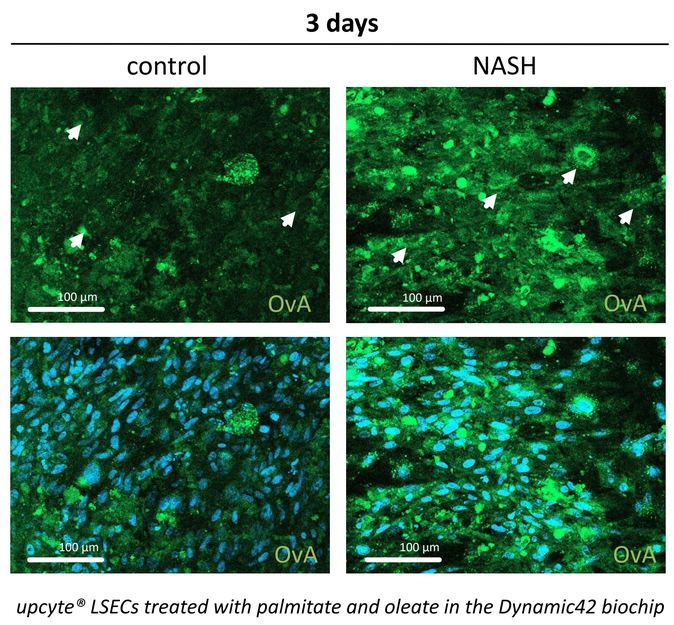
NASH treated upcyte® LSECs in micro-physiological perfused biochip



upcyte® LSECs treated with palmitate and oleate in the Dynamic42 biochip

upcyte® LSECs in micro-physiological perfused biochip were treated with 0.3 mM palmitate and oleate (1:2) for 3 and 7 days in order to mimic **nonalcoholic steatohepatitis (NASH)**. It was shown that CD31 expression of upcyte® LSECs becomes more prominent when treated with palmitate and oleate after 7 days compared to the control group. This suggests that **capillarization of LSECs** occurred within the liver organoid which plays a key role in the onset and progression of NASH. The same phenomenon is seen in patients with cirrhosis and chronic hepatitis. Furthermore, the expression of FCGR2b is significantly increased after 7 days of palmitate and oleate treatment, indicating the progression to **hepatic inflammation** seen in NASH patients.





upcyte® LSECs treated with palmitate and oleate in the Dynamic42 biochip

upcyte® LSECs co-cultured with tissue-resident macrophages were perfusion precultured with a shear rate of 0.7 dyn/cm² for 3 days, with or without NASH treatment (0.3 mM palmitate and oleate, 1:2). After 1 hour of perfused **Ovalbumin** (OvA) treatment, uptake in both control and NASH group was analyzed by immunofluorescence staining and positive cellular uptake was identified (shown by white arrows). As a result, an **elevated uptake** was observed compared to remaining cell layers.

SUMMARY & CONCLUSION

In conclusion, it is suggested that the novel Dynamic 42 biochip accompanied by the physiologically relevant upcyte® LSECs & upcyte® hepatocytes provide a reliable *in vitro* tool to study chronic liver conditions.

ACKNOWLEDGMENTS

All the data presented here were generated by Dynamic42. We thank their team for this detailed work and also for sharing it with us.

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