Generation of proliferating human liver sinusoidal endothelial cells (upcyte® LSECs) and upcyte® hepatocytes

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INTRODUCTION

Isolated liver cells, such as hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer Cells and hepatic stellate cells, are frequently used to study hepatic metabolism, toxicity and diseases. The current in vitro culture models, however, have several disadvantages, e.g. short culture longevity and artificial culture conditions that focus mainly on a single cell type in 2D culture. The use of primary cells in vitro is compromised by the limited quantity of cells that can be isolated from one donor, a lack of or very restricted proliferation capacity (hepatocytes and LSECs) and/or the change from a quiescent to an activated state (hepatic stellate cells).

Therefore, we investigated whether the transduction of proliferation-inducing genes could extend the lifespan of primary LSECs and hepatocytes without losing their primary characteristics (so-called “upcyte® technology”) as previously demonstrated with hepatocytes (upcyte® hepatocytes).

RESULTS

Generation of upcyte® LSECs and hepatocytes

Characterization: upcyte® LSECs

Why LSECs?
LSECs constitute the sinusoidal wall and can be regarded as unique capillaries, which differ from other capillaries in the body, as they possess open pores or fenestrate lacking a diaphragm and a basal lamina underneath the endothelium. Fenestrations, arranged in so-called selective “view plates”, filter fluids, solutes and particles that are exchanged between the sinusoidal lumen and the space of Disse. Among the various substances that are known to be endocyctosed by LSECs are proteins, glycoproteins, lipoproteins and glycosaminoglycans. Foreign and viable macromolecules and colloids are eliminated from the circulation mainly by receptor-mediated phagocytosis. There are only three different receptors which have been functionally observed in LSECs and are responsible for uptake of a large number of different ligands: The (E) Macrophage receptor (MR), the (2) Haptoglobin/Scavenger receptor (HA/S-R) and the (E)Fc-receptor (FcYRII, CD32)

Characterization: upcyte® hepatocytes

upcyte® hepatocytes display an adult phenotype
upcyte® hepatocytes expressed the characteristic adult marker proteins: cytochrome P450 (CYP), albumin (HSA), anti-fetoprotein (AFP), as well as endocytic markers such as a-dextrin protein (ARP). The cells further expressed E-cadherin and demonstrated marked capability for glycogen storage (PAS staining) and bile secretion (CKDIA staining).

upcyte® hepatocytes maintain metabolic activity
upcyte® hepatocytes expressed metabolizing enzymes of phase I (e.g. CYP 1A2, 2B6, 2C9 and 3A4). Cells further exhibited phase II activities (UGT, SULT & GST) close to liver as well as functional transporters (e.g. OATP1B3). upcyte® hepatocytes produced urea and secreted albumin (data not shown). Differences in performance could be detected between cells from different donors.

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Send us your primary cells - we upcyte them! We will apply our upcyte® technology and produce large batches of cells from your donor of choice. The cells will be shipped back to you as cryopreserved vials or can be stored at our facility. Do not hesitate to contact us if you would like more information on how we can upcyte your favorite batch of primary cells.

upcyte® Kupffer cells
We were able to receive a grant to generate upcyte® Kupffer cells. Currently we are in a collaboration with the University of Mannheim to isolate pure and good quality Kupffer cells, which is a big challenge. You are experienced with Kupffer cells? Please let us now and get in contact, we would love to collaborate!

SUMMARY & CONCLUSION

In conclusion, we developed a technique which causes primary human LSECs and hepatocytes to proliferate up to 40 population doublings whilst still retaining an adult phenotype when cultured at confluence. upcyte® LSECs retained important endothelial cell markers, such as CD31 and VWF, and exhibited functional uptake of LDL, as well as the ability to form tubes in Matrigel™. LSEC-specific uptake of ligands or the expression of the corresponding uptake receptors (MR, FcYRII and HA/S-R) could be detected. upcyte® hepatocytes retained typical hepatic markers such as HSA and formation of bile canaliculi. At confluence, phase I and II enzymes were detected and showed donor-specific differences. These data support that upcyte® LSECs & hepatocytes are very uniquely and applicable to cell based assays, such as co-culture and toxicity studies. Moreover, this technology allows for the generation of large batches of upcyte® cells (up to 12 x 10^9 cells per donor), enabling a reproducible and standardized experimental setting.