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

Nils Runge, Timo Johannssen, Astrid Noerenberg
*upcyte technologies GmbH, Osterfeldstr. 12-14, D-22529 Hamburg, Germany

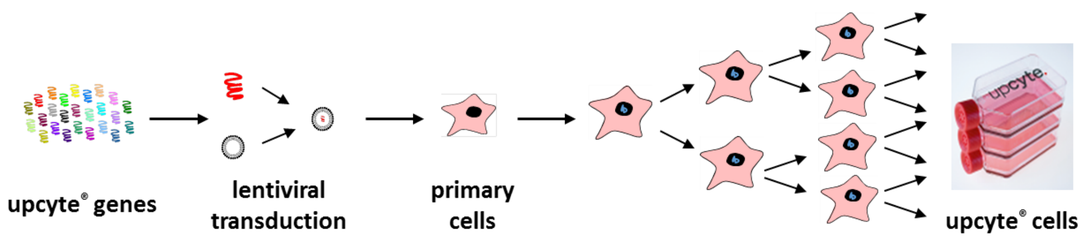
INTRODUCTION

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



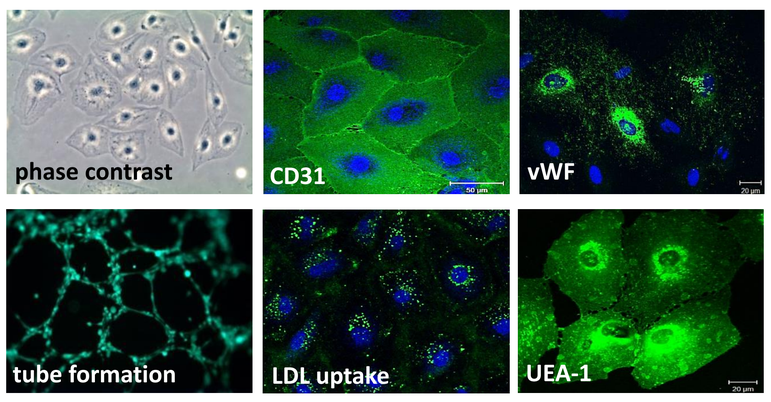
Transduction of primary cells with a defined cocktail of lentiviral vectors

Previously, a novel technique was reported which enabled the generation of non-transformed proliferating liver cells from primary human hepatocytes with maintained adult phenotype, so-called “upcyte® cells”. 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.

Characterization: upcyte® LSECs

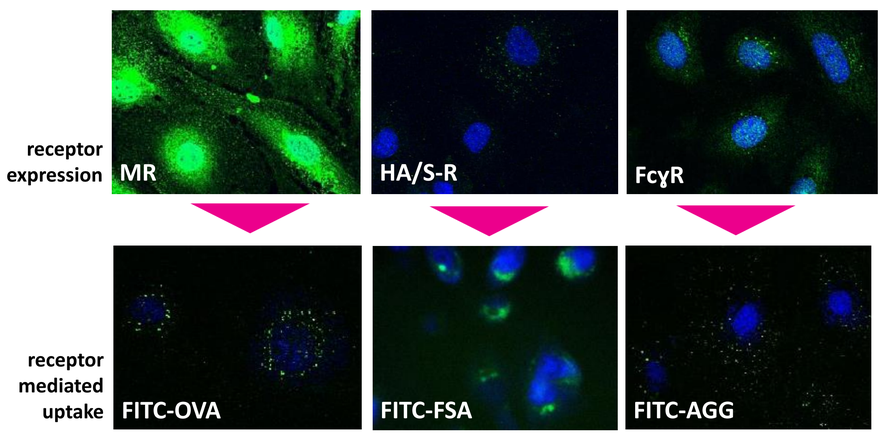
Why LSECs?

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 fenestrae lacking a diaphragm and a basal lamina underneath the endothelium. **Fenestrae,** arranged in so-called selective "sieve 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 endocytosed by LSECs are proteins, glycoproteins, lipoproteins and glycosaminoglycans. Foreign soluble macromolecules and colloids are eliminated from the circulation mainly by **receptor-mediated pinocytosis**. 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 **(1) Mannose receptor (MR)**, the **(2) hyaluronan/scavenger receptor (HA/S-R)** and the **(3) Fc-γ-receptor (FcγR2BII, CD32b)** [Smedsrod *et al.*, 2004].



upcyte® LSECs express typical endothelial cell markers.

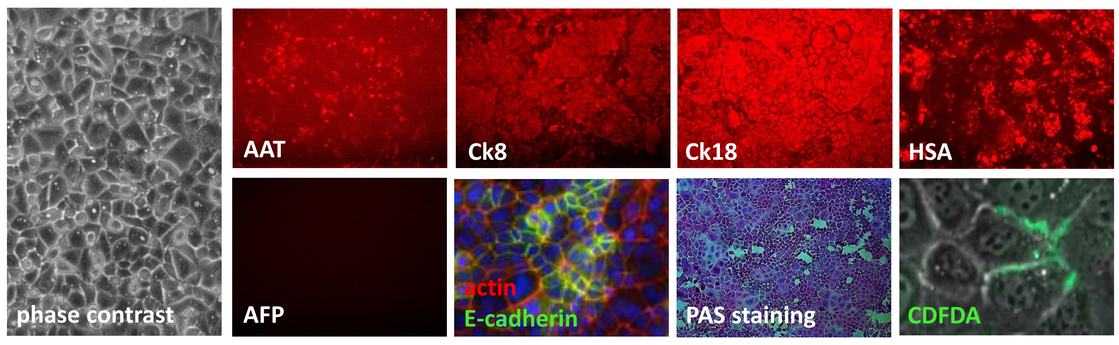
Morphology was comparable to primary cell LSECs. **CD31** was present in all cells investigated and showed a typical membrane localization. **vWF (von-Willebrand-factor)** was evident as perinuclear punctate structures, indicating its presence in the ER. upcyte® LSECs showed pronounced **tube formation** in Matrigel™ from single cells stained with Calcein-AM. **Uptake of Ac-LDL** (AF 488-AcLDL), indicating presence of SR-A (scavenger receptor A), a common endothelial cell receptor, was evident. In addition, we found strong expression of **UEA-1 (Ulex Europaeus Lectin 1)**.



LSEC-specific receptor expression and receptor-mediated endocytosis

We observed **high expression of MR and FcγR in upcyte® LSECs**. The staining of **HA/S-receptor was visible**, but weak and not in all cells. The functional test of receptor-mediated endocytosis was performed by adding fluorescent-labeled ligands to the medium. The following ligands were used: FITC-FSA (HA/S-R), FITC-AGG (FcγR2BII) and FITC-mannan, DTAF-collagen-α-chains and FITC-ovalbumin (all three for MR, just one shown). **Uptake of ligands could be shown for the MR and the FcγR with the ligands FITC Ovalbumin and FITC-AGG** (aggregated gamma globulin). **Uptake of FITC-FSA** (formaldehyde-treated serum albumin**) for the HA/S-R could not be detected in all donors** (2/3).

Characterization: upcyte® hepatocytes



upcyte® hepatocytes display an adult phenotype

upcyte® hepatocytes expressed the characteristic adult marker proteins **cytokeratin 8 (CK8)**, **cytokeratin 18 (CK18)**, **human serum albumin (HSA)**, **α-anti-trypsin (AAT)**, but no embryonic markers such as α-fetoprotein (AFP). The cells further expressed E-cadherin and demonstrated marked capability for **glycogen storage (PAS staining)** and **bile secretion (CDFDA staining)**.

Specific activity (pmol/min/mg protein)				
Cells	**CYP1A2**	**CYP2B6**	**CYP2C9**	**CYP3A4**
#10-03	3.3 ± 0.4	40.3 ± 6.5	91.8 ± 5.5	21.4 ± 9.6
#151-03	0.7 ± 1.4	71.1 ± 11.3	29.1 ± 21.4	77.8 ± 22.6
#422A-03	2.3 ± 0.1	33.6 ± 11.4	4.8 ± 3.1	42.9 ± 6.3
#653-03*	17.1 ± 0.5	68.4 ± 18.4	16.2 ± 0.9	178.3 ± 17.0
Donor 653-03-2D6 available: CYP2D6 recombinantly expressed, specific activity for 2D6* ***1627.5 ± 22.4*				

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 pHH as well as **functional transporters (e.g. OATB1B3)**. upcyte® hepatocytes produced urea and secreted albumin (data not shown). Differences in performance could be detected between cells from different donors.

Do you have a favorite donor or primary cell?

… try our upcyte® service!

Do you want to have **virtually unlimited amount** of your own selected donor? Do you need upcyte® cells from **other cell types** than what we currently offer? Do you want upcyte® cells from your **diseased donor**? Do you need cells from **other species**, e.g. monkey?

Send us your primary cells - we upcyte! 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, FcγR2BII 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 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.