Driving primary liver cells into proliferation

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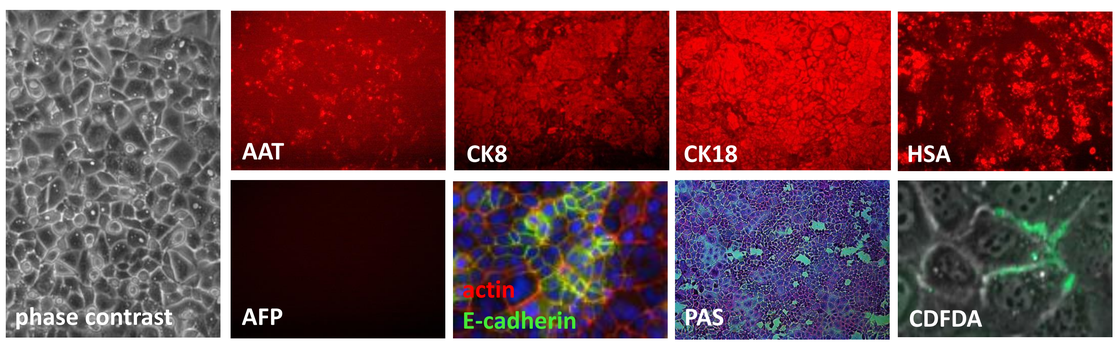
INTRODUCTION

Isolated liver cells, such as **hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer Cells** and **hepatic stellate cells (HSC)**, are frequently used to study hepatic metabolism, toxicity and diseases. However, current *in vitro* culture models exhibit several disadvantages such as short culture longevity and artificial conditions focusing on a single cell type in 2D culture. The use of primary cells *in vitro* is further compromised by the limited quantity of cells that can be isolated from one donor, a restricted proliferation capacity (hepatocytes and LSECs) and/or the change from a quiescent to an activated state (HSC). Recently developed **human upcyte® hepatocytes and LSECs** offer the advantage of combining many **features of primary cells** with the **unlimited availability** of hepatoma cells. Here we describe the latest characterization data which was performed using upcyte® hepatocytes and LSECs.

RESULTS

upcyte® hepatocytes



upcyte® hepatocytes display an adult phenotype

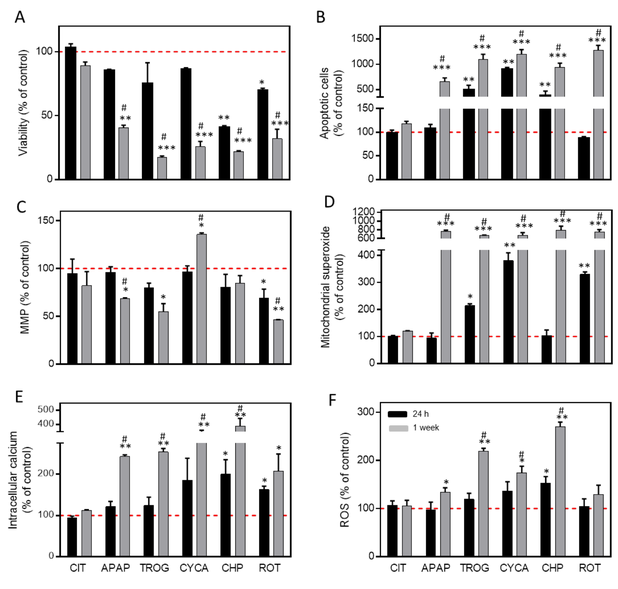
upcyte® hepatocytes expressed the characteristic adult marker proteins **cytokeratin 8 (CK8)**, **cytokeratin 18 (CK18)**, **human serum albumin (HSA)**, **α-anti-trypsin (AAT)**, but lack embryonic markers such as α-fetoprotein (AFP). The cells further expressed E-cadherin and demonstrated marked capability for **glycogen storage (PAS staining)** and **bile secretion (CDFDA staining)**.

Phase I activity [pmol/min/mg]	Donor 10-03	Donor 151-03	Donor 422A-03	Donor 653-03
CYP1A2	3.3 ± 0.4	0.7 ± 1.4	2.3 ± 0.1	17.1 ± 0.5
CYP2B6	40.3 ± 6.5	71.1 ± 11.3	33.6 ± 11.4	68.4 ± 18.4
CYP2C9	91.8 ± 5.5	29.1 ± 21.4	4.8 ± 3.1	16.2 ± 0.9
CYP3A4	21.4 ± 9.6	77.8 ± 22.6	42.9 ± 6.3	178.3 ± 17.0

Phase II activity [pmol/min/mg]	upcyte® hepatocytes	Primary hepatocytes
SULT (Hydroxycoumarin)	6-16	5-98
UGT (Hydroxycoumarin)	32-345	15-496
GST (CDNB)	15-88	21-35

upcyte® hepatocytes maintain metabolic activity

upcyte® hepatocytes (Donors #10-03, #151-03, #422a0-3 and #653-03) expressed metabolizing enzymes of **phase I (e.g. CYP 1A2, 2B6, 2C9 and 3A4)**. Cells (ufurther exhibited **phase II activities (UGT, SULT & GST)** close to primary hepatocytes as well as **functional transporters** (e.g. OATB1B3, data not shown).



Employed compounds: ***CIT**: sodium citrate (1-2 mM), **APAP**: acetaminophen (0.5-2 mM), **TROG**: troglitazone (50-100 µM), **CYC(A)**: cyclosporin A 20-50 µM, **CHP**: cumene hydroperoxide (100-500 µM), **ROT**: rotenone (0.05-1 µM), **KET**: ketotifen (1-10 µM)*

Acute and repeated-dose toxicity as determined by high content screening

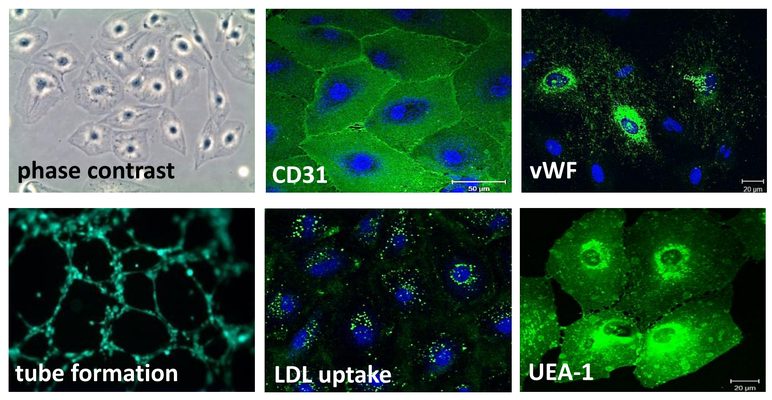
upcyte hepatocytes (donor 422a-03) were challenged with hepatotoxic compounds for 24 h or 1 week. Fluorescent probes were employed to evaluate **(A) viability**, **(B) apoptosis**, **(C) mitochondrial membrane potential (MMP)**, as well as levels of **(D) mitochondrial superoxide**, **(E) intracellular Ca^{2+} and (F) reactive oxygen species (ROS)**. Regarding **acetaminophen (APAP)**, no effect was observed after 24 h treatment, whereas **after 1 week, apoptosis and levels of intracellular Ca^{2+}, ROS and mitochondrial superoxide were significantly increased**. In general, repeated dosing over one week markedly increased the sensitivity towards hepatotoxic model compounds when compared to acute treatment. Data are expressed as mean ± SEM as percentages normalized on untreated control cells. Statistical analysis was performed using Student *t*-test (*p<0.05, **p<0.01, ***p< 0.001 vs. untreated; #p<0.05 vs. 24 h).

Tolosa *et al. 2016; Toxicological Sciences* 125 (1): 214-29

upcyte® LSECs

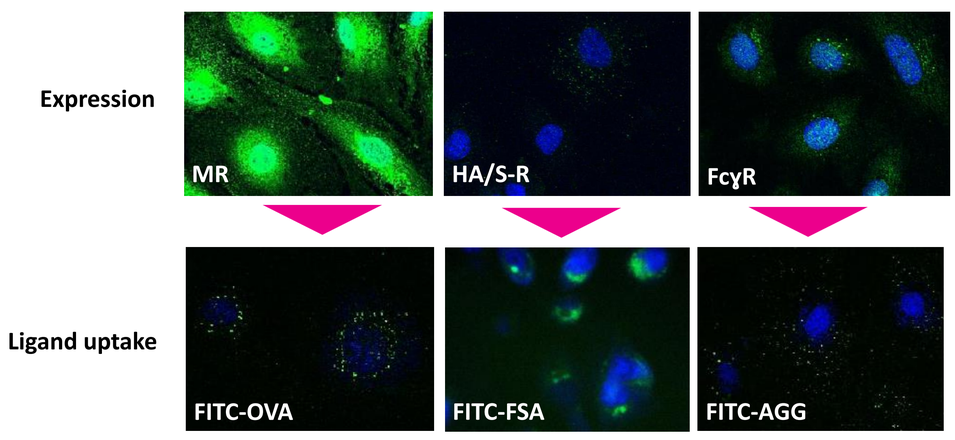
Why LSECs?

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-ɣ-receptor (FcɣR2BII, CD32b)**.



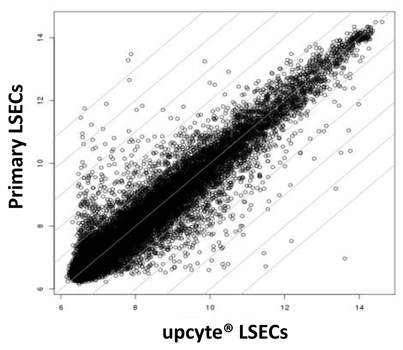
upcyte® LSECs express typical endothelial cell markers

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 FcɣR 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 (FcɣR2BII) 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 FcɣR 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).



Gene expression profile of upcyte® LSCEs

Expression profiles of upcyte® and primary LSECs were compared using Illumina whole genome BeadChip® Sentrix arrays HumanHT-12 v4. Preliminary analysis of upcyte® LSECs and primary LSECs that have been in culture for 3 days revealed very few changes in the expression profile. Only **0.45% (218 genes) of a total of 48,107 genes analyzed were found to be up- or downregulated more than 2-fold.**

What's next: upcyte® Kupffer cells

We are currently working on the development of upcyte® Kupffer cells to expand our portfolio of upcyte® liver cells. **You are working with Kupffer cells** and **looking for unlimited cell access?** Let us now and get in contact, **we would love to collaborate!**

SUMMARY & CONCLUSION

Taken together, our data suggest that **upcyte® hepatocytes** and **LSECs** are widely applicable to cell based assays, e.g. **metabolism**, **cytotoxicity** and **uptake studies**. Combining many **features of primary cells** with the **ease of handling of cell lines**, upcyte hepatocytes and LSECs offer suitable properties to be used for toxicological and metabolic assessments during drug development and biomedical research.