Expanded primary human liver sinusoidal endothelial cells (upcyte® LSECs) as a tool for complex hepatotoxicity studies

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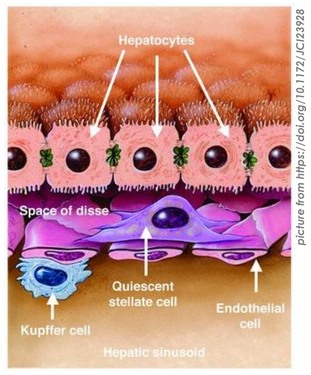
ABSTRACT

Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells critically involved in liver regeneration, the transfer of substrates between blood and liver parenchyma, rapid internalization of blood-borne macromolecules as well as immune tolerance. Despite their substantial contribution to liver homeostasis, LSECs are often overlooked during hepatotoxicity assays due to insufficient cell yields after isolation and a restricted proliferation capacity *in vitro*. To address these issues, we expanded primary LSECs derived from 3 donors by lentiviral transduction with proliferation inducing genes (upcyte® technology). Transduced LSECs performed several additional population doublings, expressed typical endothelial cell and LSEC-associated markers and revealed marked uptake of characteristic macromolecule ligands. Expanded LSECs were then used in toxicity assays including a perfused 3D liver chip system and an impedance-based cell monitoring device.

Taken together, our data suggest that upcyte® LSECs combine many characteristics of primary LSECs with the advantage of an extended lifespan, facilitating their use in hepatotoxicity assays under reproducible and standardized conditions.

INTRODUCTION

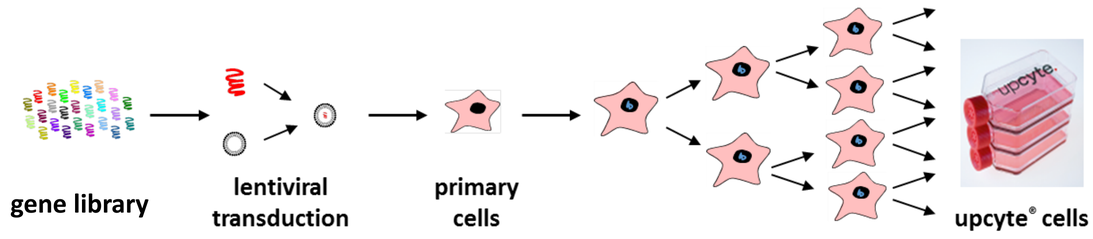
Liver sinusoidal endothelial cells (LSECs) represent unique endothelial cells located within hepatic sinusoids. In contrast to other endothelial cells, LSECs are characterized by diaphragm-lacking fenestrae, arranged in so-called sieve plates and exhibit **high scavenging potential**. In addition to their critical role in clearance of macromolecules, they act as **gatekeepers of liver homeostasis** and contribute to **immune tolerance** [Poisson *et al.,* 2017].



picture from https://doi.org/10.1172/JCI23928

Current *in vitro* models employed to predict **drug induced liver damage (DILI)** mostly focus on hepatocytes, which form a tightly controlled **sinusoidal unit together with LSECs next to Kupffer cells and hepatic stellate cells**. It is thus questionable whether hepatotoxicity can be sufficiently predicted by analyzing hepatocytes only. On the other hand the use of primary LSECs is compromised by **poor cell yields**, contamination with other endothelial cells, and a **limited proliferation capacity** after isolation. ***To circumvent these restrictions, the goal of the present study was to induce robust proliferation in primary LSECs without full immortalization as well as keeping primary cell characteristics.***

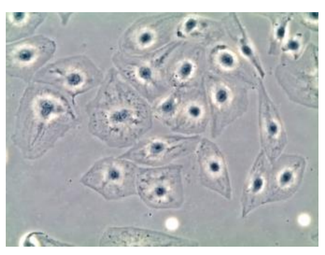
METHODS



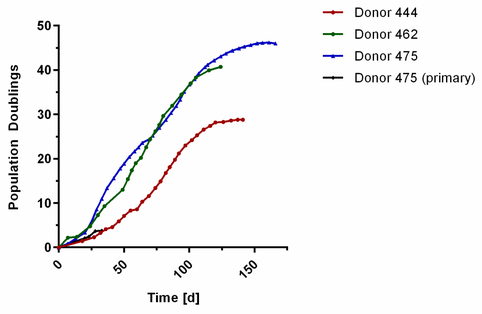
We previously reported the development of **non-transformed proliferating liver cells** from primary human hepatocytes maintaining an adult phenotype, so-called "upcyte® cells". upcyte® cells are expanded from primary cells after transduction with a **defined cocktail of lentiviral vectors** carrying proliferation inducing genes. We thus transduced primary **LSECs from three different donors**. Cells were auto-selected by senescence of primary cells after a few population doublings. Proliferating cells were expanded and frozen as Master and Working Cell Banks. Cells were thawed from Working Cell Banks (PD 25-30) and pre-cultured for 4-5 days before characterization.

RESULTS

Growth characteristics



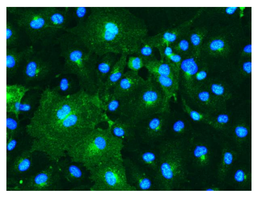
Donor 462



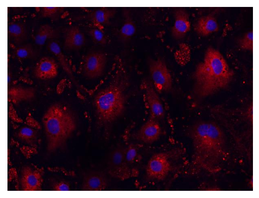
In contrast to untransduced primary cells, **all three donors** showed **extended proliferation for at least 25 population doublings**. The morphology of resulting cells was comparable to primary LSECs. Interestingly, we found that all donors were characterized by a d**elayed growth arrest when reaching higher PD numbers**. Cells consequently demonstrated signs of senescence, indicating that the cells are not fully immortalized.

Surface marker expression

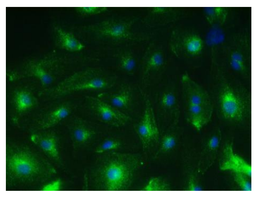
LYVE-1 DAPI



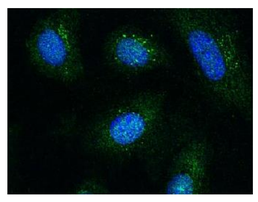
Stab-2 DAPI



MR DAPI



CD32b DAPI

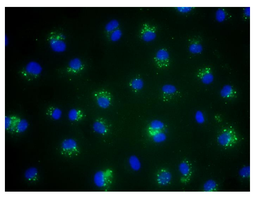


Expanded LSECs demonstrated **expression of common endothelial markers** such as CD31 and vWF (data not shown).

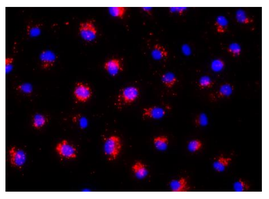
In addition, cells were positive for **LSEC-associated markers** including **LYVE-1** (lymphatic vessel endothelial hyaluronan receptor 1), **MR** (mannose receptor), **Stab-2** (Stabilin-2) and **CD32b** (Fcγ-RIIb).

Ligand uptake

OVA DAPI



ac-LDL DAPI

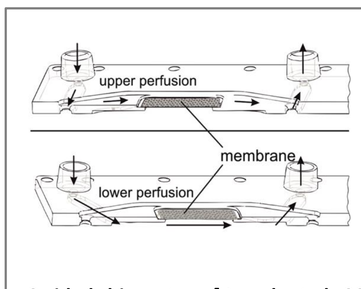


A functional test for **receptor-mediated endocytosis** (uptake function) was performed by adding fluorescent-labeled ligands to the medium in order to visualize how much of the label had been taken up. **AF488-ovalbumin (OVA)** was used as ligand for the mannose receptor, whereas uptake of **Dil-ac-LDL** was employed as target for the scavenger receptor.

Toxicity applications

Several reports in the literature have highlighted a **high sensitivity of LSECs towards hepatotoxic drugs** [DeLeve, 2013]. It has been suggested that LSECs act as an early direct target for APAP-induced toxicity, causing early swelling and loss of uptake activity and fenestrations before effects on hepatocytes are observed [McCuskey, 2006]. LSECs are further important cellular targets during sinusoidal **obstruction syndrome (SOS)**, a distinctive and potentially fatal form of hepatic injury that occurs predominantly, if not only, after drug or toxin exposure. Typical agents that cause SOS include chemotherapeutic agents, particularly alkylating agents such as cyclophosphamide. In addition to conventional toxicity readouts such as formazan formation or ATP levels, expanded LSECs have been successfully tested using chip- and impedance based systems.

3D liver chip

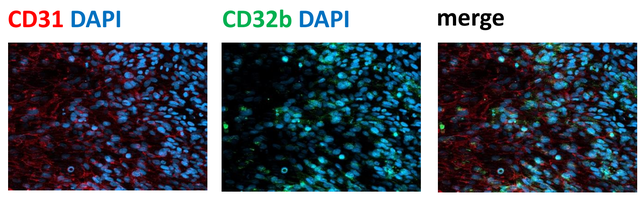


2-sided chip system [Raasch *et al.,* 2015]

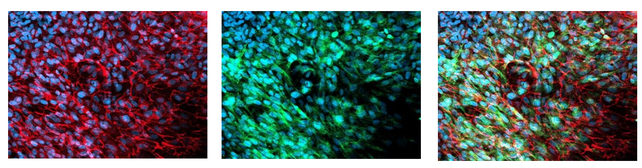
- upcyte® hepatocytes & LSECs cultured on two sides
- peripheral monocytes reflect the innate immune response
- optional: perfusion with T-cells for antigen presentation by LSECs

CD31 DAPI | CD32b DAPI | merge

Control

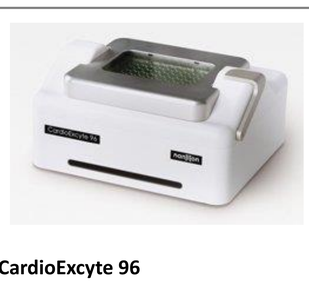


NASH



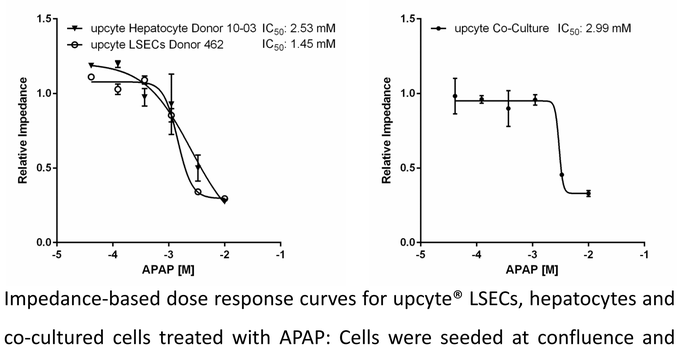
Cells were treated with **0.3 mM palmitate:oleate (1:2)** for **7 days** in order to mimic **nonalcoholic steatohepatitis (NASH). Increased CD31** & **CD32b** expression was observed. CD31 is a **capillarization marker** and was previously linked to the onset and progression of NASH, cirrhosis and chronic hepatitis. CD32b indicates the progression to **hepatic inflammation** as seen in NASH patients.

Impedance monitoring



CardioExcyte 96

- allows time-resolved impedance recordings reflecting cell status and overall morphology
- toxicity of APAP (acetaminophen) was measured in upcyte® LSECs and hepatocytes



Impedance-based dose response curves for upcyte® LSECs, hepatocytes and co-cultured cells treated with APAP: Cells were seeded at confluence and pre-cultured for 72 h before treatment with 41.15 µM-10 mM APAP for 7 days. **IC_{50} values were lower for LSECs alone**, reflecting a higher sensitivity towards APAP when compared to hepatocytes. Interestingly, hepatocytes co-cultured with LSECs were less sensitive than hepatocytes alone, indicating a protective function of LSECs.

Normalized impedance values recorded after 7 days of treatment (240 h post seeding) were used for calculation of IC_{50} values using GraphPad Prism. Data are illustrated as Mean ± SD summarized from one donor per cell type (LSECs: donor 462; white circles vs. hepatocytes: donor 10-03, triangles) or a co-culture (donors 462+10-03; black circle) in technical duplicates.

SUMMARY & CONCLUSION

In summary, we developed a technique allowing **extended expansion of primary human LSECs which retain an adult phenotype**. Our data suggest that upcyte® LSECs combine many characteristics of primary LSECs with the advantage of an extended lifespan, facilitating their use in hepatotoxicity as well as immunological assays. This technology allows for the generation of large batches of upcyte® LSECs enabling standardized and reproducible experimental settings. Future applications include e.g. *in vitro* uptake assays of ADCs (antibody-drug conjugates), antigen presentation and screening for hepatotoxicity.

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