# Expanded primary human liver sinusoidal endothelial cells (upcyte® LSECs) as a tool to complement *in vitro* hepatic studies

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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** without losing their primary characteristics (so-called "upcyte® technology") as previously demonstrated with hepatocytes (upcyte® hepatocytes).

## RESULTS

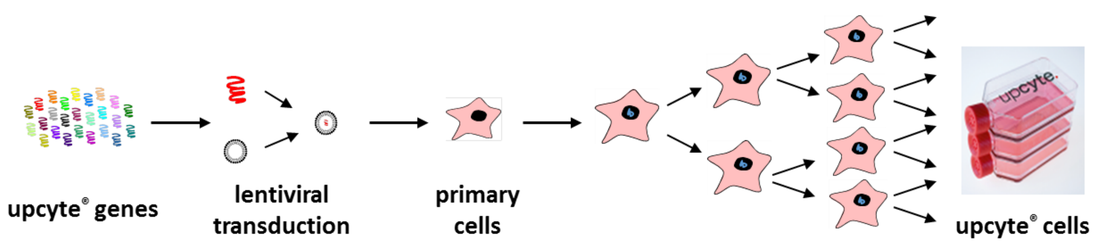
### Why LSECs?

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:

(1) The **Mannose receptor (MR)** eliminates collagen waste molecules, preventing accumulation of intravascular clotting and recruits lysosomal enzymes for degradation.
(2) The **hyaluronan/scavenger receptor (HA/S-R)** plays an important role in the physiological mechanism contributing to maintaining homeostasis and preventing atherosclerosis by binding to type I and III pro-collagen and atherogenic molecules such as oxidized LDL and advanced glycation end products.
(3) The **Fc-ɣ-receptor (FcɣR2BII, CD32b)** takes up IgG coated particles and soluble IgG immune complexes (taken up almost exclusively in the liver). Other functions include interaction with viruses (e.g. HIV-1) [Smedsrod *et al*., 2004].

### Generation of upcyte® LSECs

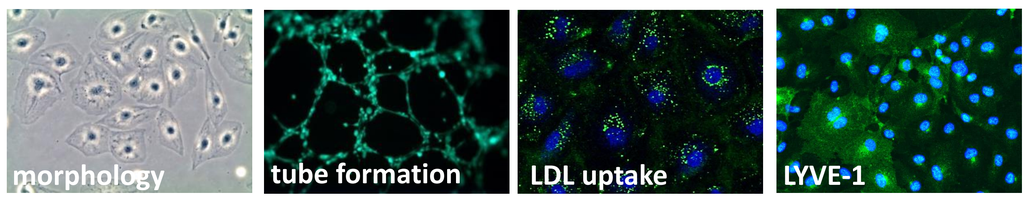



**Transduction of primary cells with a defined cocktail of lentiviral vectors**

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.

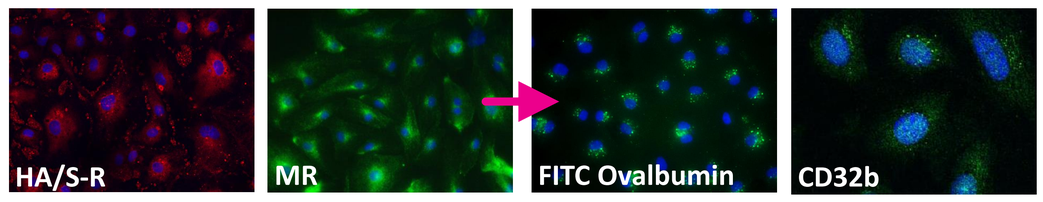
### Characterization of upcyte® LSECs: the cells express…

#### …primary endothelial cell markers




The morphology was comparable to primary LSECs. **CD31** and **vWF** were present in all cells and showed a typical membrane localization (both not shown). upcyte® LSECs can **form tubes** from single cells in Matrigel™ (Calcein AM staining). **Uptake of ligand Ac-LDL** (Alexa483-AcLDL), indicating the presence of the SR-A (Scavenger receptor A), a common endothelial cell receptor, was evident. **LYVE-1** (lymphatic vessel endothelial hyaluronan receptor 1) and **Lectin** (Ulex Europaeus Lectin1 not shown) were strongly expressed.

#### …liver specific markers




The cells express the three major uptake receptors which differ liver sinusoidal endothelial cells from other endothelial cells: **MR, HA/S-receptor** (strong) as well as the **Fcɣ-R (CD32b)**. The functional test **of 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. **FITC-ovalbumin** was used as ligand for the MR.

### Application of upcyte® LSECs

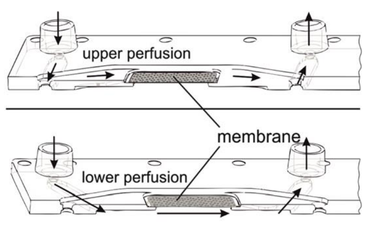
#### immunology

The liver plays an important role in the immune response. LSECs separate passenger leukocytes in the sinusoidal lumen from hepatocytes. They act as a **platform for adhesion** for Kupffer cells, innate lymphoid cells or liver dendritic cells. **LSECs cross-prime naive CD8 T cells**, causing their rapid differentiation into memory T cells and provide protection when they re-encounter the antigen during microbial infection. Cross-presentation of viral antigens by LSECs derived from infected hepatocytes triggers local activation of effector CD8 T-cells and thereby assures hepatic immune surveillance. [doi:10.1038/cmi.2016.5].

**Future applications** include antigen specific **tolerance induction** by using nanoparticles serving as carrier for antigen delivery to LSECs.

#### co & 3D cultures

The Dynamic42 biochip is made of **polyethylene terephthalate and** contains two cavities, both of which have an integrated membrane. This enables cells to be cultured under independent (upper & lower) perfusion channels. **upcyte® LSECs** were seeded together with human **monocyte-derived macrophages** in the upper channel while **upcyte® hepatocytes** were seeded on the other side (hanging fashion).




*Dynamic42 biochip (Raasch et al, 2015)*

upcyte® LSECs were treated with palmitate and oleates in order to **mimic nonalcoholic steatohepatitis (NASH)**. The expression of CD32b and CD31 (capillarization marker) is significantly increased after 7 days (not shown), indicating the progression to hepatic inflammation as seen in NASH patients.

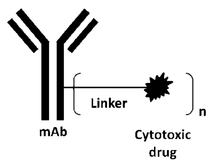
#### toxicology

**cytoxicity assay**

Further characterization with respect to different sensivities of LSECs and hepatocytes to different toxic compounds are ongoing and will be published soon. Stay tuned!

**uptake of ADCs**

ADCs (antibody drug conjugates) are complex molecules composed of an antibody linked to a biologically active cytotoxic payload or drug. **Off-target hepatic toxicities have been reported** for several ADCs (e.g. interactions/unspecific uptake for CD32b [doi:10.1002/ hep.30222] and MR [doi: 10.1007/ s00262-012-1369-3].




## SUMMARY & CONCLUSION

We developed a novel technique which causes primary human LSECs to proliferate additional population doublings whilst still retaining an adult phenotype when cultured at confluence. 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. Future applications include e.g. *in vitro* uptake assays of ADCs (antibody drug conjugates) or triggering the hepatic immune response via the inclusion of T-cells and their antigen presenting capabilities to the LSECs.

Moreover, this technology allows for the generation of large batches of upcyte® LSECs enabling a reproducible and standardized experimental setting.

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