Generation of expanded primary cells for cell-based toxicity and metabolism screenings

Timo Johannssen, Torge Evenburg, Nils Runge, Astrid Noerenberg

upcyte technologies GmbH, Osterfeldstr. 12-14, 22529 Hamburg, Germany

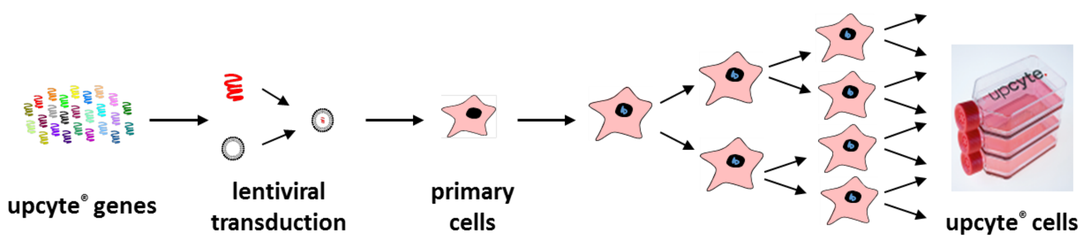
INTRODUCTION

Cell-based assays are a valuable tool to predict *in vivo effects* of drug candidates during early steps of development. Cell-based assays are performed using either cell lines or primary cells. Most **cell lines** are **easy to handle** and offer the advantage of **infinite proliferation**, allowing the generation of large cell banks and a **facilitated use in screenings or long-term experiments**. However, due to their transformed phenotype, many cell lines usually exhibit a **reduced physiological relevance**. In contrast, **primary cells** are **more representative of the *in vivo* state** when compared to cell lines. However, their use *in vitro* is hampered by **limited tissue availability**, **scarce cell yields** and a restriction or even **lack of proliferation**. Taken together, these factors may significantly compromise the scope, length and reproducibility of experiments and often circumvent their use for extended cell-based screenings.

Here, we describe the **controlled expansion of human primary cells** by lentiviral transduction with **proliferation-inducing genes**, enabling production volumes of **up to 2500 vials containing $5 \cdot 10^6$ cells** each. As a proof of principle, primary cells from several relevant target tissues (liver, skin, kidney) were transduced, subsequently demonstrating successful expansion to large master and working cell banks.

RESULTS

generation of upcyte® cells

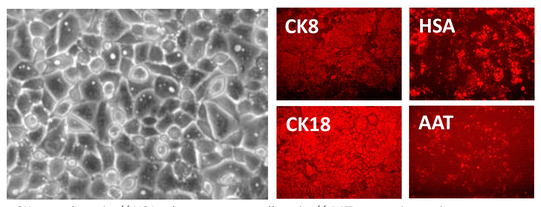


transduction of primary cells with a defined cocktail of lentiviral vectors

We first generated a library of **lentiviral vectors carrying proliferation-inducing genes**, allowing primary cells to bypass senescence. Different primary cells such as **hepatocytes**, **liver sinusoidal endothelial cells**, **keratinocytes**, **proximal tubular epithelial cells** and **bronchial epithelial cells** were transduced. Resulting upcyte® cells gained the ability to proliferate for up to 40 additional population doublings without losing functional and phenotypic characteristics of mature cells. All cells exhibited expected morphology patterns and were **restricted by the presence of specific growth factors**, **contact inhibition** and **anchorage dependence**.

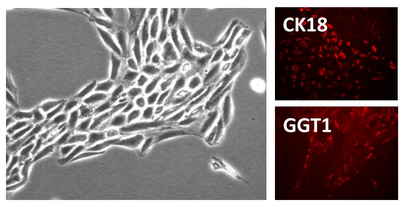
generated upcyte® cell types

hepatocytes



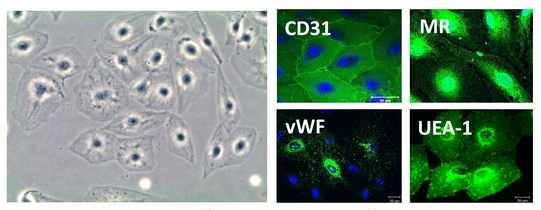
CK = cytokeratin // HSA = human serum albumin // AAT = α-anti-trypsin

proximal tubular epithelial cells (PTCs)



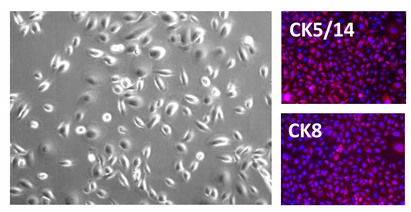
CK = cytokeratin // GGT1 = gamma-glutamyltransferase 1

liver sinusoidal endothelial cells (LSECs)



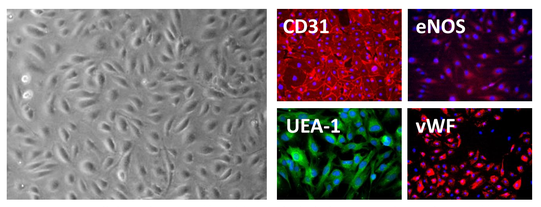
vWF = Von Willebrand Factor // MR = mannose receptor // UEA-1 = binding of Ulex europaeus agglutinin-I

keratinocytes



CK = cytokeratin

microvascular endothelial cells (mvECs)



vWF = Von Willebrand Factor // eNOS = endothelial nitric oxide synthase 3 // UEA-1 = binding of Ulex europaeus agglutinin-I

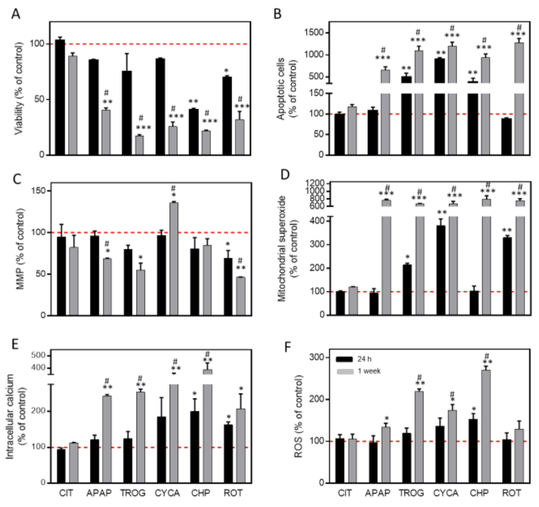
upcyte® Kupffer cells

We were able to receive a grant to generate upcyte® Kupffer cells. **You are experienced with Kupffer cells?** Please let us now and get in contact, **we would love to collaborate!**

upcyte® hepatocytes for…..

…acute and repeated-dose toxicity using sub-cytotoxic concentrations

Exposure time length had dramatic effects on the toxicity profile of a compound. For **APAP, no effect was observed after 24 h, whereas 1-week** treatment significantly induced apoptosis, mitochondrial depolarization, ROS production and intracellular Ca^{2+} levels. Other tested compounds caused some effects after 24 h, although a significant difference was detected between the two incubation periods at the lowest concentration. CIT (non hepatotoxic control) did not produce any significant effects after 24 h or 1 week treatment.

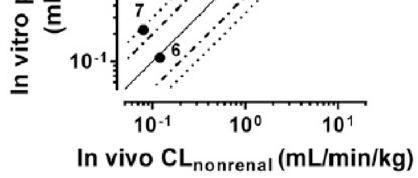


*upcyte® hepatocytes (422a-03) were exposed to test compounds for 24 h or 1 week. Fluorescent probes were subsequently employed to evaluate **(A)** viability, **(B)** apoptosis, **(C)** changes in mitochondrial membrane potential (MMP), **(D)** production of mitochondrial superoxide, **(E)** ROS **(F)** intracellular Ca^{2+} levels using HCS. 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
human upcyte® hepatocytes: characterization of the hepatic phenotype and evaluation for acute and long-term hepatotoxicity routine testing - University of Valencia, Spain

… the prediction of hepatic clearance (CL_H)

Correlation between *in vitro* predicted and *in vivo* $CL_{nonrenal}$ was demonstrated using **the well-stirred model disregarding plasma protein binding** for low and intermediate clearance compounds (donor 151-03).
Good correlation between predicted CL_H and observed *in vivo* CL values was observed for the subset of low CL drugs (shown here). **CL_H for 73%** (8 of 11 compounds) were predicted **within twofold** of *in vivo* $CL_{nonrenal}$ and **within threefold for 82%** (9 of 11 compounds) with a trend for overpredicting the actual *in vivo* rate.



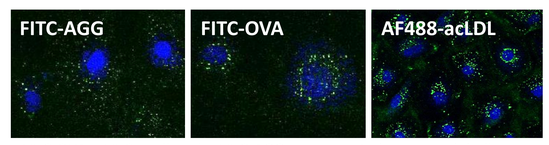
Data represent mean 6±S.D. of triplicate incubations per compound (n = 3) determined at day 7 in sandwich culture. Solid line represents conformity, and dashed lines two- and threefold error range. The set of reference drugs was subdivided into low and intermediate-cleared compounds (shown here: low): alprazolam (1), prednisolone (2), diazepam (3), voriconazole (4), tolbutamide (5), meloxicam (6), warfarin (7), glimepiride (8), riluzole (10), oxazepam (11).

Schaefer *et al.*, (2016) - Drug Metabolism & Disposition
upcyte® human hepatocytes: a potent in vitro tool for the prediction of hepatic clearance of metabolically stable compounds
Boehringer Ingelheim Pharma GmbH & Co. KG, DMPK, Biberach an der Riss, Germany

examples of functional assays using upcyte® cells

LSECs: receptor-mediated endocytosis

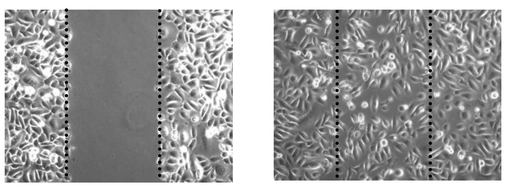
We observed **high expression of endocytic receptors, e.g. MR, FcγR and LDLR** in upcyte® LSECs. Corresponding ligand uptake could be demonstrated for respective fluorophore-conjugated ligands (FITC-OVA, FITC-AGG and AF488-acLDL).



FITC-OVA = FITC ovalbumin, FITC-AGG = aggregated gamma globulin AF488-acLDL = acetylated low density lipoprotein

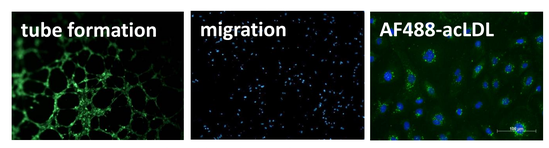
keratinocytes: wound healing

upcyte® keratinocytes exhibit a **similar migration capacity** when compared to primary cells. Migration was determined by wound healing assay. Confluent monolayers of upcyte® keratinocytes were scratched using a pipette tip. Wound closure was achieved after 8 h.



mvECs: angiogenesis

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels. upcyte® mvECs perform **tube formation** in matrigel and **uptake of LDL**. **Cell migration** is another typical feature of mvECs, showed here by transmigration through a FluoroBlok™ insert; only the cells that migrated through the membrane are detectable .



AF488-acLDL = acetylated low density lipoprotein

Do you want to expand your primary cells?

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. Please contact us if you would like more information on how we can upcyte your favorite batch of primary cells.

SUMMARY & CONCLUSION

In conclusion, we developed a comprehensive platform enabling the **controlled expansion of primary cells** derived from various tissues for **up to 40 population doublings**. Importantly, upcyte® cells maintained a **mature and primary-like phenotype** as demonstrated by expresison of marker proteins and functional assays.

We thus conclude that upcyte® expanded primary cells represent a promising model for biomedical research and drug discovery, potentially facilitating throughput and reproducibility of cell-based assays.