Generation of Expanded Primary Cells for Cell-Based Toxicity and Metabolism Screenings

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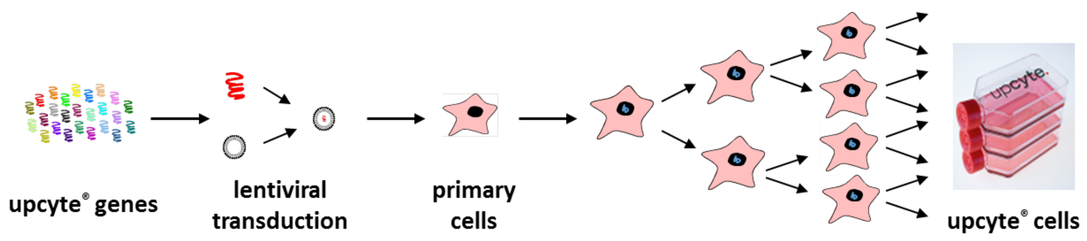
INTRODUCTION

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 often 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 5·10^6 cells** each. As a proof of principle, primary cells from several relevant target tissues (liver, skin, kidney, lung) were transduced, subsequently demonstrating successful expansion to large master and working cell banks.

RESULTS

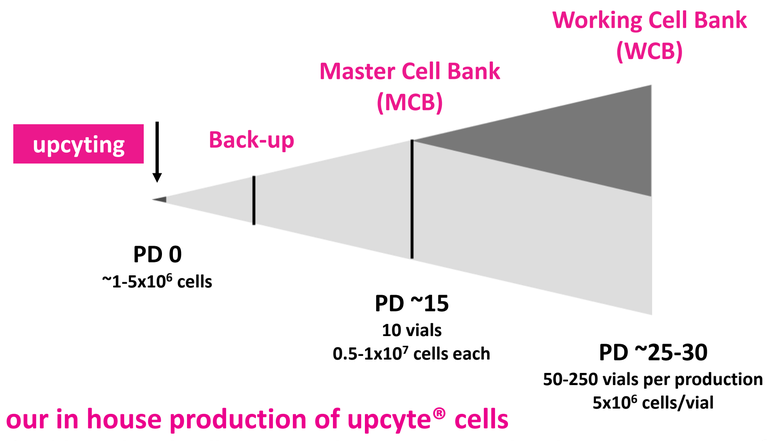
generation of upcyte® cells



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 **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 (PDs) 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**.

expansion strategy



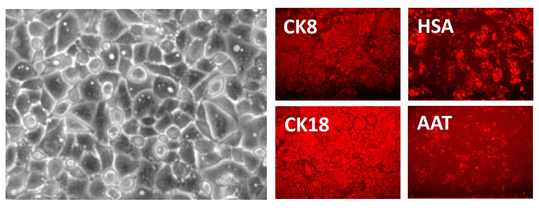
scheme for our in house production of upcyte® cells

Starting from **freshly isolated cells** or **a single cryopreserved vial** containing 1-5x10^6 cells, cells were transduced and subsequently cultured in the presence of growth factors. Depending on the cell type used, we observed **appearance of proliferating colonies at 2-3 weeks post transduction latest** (e.g. hepatocytes). After the first three expansion steps, backup cells were frozen. Cells were subsequently expanded to generate a **Master Cell Bank (MCB)** consisting of 10 vials with 0.5-1x10^7 cells each. Singe MCB vials were then used to produce a **Working Cell Bank** with **up to 250 vials containing 5-1x10^6 cells per vial**.

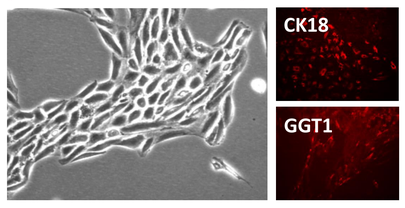
overview: generated upcyte® cell types

We next investigated the **phenotype of expanded upcyte ®cells**. Importantly, generated cells maintained **expression of characteristic marker proteins** throughout the study. For example, **hepatocytes** expressed **cytokeratin 8/18**, **human serum albumin** and **alpha-1 antitrypsin**. Accordingly, upcyte® **LSECs** were characterized by expression of **CD31** and **von-Willebrand-factor**.

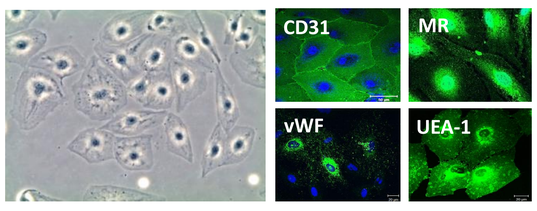
hepatocytes (HCs)



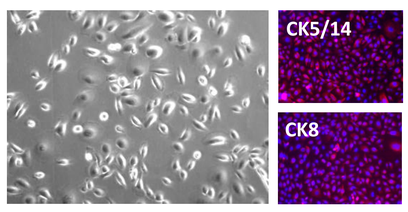
proximal tubular epithelial cells (PTCs)



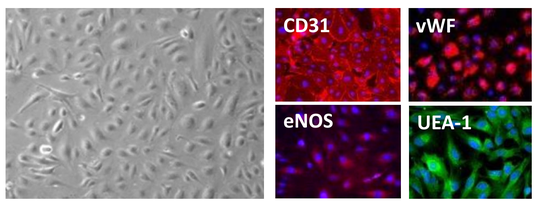
liver sinusoidal endothelial cells (LSECs)



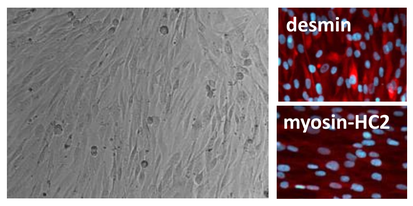
keratinocytes (KCs)



microvascular endothelial cells (mvECs)



skeletal muscle cells (SkMCs)



CK - cytokeratin, HSA - human serum albumin, AAT - alpha-antitrypsin, MR - mannose receptor, vWF - von-Willebrand-factor, UEA-1 - Ulex Europaeus Lectin 1, GGT1 - γ-glutamyltransferase 1, vWF - von Willebrand Factor, eNOS - endothelial NOS or nitric oxide synthase 3 (NOS3), myosin – myosin heavy chain 2

examples of functional assays using upcyte® cells

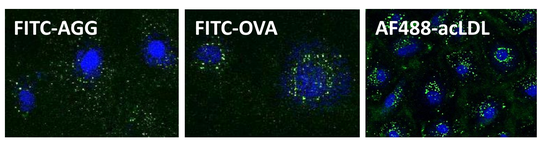
hepatocytes: metabolism

upcyte® hepatocytes expressed **metabolizing enzymes of phase I (e.g. CYP 1A2, 2B6, 2C9 and 3A4)** and further exhibited **phase II activities (UGT, SULT & GST)**. upcyte® hepatocytes further produced urea and secreted albumin (not shown). Differences in performance could be detected between cells derived from different donors.

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
HepaRG	10.0 ± 1.5	6.45 ± 0.97	4.57 ± 2.93	48.5 ± 13.9

LSECs: receptor-mediated endocytosis

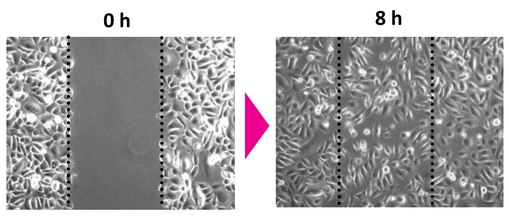
We observed **high expression of endocytic receptors, e.g. MR, FcγR and LDLR** in upcyte® LSECs. Corresponding ligand uptake could be demonstrated for respective fluorophore-conjugated ligands (FITC-OVA, FITC-AGG and AF488-acLDL).



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

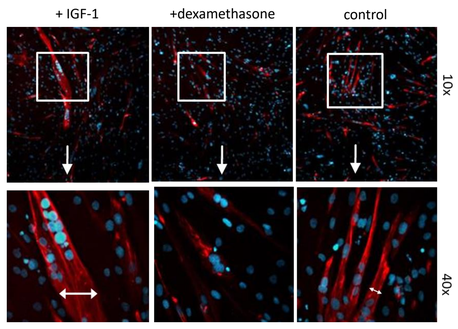
keratinocytes: wound healing

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.



SkMCs: atrophy & hypertrophy

Atrophy (decrease in the mass of the muscle) was induced by addition of dexamethasone & myostatin. The atrophic effect can be reversed through the inhibition with IGF-1 which also induces Hypertrophy (increase myofibril size). The hypertrophic effect can be reversed through the inhibition with LY294000



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. Do not hesitate to 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.