Metabolic competent expandable human upcyte® hepatocytes enable metabolism studies including CYP2D6 dependent pathways

Astrid Noerenberg*, Nils Runge*, Thomas Muerdter#, Janina Johaenning#, Maria Thomas#, Werner Schroth#

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

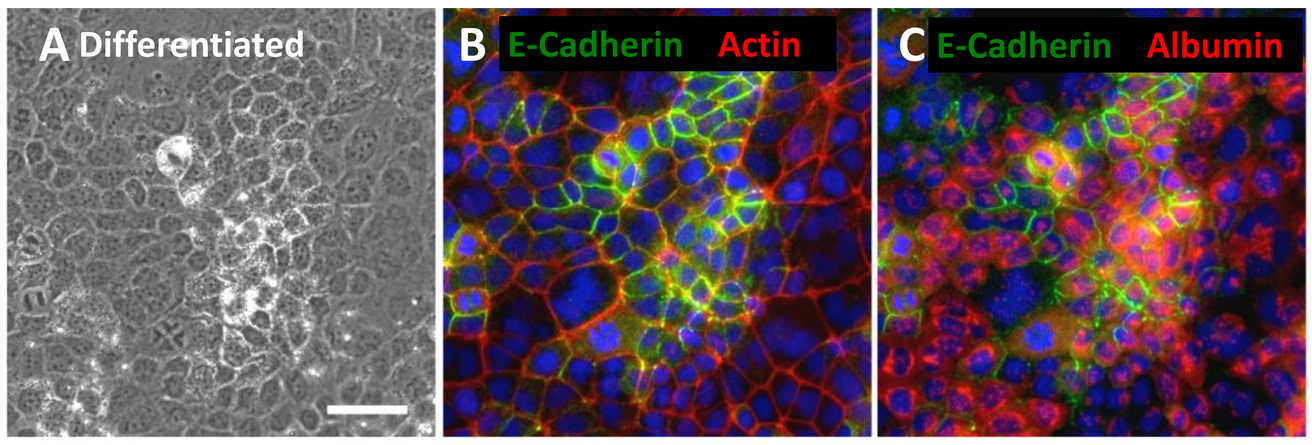
#Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Auerbachstr. 112, D-70376 Stuttgart, Germany

INTRODUCTION

Summary and novelty: Primary cultures of human hepatocytes are routinely used in drug development to evaluate metabolic fate, drug-drug interactions and drug toxicity. However, the use of hepatocytes is limited by the low availability of human liver tissue. To overcome this, we have developed a technique which causes primary human hepatocytes to proliferate up to 40 population doublings whilst still retaining an adult and metabolically competent phenotype with phase I (Cytochrome P450) and phase II activities when cultured at confluence. The resulting cells are called “upcyte® hepatocytes” and have the capability to proliferate and express sufficient drug metabolizing activities, a combination which makes them uniquely applicable to metabolism and toxicity studies.

RESULTS

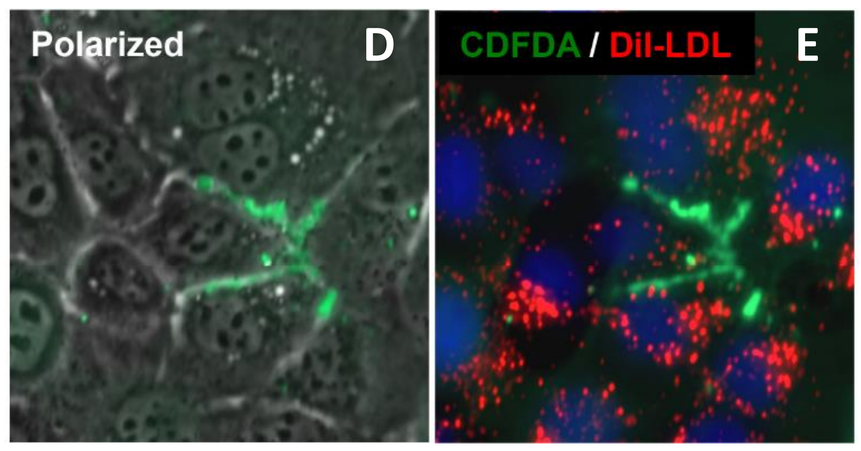
Polarization of upcyte® hepatocytes



Confluent cultures of upcyte® hepatocytes are basolaterally polarized.

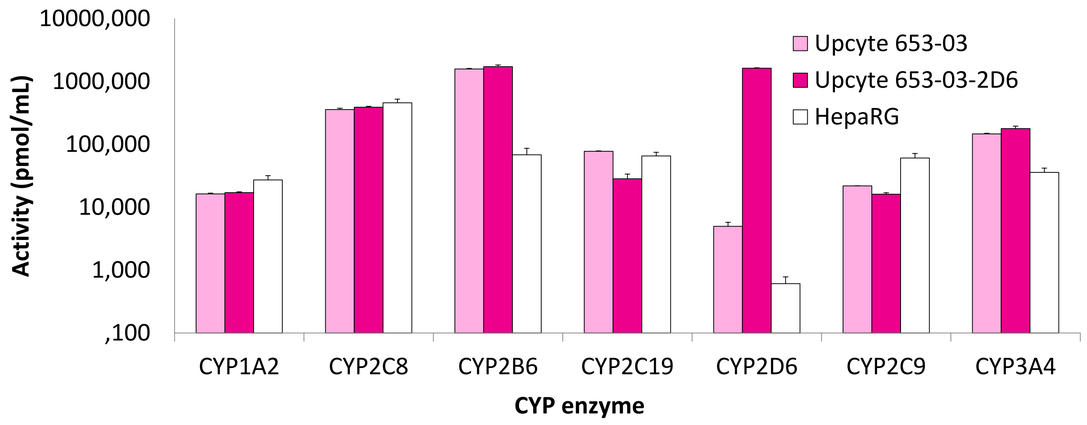
(A-C) Phase and Immunofluorescence micrographs of upcyte® hepatocytes 4 days after reaching confluence. Immunofluorescence staining of **E-Cadherin (B)**, a lateral surface marker, and **albumin (C)**, in differentiated upcyte® hepatocytes counter-stained for **actin**. Cultures show distinct polarized cell nodules amid non-polarized E-Cadherin negative cells. Both polarized and non-polarized cells show strong **albumin** staining **(C)** demonstrating a hepatocyte origin.

Analysis was performed by Micrroliver Technologies Lab of The Hebrew University of Jerusalem



(D-E) Functional polarization in differentiated upcyte® hepatocytes. **(E) CDFDA** staining shows the accumulation of green CDF in functional bile canaliculi on the apical surface of polarized cells, whereas **DiI-LDL** (low-density lipoprotein*)* is taken up primarily by the LDL-R expressed on the basal surface of polarized cells. The cultures exhibit basal-apical polarized cell nodules (*polarized*) surrounded by non-polarized cells.

Phase I activities

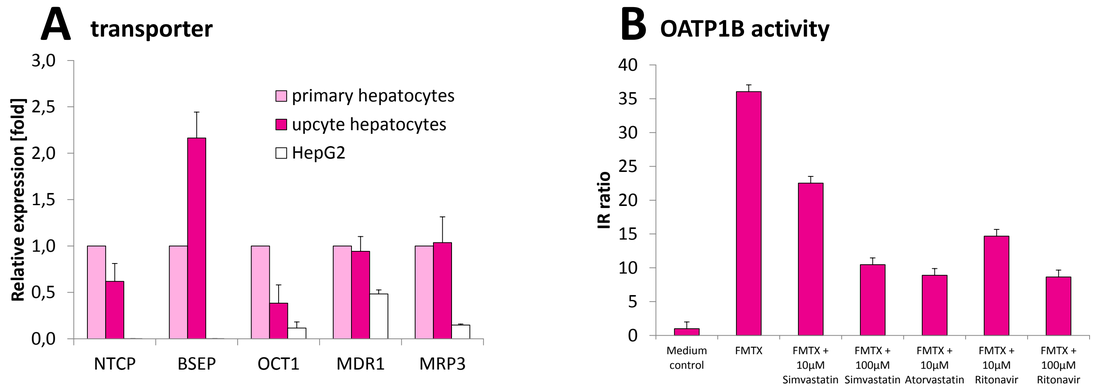


upcyte® hepatocytes show high basal activities of phase I enzymes and can be modified to express functional CYP2D6 enzymes.

In general upcyte® hepatocytes from different donors express CYP1A2 (1-17pmol/min/mg), 2B6 (19-82pmol/min/mg), 2C9 (1-96pmol/min/mg) und 3A4 (10-195pmol/min/mg). The upcyte® hepatocyte cell strain from Donor #653-03 shows moderate to high activities for a number of endogenous CYP enzymes but very low levels (5 pmol/mL) of CYP2D6 (enzyme activities were measured using a substrate cocktail incubation and Triple Quad MS analysis). In the **#653-03-2D6 cell strain, recombinant CYP2D6 is stably expressed with a basal activity of over 1600 pmol/mL**. In comparison, HepaRG cells almost completely lack CYP2D6 activity with less than 1 pmol/mL.

CYP2D6 is responsible for the metabolism and elimination of approximately 25% of clinically used drugs and of endogenous substrates including hydroxytryptamines and neurosteroids. Moreover, a considerable proportion of individuals (and their derived liver cells) lack CYP2D6 expression/activity due to genetic polymorphism. Therefore some people will eliminate certain drugs quickly (ultrarapid metabolizers) and others slowly (poor metabolizers).

Phase III, transporter expression and activities



upcyte® hepatocytes express functional transporters.

(A) Expression of transporters: We compared the mRNA expression of important hepatic transporters with primary hepatocytes and the hepatic cell line HepG2. upcyte® hepatocytes (Donor #422a-03) express a number of transporters at similar levels to those in primary hepatocytes. In contrast, HepG2 cells show low or no expression. Data are given as a ratio relative to (non-matched) PHH.

NTCP (sodium/bile co-transporter / influx); BSEP (bile salt export pump / efflux); OCTI (organic cation transporter I / influx); MDR1 (multidrug resistance protein 1 / efflux); MRP3 (multidrug resistance-associated protein 3 / efflux)

(B) OATP1B activity can be inhibited dose dependently: Activity was measured in the presence or absence of specific inhibitors (Simvastatin, Atorvastatin, Ritonavir) by incubating upcyte® hepatocytes (Donor #422a-03) with the fluorescent substrate fluorescein-methotrexate (FMTX) that is specifically taken up by OATP1B family transporters (mainly OATP1B3) and measuring fluorescence after washing and lysing the cells. The uptake was then calculated as Influx rate ratio (IR ratio). As shown in the graph, FMTX is readily taken up by upcyte® hepatocytes and uptake can be blocked by inhibitors in a dose dependent way demonstrating presence of functional OATP1B transporters.

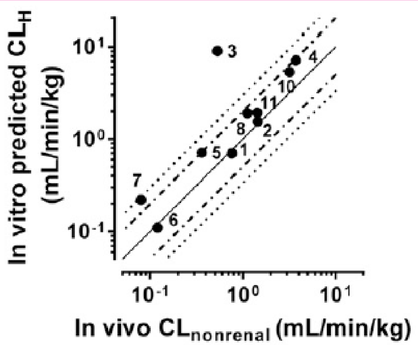
Phase II activities

Phase II activity	upcyte® hepatocytes (5 donors)	primary hepatocytes (non-matched)
SULT (Hydroxycoumarin)	6-16 pmol/min/mg	5-98 pmol/min/mg
UGT (Hydroxycoumarin)	32-345 pmol/min/mg	15-496 pmol/min/mg
GST (CDNB)	15-88 nmol/min/mg	21-35 nmol/min/mg

upcyte® hepatocytes have similar phase II activities compared to primary hepatocytes.

Phase II enzymes play a major role in the conjugation reaction of compounds with polar functional groups and therefore contribute to the clearance of many drugs. Major hepatic phase II enzymes in humans are **UDP-glucuronosyltransferase (UGT)**, **sulfotransferase (SULT)** and **glutathione S-transferase (GST).** Phase II enzyme activities in upcyte® hepatocytes generated from different donors were similar to those of freshly isolated primary human hepatocytes.

upcyte® hepatocytes for clearance prediction



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)

upcyte® hepatocytes are a potent *in vitro* tool for the prediction of hepatic clearance (CL_H)

Correlation between *in vitro* predicted and *in vivo* $CL_{nonrenal}$ was shown applying the well-stirred model disregarding plasma protein binding for low and intermediate clearance compounds and using cells from 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 over predicting the actual.

Schaefer et al., (2015) Department of Drug Discovery Support, Metabolism and Bioanalytics, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

CONCLUSION

In conclusion, upcyte® hepatocyte cultures have a differentiated phenotype and exhibit functional phase I, phase II and transporter activities. These data support the use of upcyte® hepatocytes for metabolism & toxicity screening assays. Moreover, this technology allows for the generation of large batches of upcyte® hepatocytes (up to 12 x 10^9 cells per donor) enabling a reproducible and standardized experimental setting.