upcyte technologies

upcyte® Hepatocytes - proliferating and metabolic competent human hepatocytes

Astrid Nörenberg*, Werner Schroth#, Thomas Mürdter#, Janina Johänning#, Maria Thomas#, Stefan Heinz*

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

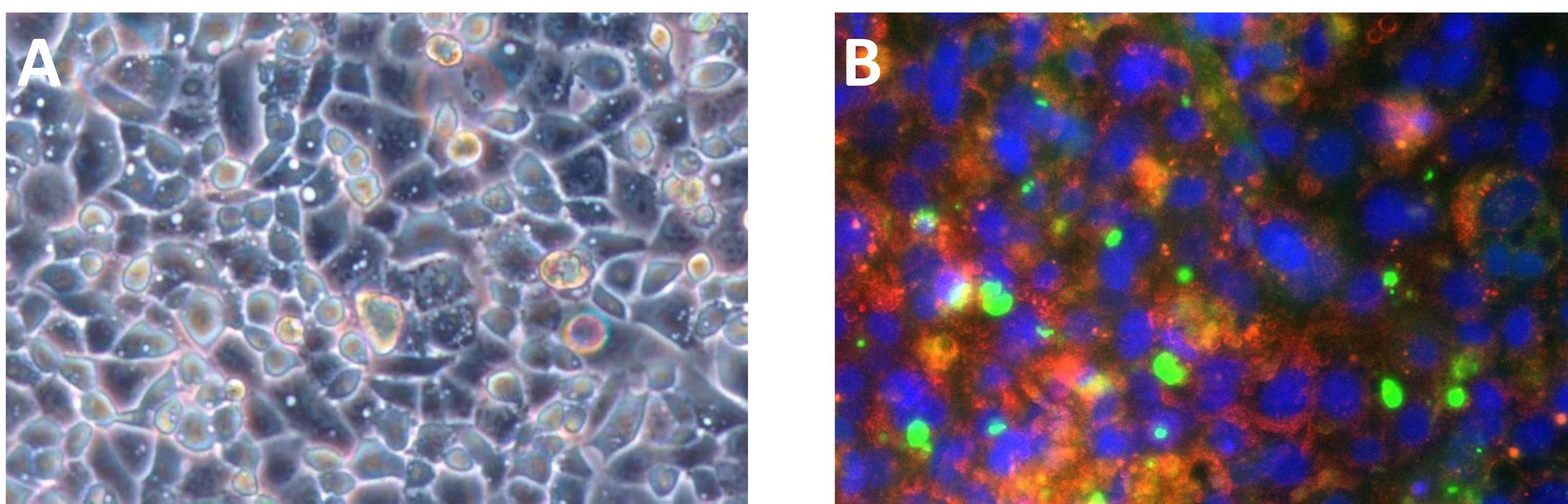
#Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Auerbachstr. 112, D-70376 Stuttgart, Germany

INTRODUCTION

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 novel technique which causes primary human hepatocytes to proliferate up to 40 population doublings whilst still retaining a metabolic competent phenotype when cultured at confluence. The resulting cells are named "upcyte® Hepatocytes". Here, we describe some of the morphological characteristics of upcyte® Hepatocytes and present data for their activities regarding drug metabolism, excretion and transport.

RESULTS

Polarization of upcyte® Hepatocytes

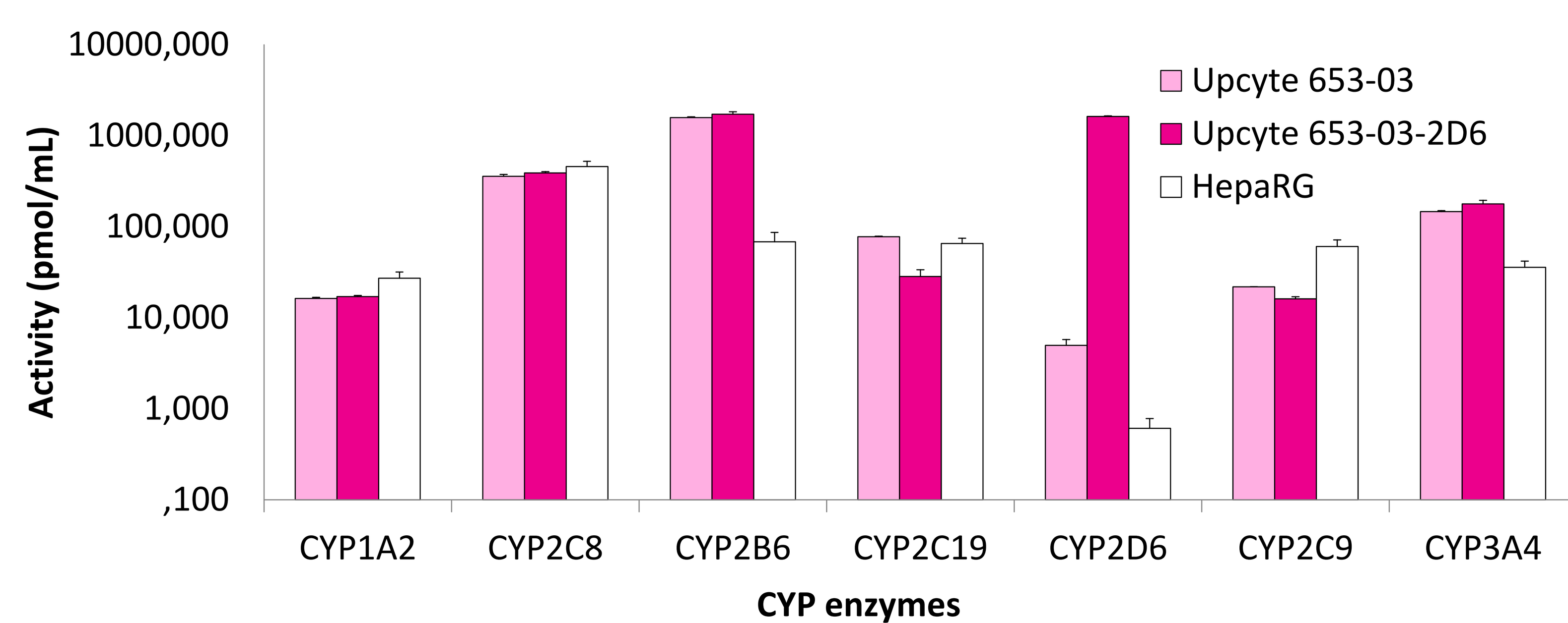


Confluent cultures of upcyte® hepatocytes are morphologically similar to primary hepatocytes and are basolaterally polarized.

(A) Morphology: Once upcyte® Hepatocytes (PD25) have been generated they can be seeded at a low density and allowed to grow to confluence. At confluence, cells adopt the well-defined, cuboidal shape of monolayer cells characteristic of primary human hepatocytes (PHH).

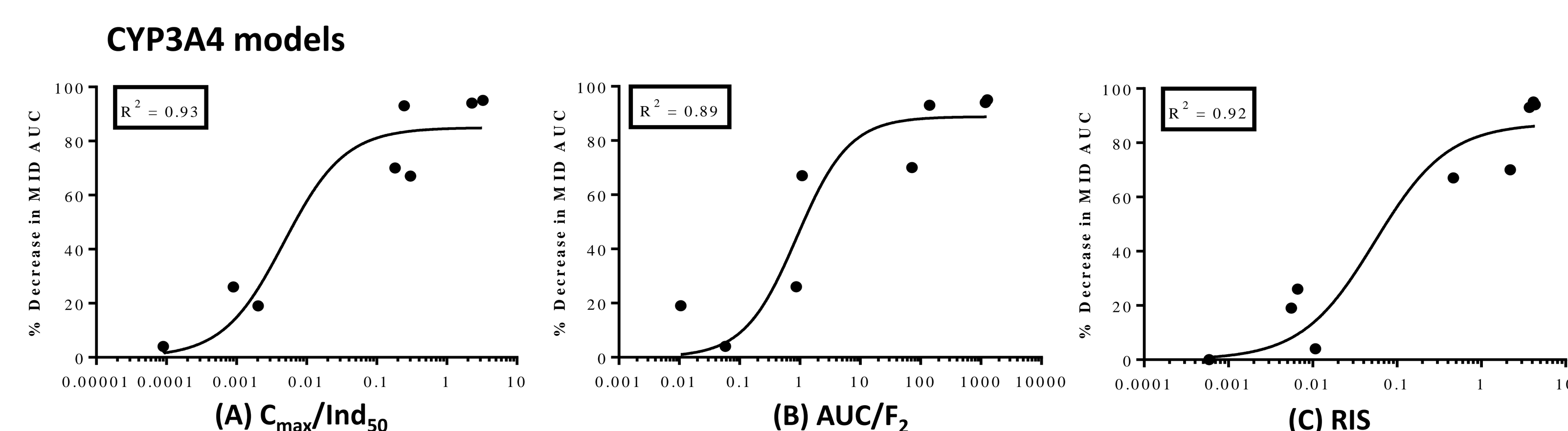
(B) Basolateral polarization: In culture, primary hepatocytes rapidly lose polarity due to the loss of tight junctions. When upcyte® Hepatocytes are cultured at confluence, they express tight junctions so that apical (canalicular) and basolateral (sinusoidal) domains characteristic for a polarized phenotype are formed. This polarity is demonstrated by the uptake of low-density lipoproteins (**LDLs**) (red staining) via LDL receptors expressed on the sinusoidal surface and by an efflux of 5-(and-6)-carboxy-2',7'-dichloro-fluorescein (**CDF**) into the bile canaliculi (intense green spots in small pockets between the cells) via multidrug resistance-associated protein 2 (MRP2). (**DAPI** = blue staining)

Phase I activities



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

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. Some people will eliminate certain drugs quickly (ultrarapid metabolizers), others slowly (poor metabolizers).



upcyte® Hepatocytes can be used to predict CYP3A4 induction *in vivo*.

upcyte® Hepatocytes have been shown to respond to various CYP inducer in a dose-dependent way and the fold induction generally reflected those of the original primary hepatocytes. Moreover, upcyte® hepatocytes predicted the *in vivo* induction potencies of known CYP3A4 inducers using the three main prediction models recommended by the FDA, EMA and PhARMA, namely, the $C_{max,u}/Ind_{50}$ (A), AUC_u/F_2 (B) and RIS (C) model (see figure). Cells were incubated daily for 3 days with 8 inducers (each point represents one) of varying induction potencies in 7 concentrations. CYP3A4 activity was measured using testosterone 6ß-hydroxylation. Of the three models, the fit was best when the RIS (R^2 = 0.92) and $C_{max,u}/Ind_{50}$ (R^2 = 0.93) were used; however, the F_2 value (R^2 = 0.89) may also be used when compounds are too toxic or insoluble to reach a maximal induction response. upcyte® Hepatocytes can therefore be used to predict CYP3A4 induction *in vivo*.

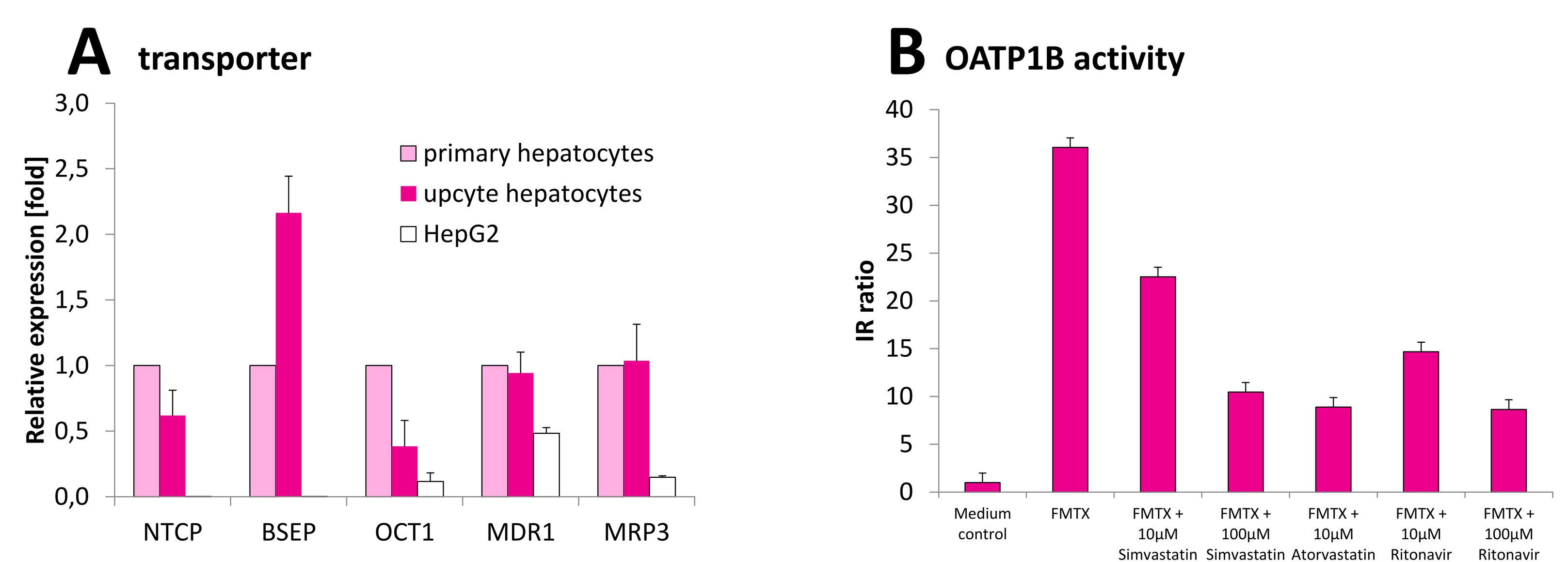
Phase II activities

Phase II activity	upcyte® Hepatocytes (5 donors)	Primary hepatocyte cultures (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 PHH (as shown in the table).

Phase III, transporter expression and activities



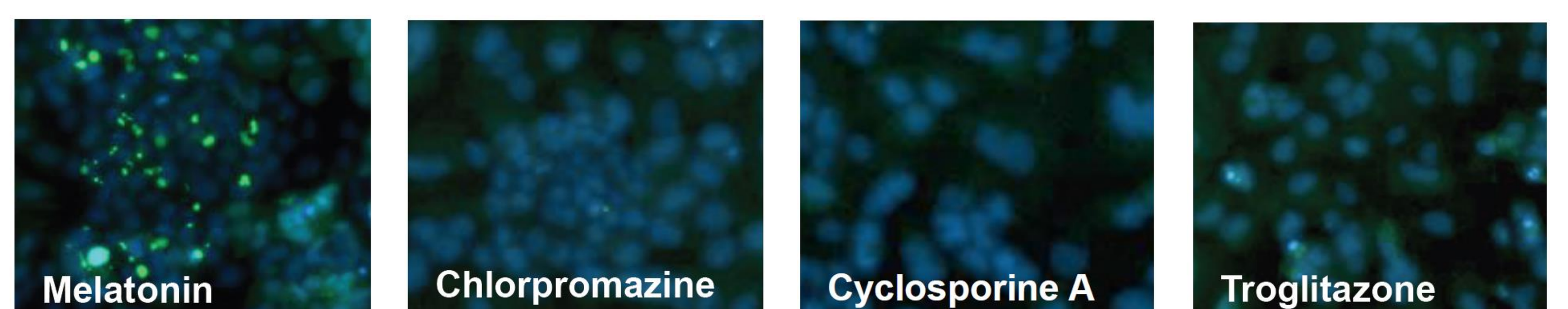
upcyte® Hepatocytes express functional transporters.

Transporters are involved in the excretion of compounds and may be subject to transporter-based drug interactions. Primary human hepatocytes express a number of uptake and efflux transporters when they are cultured in sandwich cultures.

(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 fluorescence 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.



Efflux transporters can be inhibited by cholestasis inducing compounds

Intrahepatic cholestasis can be observed in upcyte® Hepatocytes exposed to troglitazone, chlorpromazine (Thorazine) and cyclosporine A, compared to melatonin as negative control. All three drugs caused morphological changes leading to loss of bile secretion and accumulation of fluorescent CDF in the cytoplasm instead of in bile canaliculi as shown for Melatonin (green spots).

CONCLUSION

In conclusion, upcyte® Hepatocyte cultures have a differentiated, polarized phenotype and exhibit functional phase I and II activities, as well as expression and activity of important transporters. These data support the use of upcyte® Hepatocytes for induction and toxicity screening assays. Moreover, this technology allows for the generation of large batches of upcyte® Hepatocytes (up to $12x10^9$ cells per donor), enabling a reproducible and standardized experimental setting.