# Generation of proliferating human hepatocytes with enhanced CYP enzyme activities

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

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

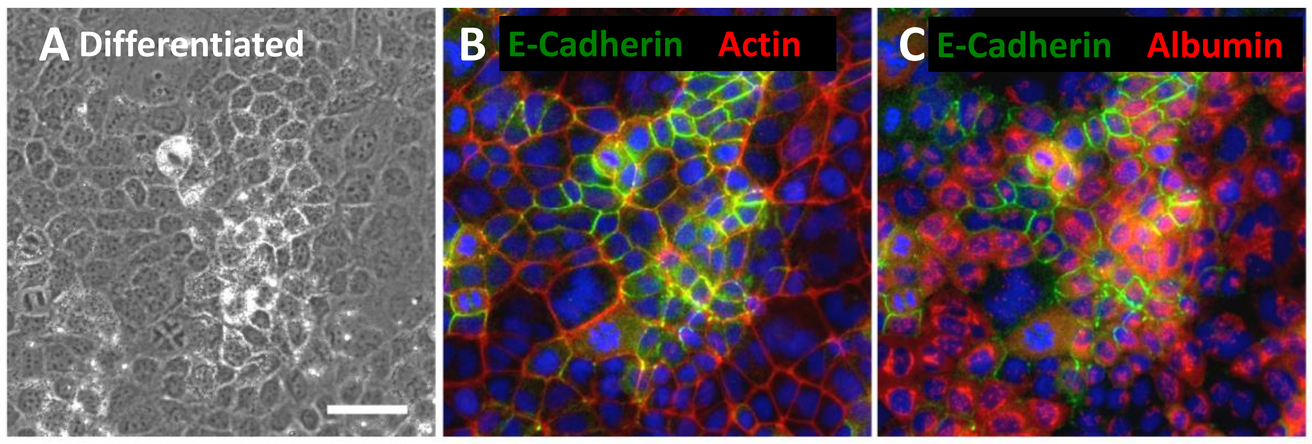
#Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Auerbachstr. 112, D-70376 Stuttgart, Germany

## INTRODUCTION

**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 novel technique which causes primary human hepatocytes to proliferate up to 40 population doublings whilst still retaining an adult and metabolic competent phenotype with phase I (Cytochrome P450) and phase II (UGT = UDP-glucuronosyltransferases, SULT= sulfotransferases, GST= glutathione S-transferases) 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.

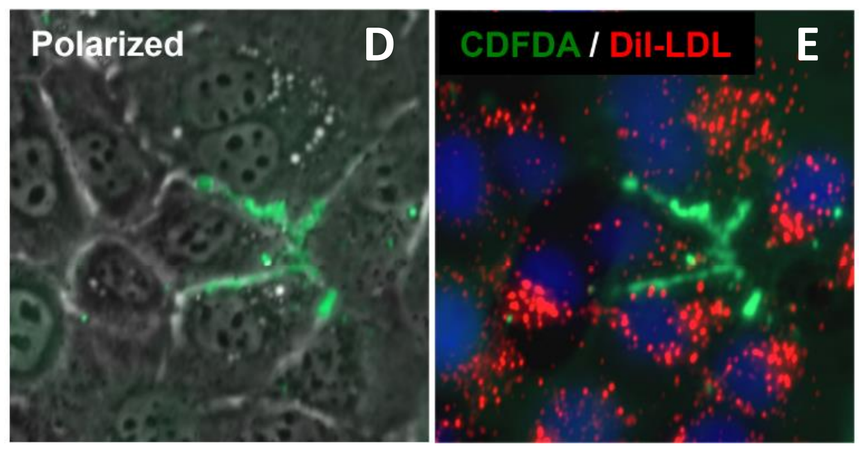
## RESULTS

### Polarization of upcyte® hepatocytes



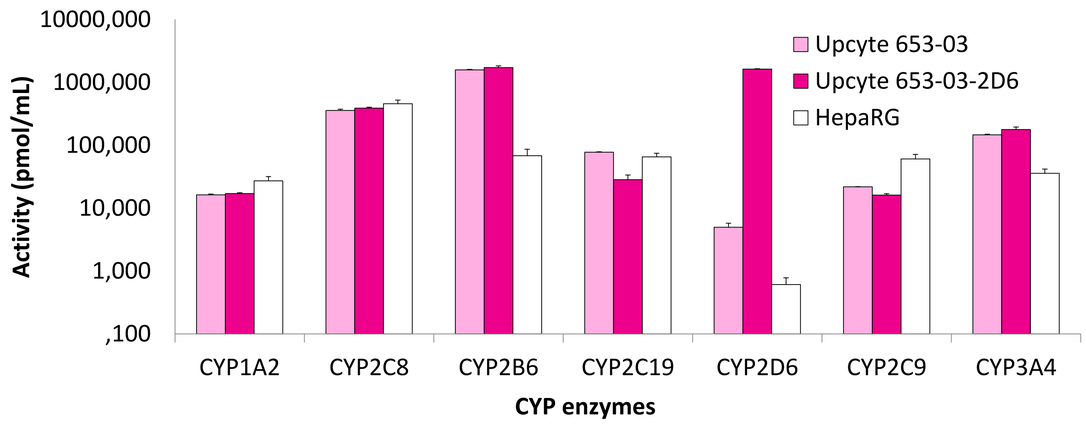

**Confluent cultures of upcyte® hepatocytes are basolaterally polarized.**

**(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 nonpolarized E-Cadherin negative cells. Both polarized and non-polarized cells show strong **albumin** staining **(C)** demonstrating a hepatocyte origin. Bar = 50 µm. Analysis was performed by Mircroliver Technologies Lab (Nahmias) 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** 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. *LDL, low-density lipoprotein*.

### 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).

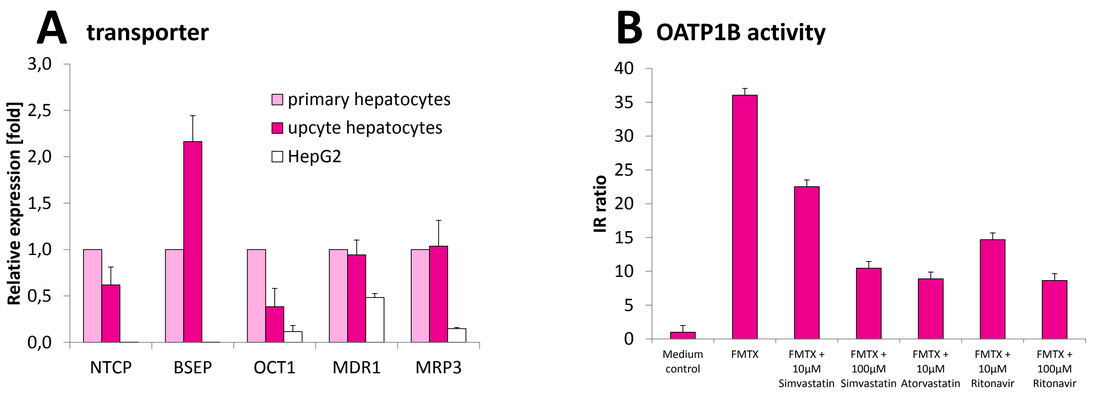
### Phase II activities

| 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

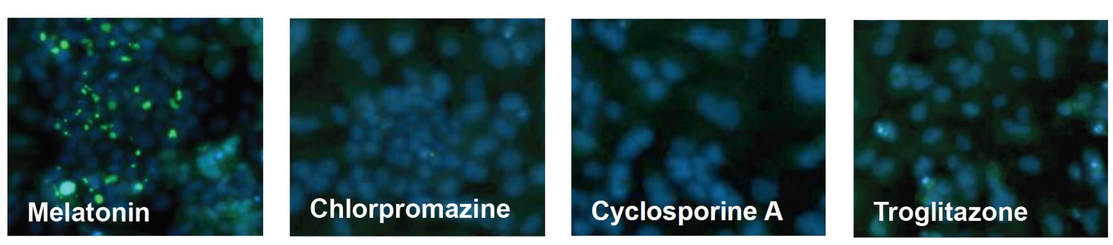



**upcyte® hepatocytes express functional transporters.**

**(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 phenotype and exhibit functional phase I, phase II and transporter activities. These data support the use of upcyte® hepatocytes for metabolism and 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.