Detection of cytokeratin 18 in proliferating, primary-like upcyte® hepatocytes to predict drug-induced hepatotoxicity

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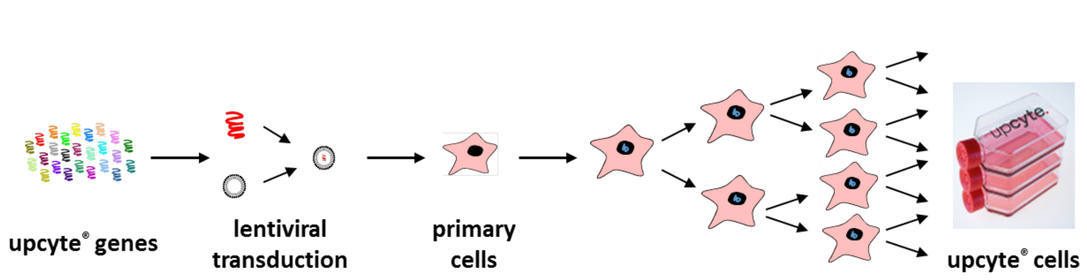
INTRODUCTION

Early detection of drug-induced liver injury (DILI) is essential during drug development to minimize the risk of adverse effects and subsequently drug withdrawal. To evaluate the safety profile of a drug candidate, there is a constant need for reliable *in vitro* test systems. While primary human hepatocytes remain the gold standard, limited throughput and a rapid loss of liver-specific functions limit their use. In the present study, we expanded primary human hepatocytes by lentiviral transduction with proliferation inducing genes. So-called upcyte® hepatocytes proliferated for up to 40 population doublings while maintaining several characteristics of primary cells, such as adult marker expression and phase I/II activities.

Recently, cytokeratin 18 (CK18) was suggested as a promising biomarker for DILI. Likewise, the M30 neoepitope generated upon caspase-dependent cleavage of CK18 is considered a robust biomarker for apoptosis. Here, we evaluated the use of upcyte® hepatocytes for M30-based *in vitro* hepatoxicity assays by analyzing their expression of CK18 and the degree of caspase-dependent cleavage upon challenge with established hepatotoxic model compounds.

RESULTS

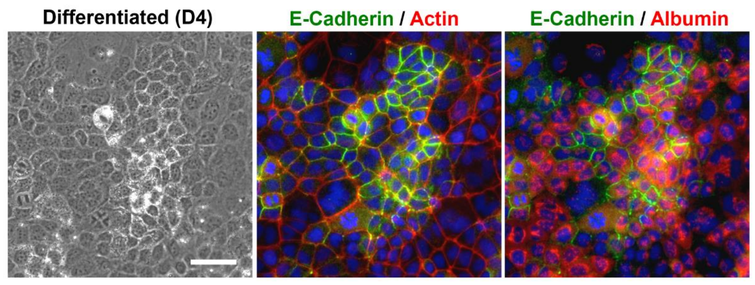
the upcyte® technology



expansion of primary hepatocytes using a defined cocktail of lentiviral vectors

We first generated a library of **lentiviral vectors carrying proliferation-inducing genes**, allowing primary human hepatocytes (pHH) to bypass senescence. Resulting upcyte® hepatocytes 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**.

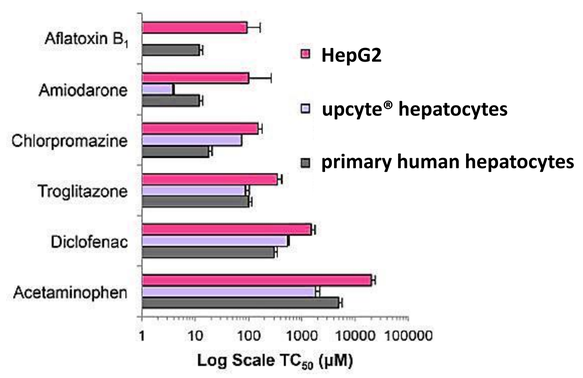
morphology and polarization of upcyte® hepatocytes



immunofluorescence analysis of differentiated upcyte® hepatocytes

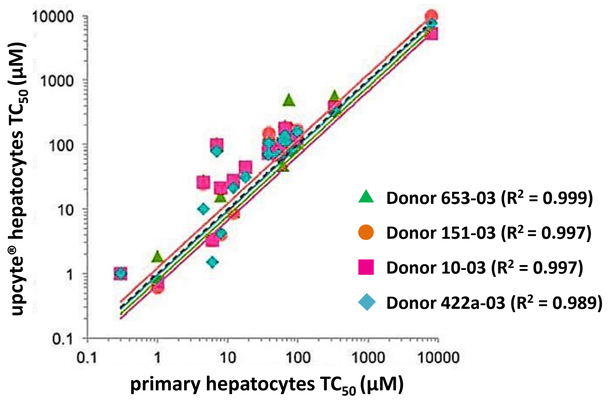
upcyte® hepatocytes were **cultured for 4 days at confluence** and analyzed by immunofluorescence microscopy for expression of the lateral surface marker **E-Cadherin (green)** counter-stained against **DAPI (blue)** and **actin or albumin (red)**. Differentiated cultures after 5 days revealed distinct polarized cell nodules amidst E-Cadherin negative cells. Both polarized and non-polarized cells show strong albumin staining, demonstrating their hepatocyte origin (bar: 50 µm).

suitability of upcyte® hepatocytes for overall toxicity and CK18-based apoptosis assays



TC_{50} comparison *vs* HepG2 and primary hepatocytes

To evaluate the suitability of **upcyte® hepatocytes** for hepatotoxicity assays, we compared respective **TC_{50}** values of six chemical compounds obtained from **dose-response studies** (24 h) with **HepG2 cells** and **primary hepatocytes**. The normalized TC_{50} toxicity profile of upcyte® hepatocytes was **not significantly different from the profile of primary hepatocytes** (p=0.466, n=4), in contrast to the respective HepG2 profile (p=0.030, n=3).



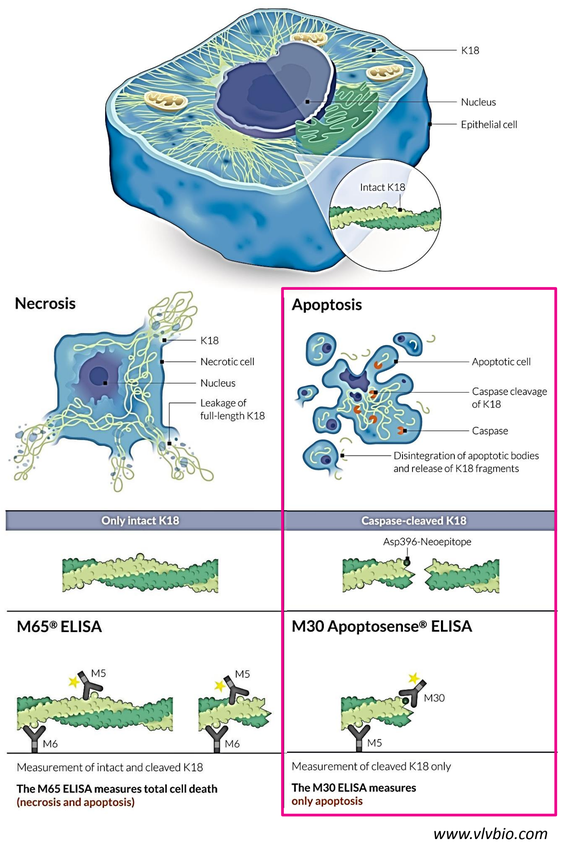
TC_{50} correlation between upcyte® and primary hepatocytes

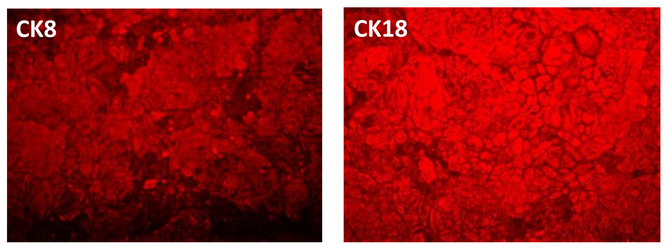
We further compared the **TC_{50} of 18 model compounds** in upcyte® hepatocytes from donors **653-03, 151-03, 10-03 and 422a-03** against TC_{50} values of primary human hepatocytes of 10 donors. Toxicity was measured using the MTS assay. **All donors showed an R^2 correlation of 0.99** (n=3).

Levy *et al.*, (2015) – Nature Biotechnology
long term culture and expansion of primary human hepatocytes The Hebrew University of Jerusalem, Israel / upcyte technologies GmbH

CK18 cleavage status during cell death

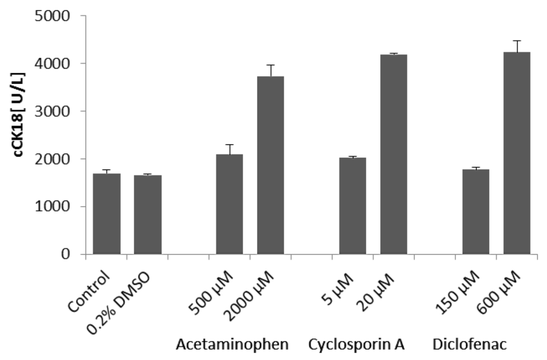
CK18 expressed by epithelial cells constitutes an important stabilizing component of the cytoskeleton. **Apoptosis** leads to activation of **caspases** which in turn **cleave CK18**, resulting in formation of the neoepitope Asp396. This epitope is selectively recognized by the **M30 antibody**. During necrosis, full-length and cleaved CK18 are released, both detectable by the M65 antibody. **VLVbio** offers both M65 and M30 based ELISA kits to detect total CK18 levels or caspase-cleaved CK18, respectively.





upcyte® hepatocytes express full-length CK8/18

upcyte® hepatocytes were previously illustrated to express the characteristic adult marker proteins **serum albumin** and **α-anti-trypsin** while lacking embryonic markers such as α-fetoprotein. Importantly, cells revealed **strong expression of CK8 and CK18**, indicating their potential use for CK18-based apoptosis assays.



drug-induced CK18 cleavage in upcyte® cells

We next investigated **basal and drug-induced cleavage of CK18** in upcyte® cells using the M30 Apoptosense® ELISA. We observed different basal levels of cleaved CK18 (cCK18) in different donors, with **donor 653-03** showing the highest signal-to-noise ratio. The majority of cCK18 was found in the supernatant of cells challenged with hepatotoxic model compounds such as **cyclosporin A** and **diclofenac**. Importantly, toxicity was also observed for substances exerting toxicity after biotransformation, such as **acetaminophen**.

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SUMMARY & CONCLUSION

In conclusion, we developed a comprehensive platform enabling the **controlled expansion of primary hepatocytes** for **up to 40 population doublings**. Importantly, upcyte® cells maintained **many features of primary hepatocytes** such as **phase I and II activities**. upcyte® hepatocytes further revealed marked expression of CK18. Exposure to **established hepatotoxic model compounds** markedly **increased caspase-cleaved CK18 levels** as determined by **M30 Apoptosense® ELISA**. We thus conclude that upcyte® hepatocytes and the M30 Apoptosense® ELISA represent a promising tool for quantification of drug-induced apoptosis, potentially facilitating throughput and reproducibility of cell-based hepatotoxicity assays.