3D and 2D In Vitro Models of Xenobiotic-Induced Hepatotoxicity for Short and Long-term Exposure Studies using iCell Hepatocytes 2.0

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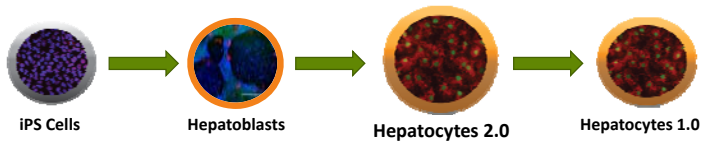
Abstract

Hepatotoxicity is a leading cause of drug withdrawal from the market, and current preclinical models are not sufficiently predictive of drug effects in humans. Causes of hepatotoxicity include intrinsic toxic effects and the enzymatic production of toxic metabolites. Development of more predictive *in vitro* model systems to identify hepatotoxicity early in drug development is critical for decision making and to avoid Drug Induced Liver Injury (DILI) in the clinic. Moreover, batch to batch and donor inconsistencies in primary human hepatocytes, as well as lack of maintained metabolic function have resulted in conflicting reports and poor predicticity. Human induced pluripotent stem cell (iPSC)-derived hepatocytes (iCell® Hepatocytes 2.0) that exhibit high purity and sustained biologically relevant functions help to address some of the needs of hepatotoxicity assessment. Here, we set out to demonstrate the functional utility of iCell Hepatocytes 2.0 (HC 2.0) to assess acute and chronic drug-induced hepatotoxicity. We evaluated HC 2.0 responses to a set of known hepatotoxins (i.e., amiodarone, acetaminophen (APAP), troglitazone, nefazadone, chlorpromazine, and FCCP) across a number of cell death readouts highlighting their capacity for mechanistic toxicity studies. In addition, the prolonged viability also enables chronic dosing *in vitro* affording the potential to detect the effects of slow to form metabolites and also perform analyses at physiologically relevant concentrations over protracted exposure periods. The short term high concentration sensitivities observed were comparable to those seen with primary human hepatocytes. However, effects seen over 48 hr and 7 day dosing are illustrative of the potential of HC 2.0 for predictive *in vivo/in vitro* toxicity correlation. With the ability to routinely access patient specific genotypes and also culture in 3D spheroids and in co-culture with other hepatic stellate cells HC 2.0 provide a biologically relevant human model system for investigating hepatotoxicity in preclinical drug development. These data illustrate how human-based iCell products offer an excellent model system for assessing compound effects in human-derived hepatocytes. In total, iPSC technology enables a reliable and predictive model systems not previously attainable, and provides new solutions, tools, and opportunities for more predictive toxicity testing.

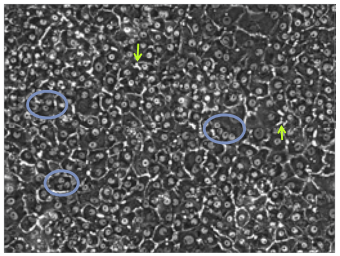
The iCell Family of Hepatocytes

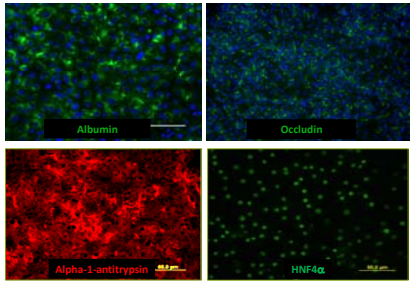


The current iCell Hepatocytes family of products derived from human iPS cells offers three different stages of hepatocyte differentiation and maturity as cryopreserved cells ready to thaw and plate. iCell Hepatoblasts represent a bi-potential cell capable of differentiating into either a hepatocyte or cholangiocyte cell population. iCell Hepatocytes 2.0 and iCell Hepatocytes 1.0 are terminally specified hepatocyte populations. Functionally, HC 1.0 and 2.0 are very similar, with the main difference being that HC 1.0 is a more mature cell type that fully expresses albumin at the time of thaw, whereas HC 2.0 requires 5 to 7 days of culture to express both albumin and alpha-1-antitrypsin (A1AT) at high levels. All data in this poster was generated with Hepatocyte 2.0.

Hepatocyte 2.0 Characterization Data

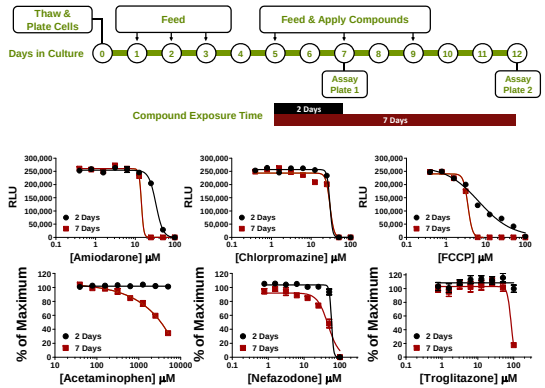
iCell Hepatocytes 2.0 display characteristic hepatocyte morphology, including polygonal shape, evidence of polynucleation (blue circles) and the formation of bile canalicular channels (green arrows). HC 2.0 have been characterized for expression of the liver cell markers including albumin, A1AT, and HNF4α. These cells form tight junctions in culture as evidenced by the expression pattern for occludin staining. Basal P450 functions have been detected in the range of primary human hepatocytes and also express functional transporters (not shown). HC 2.0 are currently employed in ADME-T applications, organotypic culture systems, hepatitis infectivity modeling (HCV and HBV), malaria and NASH disease modeling.

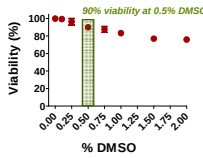




Key Hepatocyte Characteristics	
Morphology	Adherent monolayer; polarized phenotype; functional bile canalicular network; bi-nucleation
Molecular Markers	Albumin; α-1-antitrypsin; HNF family transcription factors
Intrinsic Metabolism	Glycogen storage; lipid metabolism; insulin responsiveness; urea synthesis
Phase I & II Metabolism	-CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4 (basal & induced) - UGT, ST, GSTα activity
Transporter Function	Transport via uptake (e.g. OATP, NTCP) and efflux transporters (e.g. MDR-1/P-gp, BCRP, BSEP, MRP2)
Infectivity	- Expression of hepatitis virus receptors - Viral infectivity (HCV, HBV) -Malaria parasite infection (*P. falciparum; P. vivax*)

2D Culture: Short vs. Long Term Compound Exposure



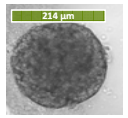


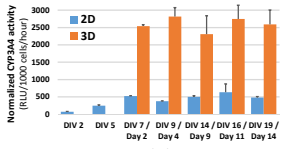
Compound	2 Days	7 Days
Acetaminophen	>5000	3080
Amiodarone	33	14
Chlorpromazine	30	30
FCCP	6.7	3.5
Nefazodone	58	47
Troglitazone	>100	83

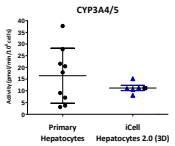
iCell Hepatocytes 2.0 can be stably cultured for more than 2 weeks, which enables their use for evaluation of liver toxicity *in vitro* over short and longer term chronic drug exposure times. In this experiment, cells were treated with compounds and then assayed at earlier (day 7) or later (day 12) time-points. Cell viability was measured using CellTiter-Glo (Promega). Two key observations were made. First, iCell Hepatocytes could tolerate up to 0.5% DMSO (vehicle control) with 90% viability remaining at day 12. Second, longer exposure times usually resulted in more compound toxicity effects (lower EC_{50} values).

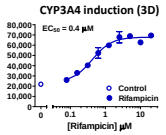
3D Spheroid Culture: Development and Toxicity Assessment

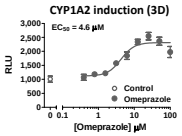
iCell Hepatocytes 2.0 can be adapted to three dimensional (3D) spheroid culture enabling a more tissue-like culture environment. 3D cultures showed improved function versus traditional 2D cultures, including improved basal CYP3A4. Treatment of 3D cultures with Rifampin and Omeprazole further increased CYP3A4 and CYP1A2 activities. 3D liver toxicity assays (CellTiter-Glo 3D, Promega) could be miniaturized to as few as 500 cells per well with robust signals. Spheroid cultures also displayed significant differences in compound sensitivity versus 2D cultures.

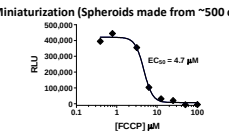


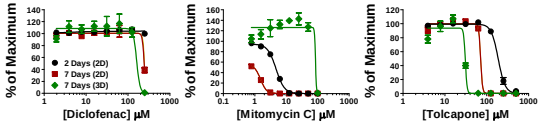












EC_{50} (µM)			
Culture Format:	2D		3D
Compound	2 Days	7 Days	7 Days
Diclofenac	>250	~240	160
Mitomycin C	4.7	1.5	85
Tolcapone	190	71	31

Summary and Conclusions

- The iCell Hepatocyte family of products provides multiple options for the study of hepatic biology
- iCell Hepatocytes 2.0 have characteristic hepatocyte morphology, marker expression and function
- iCell Hepatocytes 2.0 are useful for short and longer-term *in vitro* hepatotoxicity studies
- Three-dimensional (3D) spheroid cultures can be generated from iCell Hepatocytes 2.0 and miniaturized
- 3D cultures provide useful liver tissue-like model systems with increased metabolic function and drug sensitivities versus traditional 2D cultures