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A Novel Hepatocyte-like Cell as an *In Vitro* Screening Tool for Drug ADME/Tox Studies

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Overview

- Renewable hepatic cell model as an alternative to primary human hepatocytes is highly sought-after for drug ADME/Tox applications.
- Corning® HepatoCells are derived from primary human hepatocytes, maintain key functional characteristics of primary hepatocytes, and provide excellent lot-to-lot consistency.
- Corning HepatoCells are applicable for early-stage screening of drug-drug interactions and hepatotoxicity risk.

Introduction

Primary human hepatocytes, the current "Gold Standard" for studying metabolic fate of xenobiotics, bear inherent limitations such as large lot-to-lot variability, short life span in culture, the tendency to dedifferentiate in culture, and limited supply of high quality lots. To overcome these shortcomings, Corning HepatoCells, a renewable source of hepatocyte-like cells, were derived from primary human hepatocytes and were assessed for important ADME/Tox applications. Specifically, HepatoCells were characterized for induction response of cytochrome P450s required by FDA to test, for active expression of functional transporters that are known to be critical for drug clearance, for screening of hepatotoxic compounds in 3D spheroid culture, and for lot-to-lot consistency. The study summarized the evaluation results which demonstrate that Corning HepatoCells represent a reliable and renewable cell-based screening tool amenable for ADME/Tox high throughput screening applications.

Materials and Methods

Materials: Corning Cryopreserved HepatoCells (Cat. No. 354881), Corning Culture Medium for HepatoCells (Cat. No. 354882), Corning BioCoat™ Collagen I-coated microplate (Cat. No. 354407), Corning Ultra-Low Attachment spheroid microplate (Cat. No. 4515 for 96-well, Cat. No. 4516 for 384-well), and Corning Matrigel® matrix (Cat. No. 356237) were from Corning Life Sciences. All the chemicals for induction assays were purchased from Sigma-Aldrich. RNeasy® 96-well kit and DNase I kit (QIAGEN) were used for RNA isolation. Q-PCR master mix, high capacity reverse transcription kit, TaqMan® q-PCR primer sets for CYP1A2, 2B6, and 3A4 were purchased from Life Technologies, Promega CellTiter-Glo® 3D kit for ATP assay was purchased from Promega.

Cell culture and assay: On day 1, cryopreserved Corning HepatoCells were thawed and seeded in the Corning BioCoat Collagen I-coated plate (500,000 cells/well for 24-well plate or 100,000 cells/well for 96-well microplate). Cells were incubated in a 37ºC incubator with 5% CO_2. At 4 to 6 hours after seeding, 0.25 mg/mL cold Corning Matrigel solution was added to overlay the cell monolayer, and cells were returned to the incubator for overnight incubation.

For the induction assay, cells were treated with positive control inducers (10 µM Rifampicin, 50 µM Omeprazole, 1 mM Phenobarbital) or solvent vehicle control (0.1% DMSO) daily with freshly prepared culture medium from day 2 to day 4. On day 5, an enzyme assay was performed by incubating cells with probe substrates (100 µM Phenacetin, 250 µM Bupropion, and 200 µM Testosterone) for 1 hour and followed by RNA isolation. Metabolite formation was analyzed with LC-MS/MS and mRNA expression with qRT-PCR.

For the drug transport assay, media changes were performed daily from day 2 to day 3. On day 4, uptake was initiated by adding HBSS with Ca^{2+} and Mg^{2+} containing substrates or a combination of substrate and inhibitor after cells had been washed twice and pre-incubated with HBSS buffer at 37ºC (or 4ºC for baseline uptake) for 10 minutes. The uptake was terminated at a designated time by adding ice-cold HBSS buffer after removal of the incubation solution. Then, cells were washed twice with ice-cold HBSS buffer, lysed in M-PER for 5 minutes at room temperature. The cell lysates were then analyzed for uptake rate.

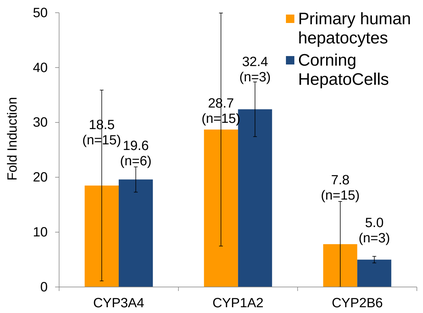
For 3D spheroid culture, cells were seeded in Corning 96-well spheroid microplate with no media change for 2 days to allow spheroid formation. Medium changes were then performed every other day. Upon treatment of cells with compounds for 24 hours, ATP levels were measured for cell viability.

Conclusion

- Corning HepatoCells show induction response to important CYP enzymes with better consistency than primary human hepatocytes.
- Corning HepatoCells retain primary human hepatocyte-like induction regulation pathway and predicted clinical CYP3A4 inducers accurately.
- Corning HepatoCells actively express functional uptake transporters important for drug clearance and drug-drug interaction study.
- Corning HepatoCells form robust 3D spheroids and respond to metabolism-based liver toxins with similar sensitivity to primary human hepatocytes.
- In conclusion, Corning HepatoCells demonstrate applicability as an alternative model to primary human hepatocytes for *in vitro* drug ADME/Tox studies.

Results

Figure 1. Corning HepatoCells Demonstrate Induction Response to Inducers for All 3 CYP Enzymes with Better Consistency than Primary Human Hepatocytes



Parameters	Lot-to-lot Variation (%CV)	
	Corning HepatoCells	Primary Human Hepatocytes
CYP3A4 fold induction	12% (n=6)	94% (n=15)
CYP1A2 fold induction	15% (n=3)	74% (n=15)
CYP2B6 fold induction	12% (n=3)	100% (n=15)

Figure 1. Corning HepatoCells showed comparable CYP induction responses to primary human hepatocytes for all 3 CYP enzymes required by FDA to test, as measured by both enzyme activity and mRNA expression of target gene (data not shown). Corning HepatoCells also show much smaller lot-to-lot variation than primary human hepatocytes.

Figure 2. Corning HepatoCells Demonstrate Primary Human Hepatocyte-like Phenobarbital-responsive Human CAR Nuclear Translocation

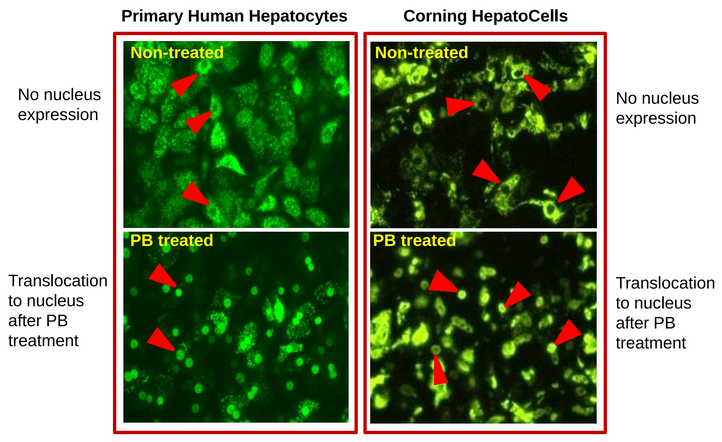
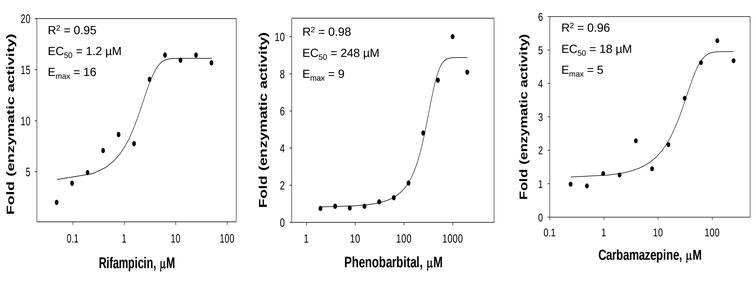


Figure 2. EYFP-hCAR expression and localization in Corning HepatoCells and primary human hepatocytes (data courtesy of Prof. Hongbing Wang, University of Maryland). HepatoCells were cultured for 3 days before infection with Ad/EYFP-hCAR for 24 hours. Infected cells were then treated with 1 mM phenobarbital for 12 hours. Before phenobarbital treatment, hCAR expression as shown by fluorescent signal was mostly in the cytoplasm. Upon phenobarbital treatment, fluorescent signal appeared in nuclei, suggesting phenobarbital responsive hCAR nuclear translocation, which is similar to what is observed in primary human hepatocytes but lost in many hepatic cell lines.

Figure 3. Corning HepatoCells Demonstrate Dose-dependent Response to a Group of Known Clinical CYP3A4 Inducers



Rifampicin induction EC_{50} for Corning HepatoCells and Primary Human Hepatocytes (PHH)

Cell Types	EC_{50}, µM
HepatoCells	0.6 - 1.4
PHH (in house)	0.2 - 1.1
PHH (literature)	0.4 - 1.3

Figure 3. Corning HepatoCells were treated with 13 known clinical CYP3A4 inducers (strong, moderate, weak, and non-inducers). Data shown are examples of strong inducers rifampicin, phenobarbital, and carbamazepine. HepatoCells demonstrated dose-dependent response similar to primary human hepatocytes, as indicated by similar EC_{50} values.

Figure 4. Corning HepatoCells Accurately Predict Clinical CYP3A4 Inducers

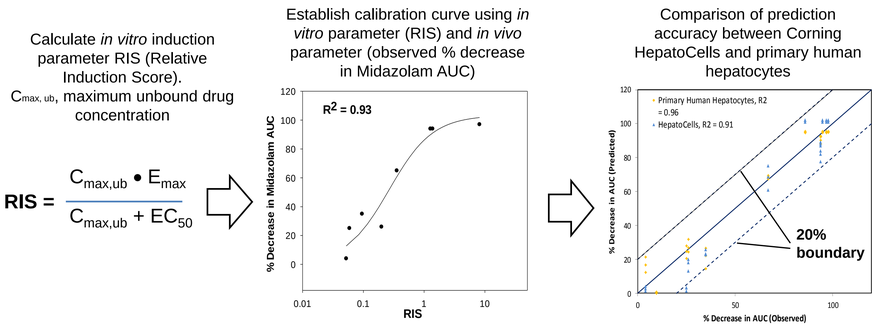
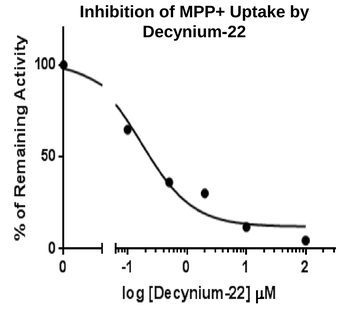


Figure 4. Calibration curve was fitted to a Hill 3 parameter model (SigmaPlot), and the corresponding equation was used to calculate predicted AUC change using *in vitro* induction parameter RIS. The predicted AUC change was then plotted against observed AUC change to determine the accuracy of prediction. Corning HepatoCells have shown similar prediction accuracy as primary human hepatocytes (for both cell models, predicted AUC changes for most of the compounds fall with 20% of prediction).

Figure 5. Corning HepatoCells Actively Express Functional Uptake Transporters

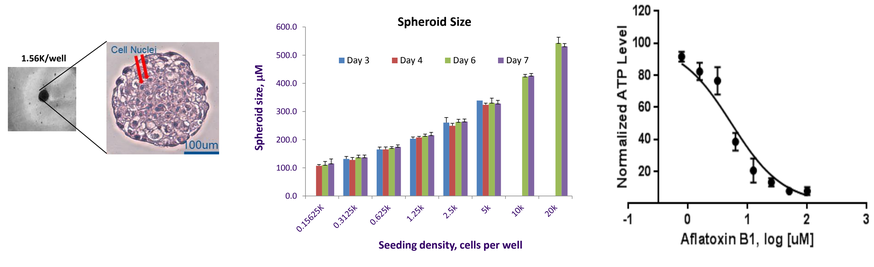
Transporter Involved	Substrate	K_m, µM	Test System
NTCP or OATP1B1/1B3	TCA	8.5	HepatoCells
NTCP or OATP1B1/1B3	TCA	8.2	Human hepatocytes
NTCP	TCA	2.1	HEK
NTCP	TCA	7.5	HeLa
NTCP	TCA	14	TransportoCells
OATP1B3	TCA	5.8	Oocyte
NTCP or OATP1B1/1B3	**Rosuvastatin**	**6.7**	**HepatoCells**
NTCP	Rosuvastatin	6.5	HeLa
OATP1B1	Rosuvastatin	7.3	HeLa
OATP1B3	Rosuvastatin	9.8	HeLa
OATP1B1	Rosuvastatin	0.8	HEK
OATP1B3	Rosuvastatin	14.2	HEK
OCT1	**MPP+**	**75**	**HepatoCells**
OCT1	MPP+	101	Human hepatocytes
OCT1	**TEA**	**1940**	**HepatoCells**
OCT1	TEA	407	Human hepatocytes
OCT1	TEA	713	TransportoCells



Test System	Substrate	IC_{50}
Corning HepatoCells	MPP+	0.3+/-0.04
Corning TransportoCells	MPP+	2.2
X. laevis oocyte	MPP+	4.7
HeLa cell line	TEA	2.7

Figure 5. Uptake assay kinetic values K_m and IC_{50} for hepatic transporters (NTCP, OATP1B1/1B3, and OCT1) are comparable between Corning HepatoCells and existing models including both native system (primary human hepatocytes) and recombinant systems (Corning TransportoCells™), indicating HepatoCells actively express functional uptake transporters important for drug clearance and drug-drug interaction study.

Figure 6. Corning HepatoCells Form Robust 3D Spheroids and Demonstrate Dose-dependent Response to Metabolism-based Liver Toxins



Toxin	EC_{50} (µM) HepatoCells, 3D	Reported EC_{50} (µM) 2D Culture
Aflatoxin B1	5.3	10 (HH)
		>100 (HepG2)
		78.4 (HepatoCells)
Acetaminophen	14,610	28,200 (HH)
		29,755 (HepG2)
Menthol	Non-toxic	Non-toxic

Figure 6. Corning HepatoCells were able to form robust 3D spheroids with consistent size in 96-well or 384-well Corning Ultra-Low Attachment spheroid microplates. Day 7 spheroid cultures (1.5K/spheroid) in 96-well format were treated for 24 hours with known liver toxins and non-toxic control for 24 hours. ATP levels were measured at the end of incubation for cell viability. Non-linear regression and EC_{50} values were calculated using GraphPad. Error bars = standard deviation, n = 3.

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