Abstract

Primary human hepatocytes are considered the "Gold Standard" for studying the metabolic fate of drugs, as they contain the full complement of drug metabolizing enzymes, cofactors, transporters, proteins, and nuclear receptor pathways for regulation of enzyme expression. However, due to large lot to lot variations and limited lot size of primary hepatocytes, significant time and resources are spent on screening a suitable lot (or lots) for desired studies. An alternative model with consistent performance and constant supply will be highly beneficial for in vitro screening and characterization of drug candidates. Here we report the characterization of cryopreserved Corning® HepatoCells (derived from primary human hepatocytes) for applications such as CYP induction, metabolism, and cytotoxicity studies. When cultured on Corning BioCoat® Collagen I-coated tissue culture plates with a Corning Matrigel® matrix overlay, Corning HepatoCells maintained mature hepatocyte morphology very similar to primary hepatocytes. Upon exposure to prototypical inducers (10 μM Rifampicin, 50 μM Omeprazole, 1 μM Phenobarbital) for 3 consecutive days, these cells demonstrated induction responses in CYP3A4, ALB, and S8B comparable to primary human hepatocytes (measured by both enzymatic activities and mRNA levels). Inter-lot and intra-lot variations were assessed, and significantly lower variations were shown than primary human hepatocytes. Corning HepatoCells also demonstrated concentration-dependent response to aflatoxin B1, a metabolism-based hepatotoxic compound; the toxic response was reverted by incubation with CYP3A4 inhibitor ketoconazole. In conclusion, this model represents a novel, renewable source of human hepatocytes with robust and consistent performance for ADME applications such as CYP induction, metabolism, and cytotoxicity.

Materials and Methods

Materials Cryopreserved Corning® HepatoCells, Corning BioCoat Collagen I-coated plates, Corning hepatocyte maintenance medium, Corning Endo-Bin® Brefeldin 200 Solution, Corning Matrigel matrix, Fetal Bovine Serum (FBS), and InstaBlue® Baked Salt solution with CO2 and Mg2+ (4% HESS B) are products from Corning Life Sciences. Mentha94 Protein Extraction Reagent (P-PEX) was purchased from Thermo Scientific. All radiolabeled chemicals were purchased from PerkinElmer or American Radiolabelled Chemicals. All the cold prepared (non-radioactive) and chemically for induction media were purchased from Sigma-Aldrich. Phenobarbital (25 μg/ml), rifampicin (10 μM), and phenytoin (25 μg/ml) were used to treat Corning® HepatoCells. Primary human hepatocytes were cultured on 24-well plates and treated with insulin (10 μg/ml), dexamethasone (25 μg/ml), and hydrocortisone (25 μg/ml) in a transwell system (Corning® Transwell®). Transwell?insert plates were used for each experiment.

Thawing and Plating: On day 1, the cryopreserved HepatoCells were cultured in a 37°C water bath. After removing the cryo-freezing media, the cell pellet was plated in shaking conditions. Then, the cell pellet was sterilized with 0.1% Human Growth Hormone, 0.05% Penicillin and Streptomycin for 5 minutes. The medium was replaced with 1% fetal bovine serum (FBS), 2% glucose, and 1% antibiotic in 100 mm plates. After 3 days, all cells were cultured with Aflatoxin B1 (1 μg/ml) for 24 hours.

Induction Assay: From day 2 to day 4, 5 cells were plated with positive control inducers (10 μM Rifampicin, 50 μM Omeprazole, 1 μM Phenobarbital) or solvent vehicle control (1% DMSO) daily with fresh, pre-warmed culture medium. On day 5, perform enzyme assay with probe substrates (100 μM Phenacetin, 250 μM Bupropion, 100 μM Timorenzine) and RNA isolation. Analysis metabolite formation with LC-HRMS and mRNA expression with qRT-PCR.

Toxicity Assay: HepatoCells plates were plated and Matrigel matrix overlay as described above in BioCoat Collagen I-coated 96-well plate. A medium change was performed on day 2. On day 4, pre-microwell of the plate with 20μL Aflatoxin B1 for 3 hours. After pre-treatment, all cells were rinsed with different aflatoxins B1 concentrations for 24 hours. On the next day, all cells were washed 4× with PBS, and cell viability was assayed using Promega CellTiter-Blue® ATP assay kit.

Spheroid Formation: Corning 16-well Ultra-Low Attachment surface spheroids were used to culture HepatoCells into single spheroids at indicated plating cell density (20 μL/mL). Half medium change was performed every other day. Cultures of spheroids were taken on day 7 with Olympus (IX). The diameter of individual spheroids were measured with QImage imaging software.

Table 1. Genotype for Cytochrome P450 (CYP) Polymorphisms

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CYP2A6*1/*1</th>
<th>CYP2A6*1/*3</th>
<th>CYP2C8*1/*1</th>
<th>CYP2C9*1/*2</th>
<th>CYP2C9*1/*3</th>
<th>CYP2C9*1/*10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
</tbody>
</table>

Conclusions

Cryopreserved Corning® HepatoCells showed 90% post thawing survival, ≥90% cell recovery, and mature hepatocyte morphology. Demonstrated robust fold induction (10-30X) for CYP3A4 and CYP3A2. Consistent intra-lot, inter-lot, and lab-to-lab performance for CYP induction assays with fold induction changes comparable to primary human hepatocytes. HepatoCells showed dose-dependent response to metabolism-based toxic compounds which can be reversed by CYP3A4 inhibition. HepatoCells can maintain hepatocyte morphology and monolayer for at least 2 weeks. HepatoCells can form spheroids with cell number dependent size. HepatoCells are a very promising system for convenient in vitro ADME/Tox research and other liver research applications.

Figure 1: Cell Culture and Morphology of Corning® HepatoCells

Figure 2: CYP3A4, ALB, and S8B in Corning® HepatoCells

Figure 3: Robust Intra-lot, Inter-lot, and Lab-to-Lab Consistency

Figure 4: The Dose-dependent Toxicity of Aflatoxin B1

Figure 5: Corning® HepatoCells for Multiple Application Endpoints

5A. Phenobarbital responsive CYP3A4 induction observed in Corning HepatoCells and Primary human hepatocytes

5B. Spheroid modeling

Figure 6: Aflatoxin B1 induction of spheroids

Figure 7: Aflatoxin B1 induction of spheroids

Figure 8: Aflatoxin B1 induction of spheroids

Figure 9: Aflatoxin B1 induction of spheroids

Figure 10: Aflatoxin B1 induction of spheroids