

High Content Screening of Corning® HepatoCells for Hepatotoxicity

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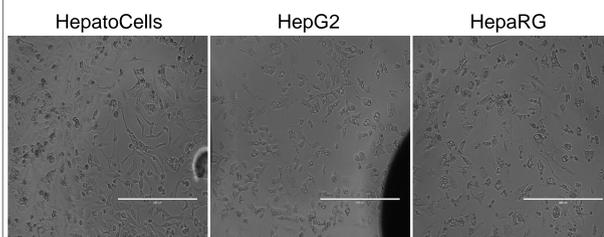
Abstract

Revealing compounds that have a negative impact on the liver early in a drug development campaign is important in drug discovery. Drug induced liver injury is one of the most cited causes of drug candidate failure, and is often the reason for withdrawal of approved drugs from the market¹. Therefore, having the correct model system and assay for screening is extremely important for detecting possible hepatotoxins. Corning HepatoCells are an immortalized alternative to primary human hepatocytes, that still retain the metabolic and enzymatic functionality of primary human hepatocytes, but without the lot-to-lot variability and reliance on human donors. Here we demonstrate how Corning HepatoCells can be a utilized to discover hepatotoxins by way of multiparameter high content screening analyses. Specifically, lipid accumulation and mitochondrial membrane potential loss were examined after exposure to a variety of known hepatotoxic compounds. High content analysis of hepatotoxicity using Corning HepatoCells plated in Corning BioCoat™ Collagen I High Content Imaging Microplates, was compared to that of alternative immortalized hepatocyte cells. These results indicate that Corning HepatoCells, together with Corning High Content Imaging Microplates, are powerful tools for reliable and reproducible *in vitro* hepatotoxicity screening to predict liver injury.

Methods

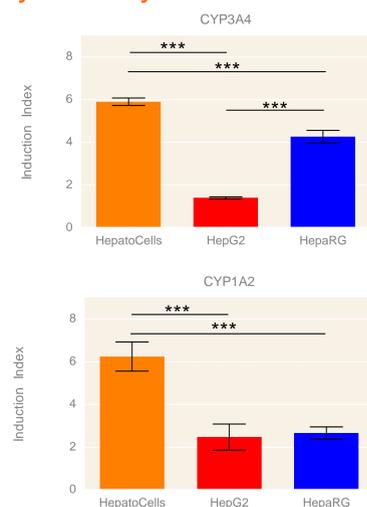
For CYP induction and bile salt transport studies, HepatoCells (Corning 354881), HepG2 cells (ATCC® HB-8065), and HepaRG™ cells (Life Technologies HPRGC10) were seeded in 96 well Corning® BioCoat™ Collagen I High Content Imaging Glass Bottom Microplates (Corning 4582) at 250,000, 100,000, and 312,500 cells/cm², respectively. HepatoCells and HepG2 cells were seeded using Corning® Culture Medium for HepatoCells (Corning 354882) containing 10% fetal bovine serum (Corning 35-010-CV). HepaRG were seeded using William's medium (Life Technologies 12551-032) supplemented to 1x with HepaRG Thaw, Plate & General Purpose Medium Supplement (Life Technologies HPRG670). Four to six hours after seeding a 0.25 mg/mL Corning® Matrigel® (Corning 356237) overlay was added. For CYP induction assays medium was changed after 24 hours to contain 10 μM rifampicin, 50 μM omeprazole, or 0.1% DMSO in either serum free Corning Culture Medium for HepatoCells or William's medium supplemented to 1x with HepaRG induction Medium Supplement (Life Technologies HPRG640). Media was changed every day for 3 days. CYP induction was quantified using Promega's P450-Glo™ Assays (V9002 and V8422). For high content imaging studies HepatoCells, HepG2 cells and HepaRG cells were seeded in 384 well Corning BioCoat Collagen I High Content Imaging Glass Bottom Microplates (Corning 4583) at 75,000 cells/cm² for HepatoCells and HepaRG. HepG2 cells were seeded 24 hours later at 40,000 cells/cm². HepatoCells and HepG2 cells were seeded using Corning Culture Medium for HepatoCells containing 10% fetal bovine serum. HepaRG were seeded using William's medium supplemented to 1x with HepaRG™ Thaw, Plate & General Purpose Medium Supplement and 1x GlutaMAX. HepaRG medium was changed to William's medium supplemented containing 1x Tox Medium Supplement (Life Technologies HPRG630) and 1x GlutaMAX 24 hours after seeding. Various hepatotoxic compounds were added 48 hours after seeding HepatoCells and HepaRG cells or 24 hours after seeding HepG2 cells. Simultaneously, HCS LipidTOX™ Red Phospholipidosis Detection Reagent (Molecular Probes H34351) was added for assessing increases in phospholipid staining. After a 48 hours cells were also stained for mitochondrial membrane potential loss via Molecular Probe's HCS Mitochondrial Health Kit (H10295) and increases in neutral lipid staining with HCS LipidTOX Green Phospholipidosis Detection Reagent (Molecular Probes H34350). High content screening was conducted with the Thermo Scientific CellInsight using 20x objective.

Cell Morphology



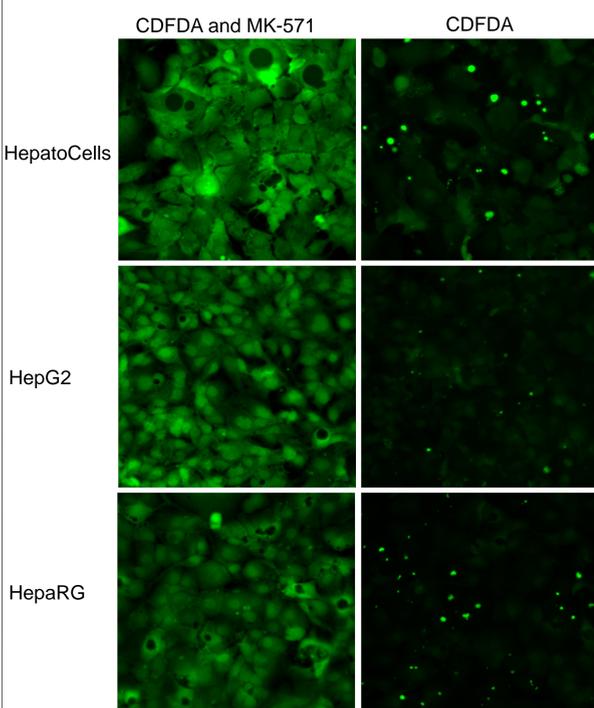
HepatoCells, HepG2 cells, and HepaRG cells seeded onto Corning BioCoat Collagen I coated microplates at 75,000 cells/cm² for HepatoCells and HepaRG, and 40,000 cells/cm² for HepG2. 100x total magnification.

CYP Enzyme Activity



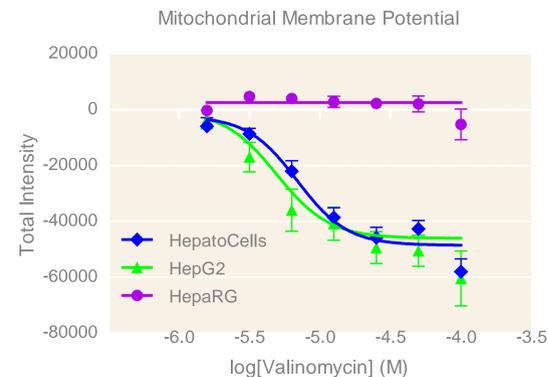
Induction index after 3 days of exposure to 10 μM rifampicin (CYP3A4) and 50 μM omeprazole (CYP1A2). N= 56 wells from 2 independent studies, *** p<0.0001 (One way ANOVA with Newman-Keuls Multiple Comparison Test)

Bile Salt Transport

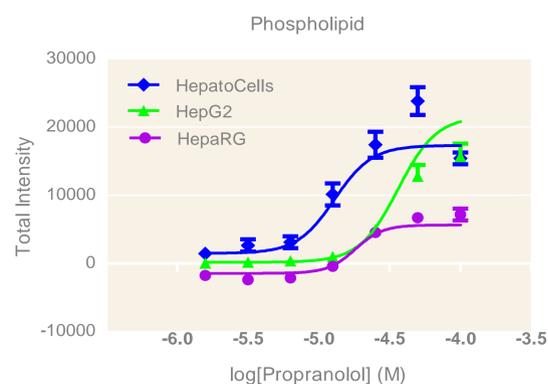


Representative photomicrographs of functional bile canaliculi of HepatoCells, HepG2, and HepaRG cells after exposure to 5 μM 5(6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) (Sigma 21884) or 5 μM CDFDA and 50 μM MK-571 (Sigma M7571). Note the lower appearance of punctate staining with the HepG2 cells.

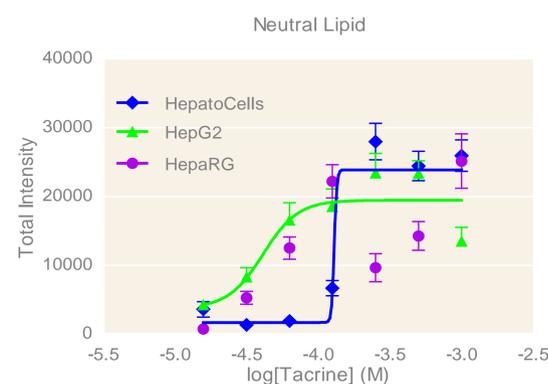
Hepatotoxicity Screen



Dose-dependent mitochondrial membrane potential (red) intensity loss of cells exposed to various concentrations of valinomycin, a drug known to depolarize mitochondria. Nuclei counterstained with hoechst 33342 (blue). N = 16 wells from 2 independent studies.



Dose-dependent increase of phospholipid staining (red) intensity of cells exposed to propranolol, a known phospholipidosis inducer. Nuclei counterstained with hoechst 33342 (blue). N = 16 wells from 2 independent studies.



Dose-dependent increase of neutral lipid staining (green) intensity of cells exposed to tacrine, a known hepatotoxin. Nuclei counterstained with hoechst 33342 (blue). N = 16 wells from 2 independent studies.

Summary/Conclusions

- Corning® BioCoat™ Collagen I High Content Imaging Glass Bottom Microplates, together with Corning HepatoCells are for an ideal tool for screening hepatotoxic compounds using high content imaging analyses.
- Corning HepatoCells demonstrated improved CYP enzyme activity over HepG2 and HepaRG when induced by rifampicin and omeprazole.
- Corning HepatoCells form bile canaliculi, and efflux is repressed by a known MRP2 inhibitor, suggesting active efflux transporter expression.

