Three Dimensional Hepatotoxicity Screening using Corning® HepatoCells, the Spheroid Microplate, and the SCREEN-WELL® Hepatotoxicity Library

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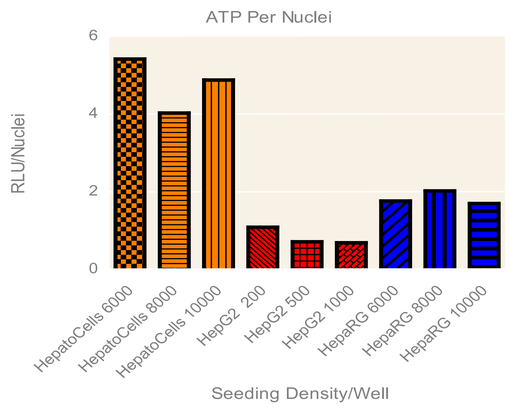
Abstract

Having the right model for drug screening is essential for predicting compounds that may cause drug-induced liver injury. Three dimensional (3D) models offer significant improvements over traditional two dimensional monolayer cell culture in terms of maintaining morphological and functional characteristics of tissue, and may provide a better representation of *in vitro* drug toxicity[1]. Here we demonstrate how Corning® HepatoCells, an immortalized alternative to primary human hepatocytes, in conjunction with Corning spheroid microplates can be a utilized for a 3D drug screen to discover potential hepatotoxins. Hepatospheres formed using Corning HepatoCells were compared to spheroids formed using alternative immortalized hepatocyte cells. Cell viability, urea, and albumin were used to assess hepatotoxicity of the hepatospheres after exposure to the SCREEN-WELL® Hepatotoxicity library from Enzo Life Sciences, a library consisting of 238 compounds with a variety of structurally and mechanistically different compound classes, as well as nontoxic controls. Selected hits identified in the 3D screen, which significantly reduced cell viability of the hepatospheres, were then assessed in a dose-dependent manner for potency analysis. These results demonstrate that Corning HepatoCells, together with Corning spheroid microplates, are powerful tools that can be used for reliable and reproducible 3D hepatotoxicity screening.

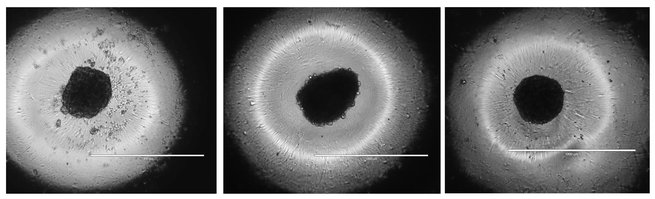
Spheroid Size Optimization

Corning HepatoCells (Corning Cat. No. 354881), HepG2 cells (ATCC® Cat. No. HB-8065), and HepaRG™ cells (Life Technologies Cat. No. HPRGC10) were seeded at various concentrations in 384-well spheroid microplates (Corning Cat. No. 3830) to optimize for the subsequent screens. HepatoCells and HepG2 cells were seeded using 50 µL HepatoCells maintenance medium (Corning Cat. No. 354882) containing 10% fetal bovine serum (Corning Cat. No. 35-010-CV). HepaRG cells were seeded using William's medium (Life Technologies Cat. No. 12551-032) supplemented to 1x with HepaRG™ Thaw, Plate & General Purpose Medium Supplement (Life Technologies Cat. No. HPRG670) and 1x GlutaMAX™ (Life Technologies Cat. No. 35050-061). Medium was changed every other day using HepatoCells maintenance medium without serum for HepatoCells and HepG2, and William's medium supplemented to 1x with HepaRG Tox Medium Supplement (Life Technologies Cat. No. HPRG630) and 1x GlutaMAX. On day 7 spheroids were either lysed for nuclei enumeration, assessed for viability using CellTiter-Glo® 3D (Promega Cat. No. 354882) or fixed in 4% paraformal-dehyde (Boston BioProducts Cat. No. BM-155) for cryostat sectioning and H&E staining (carried out at the University of New England, Biddeford, Maine). The Enzo Screen-Well Hepatotoxicity Library (Enzo Cat. No. BML-2851) and subsequent potency analysis compounds were added on day 7 and cultured for an additional 48 hours. Prior to addition of 30 µL of CellTiter-Glo for viability analysis, medium samples were taken to quantify albumin and urea production. Albumin secretion was quantified using an ELISA for human albumin (Abnova Cat. No. KA0454). Urea production was analyzed via colorimetric urea assay kit (BioAssay Systems Cat. No. DIUR-500).

Spheroid Formation



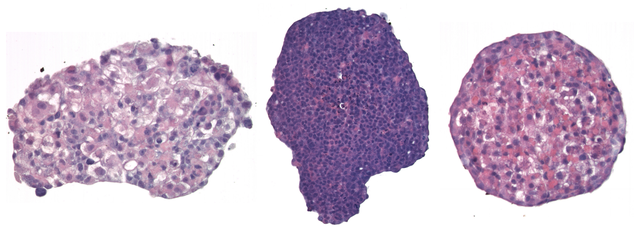
Ratio of relative luminescence units (RLU) to nuclei counts for seeding concentration optimization. Seeding densities were chosen to generate high luminescent signals for screening while avoiding necrotic core formation.



Corning HepatoCells HepG2 HepaRG

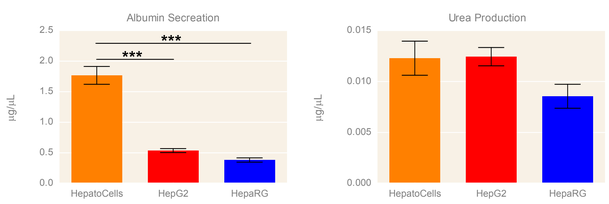
Representative photomicrographs of Corning HepatoCells, HepG2, and HepaRG spheroids after 7 days seeded at 6,000 cells/well, 200 cells/well, and 6,000 cells/well respectively (40X).

Hepatosphere Functionality



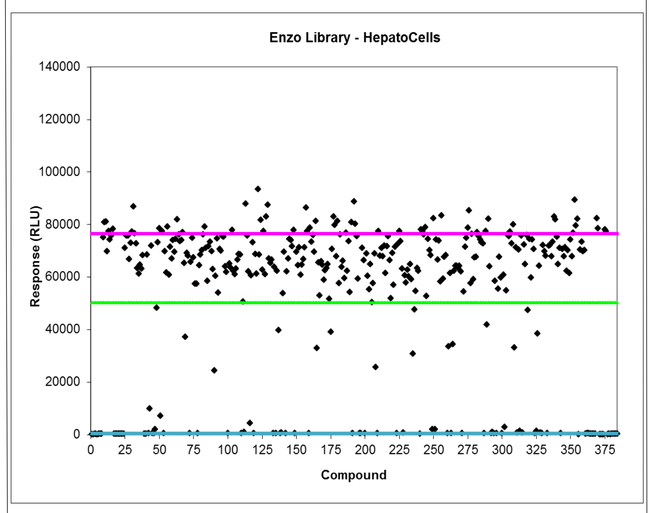
Corning HepatoCells HepG2 HepaRG

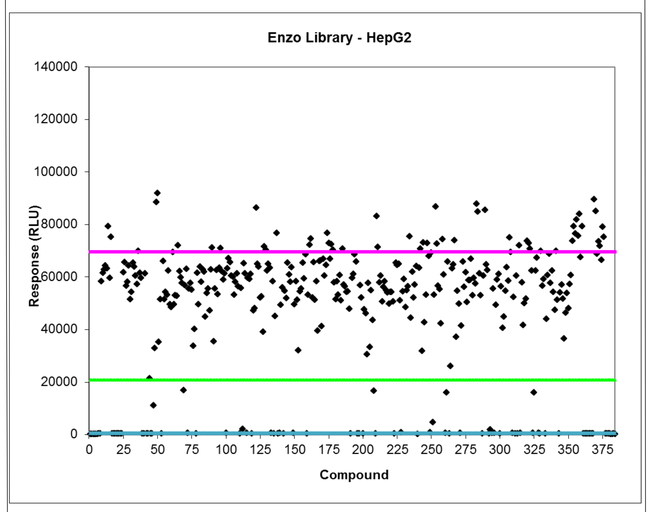
Representative H&E stained hepatospheres after 7 days of culture.

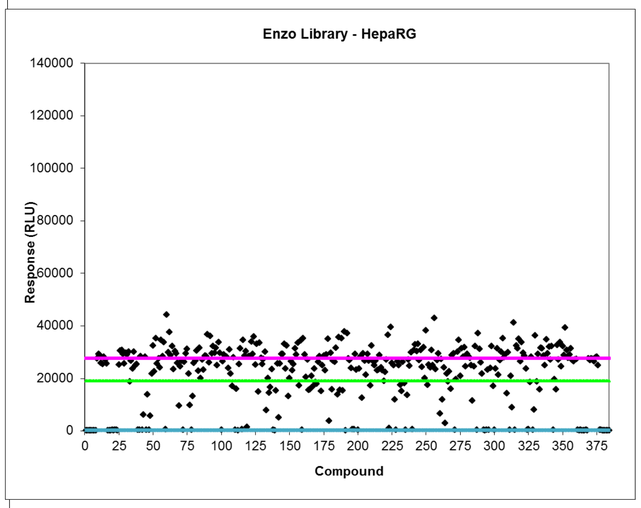


Hepatotoxicity Screen

Representative SCREEN-WELL hepatotoxicity library results for each cell. Pink line is mean response of non responders, blue line is average chlorpromazine (control) response, and anything below green line is considered a hit (>5 σ below buffer response).







Results of screen indicate many hepatotoxic hits were observed in all three cell types, highlighted by compounds displaying luminescent signal below green line on each plot. Of note, Corning HepatoCells display a much larger signal window than HepaRG cells. Z' = 0.79, 0.57, and 0.80 for HepatoCells, HepG2, and HepaRG, respectively.

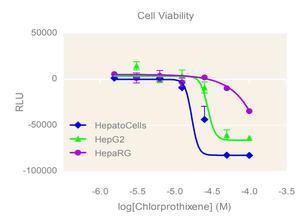
Hit Analysis

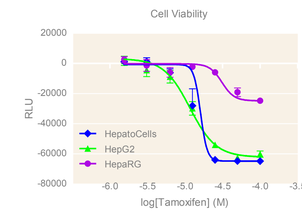
	Corning HepatoCells	HepG2	HepaRG
Lipid accumulation	7	7	7
Cholestatic effects	6	8	7
Mitochondrial toxicity	6	7	4
Toxic metabolites	3	5	5
Mallory body formation	2	2	1
Elevation of liver enzymes	22	25	16
Inhibition of BSEP	2	3	1

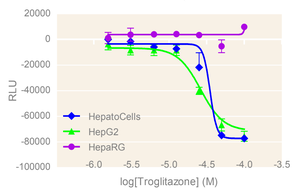
Representative SCREEN-WELL hepatotoxicity library results from 4 independent studies. Hits were considered valid if >5 σ below buffer response in at least 3 separate screens.

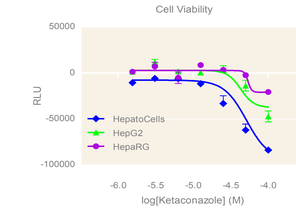
Potency Analysis of Selected Hits

Potency analysis of selected compounds from the SCREEN-WELL hepatotoxicity library as assessed by CellTiter-Glo 3D. N = 8 wells per concentration. Corning HepatoCells were equally or more sensitive to toxic compounds than either HepG2 or HepaRG when assessed in a dose-dependent fashion.









TC_{50} Values	Chlorprothixene	Tamoxifen	Troglitazone	Ketaconazole
HepatoCells	1.696e-005	1.611e-005	3.517e-005	5.12e5-005
HepG2	2.781e-005	1.164e-005	2.637e-005	4.289e-005
HepaRG	2.095e-002	3.110e-005	1.832e-004	5.380e-005

Summary/Conclusions

- Corning spheroid microplates allow for the formation of consistent sized, single spheroids in each well, available in both 96- and 384-well formats, making them a useful tool for 3D screening.
- Hepatospheres formed using the Corning spheroid microplate are amenable to histological analysis.
- The opaque walls and clear, round well-bottom of the Corning spheroid microplates allow for luminescent assays to be conducted right in the plate without the need for a transfer step.
- Corning HepatoCells offer an ideal terminally differentiated hepatic cell alternative to commonly used HepaRG cells, offering a larger assay window for luminescent ATP assays, which is amenable to screening.
- Corning HepatoCells displayed increased or equivalent sensitivity to known hepatotoxins chlorprothixene, tamoxifen, troglitazone, and ketaconazole upon potency analysis, compared to HepG2 and HepaRG cells.