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In Vitro Primary Human Hepatocyte 3D Spheroid Model for Hepatotoxicity Studies

Poster #53

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Abstract

Drug induced liver injury (DILI) remains a leading cause for drug development failure and costly post-marketing withdrawal or black box labeling. Accumulative evidence in the literature indicates that *in vitro* 3D liver models are better at mimicking human liver and more physiologically relevant than conventional 2D culture models. Spheroids made from primary human hepatocytes (PHHs) are one of the best characterized 3D models with great potential in applications such as drug metabolism, liver toxicity, as well as liver disease modeling. In this presentation, we describe a spheroid culture procedure with PHHs using Corning® spheroid microplates with an Ultra-Low attachment surface. Our results demonstrate that suitable PHH lots for 3D culture need to be pre-tested prior to their utilization for various applications. Characterization data show that major drug metabolic enzyme activity (e.g., CYP3A4) is sustained over long-term spheroid culture. A comparative (2D monolayer vs. 3D liver spheroids) hepatotoxicity study was performed with 100 known DILI and control compounds. Our results show the feasibility of conducting hepatotoxicity screening using 3D liver spheroids. Data analysis including IC_{50}, C_{max}, and margin of safety (MOS) threshold demonstrated the superior performance of liver spheroids for better prediction (2- to 3- fold more sensitive) of liver liability than 2D PHH culture.

Key Words: 3D Spheroid; Drug-induced Liver Injury; Primary Human Hepatocytes

Introduction

- 3D spheroid culture of primary human hepatocytes (PHHs) maintains *in vivo*-like phenotypes and cell viability in long-term cell culture; accumulative evidence from literature have shown that liver spheroids closely resemble the native liver (e.g., protein and gene expression).[1,2,3]
- Morphological and functional characterizations of liver spheroids made from multiple lots of PHHs indicate that these robust 3D cultures could be used for various drug discovery and development studies.
- To validate the application of liver spheroids for liver toxicity testing, a comparative study with both PHH 3D liver spheroids and 2D culture was done with a selection of 100 known DILI and control compounds.[4,5] Using bioluminescent-based ATP measurement as a readout, our results showed that PHH spheroids are superior to 2D culture to detect DILI compound induced cytotoxicity.

Results

Figure 1. 3D Liver Spheroid Culture Using Corning Spheroid Microplates

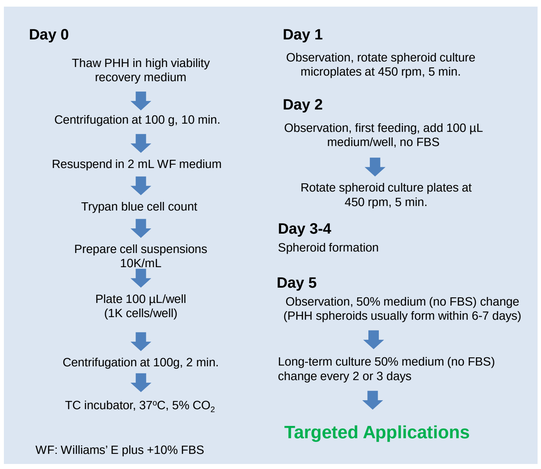

Figure 2. Size and Morphology of Liver Spheroids Remain Stable During Long-term Culture

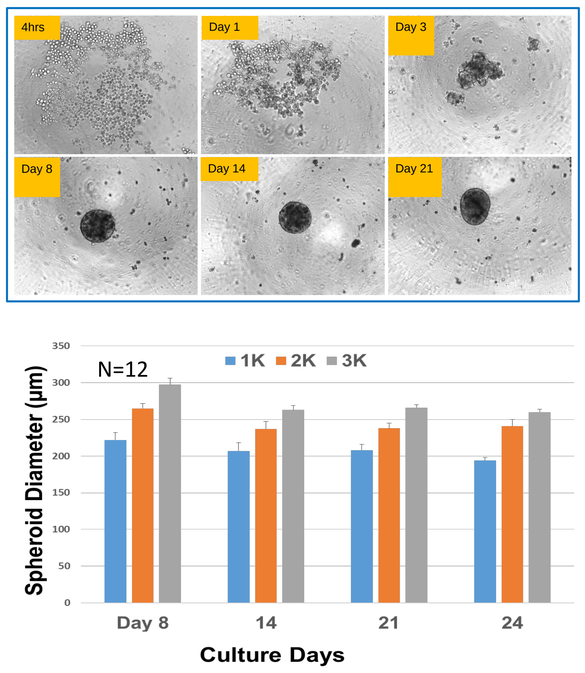

Figure 2. Liver 3D spheroid culture was set up using different seeding cell numbers of PHHs at 1K, 2K, or 3K per well on a 96-well Corning Spheroid Microplate (bar graph). Once the spheroid formed within a well on a Corning 96-well spheroid microplate, the spheroid sizes were measured over a time course of 4 weeks. Representative images (1K cells/well) were taken at the indicated time points.

Figure 3A. ATP Measurement of Day 7 PHH Spheroids

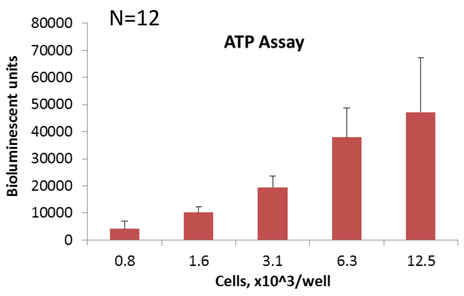

Figure 3B. H&E Staining of Day 8 PHH Spheroid (1000 cells/spheroid)

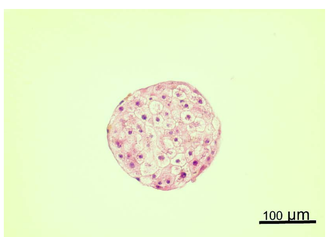

Figure 3C. Albumin Secretion of PHH Spheroids

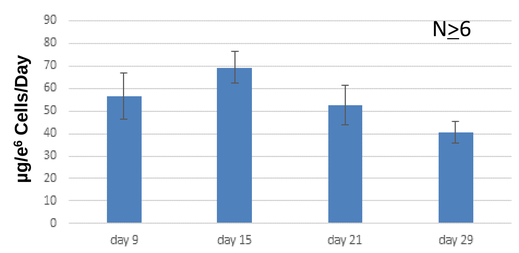

Figure 3. ATP levels of day 7 spheroids made at different seeding cell densities were measured using bioluminescent ATP assay (3A). Single spheroids were made at a lower seeding density (<5000 cells/well). Day 8 PHH spheroids (1000 cells/spheroid) were fixed in 4% PFA prior to paraffin embedding and section. H&E staining show intact core structure of a PHH spheroid (3B). Albumin secretion was monitored by ELISA assay over a 4-week culture of PHH spheroids (1000 cells/spheroid, 3C).

Figure 4.Corning Cryopreserved PHH Lots Tested for 3D Spheroid Culture

Cat. No.	Description	Lots Tested	Spheroid Forming Lots	Ranking	Spheroid Formation	Spheroid Stability
454541	Plateable, Transporter-qualified (≥5 million cells)	342, 345, 380, 393A, 393B, 396, 399, 404, 405A,	342 345 380 393A 399 404	+++ ++ ++++ +++ ++++ ++++	Day 6-8 Day 4-5 Day 6-8 Day 6-8 Day 4-6 Day 5-6	≥3 weeks Die in the 2nd week ≥3 weeks ≥3 weeks ≥3 weeks ≥3 weeks
454543	Plateable, Metabolism-qualified (≥5 million cells)	303, 304, 330, 358, 397, 410, 416	303 304 330 397 410	++++ ++ +++ ++++ ++	Day 6-8 Day 5-6 Day 8-10 Day 5-6 Day 8-10	≥3 weeks ~2 weeks ≥3 weeks ≥3 weeks ~2 weeks
454550	Inducible-qualified (2-5 million cells)	299, 338, 348A, 411A, 412	299 338 348A	+++ ++ ++++	Day 8-10 Day 4-5 Day 5-6	≥3 weeks ~2 weeks ≥3 weeks
454551	Inducible-qualified (≥5 million cells)	314, 336, 337A, 349, 363, 375, 385, 391, 395	336 349 363 391	+++ +++ +++ ++	Day 6-8 Day 3-4 Day 8-10 Day 5-6	≥ 3 weeks ~2 weeks ≥2 weeks ~2 weeks
454426	Suspension Assays, Transporter-qualified	370	No Spheroid	N/A	N/A	N/A

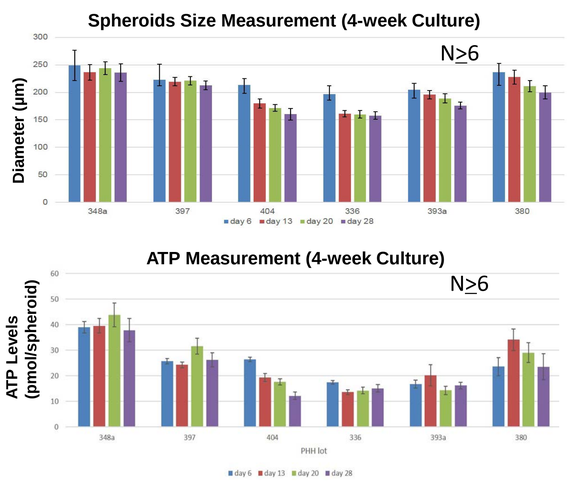

Figure 4. Cryopreserved PHH lots (Corning Gentest®) qualified for different applications were screened for 3D spheroid culture using 96-well Corning spheroid microplates. Both spheroid formation and spheroid stability were monitored over 4 weeks. Spheroid sizes and ATP levels were measured for spheroid cultures of six lots of PHHs.

Figure 5A. Selection of Testing Compounds and Dosing Regimen for 2D Culture or 3D PHH Spheroids

DILI Severity Category	No. of Compounds
1. Severe clinical DILI	17
2. High clinical DILI concern	22
3. Low clinical DILI concern	24
4. Enzyme Elevations in Clinic	17
5. No DILI	20
Total	100

DILI compounds = Categories 1, 2, and 3

Control compounds = Categories 4 and 5

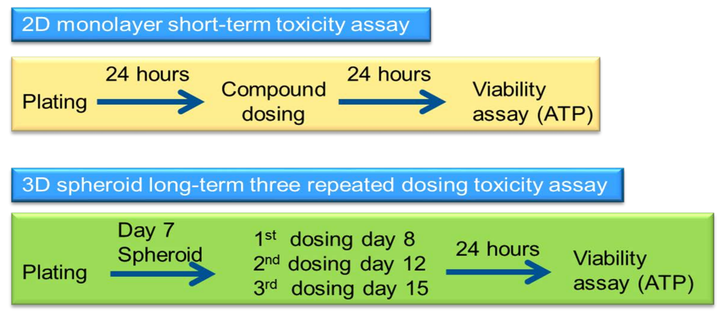

Figure 5B. PHH Spheroid Dose Response to Amiodarone

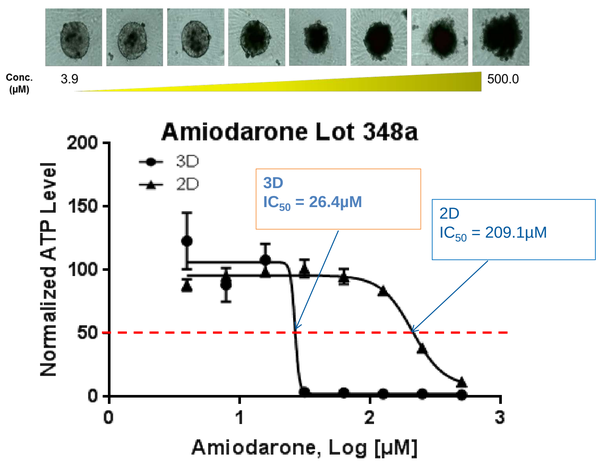

Figure 5. Selection of DILI and control compounds for hepatotoxicity assays comparing 3D and 2D PHH cultures (5A). Serial dilutions of test compounds were prepared and applied according to the dosing regimen for either 2D monolayer or 3D spheroid cultures. 5Bb. Liver spheroids were treated with a serial dilution of amiodarone at indicated concentration. Morphological changes indicated the loss of spheroid integrity and cell death. From dose response curves generated with both 2D monolayer and 3D PHH spheroids, IC_{50} values were calculated.

Figure 6. 3D PHH Liver Spheroids Show Superior Sensitivity to DILI Compound Treatment

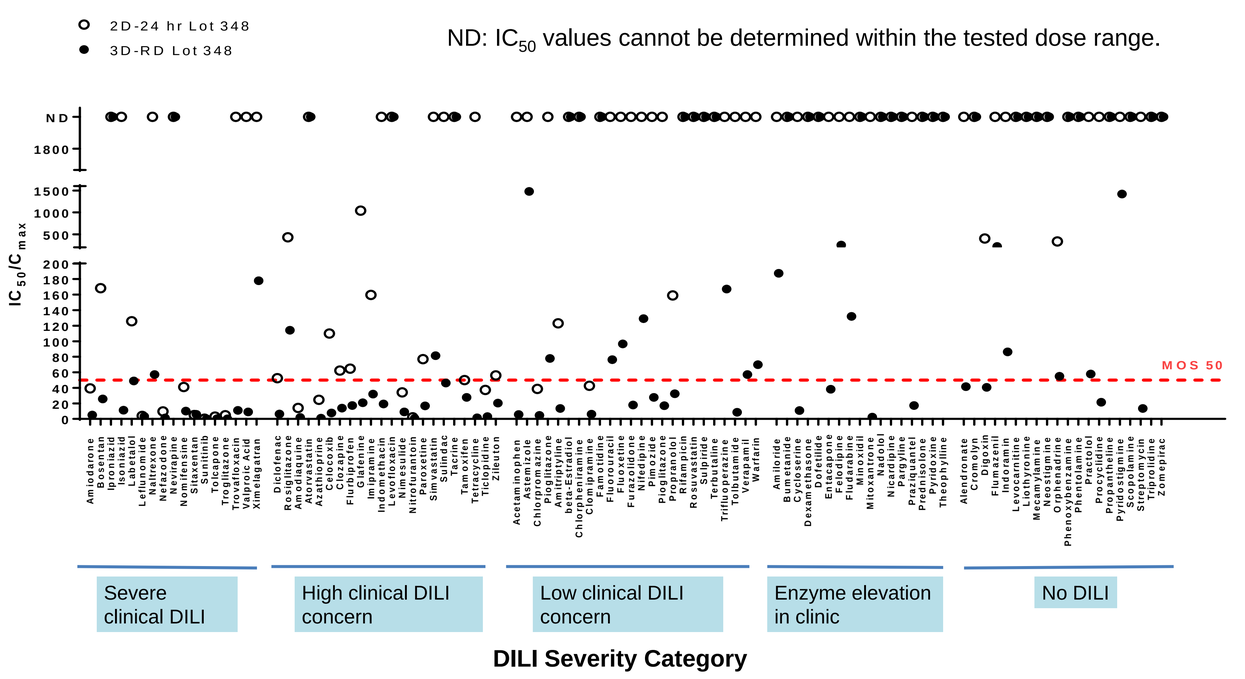

Figure 7. 2D and 3D Hepatotoxicity Assay Performance Assessment with Margin of Safety (MOS) Analysis

2D Hepatotoxicity Assay; IC_{50}/C_{max} (MOS) 50

DILI Severity Category	No. of Compounds	TP	TN	FP	FN
1. Severe clinical DILI	17	7	0	0	10
2. High clinical DILI concern	22	6	0	0	16
3. Low clinical DILI concern	24	2	0	0	22
4. Enzyme Elevations in Clinic	17	0	17	0	0
5. No DILI	20	0	20	0	0
Total	100	15	37	0	48

3D Hepatotoxicity Assay; IC_{50}/C_{max} (MOS) 50

DILI Severity Category	No. of Compounds	TP	TN	FP	FN
1. Severe clinical DILI	17	13	0	0	4
2. High clinical DILI concern	22	17	0	0	5
3. Low clinical DILI concern	24	9	0	0	15
4. Enzyme Elevations in Clinic	17	0	14	3	0
5. No DILI	20	0	17	3	0
Total	100	39	31	6	24

MOS Threshold	10X		25X		50X	
Assay	2D	3D	2D	3D	2D	3D
Sensitivity	11%	32%	14%	51%	24%	62%
Specificity	100%	95%	100%	86%	100%	84%
Positive predictive value	100%	91%	100%	86%	100%	87%
Negative predictive value	40%	45%	41%	51%	44%	56%

Figure 7. IC_{50} values measured from 2D and 3D hepatotoxicity assays are corrected with clinical C_{max} (not shown) for 100 tested drugs that belong to five DILI severity categories. Margin of Safety ratio (IC_{50}/C_{max}) for each tested compound is plotted in the graph above and the dotted red line shows the threshold of 50X MOS. Based on the DILI category and 50X MOS threshold, the resulting numbers of True Positive (TP), True Negative (TN), False Positive (FP), or False Negative (FN) predictions for each DILI category are summarized in the table for both 2D and 3D hepatotoxicity studies. To compare the performance for 2D and 3D liver spheroids, assay sensitivity and specificity are calculated using MOS thresholds 10X, 25X, and 50X, respectively. Sensitivity = TP/(TP+FN); Specificity = TN/(FP+TN); Positive predictive value = TP/(TP+FP); Negative predictive value = TN/(TN+FN).

Conclusion

- Using Corning Spheroid Microplates, we have developed a 3D liver spheroid culture protocol with qualified Corning Gentest Cryopreserved PHHs (96-well or 384-well formats, Cat. No. 4515 or 4516), which allows single spheroid formation per well and easy adaptation to cost effective in-house operations.
- We have completed a comparative hepatotoxicity study with 100 DILI and control compounds.
- Applying different MOS thresholds, statistical analysis demonstrated that PHH 3D liver spheroids are 2- to 3-fold more sensitive than 2D monolayer for predicting known DILI compounds.
- Together, these results demonstrated the feasibility and superior performance of using PHH 3D spheroids as an advanced model for hepatotoxicity studies with high throughput capacity.

References

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