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Adaptation of Corning® HepatoCells for 3D Spheroid Culture and Hepatotoxicity Studies

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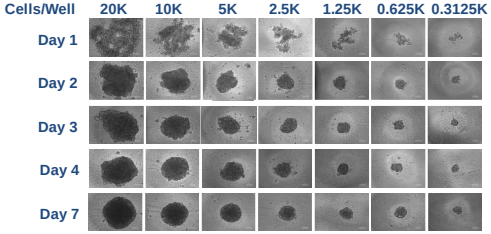
Introduction

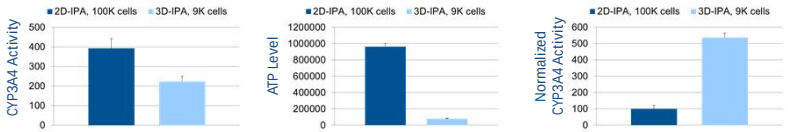
- *In vitro* hepatic 3D cultures are known to better reflect the *in vivo* behaviors of liver cells; Industry is paying increasing attention to 3D *in vitro* systems.
- Corning® HepatoCells **(Cat. No. 354881)** are a renewable hepatic cell source derived from primary human hepatocytes with consistent phenotypes, drug metabolism enzyme/transporter activities.
- Adaptation of HepatoCells to spheroid culture (96-well or 384-well format) and tests with classic liver toxins demonstrate the applications of HepatoCells for ADME research and liver cytotoxicity studies.

Correlation between Sizes of Spheroids and Initial Seeding Density



Corning HepatoCells were seeded at indicated cell densities/well in 384-well Corning Ultra-Low Attachment surface spheroid microplate (**Cat. No. 4516**). No medium changes were made for the first two days and spheroid cultures were then maintained with half medium change every other day. Bar = 100 µm.

Higher CYP3A4 Activity in Corning HepatoCells Spheroid Microplate vs. 2D Culture (Normalized to ATP Levels)



Corning HepatoCells were seeded at 1.5K/well for spheroid culture in 96-well Corning Ultra-Low Attachment surface microplates (**Cat. No. 4515**). 100K/well HepatoCells were used to set up 2D culture in 96-well Corning BioCoat™ Collagen I microplate. CYP3A4 activity was measured with Promega Luciferin-IPA kit for day 7 spheroids (pool of 6 spheroids = 9K cells or 2D (100K cells/well) culture (chart, left). ATP levels (chart, middle) were measured for spheroids and 2D cultures and used for normalizing the CYP3A4 activity (chart, right). Error bars = Standard Deviation, n = 3.

Inducible CYP3A4 Activity in Corning HepatoCells Spheroids

	Single Spheroid		5 spheroids	
	DMSO	RIF	DMSO	RIF
CYP3A4 Activity	167.1 (n=5)	535.7 (n=4)	983.9	3483.8
Fold Change*		3.2		3.5

CYP3A4 3-day induction was performed with 10 µM Rifampicin (RIF) for day 4, 5 and 6 for single or pooled 5 spheroids. 0.1% DMSO was included as negative control. CYP3A4 activity was measured on day 7 with Luciferin-IPA probe.

* Corning HepatoCells 2D culture shows a CYP3A4 induction fold change of 19.6 (Poster 115 by Rongjun Zuo, et al).

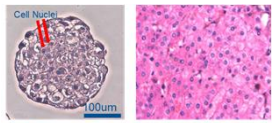
* CYP3A4 induction fold change in 3D culture (e.g., primary hepatocytes) is known to be lower than 2D culture due to increased basal enzyme activity.

Conclusion

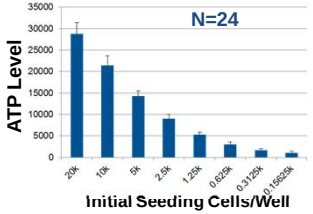
- **Corning HepatoCells were successfully adapted to a high throughput 3D culture system using Corning Ultra-Low Attachment surface spheroid microplates.**
- **Tests with classic liver toxins such as Aflatoxin B1 and Acetaminophen, demonstrate that HepatoCells spheroids are a promising 3D model system for liver cytotoxicity studies.**

Uniform Corning HepatoCells Spheroid Formation on a Corning Ultra-Low Attachment Surface Spheroid Microplate

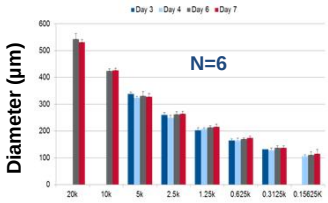
Hematoxylin and Eosin Staining Shows Healthy Core Structure of a Corning HepatoCells Spheroid



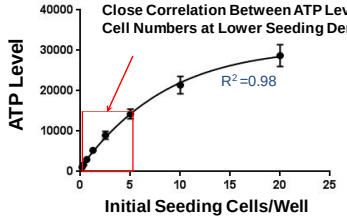
ATP Assay for Day 7 Spheroids



Spheroid Size Measurement Over a 7-day Culture



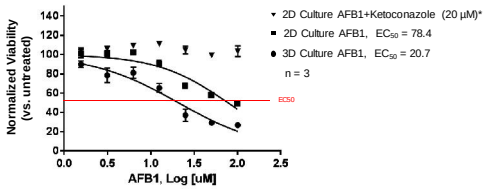
Close Correlation Between ATP Levels and Cell Numbers at Lower Seeding Density



Corning HepatoCells were seeded at indicated cell densities/well in a 384-well Corning Ultra-Low Attachment surface spheroid microplate. For H&E staining, day 7 spheroids from HepatoCells (1.5K/spheroid) were collected and fixed in 10% PBS buffered formalin for >24 hours prior to paraffin embedding and 10 µm sections were prepared and stained. Spheroid images were taken and sizes (diameter, µm) were measured during the culture period. ATP Levels (viability) were measured with a Promega CellTiter-Glo® 3D kit.

Corning HepatoCells Spheroid Treatment with Classic Liver Toxins

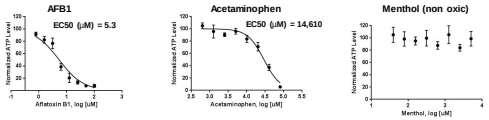
Day 4 Spheroid Culture vs . 2D Culture to Aflatoxin B1 (AFB1) Treatment



- 2D (100K/well) or 3D cultures (1.5K/spheroid) in 96-well format were treated with AFB1 for 24 hours.
- 3D spheroid culture increases the sensitivity of Corning HepatoCells to AFB1 toxicity, consistent with higher CYP metabolic activity in 3D culture format than 2D culture.
- AFB1 induced cytotoxicity in liver cells depends on drug metabolism by CYP enzymes.

*Ketoconazole (20 µM) inhibits CYP activity, and therefore, prevents AFB1 toxicity to HepatoCells.

EC_{50} Values of Day 7 Corning HepatoCells Spheroid to Classic Liver Toxins and Comparisons to Literature



Toxin	EC50 (uM) HepatoCells, 3D	Reported EC50 (uM) (2D culture)	References
Aflatoxin B1	5.3	10 (HH, 2D) >100 (HepG2, 2D) 78.4 (HepatoCells, 2D)	Avior Y. et al, 2015 In-house data
Acetaminophen	14610	28200 (HH), 29755 (HepG2)	Jemnitz et al, 2008 Wang et al, 2002
Menthol	Non toxic	Non toxic	Avior Y. et al, 2015

HH, human hepatocytes

Day 7 spheroid cultures (1.5K/spheroid) in 96-well format were treated for 24 hours with classic 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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