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Primary Human Hepatoctye 3D Spheroids for Studying Hepatic Function and Drug Toxicity

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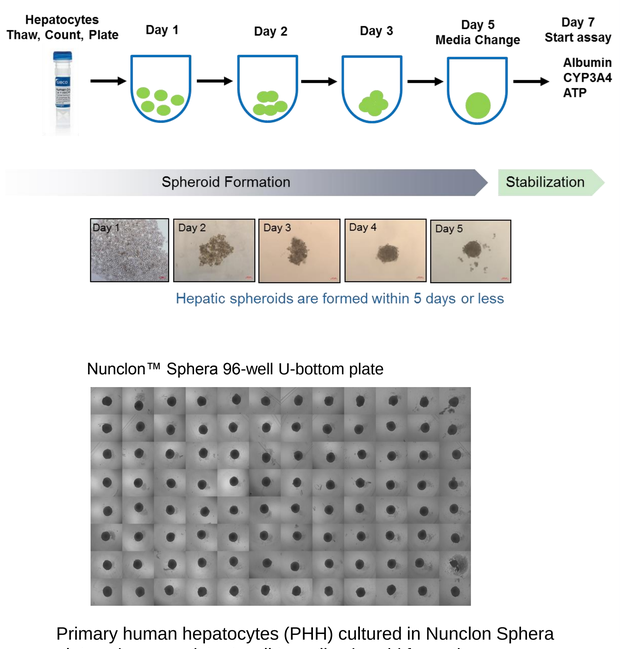
INTRODUCTION

Primary Human Hepatocyte (PHH) culture provides the closest *in vitro* model to human liver that can produce a metabolic profile of a given drug very similar to that found *in vivo*. Hence, PHH culture is the gold standard for studying the *in vitro* hepatic biology, liver function, and drug induced hepatotoxicity. The conventional 2-dimensional (2D) PHH culture is limited by de-differentiation and rapidly loss of hepatic specific functions. Therefore, there is a need for more robust *in vitro* models that reflects *in vivo* liver biology with better culture longevity. Recently, 3-dimensional (3D) *in vitro* models for hepatocytes have gained a lot of attention for their ability to recapitulate the hepatic function and greater longevity. Recently we have developed an easy-to-assemble *in vitro* PHH 3D-spheroid model. Our initial work shows that PHH can assemble into spheroids using Nunc™ Sphera™ super low attachment 96-well U-bottom plates and standard centrifugation method within 5 days of seeding. Interestingly, we have also found that not every lot of PHH can assemble into 3D-spheroids. We have shown that seeding 1,500 PHH/well resulted in spheroid formation with homogenous morphology and consistent size (~200 µm diameter). The PHH spheroids can live up to 28 days in culture and can retain hepatocyte-specific functions. To assess whether hepatocyte-specific functions were maintained in the PHH spheroids during prolonged culture, albumin secretion, CYP3A4 activity and levels of ATP synthesized were analyzed. These parameters were found to remain stable during prolonged culture period. Also, gene expression profiles at 5, 7, 14 and 21 days showed a relatively higher expression of hepatocyte specific genes, such as albumin and CYP3A4, compared to that of the 2D-culture. Finally, we have performed cytotoxicity assay using compounds causing drug induced liver injury (DILI), such as Chlorpromazine and Diclofenac, and found comparable IC50 values between the 2D and 3D cultures using PHH. These results indicate that the PHH 3D-spheroid system developed by us constitutes a promising *in vitro* tool to evaluate hepatic function. As part of our future work, we are investigating the possibility of introducing nonparenchymal liver cells like Kupffer and Stellate cells to the spheroid system to assess feasibility of creating various liver disease models.

RESULTS

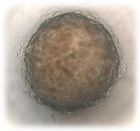
Figure 1. Formation of Hepatic Spheroids



Primary human hepatocytes (PHH) cultured in Nunclon Sphera plates show consistent well to well spheroid formation

Figure 2. 3D-Qualified Hepatocytes

Lot #	3D - culture	Spheroid formation
HuXX56		X
HuXX96		✓
HuXX24		X
HuXX80		✓

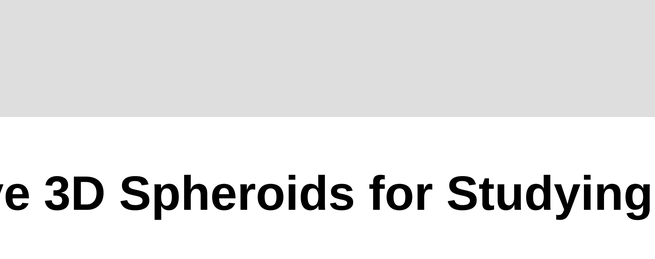


- Not all Hepatocyte lots make Spheroids.
- Functional Qualification required

Advantage of the Hepatic Spheroids

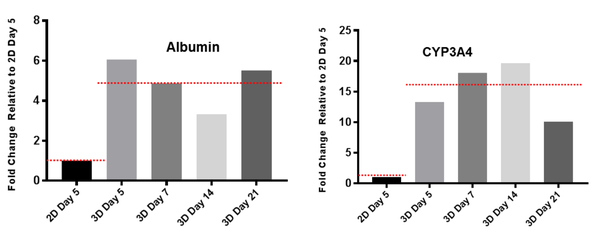
- Longevity: 2D cultures (4-6 days) vs. 3D cultures (weeks)
- Higher levels of CYP activity
- Better *in vivo-like* gene and protein expression

Figure 3. Variability in Spheroid formation from Microwell Plates (*or "plastics" matter*)

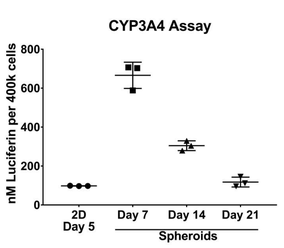


Nunclon™ Sphera™ Microplates with the **super low cell attachment** surface consistently promote 3D spheroid and organoid culture

Figure 4. Primary Human Hepatocyte Spheroid Cultures Show Improved Albumin and CYP3A4 Levels

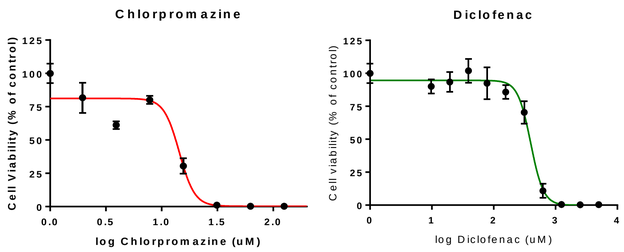


Gene expression levels of Albumin and CYP3A4 were consistently higher in the 3D-spheroids than that of the 2D-culture.



CYP3A4 activity in 3D-spheroids was higher than that of 2D-culture.

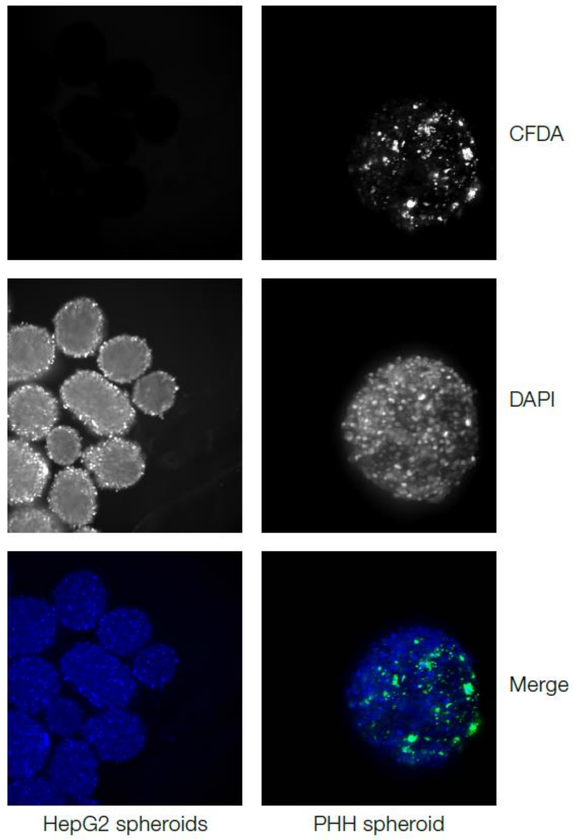
Figure 5. Hepatic Spheroids Show Altered Sensitivity to Reference Compounds Relative to 2D controls



Drugs	IC_{50} - 2D culture	IC_{50} - 3D culture
Chlorpromazine	34.22 µM	14.39 µM
Diclofenac	331 µM	396.2 µM

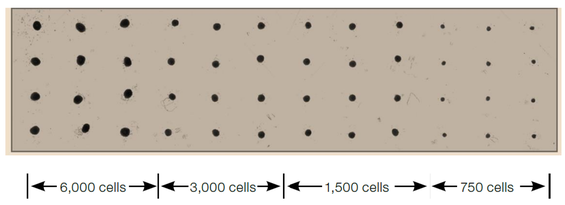
Drug-induced cytotoxicity assay using 3D spheroid hepatic cultures

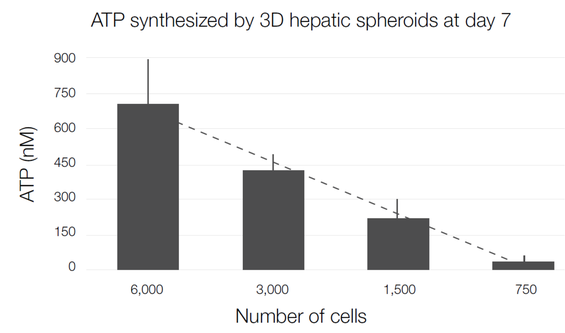
Figure 6. Evaluation of Bile Canaliculi Formation in Hepatic Spheroids



HepG2 spheroids on day 14 (left) and hepatic spheroids on day 7 (right) were stained with CFDA and DAPI and imaged using the Thermo Scientfic™ CellInsight™ CX7 platform at 10x magnification. Hepatic spheroids show clear formation of bile ducts in comparison to the HepG2 spheroids (used as the negative control).

Figure 7. Spheroid Size and ATP Production is Directly Proportional to the Number of Cells Seeded





CONCLUSIONS

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Collectively, these data support that 3D spheroid–qualified human hepatocyte cultures have been characterized to show stable morphology, viability, and hepatocyte-specific functions for at least 21 days. We have demonstrated that our 3D spheroid–qualified hepatic cultures are functional as indicated by formation of bile ducts (Figure 6) as well as sustained albumin expression (Figure 4). In comparing CYP3A4 activity between day 5 of 2D hepatic cultures and day 7 of 3D spheroid hepatic cultures we have shown that 3D spheroid cultures have significantly higher levels of activity (Figure 4). We also show that this 3D spheroid hepatic culture system can be used to analyze druginduced cytotoxicity in hepatocytes (Figure 5). Ultimately, these data indicate that the reduced cell number required for 3D spheroid formation as well as the sustained longevity of these cultures may better support high-throughput assays and long-term studies of hepatocyte functions.

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