HepatiCult™ for Human Hepatic Organoids: A Validated Culture System for Drug Toxicity Screening

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INTRODUCTION

Hepatic organoids are three-dimensional (3D) cell culture systems that serve as a valuable model for studying liver cell biology. They retain key features of in vivo liver cells and can recapitulate donor heterogeneity. They serve as a proliferative and physiologically relevant alternative to conventional 2D cell culture for studying liver biology, disease modeling, and drug screening. HepatiCult™ Organoid Kit (Human) supports a complete hepatic organoid workflow that includes robust organoid establishment from tissue, long-term expansion, and differentiation to mature hepatobiliary cell types for downstream applications. Proliferating organoids remain viable following cryopreservation and retain their capacity for propagation and maturation, providing flexibility and convenience when working with human tissue-derived samples.

METHODS

HepatiCult™ Organoid Kit (Human) Workflow:

1. Human liver tissue was minced mechanically and then sequentially digested using Collagenase IV for up to 1 hour at 37°C to isolate intrahepatic ductal fragmented cellular material.
2. Ductal fragments were embedded in 30-µL Matrigel® domes and cultured in HepatiCult™ Organoid Initiation Medium (OIM) for up to 2 weeks with medium changes every 2 - 3 days.
3. Established organoids were then cryopreserved or maintained/expanded in HepatiCult™ Organoid Growth Medium (OGM) and passaged every 4 - 10 days.
4. To generate organoids comprising mature hepatic cell types, organoid fragments were seeded in OGM, cultured for 5 days and then switched to HepatiCult™ Organoid Differentiation Medium (ODM) for at least 10 days with medium changes every 2 - 3 days.

Hepatic Organoid Characterization:

Hepatic organoids were characterized by immunocytochemistry (ICC) and quantitative real-time PCR (qPCR) for hepatic proteins and genes, respectively.

Drug Screening Assay:

1. Seven drugs were tested in proliferative (maintained in HepatiCult™ OGM) and mature (differentiated in ODM) hepatic organoids in a viability assay and the results were compared to those of HepG2 cells and primary human hepatocytes (PHH) under similar conditions.
2. Organoid fragments embedded in 6-µL Matrigel® domes per well of a 96-well plate were cultured for 4 - 5 days in HepatiCult™ OGM until organoids formed.
3. Cultures in HepatiCult™ OGM were then switched to ODM and incubated for an additional 7 days to induce maturation
4. Proliferative or mature organoids were treated with drugs or DMSO vehicle control for 72 hours with full-medium changes containing fresh drugs every 24 hours, and viability was assessed using CellTiter-Glo® 3D Cell Viability Assay (Promega).
5. Coefficient of variation (CV) and Z' factor were calculated for data quality estimation.

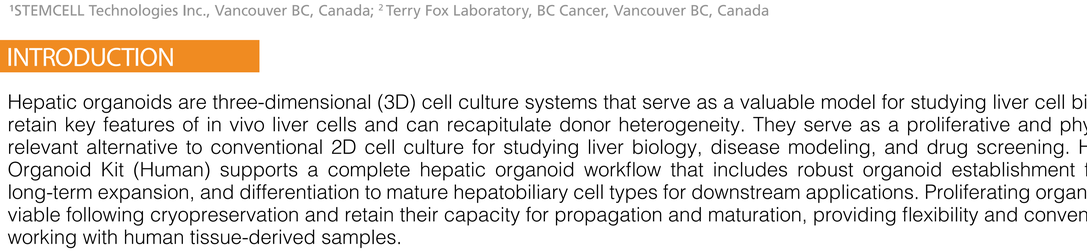


FIGURE 1. Schematic of HepatiCult™ Organoid Kit (Human) workflow

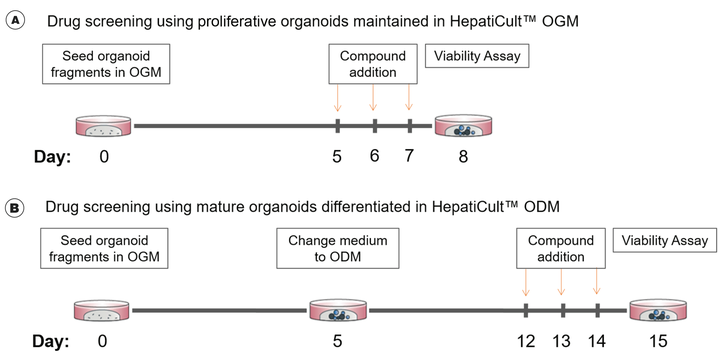


FIGURE 2. Schematic of drug screening and cell viability assay protocol

TABLE 1. Z' Factors from 3 independent experiments calculated using viability values from DMSO- and 750 µM troglitazone-treated wells.

Run	Z' Factor	
	OGM	ODM
1	0.67	0.43
2	0.86	0.81
3	0.78	0.64

TABLE 2. Coefficient of variation (CV) of viability assay in proliferative and mature organoids calculated by dividing the standard deviation by the mean of untreated wells (n = 48 and 60 technical replicates respectively.

	CV
Proliferative Organoids	12%
Mature Organoids	14%

RESULTS

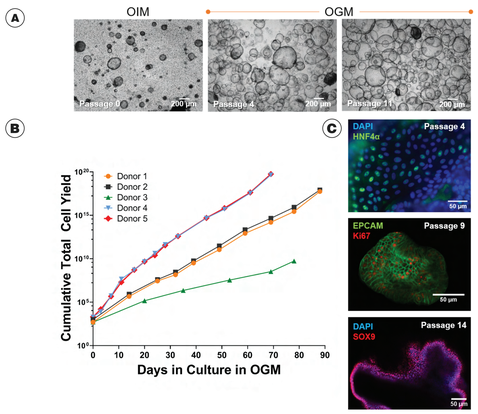


FIGURE 3. Human hepatic organoids can be expanded efficiently in Hepaticult™ OGM and exhibit characteristics of hepatic progenitor cells.

(A) Phase contrast images of hepatic organoids in HepatiCult™ OIM (Passage 0) and HepatiCult™ OGM (Passage 4 and 11). **(B)** Cumulative expansion of hepatic organoids in HepatiCult™ OGM from 5 donors. **(C)** ICC of hepatic organoids in HepatiCult™ OGM stained for hepatic transcription factor HNF4a, ductal progenitor marker SOX9, epithelial marker EPCAM, and proliferation marker Ki67. (n = 2 - 5)

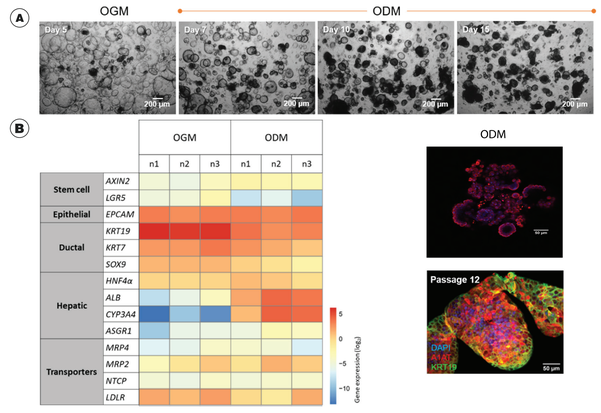


FIGURE 4. Human hepatic organoids maintained in Hepaticult™ OGM and differentiated in Hepaticult™ ODM display distinct morphology, gene expression, and immunostaining.

(A) Phase contrast images of hepatic organoids in HepatiCult™ OGM and ODM. Days listed indicate the number of days post-passaging; organoids were switched from HepatiCult™ OGM to ODM on Day 5. **(B)** Gene expression analysis of proliferative organoids (OGM) versus mature organoids (ODM) by qPCR **(C)** ICC of differentiated organoids stained for mature hepatic markers CYP3A4 and A1AT, and ductal marker KRT19. (n = 1 - 5)

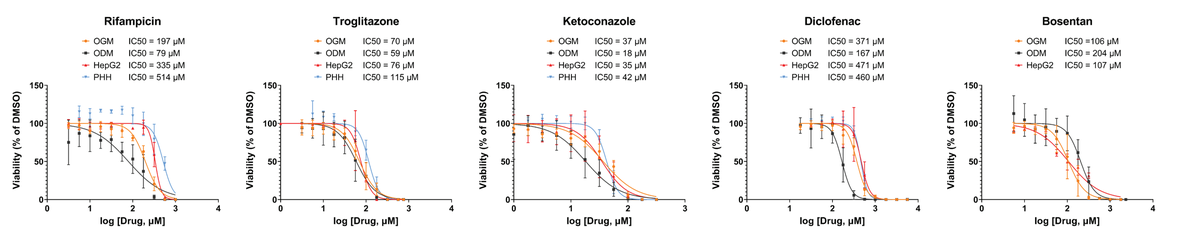


FIGURE 5. Human hepatic organoids have high sensitivity to toxic effects of rifampicin, troglitazone or ketoconazole and display IC50 values < 200 µM.

Non-linear regression curves of proliferative organoids (OGM), mature organoids (ODM), HepG2 cells, and PHH treated with rifampicin, troglitazone, or ketoconazole and their respective IC50 values (n = 3 - 7).

FIGURE 6. Human hepatic organoids have moderate sensitivity to toxic effects of diclofenac or bosentan and display IC50 values between 100 - 400 µM.

Non-linear regression curves of proliferative organoids (OGM), mature organoids (ODM), HepG2 cells, and PHH treated with diclofenac or bosentan and their respective IC50 values (n = 3 - 7).

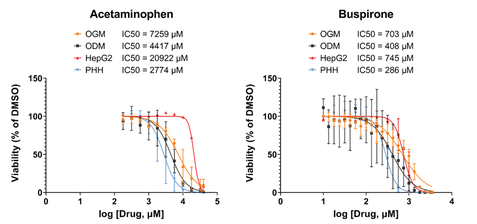
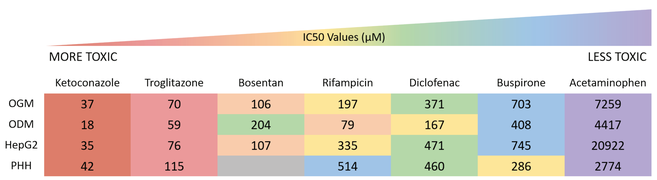


FIGURE 7. Human hepatic organoids have low sensitivity to toxic effects of acetaminophen or buspirone and display high IC50 values of > 400 µM.

Non-linear regression curves of proliferative organoids (OGM), mature organoids (ODM), HepG2 cells, and PHH treated with acetaminophen or buspirone and respective IC50 values (n = 3).



	Ketoconazole	Troglitazone	Bosentan	Rifampicin	Diclofenac	Buspirone	Acetaminophen
OGM	37	70	106	197	371	703	7259
ODM	18	59	204	79	167	408	4417
HepG2	35	76	107	335	471	745	20922
PHH	42	115		514	460	286	2774

FIGURE 8. Summary of drug toxicity effects in human hepatic proliferative (OGM) and mature (ODM) organoids, HepG2 cells, and PHH.

Summary

- Human hepatic organoids are uniquely suited to the development of drug screening assays; they readily form in culture and can be expanded and differentiated. They are composed of several hepatic cell types, and better recapitulate the complexities of in vivo liver function when compared to current conventional models.
- Drug dose-response curves of organoid viability generated in proliferative and mature organoids were validated and compared to conventional 2D models, including HepG2 cells and primary human hepatocytes (PHHs). This further demonstrates the utility of human-derived hepatic organoids for clinical research, drug discovery, and advancement of personalized medicine practices.
- Mature differentiated organoids appeared more sensitive to the drugs tested, indicating that these compounds may preferentially affect a mature cell population, rather than proliferative progenitor cell types of the liver.
- The donor variability observed in organoids may reflect the idiosyncratic nature of patient responses and would therefore be beneficial in detecting hepatotoxicity during drug development and development of personalized medicine treatments.