Characterization and Application of Self-Organizing Multicellular Human Liver Organoid Model

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INTRODUCTION

Conventional 2-dimensional hepatocyte culture systems are not suitable for long term studies of hepatic functions, biology, drug-induced liver injury (DILI) and liver diseases due to rapid loss in viability and function. Recent advances in 3D culture methods have shown feasibility of long-term maintenance of hepatic structure, function and viability along with attributes such as multi-cellular tissue formation and physiologically relevant cell-cell interactions and scalability. Here, we describe characterization of a self-organizing 3D liver organoid system comprising of major liver cell populations that offers distinct advantages over the conventional hepatocyte culture systems. The liver organoids were analyzed for morphology, viability, hepatic functions, basal CYP450 activity and expression, transporter activity, inflammatory response and DILI.

RESULTS

Long term maintenance of size, viability and function

To generate a relevant in vitro hepatic system, four major liver cell types namely hepatocytes, Kupffer cells, stellate cells and liver sinusoidal endothelial cells were seeded in ultra-low attachment spheroid plates and cultured for up to 28 days. Size (Diameter), viability (ATP) and function (Albumin) were measured weekly. Histological analysis was performed after paraffin embedding and processing of the organoids.

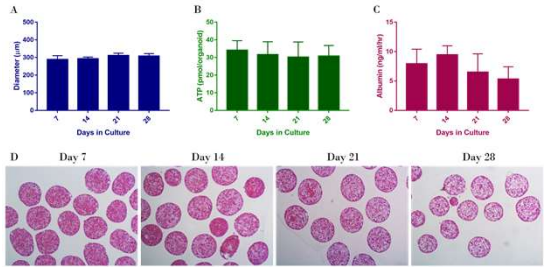


Figure 1. Validation of long term maintenance of liver organoids. (A) Spheroid diameter measured by capturing spheroid images every week (n=6). (B) Total cellular ATP was measured weekly using Promega Cell Titer Glo 3D assay (n=8). (C) Albumin secretion in the culture medium was measured using an ELISA (n=8).

Liver organoids represent 4 major liver cell types

The self-organizing liver organoids consisted of four different liver cell types. The organization of these cells within the organoid was characterized using specific markers for each cell type by immunofluorescence staining.

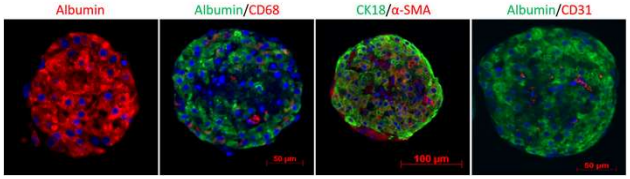


Figure 2. Immunohistological characterization of liver organoids. Organoids were stained for Albumin & Cytokeratin 18 (hepatocytes), CD68 (Kupffer cells), alpha-smooth muscle actin (hepatic stellate cells) and CD31 (endothelial cells). Nuclei were stained using DAPI.

Organoids maintain metabolic enzyme expression and activity

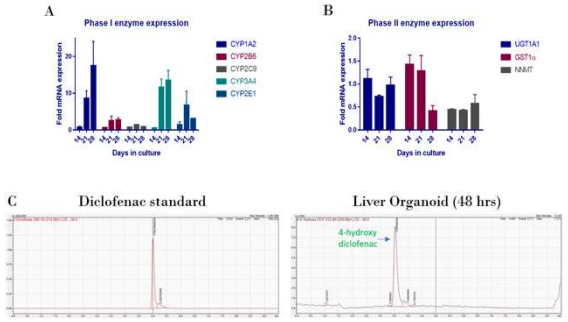


Figure 3. Metabolic enzyme expression and activity in liver organoids. (A) Organoids express key Phase I CYP enzymes for up to 4 weeks, critical for long term metabolic studies. (B) Major Phase II enzymes are expressed in the organoids throughout their culture period of 28 days. (C) The organoids metabolized diclofenac into 4-hydroxy diclofenac via CYP2C9 pathway after 48 hour incubation.

Organoids show inflammatory response in presence of LPS

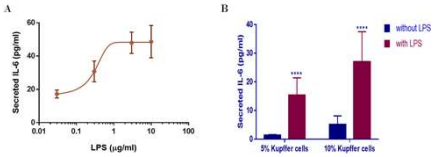


Figure 4. Inflammatory response in organoids. (A) Dose response curve of IL-6 secretion by the organoid highlighting the capability to respond to inflammatory stimulus. IL-6 secretion was measured using ELISA (B) The intensity of inflammatory response (IL-6 secretion) in presence or absence of LPS is significant in inflamed state of liver (10% Kupffer cells) compared to normal state of liver (5%) Kupffer cells. p****<0.0005 (n=8 organoids, student's t-test)

Organoids exhibit Bosentan induced cholestasis

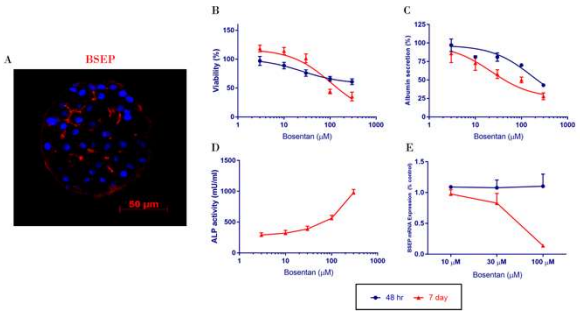
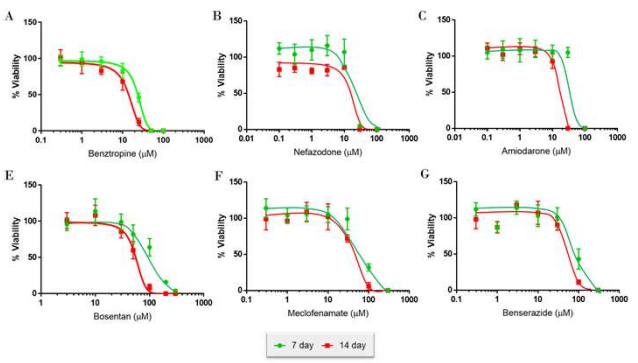


Figure 5. Drug induced cholestasis in liver organoids. (A) Hepatocytes within the organoids express Bile Salt Export Pump (BSEP) shown in red. Nuclei stained blue using DAPI. (B) Organoids treated with Bosentan show dose-dependent hepatotoxicity. (C) Hepatic function is reduced over time in presence of Bosentan as measured by albumin secretion. (D) Increased Alkaline Phosphatase activity suggesting cholestatic stage of liver resulting from 7 day Bosentan treatment (48 hour not measured). (E) BSEP expression is reduced in presence of Bosentan over time.

Liver organoids are suitable for chronic DILI studies



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		IC50 (µM)	
Compound	In vivo Cmax (µM)	7 day	14 day
Benztropine	0.59	23.56	14.05
Nefazodone	4.26	26.86	15.88
Amiodarone	5.27	68.01	11.53
Bosentan	7.43	101.70	55.72
Meclofenamate	81.07	97.06	31.27
Benserazide	3.89	98.72	33.55

Figure 6. Liver organoids support 14-day drug induced liver injury (DILI) studies. Organoids were treated with (A) Benztropine (B) Nefazodone (C) Amiodarone (D) Bosentan (E) Meclofenamate and (G) Benserazide for 14 days. Viability was measured at day 7 and 14 by measuring cellular ATP content (n=6 spheroids for each time point). (H) IC50 values were calculated by plotting a log inhibitor vs normalized response curve in Graph Pad.

SUMMARY

We have developed a self-organizing liver organoid system comprising of four major liver cell types that maintains size, structure, viability and function for up to 4 weeks in culture. Gene expression analysis demonstrated that the organoids maintained expression of major drug metabolizing enzymes and CYP2C9 activity was confirmed via biotransformation of diclofenac. In presence of LPS, organoids showed inflammatory response via increased secretion of IL-6. Presence of bile canaliculi was established by BSEP expression and cholestatic response was reported using Bosentan treatment in the organoids. The organoids accurately demonstrated drug induced hepatotoxicity. The liver organoid described herein represents a scalable model for studying hepatic function and structure, a screening platform for hepatic pharmacotoxicology and a system for creating liver disease models.

CONTACT

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