Development of iPSC-Derived Human Liver Organoids for Preclinical Drug Testing and Toxicology Studies

Fong Cheng Pan[1], David Austin[1], Stephan Krieg[2], Luisa-Marie Pfeifer[2], Philip Hewitt[2], Steven Johnston[2], Willem Kools[3], Vi Chu[1]

[1]MilliporeSigma, Temecula CA; [2]Merck KGaA Darmstadt, Germany;
[3]MilliporeSigma, Burlington MA

Abstract

Over 90% of drug candidates fail during clinical trials, often due to insufficient efficacy and unmanageable toxicity. Drug-induced liver injury (DILI) is frequently missed in preclinical testing due to a lack of robust and predictive liver models. We report the de novo development of iPSC-derived human liver organoids as scalable, reliable, and physiologically relevant preclinical models. Using an efficient and reproducible protocol, we generated expandable, cryopreservable bipotential 3dGRO™ Human iPSC-derived Liver Progenitor Organoids, which differentiate into mature liver organoids (MLOs) containing multiple liver cell types. These MLOs display long-term stability, secrete albumin and urea, and express key biomarkers of mature hepatocytes (albumin, CYP3A4, HNF4a, PCK2) and cholangiocytes (Sox17, Sox9, CK7, MRP2). They also exhibit functional Phase I/II liver enzymes (CYP3A4, CYP2C9, CYP1A2, ALT, AST, GST) and active bile salt/drug transporters. Compared to primary hepatocytes, our 3dGRO™ Human iPSC-derived Liver Progenitor Organoids demonstrate comparable functionality and outperform HepG2 cells and liver spheroids. In DILI assays, MLOs responded to drug treatment with elevated liver enzymes, confirming their potential for toxicology screening. These organoids represent a powerful tool for liver disease modeling and high-throughput drug screening, with future applications including models of hepatocellular carcinoma, MASLD, and NASH.

Introduction

- High demand for liver organoids following FDA approval on using *ex vivo* models for preclinical drug screening/evaluation in lieu of *in vivo* models.
- iPSC-derived liver organoids as unlimited source of isogenic liver organoids with more genetic homogeneity compared to patient-derived organoids.
- **Advantages of 3D liver organoids** over 2D primary hepatocytes (PHH)/HepG2 cells:
  - 2D PHH/HepG2 cells failed to maintain metabolic function after prolonged *in vitro* culture and limited expansion.
  - 3D liver organoids with multicellular characteristic enable recapitulation of *in vivo* physiological functions.

Fig. 1 Liver Organoids applications (De Siervi and Turato, 2023).

Purpose

- Large scale production of polarized and expandable bipotential progenitor state liver organoids (LPO) that can be cryopreserved..
- Generation of mature, functional, and assay ready liver organoids (MLO) that can be used for DMPK and toxicological studies in drug discovery and development.

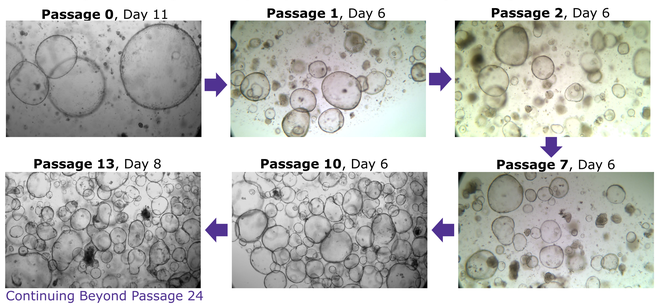
Generation of robust, long-term expandable and cryopreservable iPSC-derived liver organoids

A. Workflow for differentiation of iPSC to mature liver organoids

iPSCs → Definitive Endoderm → Hepatic Endoderm → Liver progenitor organoids → Mature liver organoids

3dGRO™ Human iPSC-derived Liver Progenitor Organoids (Expansion Media)
Cat# SCC572 (SCM313) Launched July 2025

B. Liver Progenitor Organoids (LPO) show robust, long-term growth



Continuing Beyond Passage 24

C. Liver Progenitor Organoids are cryo-preservable and showed post-thaw growth

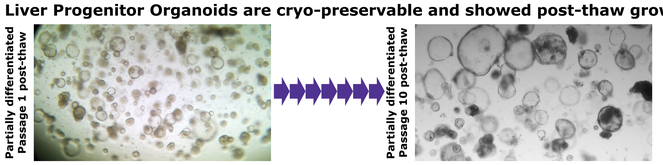


Fig. 2 Generation of iPSC-derived liver progenitor organoids with robust growth, long-term expandable and cryopreservable. (A) iPSCs were differentiated to definitive endoderm, hepatic endoderm, bipotential liver progenitors and mature liver organoids in 3D culture using defined media containing growth factors for each differentiation steps. **(B)** iPSC-derived liver progenitor organoids showed persistent, robust long-term expansion for over 20 passages in modified liver expansion media. (C) Liver progenitor organoids (LPO) were frozen, thawed and passaged using standard protocol. These organoids continued to grow for over 10 passages post thaw without morphological changes.

Successful generation of fully differentiated, mature liver organoids (MLO)

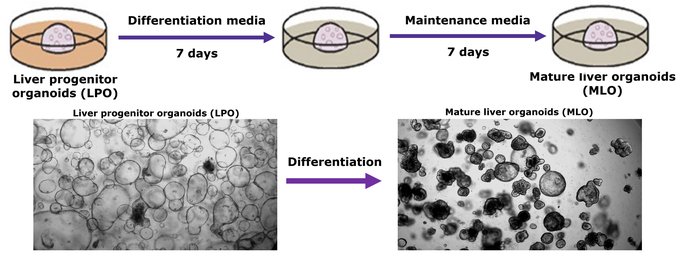


Fig. 3 Fully differentiated mature liver organoids (MLO) can be generated from liver progenitor organoids. Liver progenitor organoids were cultured in differentiation media (modified from Mun et al., *Mol Cell Biology,* 2019) for 7 days followed by 7 days culturing in maintenance media to generate fully differentiated mature liver organoids.

Mature liver organoids (MLO) expressed key mature hepatocyte and cholangiocyte markers

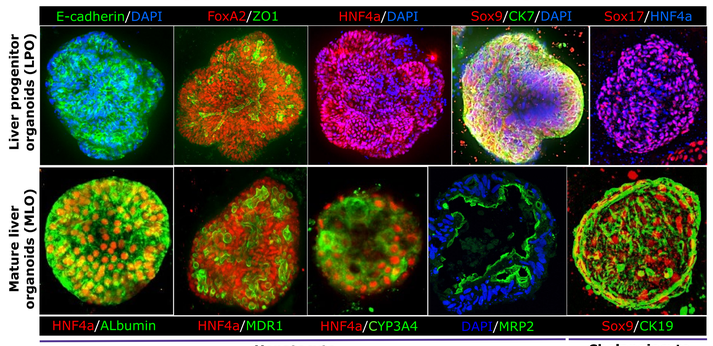


Fig. 4 Liver progenitor organoids (LPO) are polarized and can be differentiated to mature liver organoids (MLO) expressing key maturation and functional markers of hepatocytes and cholangiocytes. Immunofluorescence data showed the liver progenitor organoids (LPO) are polarized and expressing liver progenitor markers (FoxA2, HNF4a, Sox9, and Sox17). Differentiated mature liver organoids (MLO) expressed mature functional hepatocyte (albumin, CYP3A4, MDR1 and MRP2) and cholangiocyte (Sox9, CK7, CK19) markers. Mature liver organoids contain hepatocytes and cholangiocytes as two main cell types.

Mature liver organoids are functional, secreting albumin and urea

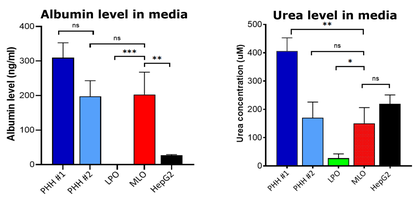


Fig. 5 Differentiated liver organoids secrete albumin and urea into the culture medium. Albumin and Urea secretion were detected in the media from differentiated liver organoids (**MLO**) at the level comparable to primary hepatocytes (**PHH**) and HepG2 cells. Liver progenitor organoids (**LPO**) do not secrete albumin or urea. Kits: Albumin ELISA assay and Urea assay kits (both from MilliporeSigma). All assays were normalized to viable cells determined by CellTiter Fluor assay (Promega). ns, non-significance.

Mature liver organoids possess functional and inducible Cytochrome P450 activities

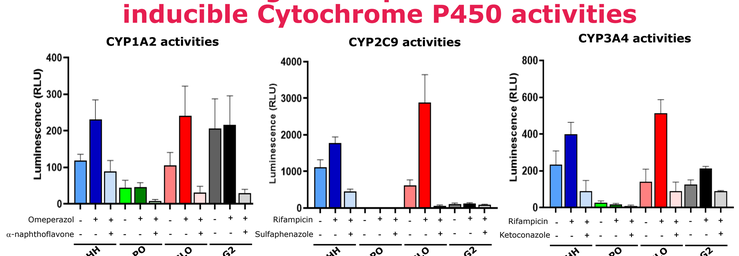


Fig. 6 Differentiated liver organoids possess endogenous and inducible Cytochrome P450 activities (CYP1A2, CYP2C9 and CYP3A4). Differentiated liver organoids (**MLO**) possess CYP1A2, CYP2C9 and CYP3A4 activities that are inducible (by Omeperazol and Rifampicin) at a comparable level to primary hepatocytes (**PHH**). The CYP450 activities in MLO and PHH were also inhibited by CYP450-specific inhibitors (α-naphthoflavone for CYP1A2, Sulfaphenazole for CYP2C9 and Ketoconazole for CYP3A4). MLO also possess higher CYP2C9 and CYP3A4 activities compared to HepG2 cells. Liver Progenitor Organoids (LPO) possess very low to no CYP450 activities. Kits: P450-GLO™ CYP1A2, CYP2C9 and CYP3A4 assay (Promega). All assays were normalized to viable cells determined by CellTiter Glo 3D (Promega).

Not intended for marketing purposes

Differentiated liver organoids possess important liver enzymes activities (ALT, AST and GST)

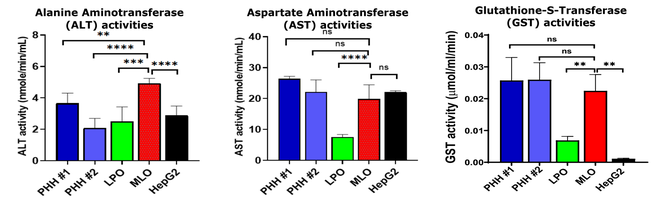


Fig. 7 Differentiated liver organoids possess important liver enzymes (ALT, AST and GST) activities. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutathione-S-transferase activities were detected differentiated liver organoids (**MLO**) at the level comparable to primary hepatocytes (**PHH**). Liver progenitor organoids (**LPO**) possess lower activity level for these 3 enzymes. Kits: ALT, AST and GST assay kits (MilliporeSigma). All assays were normalized to viable cells determined by CellTiter Fluor assay (Promega).

Mature liver organoids possess functional bile salt and drug transporters

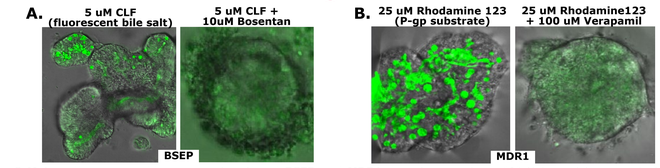
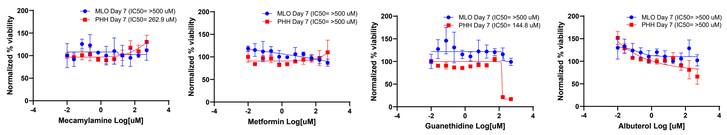


Fig. 8 Mature liver organoids possess functional BSEP and MDR1 drug transporters. (A) Uptake of fluorescent bile salt analog CLF into the mature liver organoids followed by secretion into the bile canaliculi indicate a functional BSEP transporter, which can be inhibited by Bosentan. **(B)** Functional MDR1/P-gp transporters are indicated by the uptake and accumulation of Rhodamine 123 in the bile canaliculi. Inhibition of MDR1 by Verapamil diminished the uptake and accumulation of Rhodamine 123 in mature liver organoids.

Mature liver organoids are responsive to drug-induced liver injuries (DILI)

A. Non-DILI drugs



B. DILI drugs

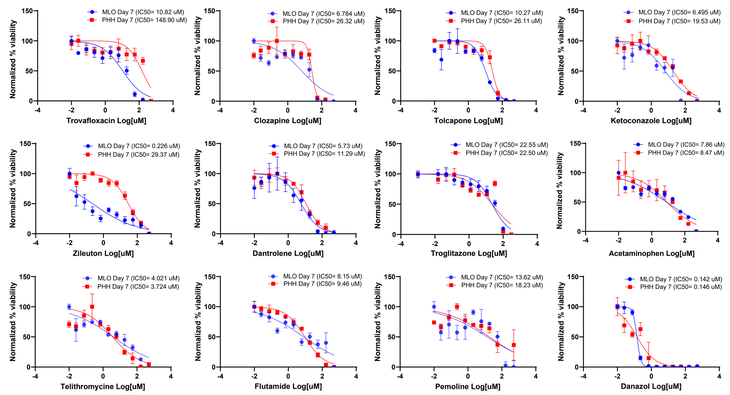


Fig. 9 Mature liver organoids can be used as drug-induced liver injury (DILI) model. Mature liver organoids are treated with known (A) non-DILI drugs and (B) DILI drugs. Realtime MT Glo cell viability assay indicated that the non-DILI drugs do not have effects on cell viability on both mature liver organoids (MLO) and primary hepatocytes (PHH) (A). Treatment of MLO with known DILI drugs resulted in decreased cell viability in both MLO and PHH. MLO showed better or comparable sensitivity than PHH in response to most of the DILI drugs.

Summary

- We successfully generated robust, long-term expandable and cryopreservable liver progenitor organoids.
- The liver progenitor organoids (LPO) can be differentiated into mature functional liver organoids (MLO).
- These mature liver organoids (MLO) secrete albumin and urea into the media and possess key phase I (Cytochrome P450), phase II (ALT, AST and GST) liver enzymes activities, and functional drug transporters.
- Proof-of-concept drug-induced liver injury (DILI) studies showed these MLO are responsive to drug-induced injuries to known non-DILI and DILI drugs.
- The LPO can be easily and reproducibly differentiated into functional MLO by new organoid users and data obtained from functional assays are consistent between different passages.
- The 3dGRO Human iPSC-derived Liver Progenitor Organoids (cat# SCC572) is an excellent tool for drug screening, DMPK and toxicology studies.

www.sigmaaldrich.com