Exploring the performance of HepatoXcell™ in two liver-chip platforms

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Introduction

The liver is the largest internal organ in the human body and is responsible for more than 500 metabolic functions. One of its primary roles in pharmacology is to metabolize drugs into forms that the body can utilize or excrete. In vitro liver models are essential tools during the discovery and preclinical phases of drug development. However, conventional monolayer and suspension cultures of hepatocytes often rapidly lose metabolic activity over time.

To address this limitation, liver-chip technology integrates microfluidics to simulate the liver's dynamic microenvironment, which includes blood flow and complex cell-cell interactions that replicate the three-dimensional structure of liver tissue. This technology supports hepatocyte cultures for extended periods while preserving their functional activity.

In this study, we evaluate the performance of HepatoXcell™ culture within liver-chip technologies. Using a standardized protocol, we cultured cells in two microfabricated liver-chip designs: a flat-bed design and a 3-D meshed-bed design. The flat-bed design features hepatocytes interfacing with non-parenchymal cells through a porous membrane in a microfluidic channel. In contrast, the meshed-bed design employs a silicon perforated scaffold situated on a microporous filter, with primary hepatocytes and non-parenchymal cells seeded on the filter within the scaffold. In this setup, fluid flows through the cell aggregates.

Table 1: ATCC® Inventory for drug development and toxicity test

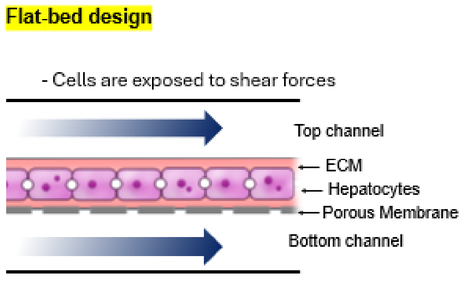
Product Name	ATCC® No.	Format	Amount
HepatoXcell™ Eco	PCS-450-012™	Suspension	1 vial, ≥ 4 x 10^6 cells/vial
HepatoXcell™ Plus	PCS-450-010™	3-Day Plateable	1 vial, ≥ 4 x 10^6 cells/vial
HepatoXcell™ Pro	PCS-450-011™	7-Day Plateable	1 vial, ≥ 4 x 10^6 cells/vial
HepatoXcell™ Thawing Medium	PCS-450-032™	1 bottle	250 mL
HepatoXcell™ Maintenance Medium	PCS-450-034™	1 bottle	500 mL
HepatoXcell™ Plating Medium	PCS-450-038™	1 bottle	100 mL

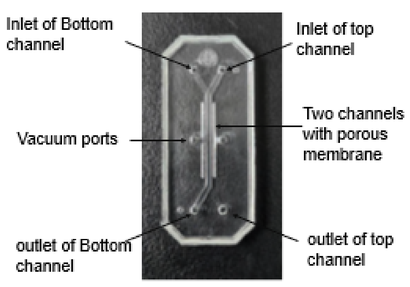
The quality and functionality of primary hepatocytes are crucial for the success and relevance of liver-chip models. Freshly isolated cryopreserved hepatocytes can exhibit varying functions depending on their source and the freezing process used. ATCC®, a leading resource for biological materials, has recently introduced high-quality HepatoXcell™ primary human hepatocytes along with validated liver media kits specifically designed for use in microphysiological systems (MPS) technologies. These resources provide a comprehensive system for human liver culture.

This study demonstrates the high quality of HepatoXcell™ across two distinct liver-chip platforms, highlighting their applicability in microfluidic systems. The data supports their use in pharmacological research, including drug discovery, toxicity testing, and disease modeling.

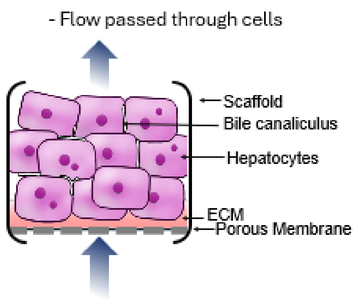
Two liver-chip platforms

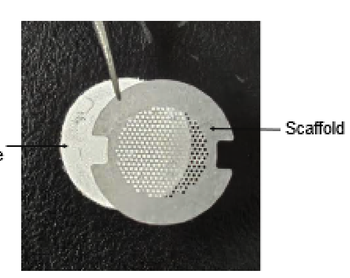
Flat-bed design





3D Meshed-bed design





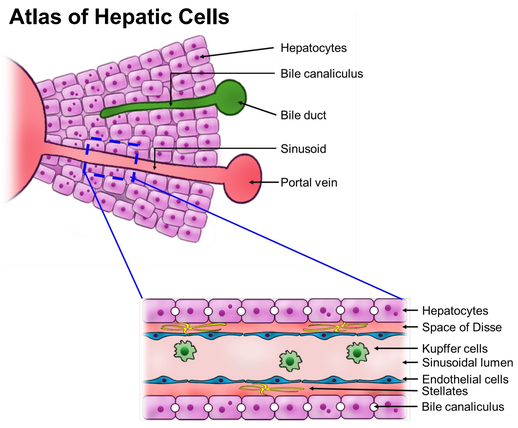
Schematic representation of two different MPS platforms for liver models: Flat-bed design (Top; e.g., Emulate) to flow through cell aggregates and 3D Meshed-bed design (Bottom; e.g., CN-Bio) to be exposed to shear forces.

Summary

- ATCC® HepatoXcell™ Pro is meticulously isolated and characterized to ensure the highest quality and performance
- ATCC® HepatoXcell™ Pro is applicable for different formats of 3-D culture and demonstrated liver functional activities
- ATCC® HepatoXcell™ Pro has the potential to implement two different types of MPS platforms
- ATCC® HepatoXcell™ Pro can be cultured for a long term in spheroid culture and fluidic culture
- ATCC® HepatoXcell™ Pro has prolonged metabolic activity and better liver-specific functions in a Flat-bed designed platform

Learn more about HepatoXcell™

Results



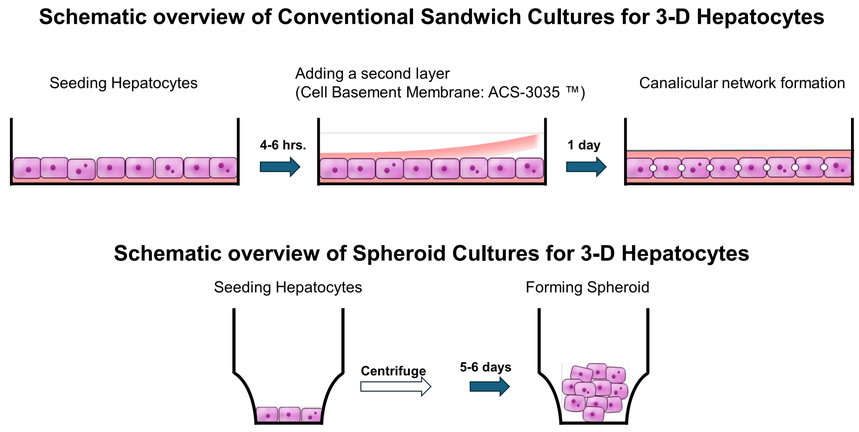


Figure 1: Schematic overview of hepatocytes in conventional sandwich culture and spheroid culture. Hepatocytes maintain more stable hepatic functions and morphologies as compared to traditional 2-D cultures, mimicking the in vivo liver environment more closely. Sandwich cultures involve hepatocytes sandwiched between layers of extracellular matrix, while spheroids are 3-D aggregates of hepatocytes.

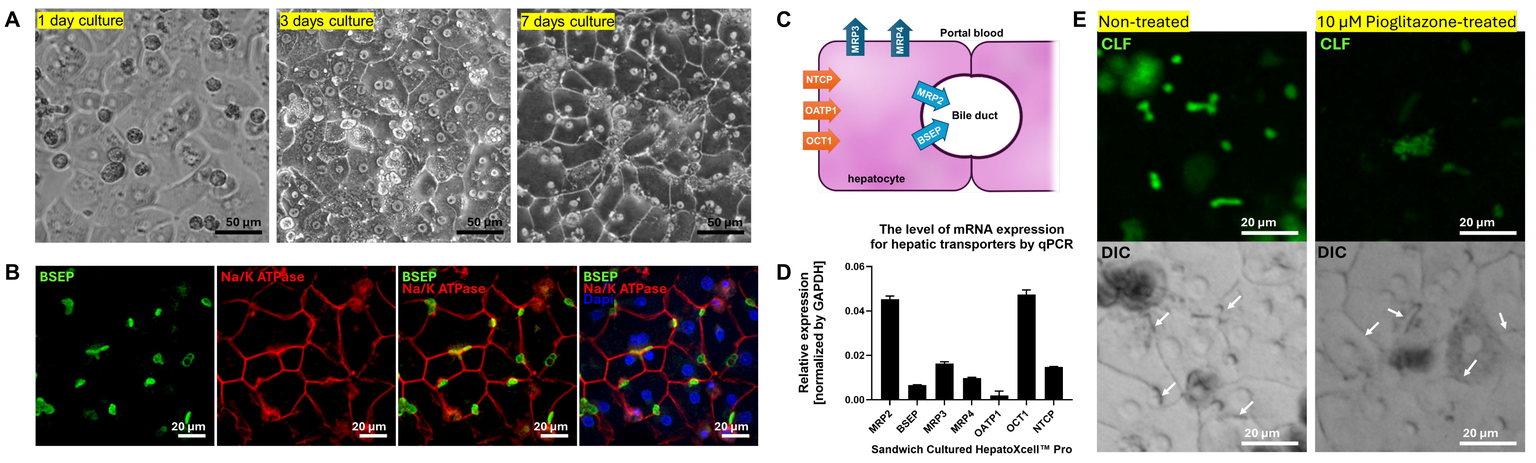


Figure 2: Sandwich-cultured HepatoXcell™ Pro. (A) Phase-contrast and (B) confocal images (7 days culture) show polarized distribution of hepatocytes and the formation of canalicular membranes in sandwich culture. (C, D) Hepatocytes express various transporters on the surface membranes to facilitate the uptake and efflux of substances. (E) CLF efflux assay to demonstrate hepatic transporters and functional efflux of bile canaliculi. Hepatocytes were exposed to 5 µM CLF in HBSS with Ca^{2+}/ Mg^{2+} for 30 min; results were observed using confocal microscopy. *BSEP (Bile Salt Export Pump); located on the canalicular membrane and transport to bile salts. Na/K ATPase; located on the lateral membrane. CLF (Cholyl-Lysyl-Fluorescein); fluorescent bile acid analog. White arrows to point the canaliculus.

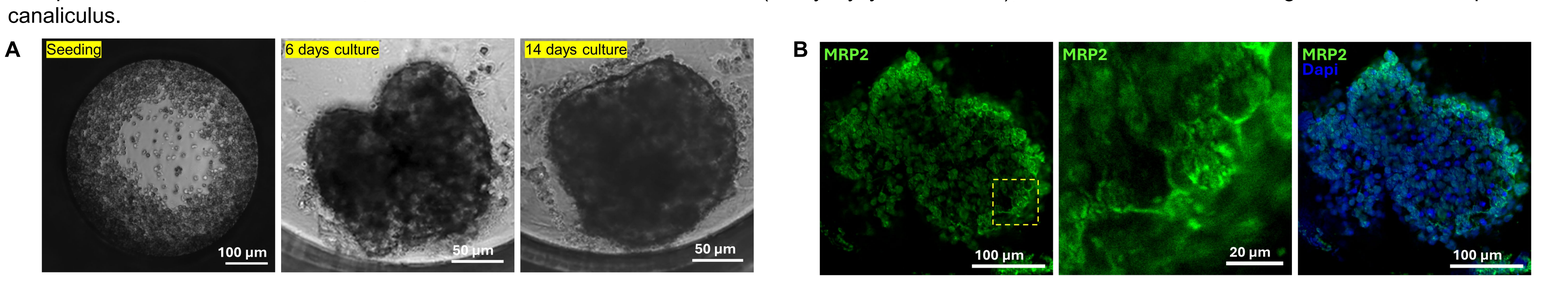


Figure 3: Spheroid-cultured HepatoXcell™ Pro in Akura 96 Spheroid Microplate (InSphero). (A) Phase-contrast images showing spheroid formation and (B) confocal images showing expression of MRP2 (14 days culture) . *MRP2 (Multidrug Resistance-Associated Protein 2); located on the canalicular membrane.

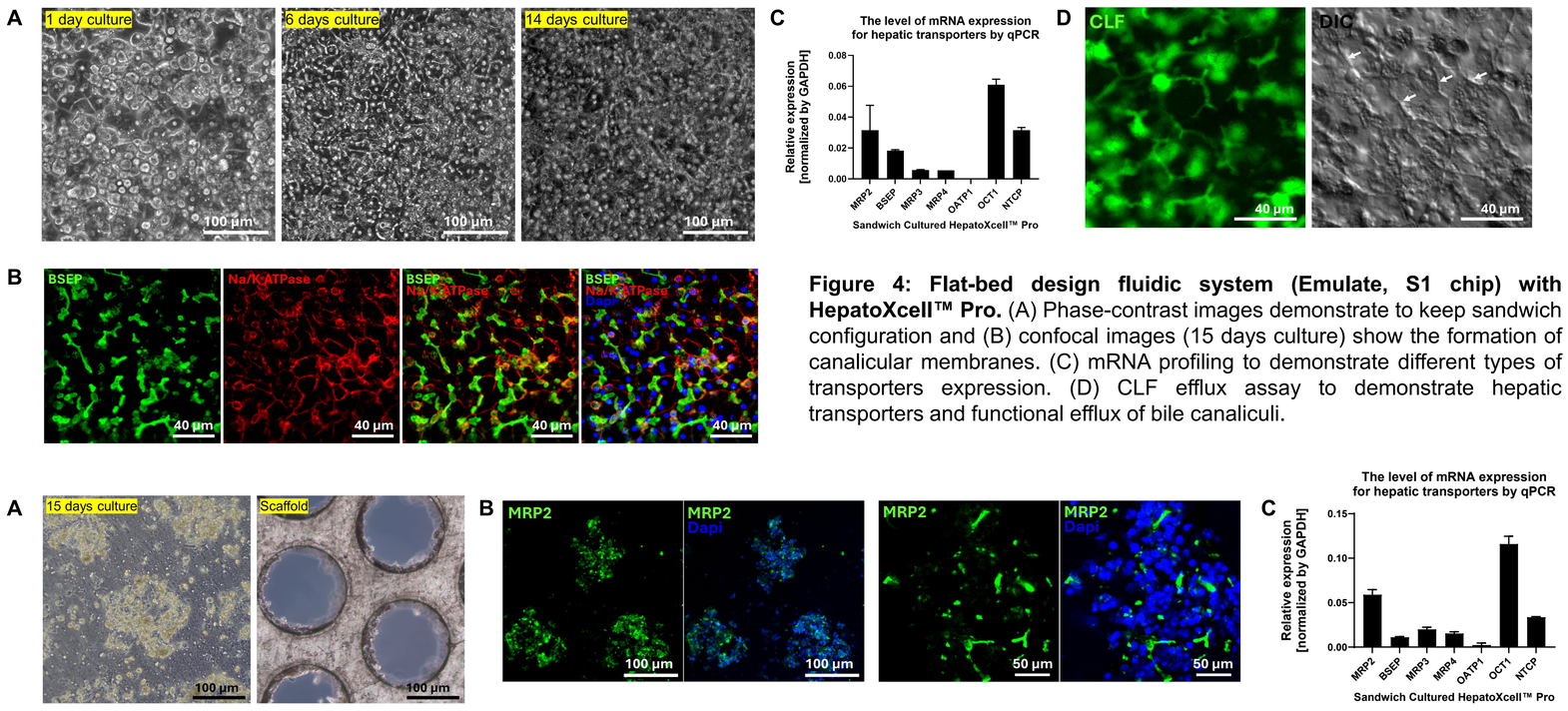


Figure 4: Flat-bed design fluidic system (Emulate, S1 chip) with HepatoXcell™ Pro. (A) Phase-contrast images demonstrate to keep sandwich configuration and (B) confocal images (15 days culture) show the formation of canalicular membranes. (C) mRNA profiling to demonstrate different types of transporters expression. (D) CLF efflux assay to demonstrate hepatic transporters and functional efflux of bile canaliculi.

Figure 5: 3-D Meshed-bed design fluidic system (CN-Bio, CN-Bio Liver) with HepatoXcell™ Pro. (A) Phase-contrast images show hepatocyte aggregation on the membrane and the scaffold after taking out from a flow chamber. (B) Confocal images (15 days culture) show MRP 2 expression in the aggregation of hepatocytes. (C) Hepatocytes express various transporters on surface membranes to facilitate the uptake and efflux of substances.

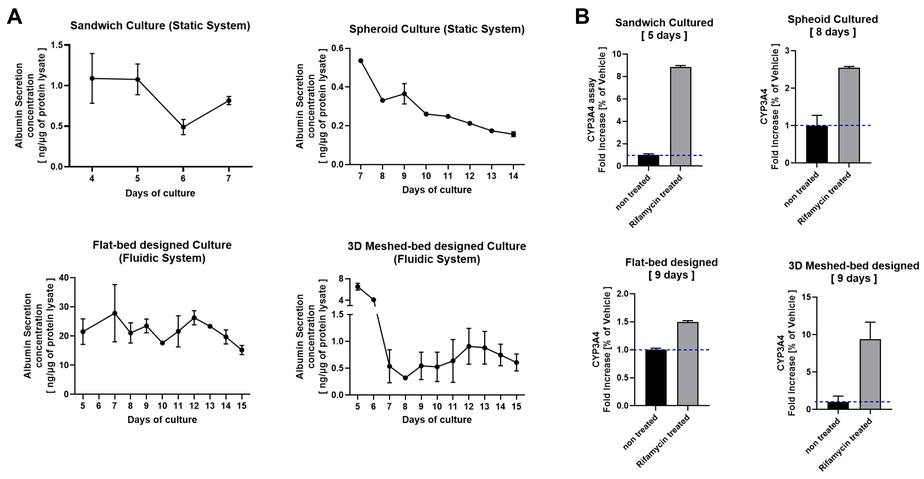


Figure 6: Albumin secretion and CYP3A4 induction to show healthy liver function in the different types of cultured HepatoXcell™ Pro. The levels of albumin in daily collected culture media were measured using ELISA (R&D systems; Human Serum Albumin DuoSet ELISA) and induced CYP3A4 was determined (Promega; P450-Glo CYP3A4 Assay) after a 48 hr incubation with 10 µM Rapamycin. *CYP3A4 (cytochrome P450 3A4); a drug-metabolizing enzyme.

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