Abstract # 4136

Human Liver-on-a-Chip Systems for Enhanced Mechanism-Based Toxicity Screening

Changsuk Moon, PhD; Ajeet Singh, PhD; Fernanda Ventura, BS; Ash Lee Manley, BS; Emma Todd, BS; Amber Sieffert, BS; and Carolina Lucchesi, PhD

ATCC, Manassas, VA 20110

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Introduction

Lipid nanoparticles (LNPs) are leading non-viral carriers for nucleic acid therapeutics due to their modular composition, which supports efficient payload encapsulation, cellular uptake, and endosomal escape. A major design consideration is their strong tendency to accumulate in the liver after systemic administration. While useful for liver-targeted delivery, it also raises concerns about hepatotoxicity to induce liver damage and inflammation.

Predicting drug efficacy and toxicity requires liver models that closely mirror human physiology. Traditional immortalized hepatocyte lines lack critical metabolic enzymes and transporter activities, limiting their utility for mechanistic or translational studies. In contrast, ATCC's high-quality HepatoXcell™ primary human hepatocytes retain native metabolic function, lipid processing capability, and uptake pathways essential for accurate evaluation of LNP-based therapeutics. To ensure consistency and performance, ATCC provides optimized thawing, plating, and maintenance media, and each HepatoXcell™ lot undergoes MPS-specific qualification. Moreover, the liver MPS platform sustains hepatocyte phenotype and metabolic activity over extended culture periods, enabling assessments of durability, repeat-dose toxicity, and immune-mediated effects that cannot be captured in conventional static cultures.

Table 1: HepatoXcell™ products

Product Name	ATCC® No	Format	Amount
HepatoXcell™ Eco	PCS-450-012™	Suspension	1 vial, ≥ 4 x 10^6 cells
HepatoXcell™ Plus	PCS-450-010™	3-Day Plateable	1 vial, ≥ 4 x 10^6 cells
HepatoXcell™ Pro	PCS-450-011™	7-Day Plateable	1 vial, ≥ 4 x 10^6 cells
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

Media optimization further enhances LNP evaluation in MPS. Components such as serum and ApoE shape the LNP protein corona and influence hepatocyte uptake. Media that promote physiologic apolipoprotein binding better replicate in vivo hepatic distribution. Together, advanced MPS platforms, HepatoXcell™, and optimized culture environments create a more predictive system for studying LNP delivery, potency, and hepatotoxicity over extended timeframes, improving translation from in vitro results to clinical outcomes.

In this study, we achieved efficient LNP-mediated delivery of GFP mRNA in a liver-on-a-chip platform cultured under serum-free, 10% serum, and ApoE-supplemented conditions to assess transfection efficiency and toxicity over 19 days. Our findings demonstrate that the HepatoXcell™ system provides a physiologically relevant liver microenvironment suitable for evaluating pharmaceutical LNP-encapsulated therapeutics and conducting long-term liver toxicity studies.

Materials and methods

HepatoXcell™ Pro Culture: HepatoXcell™ Pro cells were thawed using HepatoXcell™ Thawing Medium, centrifuged, and plated onto either a 24-well plate or a Chip-R1 (Emulate). Following plating, cells were overlaid with Cell Basement Membrane (ATCC® ACS-3035™). Hepatocytes were then maintained under continuous culture conditions using HepatoXcell™ Maintenance Medium.

LNP-GFP mRNA Delivery: LipidLaunch SM-102 LNP-encapsulated GFP mRNA (0.5 mg/mL; Cayman Chemical, 39320) was diluted in HepatoXcell™ Maintenance Medium supplemented with either 15 µg/mL ApoE3 (Thermo Fisher Scientific, 350-02), 10% Fetal Bovine Serum (FBS; ATCC® 30-2020™), or no additional supplement. The formulations were introduced into the top channel of the chip for treatment.

Characterization of HepatoXcell™ Pro: Phase-contrast imaging and immunostaining for BSEP (Bile Salt Export Pump) and Na/K ATPase were performed to assess hepatocyte polarity and formation of bile canalicular networks. Albumin immunostaining was used to evaluate functional metabolic activity. A CLF (Cholyl-Lysyl-Fluorescein; AAT Bioquest,36701) efflux assay was performed to confirm activity of hepatic transporters and functional canalicular efflux. Additionally, HepatoXcell™ Pro cultured under 2-D sandwich conditions was subjected to RNA-seq analysis to profile mRNA expression of hepatic enzymes and transporters.

Functional Assays of HepatoXcell™ Pro: Effluent from the chip's top channel was collected daily to quantify albumin secretion using an ELISA (R&D Systems, DY1455) and urea production using a Urea Assay Kit (Abcam, ab83862). Cell viability and cytotoxicity were assessed using the CellTiter-Glo 2.0 Cell Viability Assay (Promega, G9241) and the LDH-Glo Cytotoxicity Assay (Promega, J2380).

Results

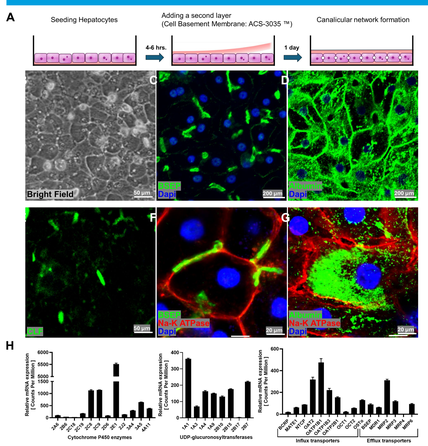

Figure 1: Qualification of HepatoXcell™ Pro in 2-D sandwich culture (Day 5). (A) Schematic illustration of hepatocytes maintained in a conventional 2-D sandwich configuration. (B) Bright-field imaging demonstrates typical hepatocyte morphology. (C, F) BSEP immunostaining confirms proper localization of canalicular transporters, indicating polarized hepatocyte architecture and formation of functional canalicular networks. (D, G) Albumin staining serves as a marker of hepatocyte viability and metabolic activity. (E) CLF efflux assay validates functional hepatic transporter activity and bile canalicular efflux. (H) RNA-sequencing analysis shows sustained expression of key drug-metabolizing enzymes and transporters, indicating preservation of hepatic functional profiles in 2-D sandwich culture. *n=5

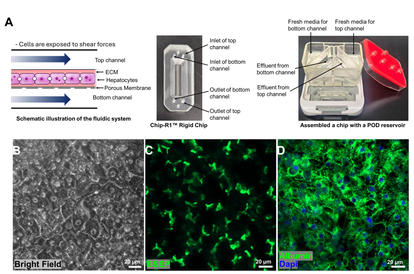

Figure 2: MPS-qualified HepatoXcell™ Pro (A) Schematic illustration of the microfluidic system and overview of the fluidic culture system (Emulate, Chip-R1 Rigid Chip) showing continuous media perfusion through the top and bottom channels, exposing cells to physiologically relevant shear forces. (B–D) Confocal imaging after 6 days of fluidic culture reveals well-defined canalicular membrane structures and robust albumin expression, indicating functional polarization and maturation of HepatoXcell™ Pro under flow conditions.

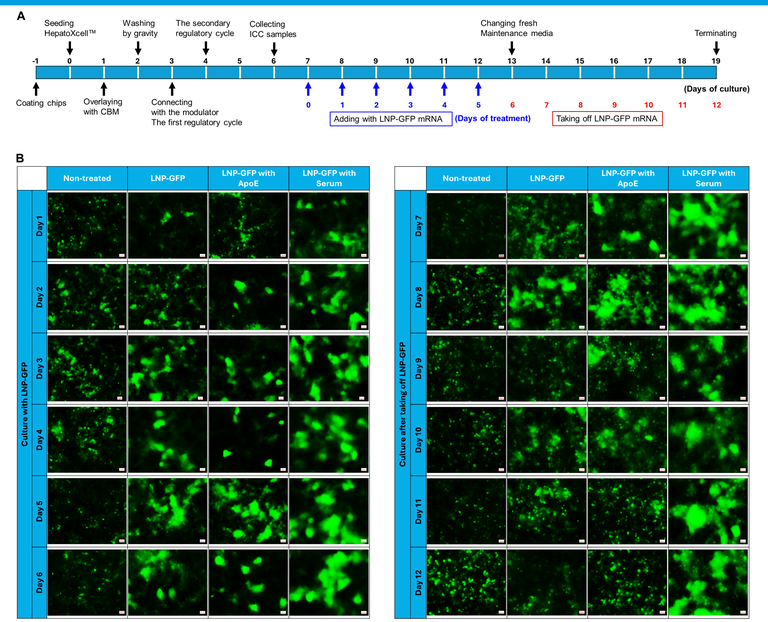

Figure 3: LNP-GFP mRNA delivery and expression dynamics in the liver-on-a-chip system. (A) Timeline of LNP-GFP mRNA administration in HepatoXcell™ Pro cultured under fluidic conditions over a 19-day period.(B) Fluorescence microscopy images showing GFP expression following LNP-GFP mRNA delivery, highlighting supplement-dependent transfection efficiency. Serum-supplemented media produced detectable GFP expression by Day 1, with signal maintained for more than 12 days. ApoE-supplemented media generated GFP expression beginning on Day 2, whereas unsupplemented media showed signal onset on Day 3. Following removal of LNP treatment, GFP expression persisted for an additional 4 days in both ApoE-supplemented and unsupplemented conditions.

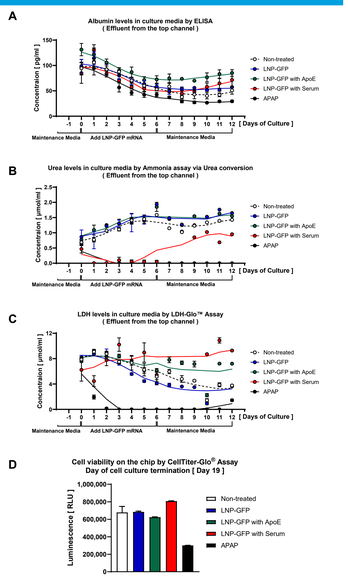

Figure 4: Assessment of HepatoXcell™ Pro metabolic function and cytotoxicity under LNP treatment. (A) Albumin secretion remained stable throughout the period, showing no significant changes following exposure to LNP-GFP mRNA. (B) Urea production decreased during LNP-GFP mRNA treatment in serum-containing media but recovered after treatment cessation. (C, D) Exposure to 0.5 mg/mL LNP-GFP mRNA did not induce detectable toxicity. As a positive control, 10 mM acetaminophen (APAP) was used to induce hepatocyte toxicity.

Conclusions

- HepatoXcell™ Pro maintains strong hepatic phenotype in fluidic culture, preserving polarity, canalicular networks, and key metabolic markers.
- HepatoXcell™ Pro remains stable and functional under shear stress, confirming suitability for long-term fluidic culture applications.
- LNP-GFP mRNA transfection is efficient and supplement-dependent, with sustained GFP expression across conditions.
- Minimal impact on albumin, urea, LDH, and ATP levels demonstrates excellent viability and low toxicity during 0.5 mg/ml LNP treatment.
- Overall, HepatoXcell™ Pro provides a reliable and physiologically relevant platform for evaluating LNP mRNA delivery, and safety in microfluidic liver-on-a-chip models.

Learn more about HepatoXcell™

ATCC 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** sales@atcc.org **Web:** www.atcc.org