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Modeling MASLD in a Dynamic Human Liver Microphysiological System

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Introduction

Metabolic dysfunction–associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease worldwide, with global prevalence rising from approximately 25.3% in 1990–2006 to 38% in 2016–2019. Although only a subset of patients (3–5%) progress to inflammatory disease with fibrosis or cirrhosis, increasing incidence at younger ages suggests a growing long-term risk of disease progression. MASLD is defined by hepatic lipid accumulation, insulin resistance, and metabolic dysfunction in the absence of clinically significant alcohol consumption, with progression driven by the severity and coexistence of metabolic risk factors leading to fibrosis, cirrhosis, or hepatocellular carcinoma.

Proper in vitro modeling of MASLD requires validated, physiologically relevant hepatocytes and culture systems capable of recapitulating key aspects of the liver microenvironment. HepatoXcell™ Pro 7-day plateable primary human hepatocytes (PCS-450-011™) provide consistent and reliable metabolic activity, sustained differentiation, and viability, making them well suited for steatosis induction. While these hepatocytes can be maintained in conventional 2-D sandwich culture, such static systems fail to reproduce essential physiological features—including fluid-induced shear stress, dynamic nutrient and oxygen gradients, cellular crosstalk, and long-term culture support—thereby limiting their relevance for modeling MASLD. To overcome these constraints, a 3-D microphysiological fluidic system enables long-term co-culture and chronic oleic acid exposure under perfused conditions, supporting sustained lipid accumulation and MASLD-relevant cellular responses under more physiologically representative conditions.

In this study, we established a 3-D MASLD model using cryopreserved HepatoXcell™ Pro hepatocytes cultured in a microphysiological chip platform. Steatosis was induced through optimized oleic acid supplementation while preserving hepatocyte morphology and viability. Lipid accumulation was monitored over time, demonstrating a stable and reproducible in vitro MASLD model suitable for mechanistic studies and translational toxicology and drug development applications.

HepatoXcell™

Primary human hepatocytes (PHH) are the gold standard for in vitro liver models, offering high predictive value for drug metabolism and toxicity.

ATCC hepatocytes are carefully isolated and characterized to deliver reliable, high-quality performance for drug development.

Learn more about HepatoXcell™

Materials and Methods

HepatoXcell™ Pro–induced steatosis: 2-D sandwich configuration

HepatoXcell™ Pro hepatocytes were thawed using HepatoXcell™ Thawing Medium (ATCC® PCS-450-032™) and plated in HepatoXcell™ Plating Medium (ATCC® PCS-450-038™). Four hours after seeding, a second layer of Cell basement membrane (ATCC® ACS-3035™) was added to establish a sandwich culture. Cells were then maintained in HepatoXcell™ Maintenance Medium (ATCC® PCS-450-034™) and treated with varying concentrations and oleic acid (OA; Cayman Chemical, 29557) to identify conditions that induced steatosis while maintaining cell viability.

HepatoXcell™ Pro–induced steatosis: 3-D microfluidic platform

HepatoXcell™ Pro Hepatocytes were seeded into the top channel of a chip (Emulate, S1), and a second layer of cell basement membrane was added the following day. Liver sinusoidal endothelial cells (LSECs; Cell Systems, ACBRJ 566) were subsequently seeded into the bottom channel. The chips were then connected to flow at a rate of 30 seconds per hour. OA was applied in HepatoXcell™ Maintenance Medium to the top channel to induce steatosis, while Endothelial cell medium (ATCC® PCS-100-030™ and ATCC® PCS-100-041™) without OA was perfused through the bottom channel.

Results

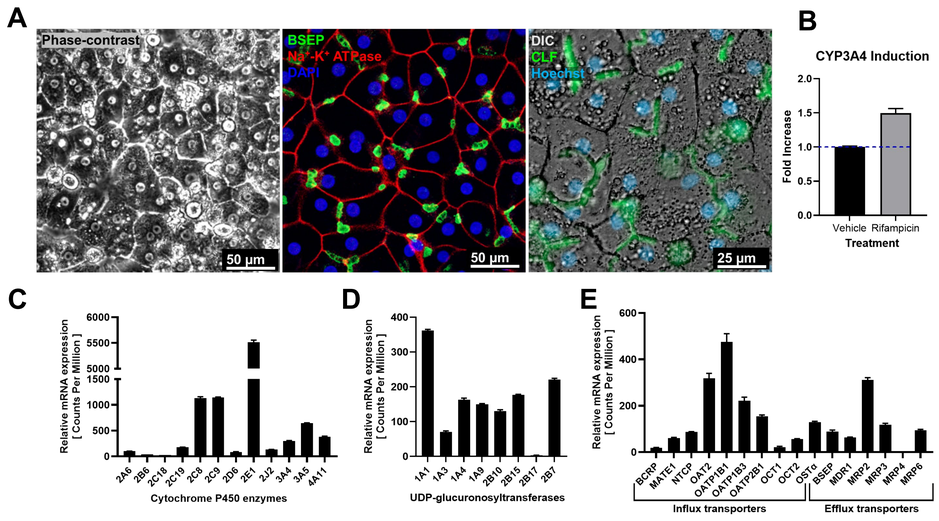

Figure 1: Functional characterization of HepatoXcell™ Pro Hepatocytes. (A) Sandwich-culture HepatoXcell™ Pro hepatocytes were imaged under phase-contrast microscopy to assess cellular morphology and structural integrity. (B) Immunofluorescent staining for BSEP (bile salt export pump; Santa Cruz biotechnology, sc-74500) and Na^+/K^+-ATPase (Abcam, ab76020) demonstrates hepatocyte polarization, differentiation, and transporter functionality. (C) Hepatocytes were exposed to 5 µM CLF (Cholyl-Lysyl-Fluorescein; AAT Bioquest, 36701) to examine the functional activity of BSEP. (D) Functional cytochrome P450 activity was further confirmed by a CYP3A4 induction assay (Promega, V9001) following 10 µM Rifampicin (Selleck Chemicals, S1764) treatment for 24 hr, demonstrating metabolic competence of HepatoXcell™ Pro hepatocytes (n=2). (E) Gene expression profiling of cytochrome P450 enzymes, (F) UDP-glucuronosyltransferases, and (G) hepatic uptake and efflux transporters further confirms preservation of key hepatocyte metabolic and transport pathways (n=5).

Results

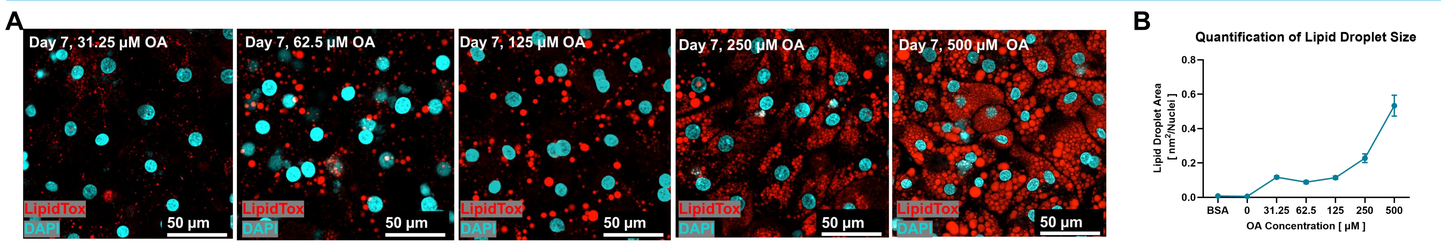

Figure 2: Dose-dependent OA–induced lipid accumulation in HepatoXcell™ Pro. (A) Representative confocal images showing dose-dependent lipid accumulation in HepatoXcell™ Pro cultured in sandwich culture and treated with increasing concentrations of OA at defined different concentrations for 7 days. Lipid droplets were stained with LipidTOX (Thermo Fisher Scientific, H34476). (B) Quantification of lipid droplet size was performed using ImageJ analysis to identify optimal conditions for lipid accumulation. The graph demonstrated the strongest dose-dependent increase in lipid droplet size (n=3). BSA was included as a control condition for BSA–fatty acid complexes (Cayman Chemical,29556).

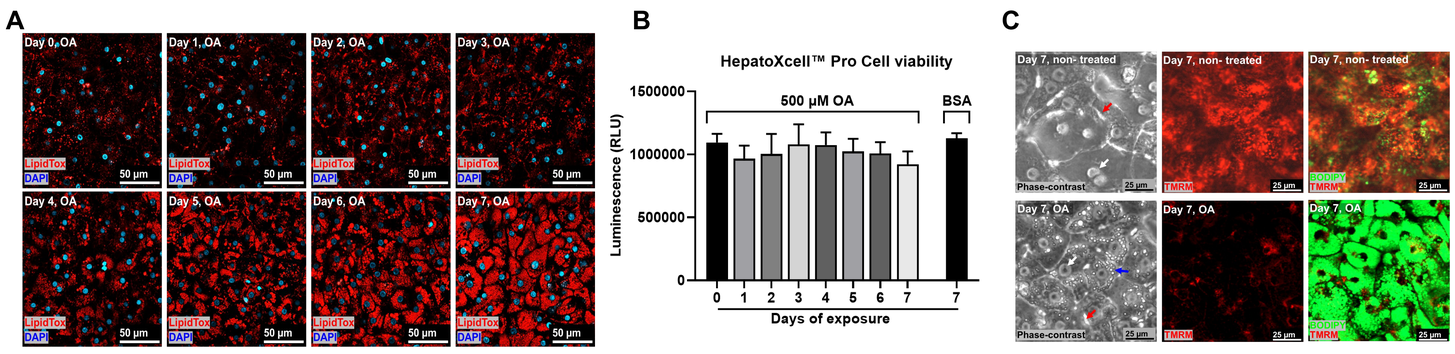

Figure 3: Modeling steatosis in HepatoXcell™ Pro Hepatocytes: Temporal lipid accumulation and mitochondrial impairment. (A) Sandwich culture HepatoXcell™ Pro cells were treated with 500 µM OA for 1–7 days or left non-treated control to evaluate time-dependent lipid accumulation. Representative images show a progressive increase in lipid droplet size and abundance with longer OA exposure. (B) Cell viability following different durations of OA exposure (0–7 days) was assessed using CellTiter-Glo® (Promega, G9241). No significant change in viability was observed relative to non-treated/ BSA-treated controls as well (n=3). (C) Bright-field imaging demonstrates that the sandwich culture maintains structural integrity through day 7 following treatment with 500 µM OA. The canalicular network (red arrow) remains preserved during OA induction over this period, accompanied by increased lipid accumulation (blue arrow). HepatoXcell™ Pro hepatocytes were stained with TMRM (Thermo Fisher Scientific, T668) to assess mitochondrial membrane potential and with BODIPY (Thermo Fisher Scientific, D3922) to label neutral lipids. Compared with non-treated controls, OA-treated cells showed reduced TMRM fluorescence and increased BODIPY signal, indicating mitochondrial dysfunction and enhanced lipid accumulation.

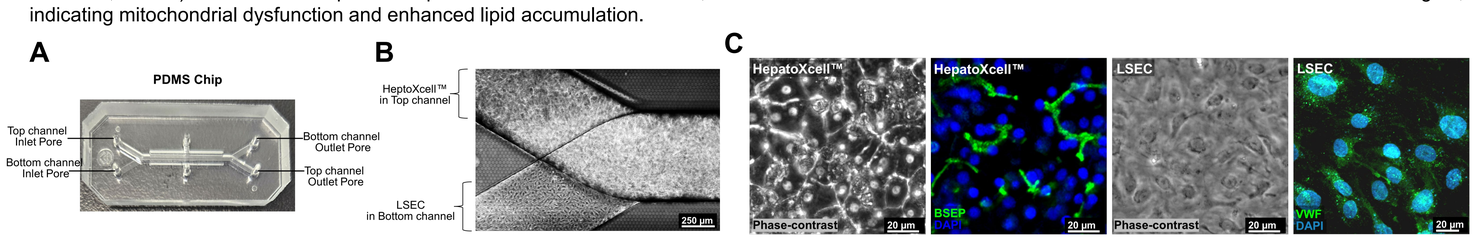

Figure 4: Liver-on-chip co-culture setup and characterization. (A) The PDMS (polydimethylsiloxane) chip features a design with two crossing channels. (B) Phase-contrast images demonstrate that HepatoXcell™ Pro hepatocytes were seeded in the top channel, while liver endothelial cells occupied the bottom channel. (C) Phase-contrast microscopy assessed morphology and integrity of HepatoXcell™ Pro hepatocytes and endothelial cells. Immunofluorescence staining for BSEP indicated hepatocyte polarization, and VWF (Abcam, ab287962) marked endothelial cells.

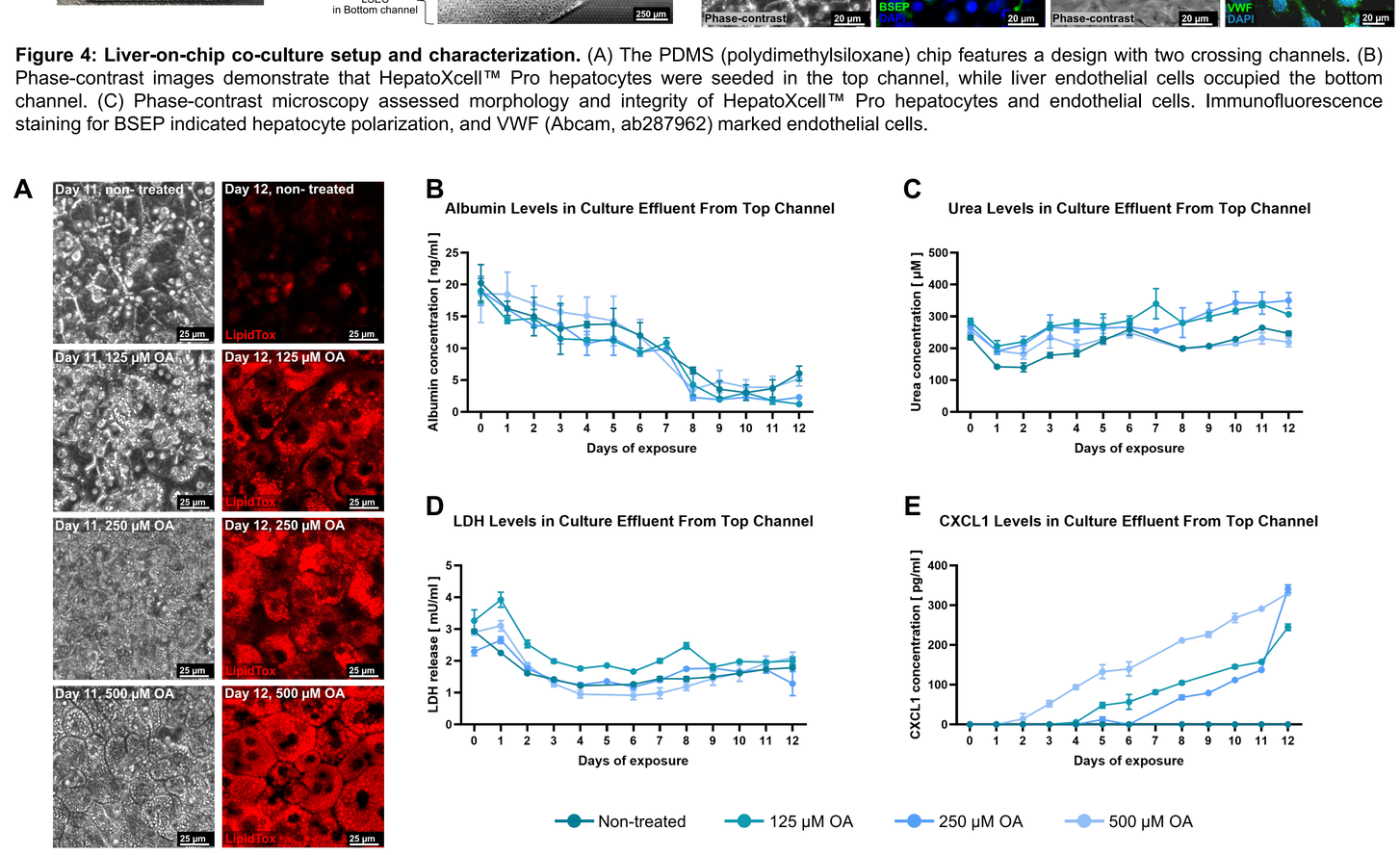

Figure 5. OA–induced lipid accumulation preserves hepatocyte function while promoting inflammatory responses in HepatoXcell™ Pro liver chips. (A) Phase-contrast images and confocal images showing increased lipid accumulation in HepatoXcell™ Pro hepatocytes treated with 125, 250, and 500 µM OA on the chips compared to non-treated controls. Cells exhibit progressive enlargement with increasing fatty acid concentration while maintaining hepatocyte morphology. (B) Albumin secretion measured by DuoSet ELISA (R&D systems, DY1455) over 12 days demonstrates maintained hepatic protein production across treatment conditions. (C) Urea production measured by ammonia assay after urea conversion (Abcam, ab83362) over 12 days showed minimal differences between OA-treated and control groups, indicating preserved hepatocyte function. (D) LDH release measured in culture effluent over 12 days showed similar trends across all groups, indicating comparable OA-induced cytotoxicity (Promega, J2380). (E) CXCL1 secretion, measured using the DuoSet ELISA kit (R&D Systems, DY275), increased over a 12-day period in oleic acid (OA)–treated hepatocytes compared to controls, indicating an inflammatory response. (n=2)

Conclusions

- ATCC-provided HepatoXcell™ Pro hepatocytes exhibit high viability, robust metabolic activity, reliable physiological performance, structural integrity, functional activity, and relevant gene expression in a single platform.
- Fatty acid–exposed HepatoXcell™ Pro hepatocytes exhibit key features of MASLD, including increased lipid droplet accumulation, reduced mitochondrial function, and elevated inflammatory signaling.
- HepatoXcell™ Pro hepatocytes are well suited for establishing a MASLD model in a microphysiological platform, maintaining integrity during long-term culture, co-culture, and shear stress flow conditions.
- ATCC provides guidance for developing 3D liver architectures using HepatoXcell™ Pro Human Hepatocytes, enabling the study of liver disease mechanisms for drug discovery and screening applications..

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