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Modeling MASLD with Primary Human Hepatocytes in a Physiologically Relevant Microfluidic Platform

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease worldwide, with a rapidly increasing global prevalence driven by obesity, insulin resistance, and type 2 diabetes. Characterized by excessive hepatic lipid accumulation and metabolic dysfunction, MASLD can progress to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma, highlighting the need for clinically relevant therapeutic strategies. Development of predictive in vitro MASLD models is essential for understanding disease mechanisms and evaluating potential therapeutics. Primary human hepatocytes provide a physiologically relevant platform for modeling hepatic metabolism and lipid accumulation. However, conventional static culture systems do not fully reproduce the liver microenvironment and often exhibit limited long-term functionality. The HepatoXcell™ Pro cryopreserved primary human hepatocyte system enables sustained hepatocyte differentiation, metabolic activity, and viability, supporting the development of reproducible steatosis models. The dual GIP/GLP-1 (Glucose-Dependent Insulinotropic Polypeptide/Glucagon-Like Peptide-1) receptor agonist tirzepatide has demonstrated significant clinical benefits in obesity and metabolic disorders. Emerging evidence suggests that this agent may also reduce hepatic steatosis; however, its direct effects on human hepatocytes remain incompletely understood. The objective of this study was to establish a primary human hepatocyte-based in vitro MASLD model and evaluate the effects of tirzepatide on oleic acid (OA)-induced lipid accumulation. Hepatic steatosis was induced through OA supplementation, and lipid accumulation was quantified to assess the ability of tirzepatide to mitigate the steatotic phenotype while maintaining hepatocyte viability and function.

Materials and Methods

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

HepatoXcell™ Pro hepatocytes (ATCC® PCS-450-011™) 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, S-1), 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

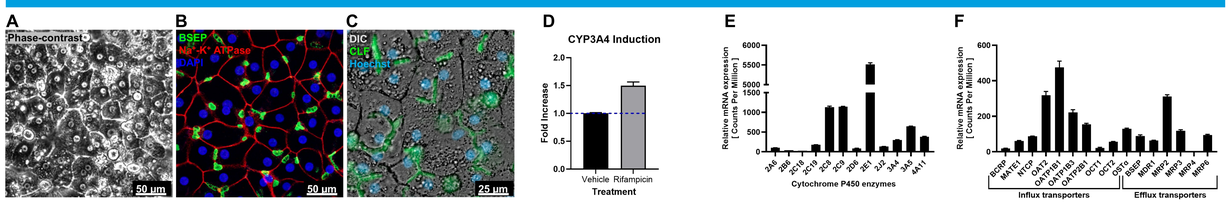

Figure 1: Functional characterization of HepatoXcell™ Pro Hepatocytes. (A) Sandwich-culture HepatoXcell™ Pro hepatocytes were imaged under phase-contrast microscopy to assess cellular morphology and structural integrity. (B) Immunofluorescent staining for BSEP (bile salt export pump; Santa Cruz Biotechnology, 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 and (F) hepatic uptake and efflux transporters further confirms preservation of key hepatocyte metabolic and transport pathways (n=5).

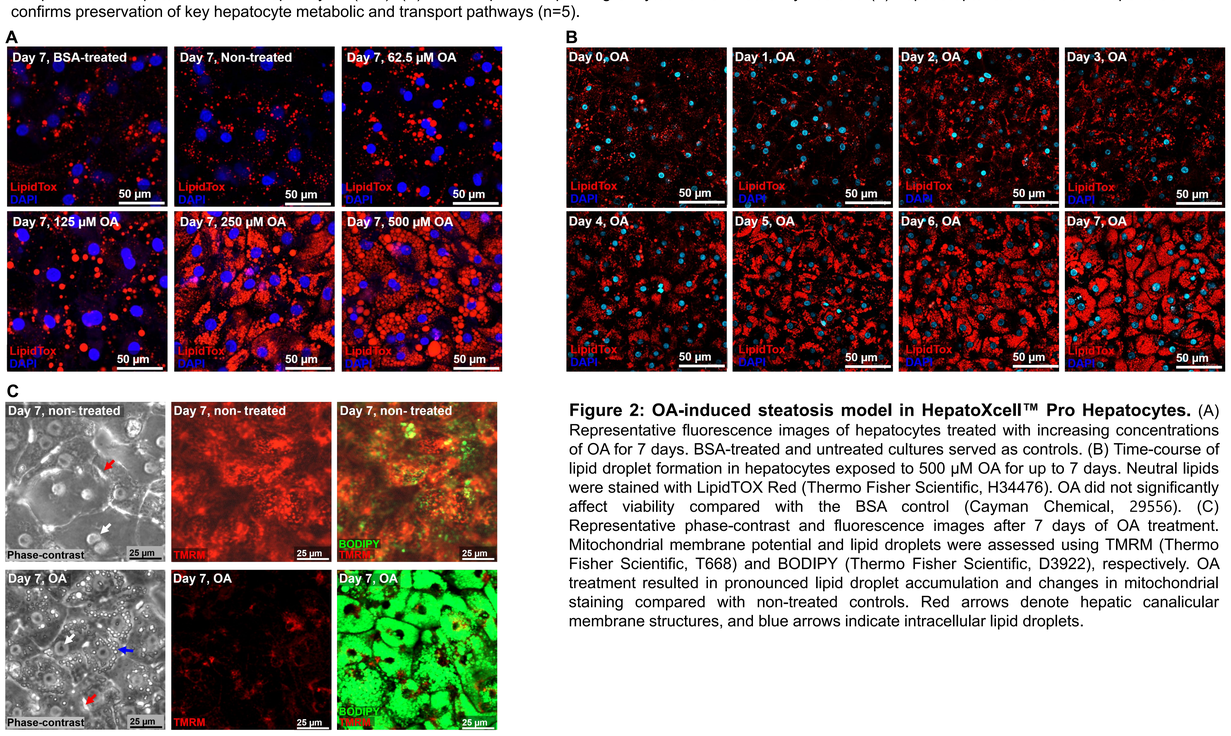

Figure 2: OA-induced steatosis model in HepatoXcell™ Pro Hepatocytes. (A) Representative fluorescence images of hepatocytes treated with increasing concentrations of OA for 7 days. BSA-treated and untreated cultures served as controls. (B) Time-course of lipid droplet formation in hepatocytes exposed to 500 µM OA for up to 7 days. Neutral lipids were stained with LipidTOX Red (Thermo Fisher Scientific, H34476). OA did not significantly affect viability compared with the BSA control (Cayman Chemical, 29556). (C) Representative phase-contrast and fluorescence images after 7 days of OA treatment. Mitochondrial membrane potential and lipid droplets were assessed using TMRM (Thermo Fisher Scientific, T668) and BODIPY (Thermo Fisher Scientific, D3922), respectively. OA treatment resulted in pronounced lipid droplet accumulation and changes in mitochondrial staining compared with non-treated controls. Red arrows denote hepatic canalicular membrane structures, and blue arrows indicate intracellular lipid droplets.

Results

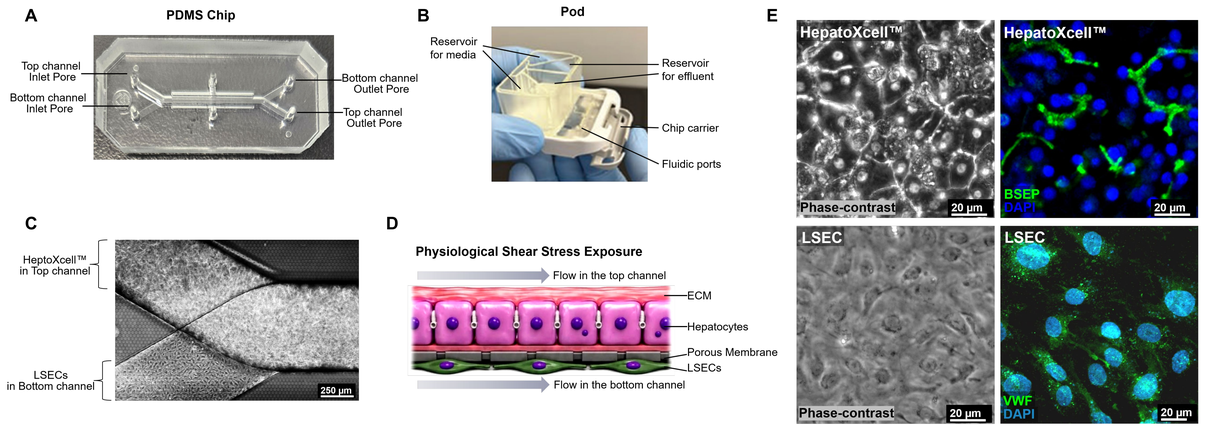

Figure 3: Microfluidic co-culture platform for HepatoXcell™ hepatocytes and liver sinusoidal endothelial cells (LSECs) under physiological shear stress. (A) Image of the chip showing the dual-channel architecture with independent inlet and outlet ports. (B) Microfluidic pod assembly used for continuous perfusion culture. (C) Bright-field image of the co-culture device with HepatoXcell™ hepatocytes seeded in the upper channel and LSECs seeded in the lower channel. (D) Schematic representation of the 3-D microfluidic platform, in which hepatocytes and LSECs are cultured on opposite sides of a porous membrane and subjected to physiologically relevant shear stress under continuous flow. The image was created by AI. (E) Representative phase-contrast and immunofluorescence images of HepatoXcell™ hepatocytes and LSECs and immunofluorescence staining for BSEP indicated hepatocyte polarization, and VWF (Abcam, ab287962) marked endothelial cells.

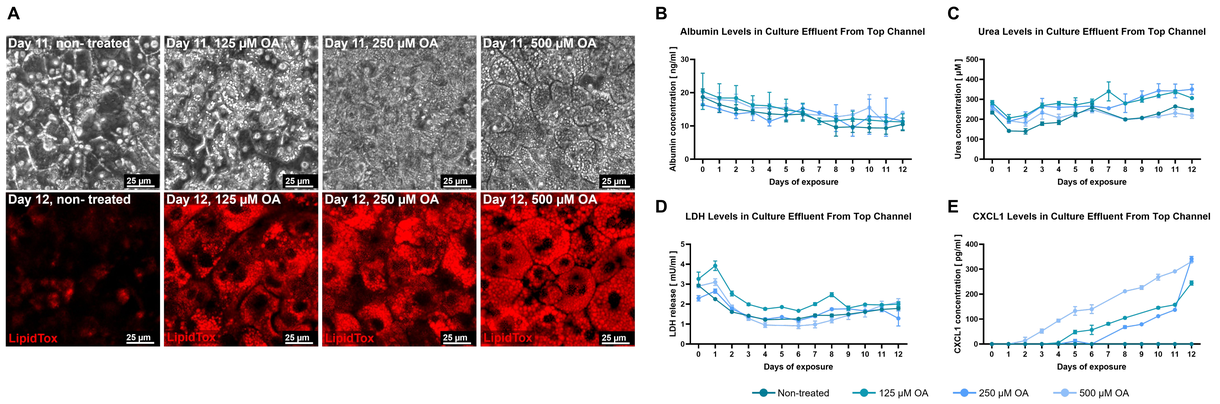

Figure 4: A functional liver chip co-culture model of OA-induced steatosis using HepatoXcell™ Pro hepatocytes. (A) Phase-contrast images and confocal images showing increased lipid accumulation in HepatoXcell™ Pro hepatocytes treated with 125, 250, and 500 µM OA on the chips compared to non-treated controls. 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 per group.

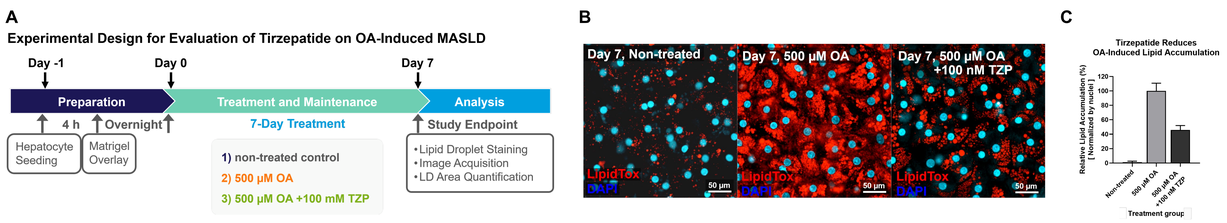

Figure 5: Tirzepatide reduces OA-induced lipid accumulation in HepatoXcell™ Pro hepatocytes. (A) Preventive study design for evaluating the effect of tirzepatide (TZP; Slleckchem, P1206) on OA-induced steatosis in primary human hepatocytes. Hepatocytes were seeded on day −1, overlaid with Matrigel on day 0, and co-treated for 7 days with 500 µM OA in the presence or absence of 100 nM TZP. On day 7, intracellular lipid accumulation was assessed by LipidTOX staining and image-based quantification. (B) Representative fluorescence images of intracellular lipid accumulation in primary human hepatocytes following treatment with control medium, 500 µM OA, or 500 µM OA plus 100 nM TZP. Neutral lipid droplets were visualized by LipidTOX staining. (C) Quantification of LipidTOX-positive area showed that TZP reduced OA-induced lipid accumulation by approximately 50% compared with OA treatment alone. *n = 3 per group.

Conclusions

- ATCC HepatoXcell™ Pro hepatocytes provide a consistent, high-quality human hepatocyte platform with sustained viability and hepatic function, supporting reproducible liver research and disease modeling.
- OA treatment successfully induced key features of MASLD, including lipid accumulation, mitochondrial dysfunction, and increased inflammatory signaling, establishing a robust in vitro steatosis model.
- HepatoXcell™ Pro hepatocytes maintained performance under long-term culture, hepatocyte-endothelial co-culture, and fluidically perfused liver-on-a-chip conditions, demonstrating suitability for advanced MPS.
- TZP reduced OA-induced lipid accumulation by approximately 50%, confirming the model's utility for detecting pharmacological responses.
- HepatoXcell™ Pro provides a physiologically relevant liver-chip platform for drug discovery, lead optimization, and mechanism-of-action studies in metabolic liver diseases, including MASLD/MASH and related hepatic disorders..

Learn more about HepatoXcell™

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