Liver-Chip: A Model for Understanding Diet-Induced Liver Disease and Drug Efficacy Assessment

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Abstract

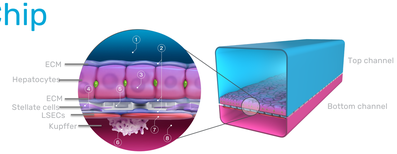
Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is a progressive condition initially characterized by increased lipid accumulation in the liver (steatosis) and can develop into nonalcoholic steatohepatitis (NASH). There is an unmet need for a human-relevant *in vitro* model to enable successful development of therapies. **Methods**: To address this unmet need, we utilized our human Liver-Chip, which retains key characteristics of native liver function over long-term culture. To induce steatosis, chips were treated with saturated (palmitate) or unsaturated (oleate) fatty acids, alone or in combination. TGF-beta was used as a positive control for hepatocellular injury and stellate cell activation. To assess therapeutic efficacy against steatosis, chips were treated for two days after initiating steatosis (therapeutic), or co-treated (prophylactic) with a liver-targeted analogue of firsocostat, a known inhibitor of acetyl-CoA carboxylase (ACC-i). Morphological evaluation of the hepatocytes and AdipoRed™ staining was used to evaluate steatosis. Quantification of triglycerides released in the media was used to evaluate lipid removal, and alpha-SMA staining was used to assess stellate cell activation. **Results**: We demonstrated induction of steatosis in hepatocytes in a concentration-dependent manner following continuous exposure to oleate, palmitate, or in combination. Withdrawal of fatty acids significantly diminished the steatotic phenotype as well as levels of triglycerides released in accordance with relevant human *in vivo* data. Administration of TGF-beta resulted in increased stellate cell activation, hepatocellular injury, and lipid accumulation compared to the vehicle controls. Chips treated with the ACC-i demonstrated a concentration-dependent reduction in lipid accumulation in both the therapeutic and prophylactic paradigms when compared to steatosis induced controls. **Conclusions**: In this study we provide preliminary data supporting the potential application of the Liver-Chip for modeling NAFLD-like phenotypes and conducting human-relevant therapeutic efficacy assessment using clinically relevant endpoints.

Combining Design, Engineering, and Biology

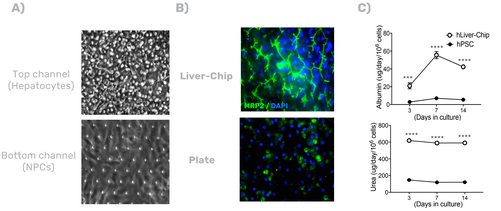
Recreating the Cellular Microenvironment in Our Chips

- Extracellular matrix and cell interactions
- Cell shape and cytoarchitecture
- Tissue-tissue interactions
- Mechanical forces
- Dynamic system — flow
- Resident or circulating immune cells

Liver-Chip



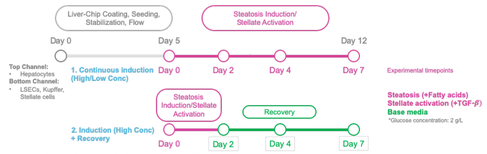
The Liver-Chip is made of polydimethylsiloxane (PDMS) and contains an upper channel (1 mm high × 1 mm wide) and a lower channel (0.2 mm high × 1 mm wide) that are separated by a porous PDMS membrane that is coated by hepatic extracellular matrix (ECM). In our design, primary hepatocytes were cultured in the upper channel and on the top of the ECM-coated membrane, and primary liver sinusoidal endothelial cells (LSECs), Kupffer cells, and hepatic stellate cells, collectively known as non-parenchymal cells (NPCs) were cultured in the lower channel and on the opposite side of the membrane.



Liver-Chip Morphology and Function
A) Brightfield images of Liver-Chip top channel (hepatocytes) and bottom channel (NPCs) maintained up to 14 days in culture. B) Liver-Chips showed localization of MRP2 at the canalicular / efflux membrane of hepatocytes vs. hepatocytes cultured on plate after 14 days in culture. C) Rates of albumin and urea secretions in human Liver-Chip vs. plate culture over 14 days.

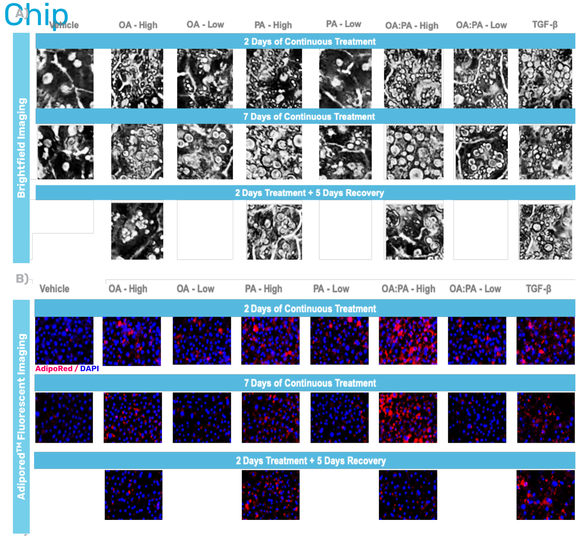
Results

Fatty Acid Treatment Schematic



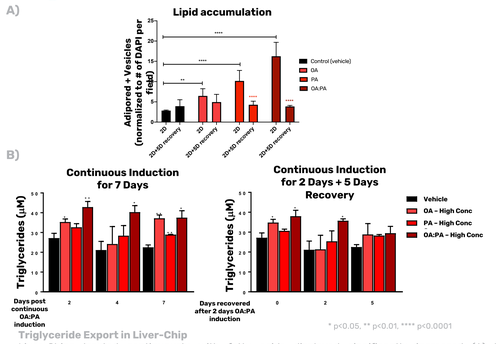
Experimental Design for Lipid Accumulation in Liver-Chip
Fatty acid treatment schematic showing the experimental timelines for steatosis induction in Liver-Chip. To model continuous induction, Liver-Chips were treated with oleic acid (OA) at 100 µM (low) and 300 µM (high), palmitic acid (PA) at 50 µM (low) and 300 µM (high), or the combination of OA and PA at 150 µM (OA:PA=100:50, low) and 600 µM (OA:PA=300:300, high) for 7 days. To model induction followed by recovery, liver-chips were treated with OA, PA, or OA:PA at high concentrations for 2 days then maintained in non treated culture media for 5 days. TGF-β was treated separately at 2.5 ng/mL. Both Top and bottom channels were dosed with fatty acids and TGF-β. Non treated Liver-Chip was used at controls.

Induction of Lipid Accumulation in Liver-Chip



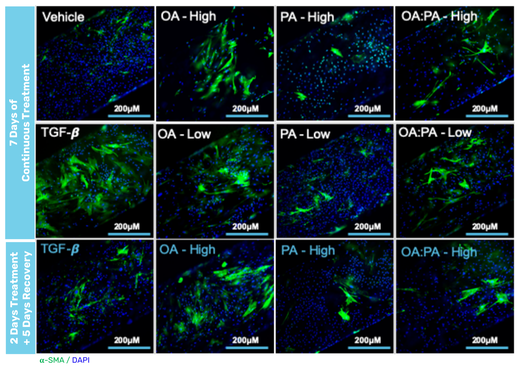
Induction of Lipid Accumulation in Liver-Chip
A) Brightfield imaging of hepatocytes in Liver-Chip showing increased incidence and sizes of lipid droplets after continuous treatment with fatty acids for 2 and 7 days vs. vehicle controls. B) Increased expression of lipophilic stain, Adipored™ confirming increased lipid accumulation in hepatocytes within Liver-Chips treated continuously with fatty acids for 2 and 7 days. C) Morphometric analysis of Adipored™ and DAPI staining of hepatocytes revealed significantly increased lipid accumulation in fatty acid treated Liver-Chips as well as TGFβ treatment vs vehicle controls. TGFβ resulted in toxicity as evident by significantly reduced hepatocyte nuclei vs vehicle controls.

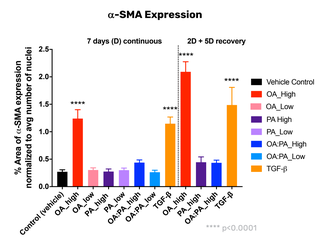
Triglyceride Export in Liver-Chip



Triglyceride Export in Liver-Chip
Liver-Chips treated continuously with fatty acids displayed significantly increased (A) lipid accumulation and (B) triglyceride (TG) export over time vs. vehicle controls. Removal of fatty acid stimuli for 5 days after 2 days overload with fatty acids resulted in significantly reduced accumulated lipids and reduced export of fatty acids over time concomitant with the reduced lipids seen following recovery.

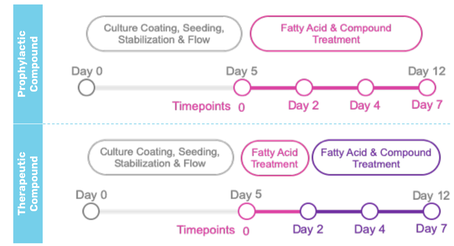
Hepatic Stellate Cell Activation in Liver-Chip





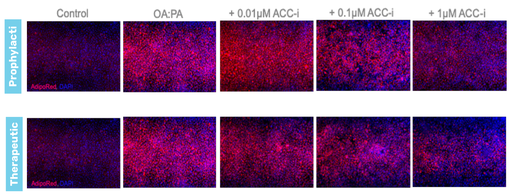
Hepatic Stellate Cell Activation in Liver-Chip
Liver-Chips treated continuously with TGFβ and high concentrations of OA for 7 days revealed significantly increased α-SMA expression (marker for hepatic stellate cell activation) vs. vehicle controls. Stellate cells treated with high concentrations of OA and TGFβ remain significantly activated following 2 days induction then 5 days recovery without stimuli.

Fatty Acid and Compound Treatment Schematic



Experimental Design for Compound Efficacy testing in Fatty Liver-Chip
To model a prophylactic treatment regimen, liver-chips were simultaneously treated with the high concentration (600 µM) of OA:PA and the ACC-i for 7 days. To model a therapeutic approach, liver-chips were treated with high concentration (600 µM) of OA:PA for 2 days followed by treatment with ACC-i and OA:PA for 5 days. Non treated and OA:PA only treated liver-chips were used at controls.

Compound Efficacy Assessment in Liver-Chip



Compound Efficacy Assessment in Liver-Chip
Liver-Chips continuously treated for 7 days with high concentrations of OA:PA displayed increased lipid accumulation. ACC-I treatment in both the prophylactic (top panel) and therapeutic (bottom panel) treatment regimens resulted in a concentration dependent decrease in lipid accumulation when compared to vehicle controls.

Conclusions

In this study we provide preliminary data supporting the potential application of the Liver-Chip for modeling NAFLD-like phenotypes and conducting human-relevant therapeutic efficacy assessment using clinically relevant endpoints such as the histological assessment of hepatic lipid accumulation.