Liver-Chip Model for Human Fatty Liver Disease

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Disclosure:

1- DBP is an employee of Emulate, Inc., and holds employee stock options

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Introduction

Liver lipid accumulation (fatty liver) is an early step in the development of drug-induced, alcoholic (ASH) and non-alcoholic steatohepatits (NASH) that may progress into cirrhosis and hepatocellular carcinoma.

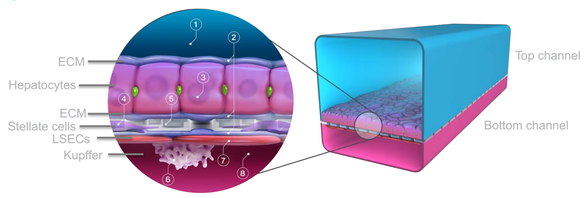
A wealth of animal studies and *in vitro* models have elucidated molecular mechanisms associated with NASH, whereas translation of these findings to effective therapeutics for humans, remains an unmet medical need. In the effort to establish more predictive and human-relevant models for fatty liver disease/steatosis, a microengineered Liver-Chip was developed to include hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer, and stellate cells for more accurate representation of the *in vivo* tissue. This Liver-Chip has the potential for long-term maintenance of cell viability to enable repeated drug exposure and exhibited hepatic functions that recapitulate *in vivo* metabolic capabilities. The Liver-Chip has well-characterized toxicology endpoints sensitive for cell-specificity over time and is capable of demonstrating the diverse mechanisms of drug-induced liver injury. The model incorporates relevant cell-ECM interactions, a hepatocyte and LSEC interface, with relevant cytoarchitecture and physiological flow. Liver-specific functions were measured for all three species and demonstrated maintenance of *in vivo* relevant levels of functionality including albumin secretion, CYP450 enzyme activity, and gene expression of certain markers that demonstrated improved function over conventional monolayer models. We evaluated the ability of the human Liver-Chip to respond to steatosis inducers (e.g., free fatty acid, glucose and ethanol).

Combining Design, Engineering, and Biology

Recreating the Cellular Microenvironment in Our Chips

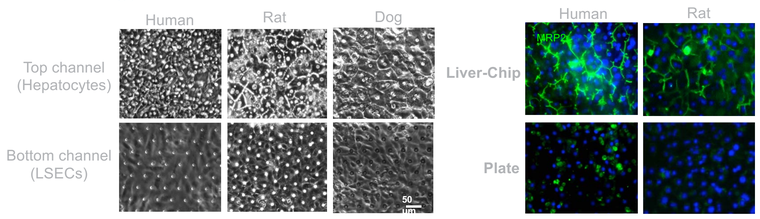
- Extracellular matrix and cell interactions
- Cell shape and cytoarchitecture
- Tissue-tissue interactions
- Mechanical forces
- Dynamic system — flow
- Resident or circulating immune cells

Liver-Chip



To construct 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) were cultured in the lower channel and on the opposite side of the membrane in combination or not with primary Kupffer cells and stellate cells. This arrangement recapitulates the hepatocyte–sinusoidal endothelial interface.

Morphology

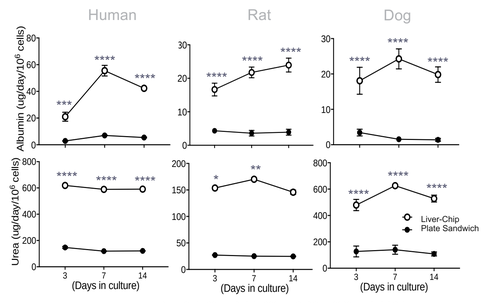


Liver-Chip Morphology
Co-cultured primary hepatocytes and LSECs maintained their stereotypical morphologies in the human, rat, and dog Liver-Chips for up to 14 days in culture.

MRP2 Localization
Liver-Chips showed localization of MRP2 at the canalicular wall of hepatocytes upon 14 days in culture, whereas plates showed only background staining of non-polarized or dead cells.

Results

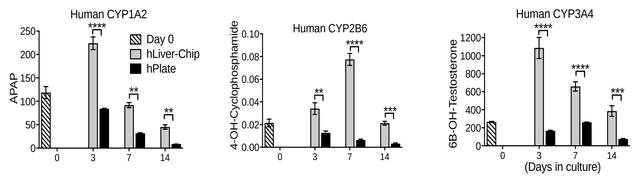
Liver Function



* p<0.1, ** p<0.01, *** p<0.001, **** p<0.0001

Liver Function
Rates of albumin and urea secretions in human, rat, and dog Liver-Chips were evaluated and compared to conventional plate culture over 2 weeks. All three species of Liver-Chips maintained albumin and urea secretions during the 2 week time course at robust levels compared to plate sandwich cultured hepatocytes

CYP450 Enzyme Activity

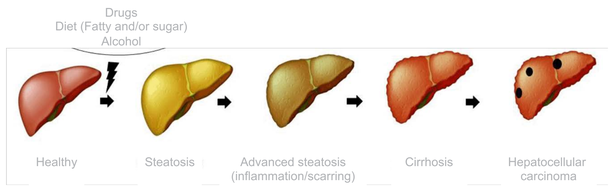


Unit : pmol/min/10^6 cells
* p<0.1, ** p<0.01, *** p<0.001, **** p<0.0001

Cytochrome P450 enzyme activity
CYP450 enzyme activity in the Liver-Chips was evaluated and compared to conventional plate culture and day 0 hepatocyte suspension over 2 weeks. A cocktail substrate, mixture of phenacetin, bupropion, and midazolam was used to measure CYP1A, CYP2B, CYP3A respectively . Overall, all three species Liver-Chips maintained CYP1A, 2B, and 3A activity during the 2 week time course at robust levels compared to plate sandwich cultured hepatocytes.

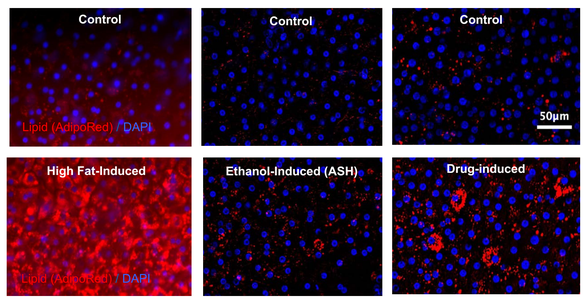
Liver-Chip Fatty Liver Disease Approach

Inducers:



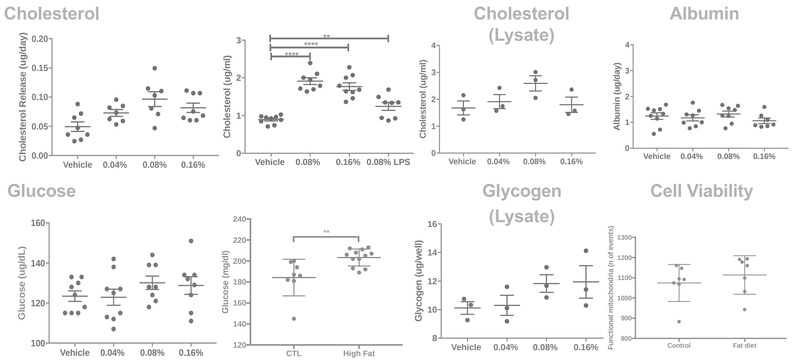
Phenotype:

Lipid Accumulation



Lipid Accumulation
Lipid accumulation was observed and quantified by live or fixed staining with AdipoRed (Lonza).
As expected, the presence of high fat or ethanol on the Liver-Chip significantly increased lipid droplet accumulation in hepatocytes.

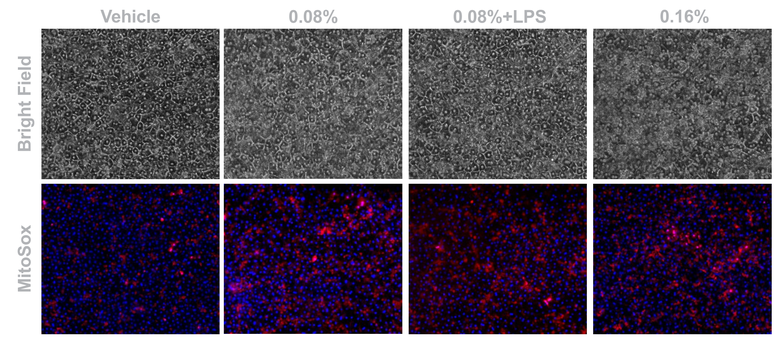
Energy Metabolism Read-Outs

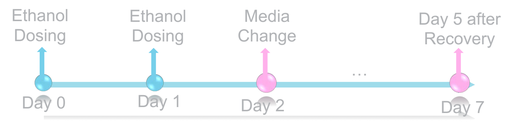


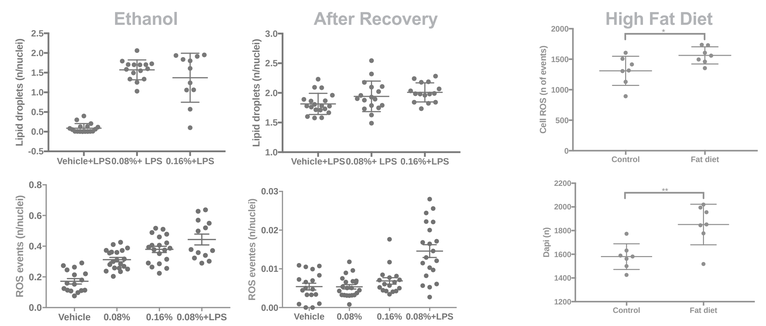
Liver-Chip demonstrated increase of cholesterol (effluent and lysate) and glycogen when incubated with ethanol at physiologically relevant blood alcohol concentration (BAC). Modifications in glucose release levels were only observed with ethanol concentration above 0.32%.

Liver-Chip in high fat diet showed increased cholesterol and glucose release and glycogen storage.

Oxidative Stress Read-outs







Representative time-line of an ethanol or high fat dosing Liver-Chip study plan.
Oxidative stress was measured by staining of ROS-positive hepatocytes.
Ethanol consumption is well known to modify gut permeability, increasing the levels of LPS in the blood. Here we demonstrate how the Liver-Chip can recapitulate similar changes.
As expected based on clinical data, the Liver-Chip alcohol-induced steatosis reverted after ethanol-free recovery.
LPS presence significantly increased oxidative stress even after 5 days of recovery demonstrating to be play an part of alcoholic liver disease mechanisms of action.

Conclusions

We were able to demonstrate hepatocyte lipid droplets accumulation, increase of cholesterol and/or glucose release on the Liver-Chip upon the presence of steatosis inducers like ethanol and high fat. Moreover, the Liver-Chip under fat diet also led to a significant increase on hepatocytes polyploidy and induction of oxidative stress, in line with data in human patients and *in vivo* experimental disease models. In summary, our findings indicate that the Liver-Chip has the potential to provide a more human-relevant model to study the complex pathogenic mechanisms driving liver steatosis. Although further characterization and validation of the model is required in future it could potentially provide a platform to assess efficacy and safety of new drugs, enable identification of biomarkers, and potentially be applied for new target validation and identification.

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