Developing a Human Liver-Chip Model of Alcoholic Steatosis

Sushma Jadalannagari[1], Jake Chaff[1], Michael Carleton[2], Heather Hsu[2], James Velez[1], Sannidhi Joshipura[1], David Conegliano[1], S. Jordan Kerns[1], Lorna Ewart[1]

[1] Emulate Inc., 27 Drydock Avenue, Boston, MA 02210, USA
[2] Inipharm Inc., 500 108th Ave NE, Bellevue, WA 98104, USA

Purpose

Non-alcoholic and alcoholic fatty liver disease (NAFLD and AFLD) are a growing public health concern[1], especially in the United States where it affects one quarter of the adult population[2]. The accumulation of excess fat in the liver causes progressive cell damage and inflammation which in severe forms of steatohepatitis can be toxic and lead to end-stage liver disease. Due to the lack of human relevant preclinical disease models to test lead candidate drugs, there are no clinically approved therapies targeting these diseases. To overcome this, we are developing a model for studying alcoholic steatosis using the Human Liver-Chip to induce cytotoxic levels of lipid accumulation by treating with increasing concentrations of ethanol, an important mediator of disease pathogenesis in AFLD patients.

Methods

Human Quad-Culture Liver Chips (n=4 for each condition) were coated with a mixture of rat tail collagen type I and bovine fibronectin (Figure 1). Primary human hepatocytes from donor CYC were seeded at a density of 3.5 million cells/mL in the upper parenchymal channel and later overlaid with Matrigel and incubated at 37°C with 5% CO_2. In the lower vascular channel on the opposite side of the porous membrane, human liver sinusoidal endothelial cells (LSECs) (3 million cells/mL), human liver Kupffer (0.5 million cells/mL) and stellate cells (0.1 million cells/mL) were seeded. Two days later, the Chips were connected to the Zoë® Culture Module (Human Emulation System®), and both the Chip channels were perfused at a constant flow of 30 µL/h. On Day 7 post seeding, the Liver-Chips were treated with ethanol at 0.5%. The Chips were maintained for 11 days with imaging and effluent collection on days 1, 3, 7, 10 and 11. On Day 11 post-treatment, the experiment was terminated, and Chips were fixed for immunofluorescent imaging with AdipoRed™ for lipid droplet accumulation and DAPI in the top channel and α-Smooth muscle actin (SMA) for activated stellate cells and DAPI in the bottom channel.

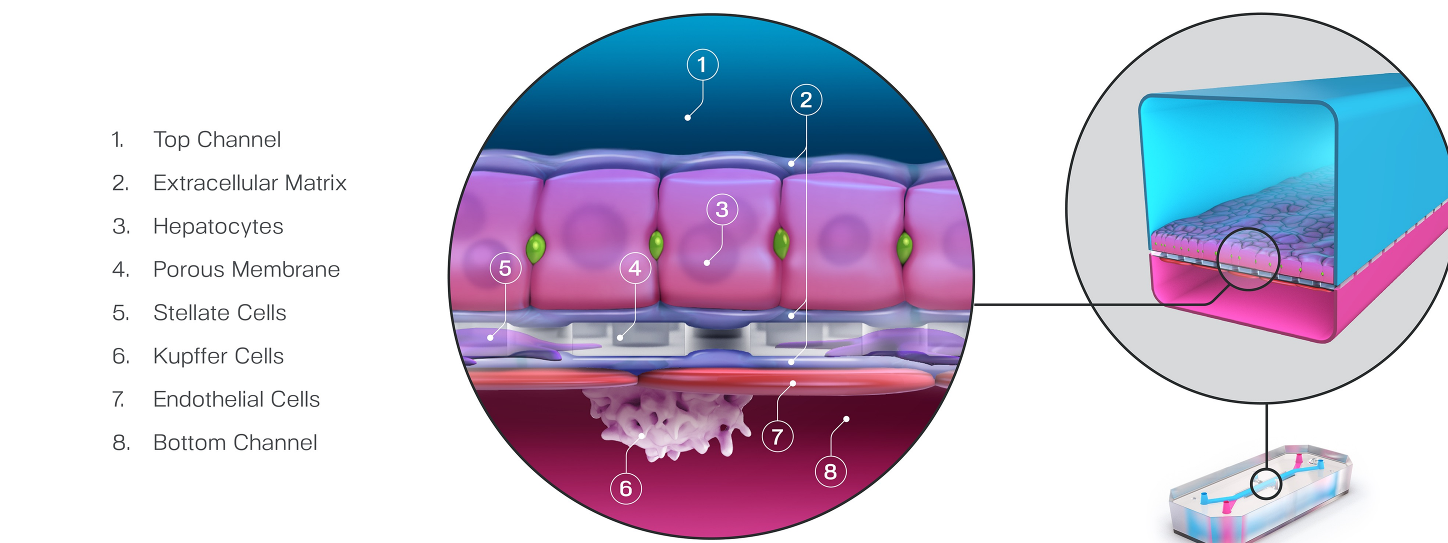

Figure 1 : Schematic of Human Quad Culture Liver-Chip
The Human Liver-Chip is made of polydimethylsiloxane (PDMS) and split into two channels. The upper channel (1mm high x 1mm wide) contains hepatocytes while the bottom channel (0.2mm x 1mm) contains Non-Parenchymal Cells (NPCs) such as Human stellate, Kupfer and endothelial cells. Human hepatocytes are in a sandwich culture between ECM components bovine fibronectin, rat tail collagen I and Matrigel®. A porous membrane separates the top channel from the bottom channel and media specific to hepatocytes and NPCs is flown in their respective Channels.

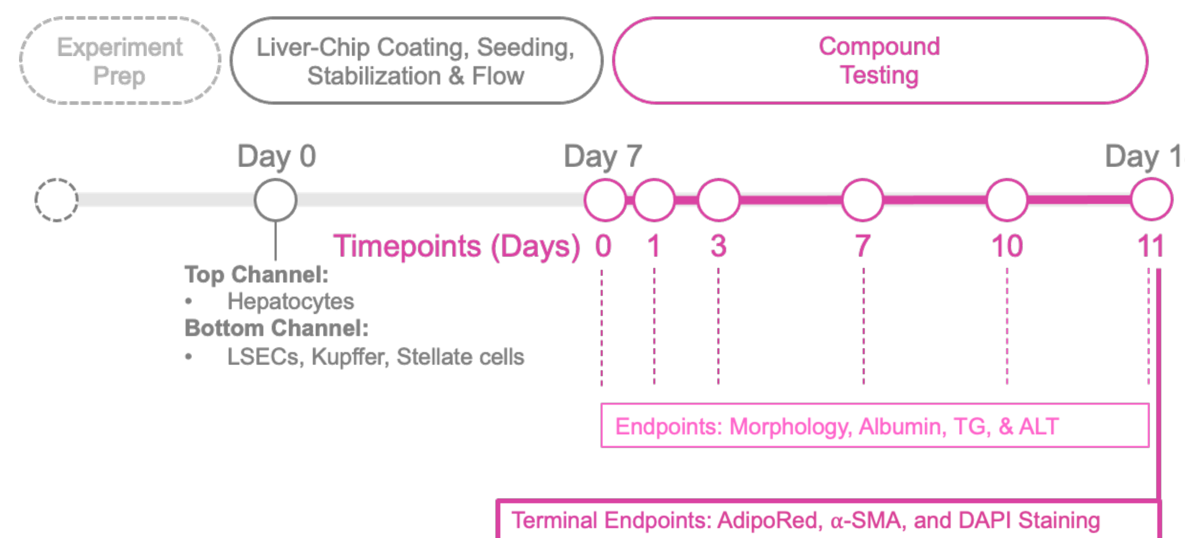

Figure 2 : Ethanol Treatment Study timeline on Human Quad Culture Liver-Chip
The Liver-Chip requires 3 days of preparation before seeding the Hepatocytes to the top channel. After 7 days of maintenance, the Liver-Chips were either untreated, or treated with ethanol in both the Channels. Effluent was collected from both the Channels on days 0,1,3,7,10 and 11. From the Top Channels effluents, assays such as Albumin, ALT and Triglycerides were run in triplicated. At the terminal end-point, the Liver-Chips were fixed and stained for AdipoRed, α-Smooth Muscle Actin and DAPI.

Results

- A severe time-dependent toxic response was observed in the hepatocytes, post ethanol treatment (Figure 3A). Through phase contrast imaging, the 0.5% ethanol treated hepatocytes began to show a decline in health from day 3 onwards. All NPCs appeared healthy until day 10 when the 0.5% ethanol treated groups declined in health. On day 11 the ethanol treated NPCs appeared less healthy than the control NPCs.
- The ethanol treated group demonstrated an increased lipid accumulation in comparison to the vehicle control (Figure 3B). The NPCs in both groups demonstrated similar activation of stellate cells.
- Additionally, the 0.5% ethanol treated group showed a significant decrease in albumin release (Figure 6) and an increase in ALT (Figure 5) and triglyceride export (Figure 4) in the suggesting cytotoxicity from day 7 post-treatment

Image Analysis

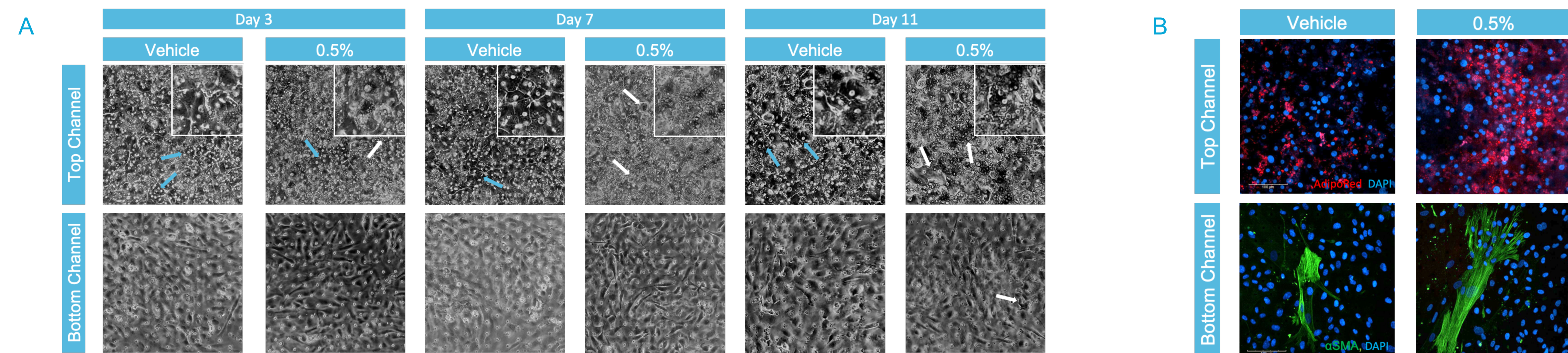

Figure 3: Representative Morphology and Immunofluorescent imaging of the treated groups on Human Quad Culture Liver-Chip
A. Phase contrast images were taken at 10x magnification at every timepoint in both the top and bottom channels (n=4). Images at day 3, 7 and the terminal endpoint (day 11) are shown above. Hepatocytes were cultured in the top channel and stellate, Kupfer and endothelial cells were cultured in the bottom channel. Images of hepatocytes were taken in the middle of the channel whereas images of the bottom channel were taken at both the inlet and outlet ports. Image inserts represent magnifications to show points of damage. The blue arrows represent healthy, characteristic morphology (binucleated, cuboidal cells, define bile canaliculi). The white arrows also represent points of toxicity or cellular damage.
B. Immunofluorescent images were captured with a 20x magnification on day 11 (n=1). Cells in the top channel were stained for DAPI (blue), and AdipoRed (red), and cells in the bottom channel were stained with DAPI (blue), and α-SMA (green) after 11 days of continuous treatment.

Liver-Chip Markers

Triglycerides Quantification in Human Liver-Chip

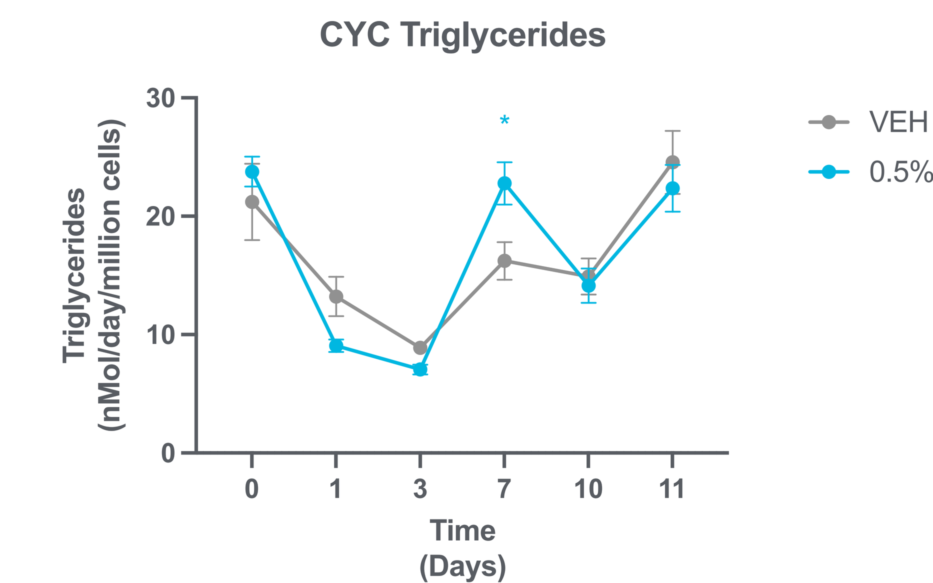

Figure 4: Triglycerides Assay from sample effluent
Triglycerides were measured (Abcam, ab65336) from effluent collected on days 0, 1, 3, 7, 10, and 11 (n=4). The CYC donor received the ethanol treatment for 11 days, with constant flow at 30µL/hr. Analysis was done with a Fisher's LSD Test.

Alanine Aminotransferase Secretion in Human Liver-Chip

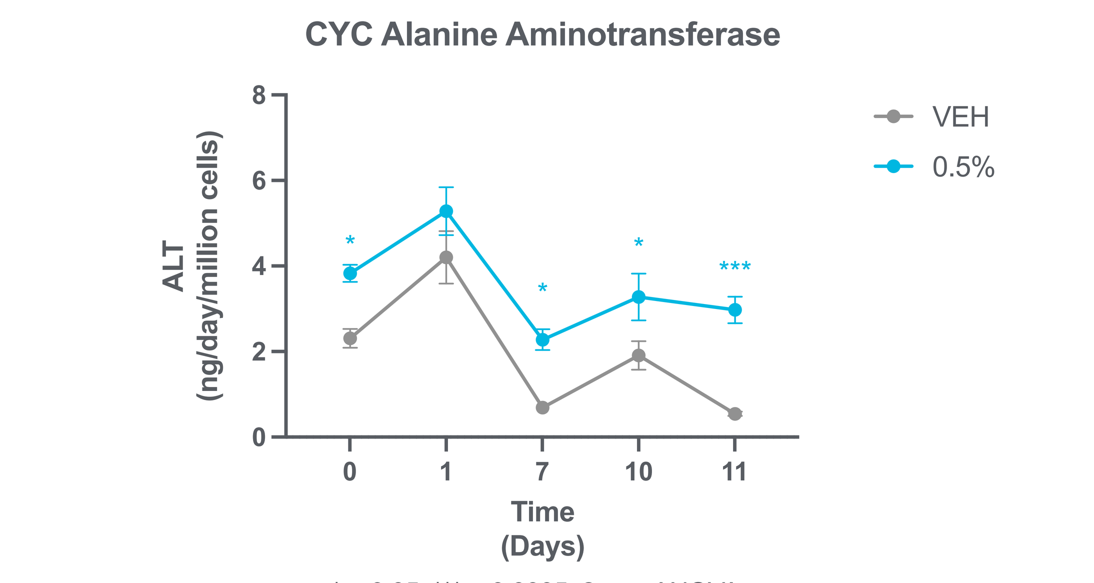

Figure 5: Alanine Aminotransferase assay from sample effluent
Alanine Aminotransferase was measured (Abcam, ab234578) on days 0, 1, 7, 10, and 11 (n=4). The CYC donor received ethanol treatment for 11 days, with constant flow at 30µL/hr. Analysis was done with a Fisher's LSD Test.

Albumin Secretion in Human Liver-Chip

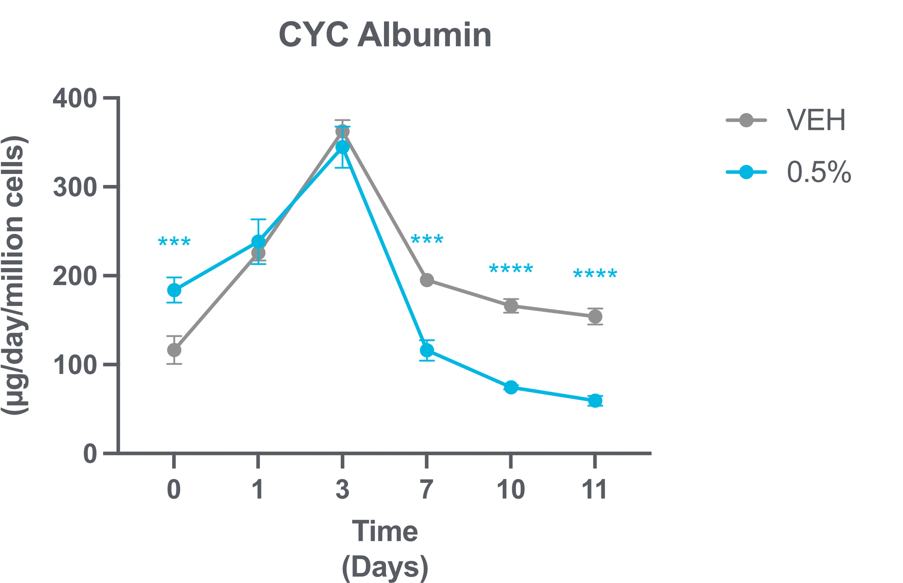

Figure 6: Albumin assay from sample effluent
Albumin was measured (Abcam, ab179887) on days 0, 1, 3, 7, 10, and 11 (n=4). The CYC donor received ethanol treatment for 11 days, with constant flow at 30µL/hr. Analysis was done with a Fisher's LSD Test.

Conclusion

The Human Quad-Culture Liver-Chip model demonstrated a time-dependent increase in intracellular hepatic lipid accumulation following ethanol treatment leading to the pathogenesis of a steatosis-like toxic response. Albumin secretion, ALT release and triglyceride export were also affected by ethanol treatment. Thus, based on the promising preliminary results from this study, further investigation is needed to enable evaluation of therapeutic agent efficacy in this Liver-Chip model of alcohol induced steatosis.

References

1 Fazel et al., Epidemiology and natural history of non-alcoholic fatty liver disease. Metabolism. 2016 Aug;65(8):1017-25. Epub 2016 Jan 29.
2 Perumpail et al., Clinical epidemiology and disease burden of nonalcoholic fatty liver disease, World J Gastroenterol. 2017 Dec 21; 23(47): 8263–8276.

emulate inipharm

© 2021 Emulate, Inc | 27 Drydock Ave. 5th Floor | Boston, MA 02210