A high throughput automatable microfluidic liver organoid chip for long-term dili modelling

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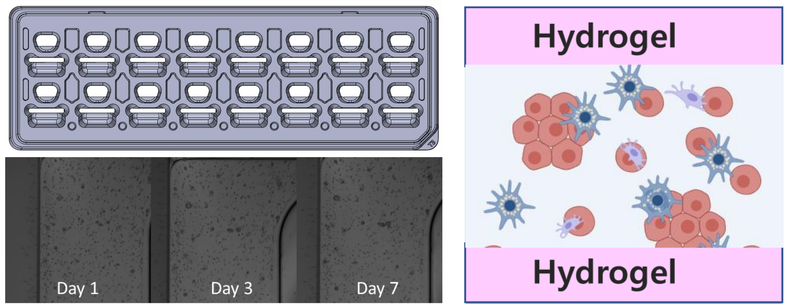
Introduction

Recently, extensive research has demonstrated the superiority of iPSC-derived liver organoids and microfluidic liver chips models over classic hepatocyte cultures in terms of cell maintenance and physiological relevancy. These models have pioneered a new generation of liver disease research, including drug-induced liver injury (DILI). However, limitations arise in cost, scalability, and consistency; crucial factors regarding the higher throughput needs in drug discovery. Herein, we describe a long term high-throughput microfluidic liver chip for studying DILI amenable to widely available lab automation technologies.

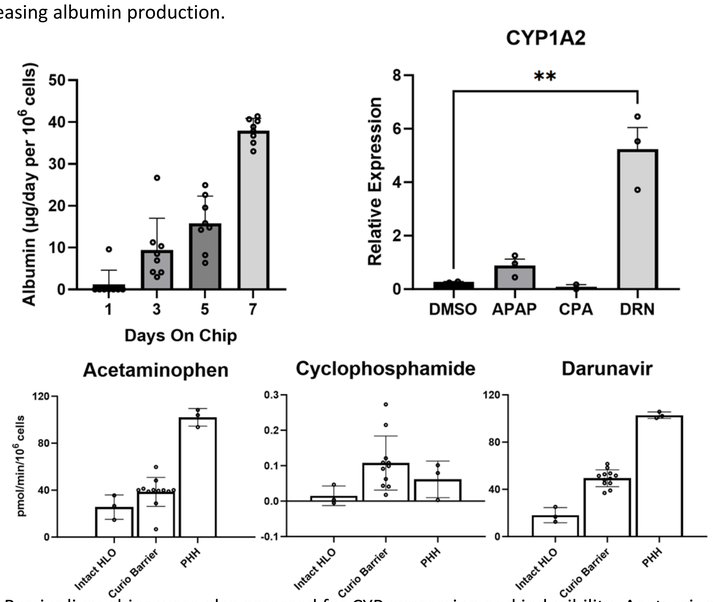
Goal

In an attempt at devising a system that overcomes all these issues, we adapted iPSC-derived human liver organoids (HLOs) to an engineered high-throughput microfluidic system emphasizing on consistency across replicates and minimal inherent drug interactions for robustness when applied to large-scale compound testing

Curio Barrier liver chip development



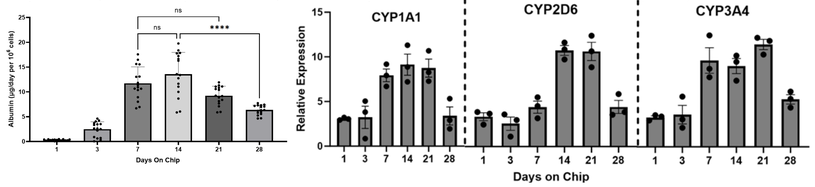
Curio Barriers were designed to incorporate 16 individual three-compartment cultures on a single microfluidic device. For our liver chip cultures, human liver organoids (HLOs) differentiated from a previously described protocol were embedded in between two layers of hydrogel containing a mixture of collagen I and Matrigel. Curio Barrier liver chips were originally assessed for viability across 7 days of culture and monitored with brightfield imagin; Notably, liver chips maintained viability and continued to proliferate across 7 days with increasing albumin production.



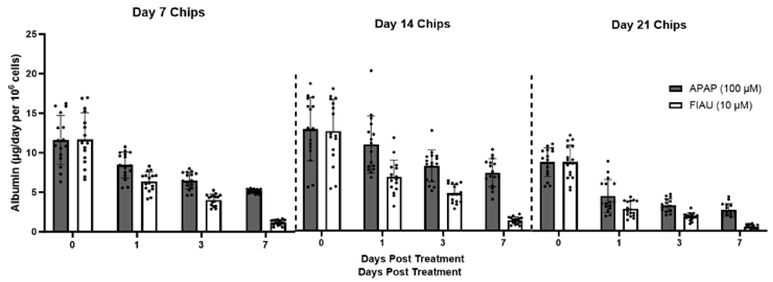
Curio Barrier liver chips were also assessed for CYP expression and inducibility. Acetaminophen (APAP), cyclophosphamide (CPA) and darunavir (DRN) were used to probe CYP 1A, 2D and 3A family enzymes. Additionally, CYP1A2 expression increased significantly with overnight treatment of DRN.

28-day liver culture on chips

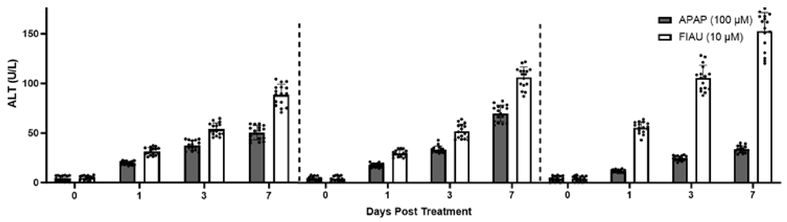
While both Curio Barrier liver chips and previously described microfluidic systems showed capability in further development of iPSC-derived liver organoids, few liver systems have shown ability to maintain liver physiology over an extended period. To this end, Curio Barrier liver chips were cultured for 28-days post seeding and periodically benchmarked for Cyp expression, albumin production, and ability to model APAP and fialuridine (FIAU)-induced DILI.



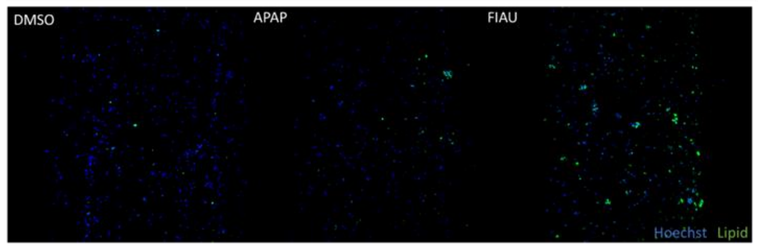
qPCR measurement of CYP 1A1, 2D6, and 3A4 expression shows consistent expression across 28-days of Curio Barrier liver chip culture. Our findings suggest a gradual increase of CYP expression peaking at around days 14-21 with a drop off at day 28.



As CYP expression and albumin production is shown to be continuously increasing up to day 21, Curio Barrier chips at Day 7, 14, and 21 were treated with known hepatotoxins APAP and FIAU for an additional 7 days. Both APAP and FIAU treatment resulted in a consistent loss of albumin production across 7 days of treatment with identical response patterns between 7, 14, and 21 day-aged liver chips.

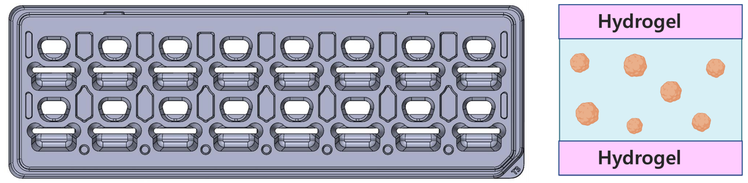


Alanine transferase (ALT) was assayed from media as a measure of hepatocyte-specific death. While both APAP and FIAU elicit a hepatocyte death response across 7, 14, and 21 day aged Curio Barrier liver chips, day 21 chips exhibit a noticeably lesser response to APAP. We suspect that this decreased response to APAP is a result of diminished CYP expression as APAP-mediated hepatotoxicity is well known to be reliant on metabolism into NAPQI.

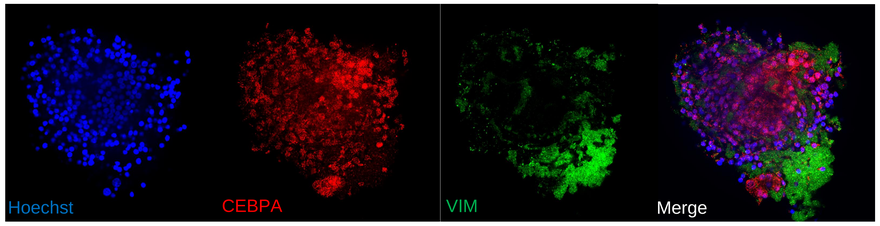


Lastly, control and treated Curio Barrier chips were stained for lipid content with LipidTox Green. Interestingly, while APAP resulted in a minimal increase in lipid accumulation, lipid accumulation was exacerbated with FIAU treatment. These findings are consistent to previous reports in literature.

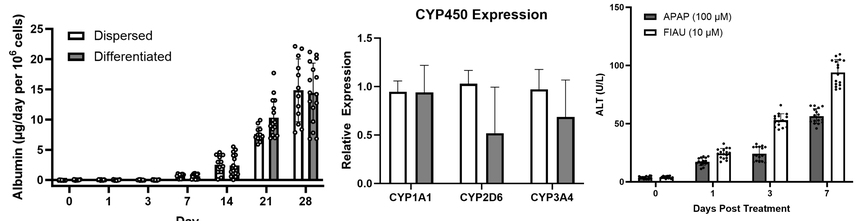
Liver organoid differentiation on chip



As the Curio Barrier chip allows for culture of cells in a 3-dimensional hydrogel embedding, we next tested the ability to differentiate HLOs directly on the microfluidic device rather than in a separate embedding followed by a cell dispersion and transfer. For this system, definite endoderm spheroids were seeded directly into the Curio Barrier chip and cultured for an additional 14 days on a hepatocyte growth media as previously described.



Curio Barrier chips demonstrated compatibility for high-content imaging with a Yokogawa CV8000 water-immersion platform. Immunostaining showed that organoids were doubly positived for hepatocyte (CEBPA) and stellate (VIM) markers. However, marker expressions showed minimal colocalization suggesting the formation of definitive cell types as in differentiation in standard Matrigel embeddings.



As with HLOs differentiated in separate Matrigel embeddings and transferred to Curio Barrier chips, HLOs differentiated directly on the platform showed consistent results in terms of albumin production, CYP450 expression, and response to DILI compounds.

Future Directions

Future studies include using this microfluidic system to study long-term, chronic dose, DILI, and increased model complexity for idiosyncratic DILI research. In addition, BarrierChip HLOs may see utilization for other disease models necessitating long-term viability, such as for Hepatitis B and NAFLD research.

Conclusions

- HLOs maintain functionally and continue development on Curio Barrier chips
- Curio Barrier chips are low cost and high-throughput platforms for advanced liver cultures
- Chips maintain liver physiology and DILI responsiveness across 28-days of culture as measured by albumin production, CYP expression, ALT release, and fluorescent staining.
- HLOs can also be differentiated directly on the Curio Barrier platform, further supporting its capabilities as an automatable high-throughput platform

References and Acknowledgement

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1. Ouchi, R. et al. Modeling Steatohepatitis in Humans with Pluripotent Stem Cell Derived Organoids. Cell Metab. (2019) doi:10.1016/j.cmet.2019.05.007.
2. Zhang, C. et al. A Human Liver Organoid Screening Platform for DILI Risk Prediction. bioRxiv (2021) doi:10.1101/2021.08.26.457824

ANOVA with multiple comparison was done to determine significance with *, **, ***, *** denoting P < 0.05, 0.01, 0.01, and 0.001 respectively.