Human Liver-Chip for Drug Metabolism and Liver Safety Assessment

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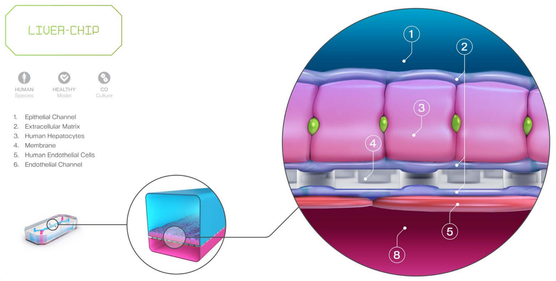
Introduction

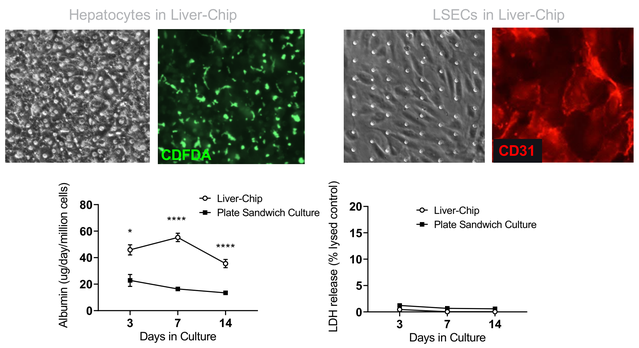
The pharmaceutical industry has an unmet need for predictive human models for drug metabolism and pharmacokinetics, drug-drug interactions, and drug-induced liver injury (DILI) that can better emulate human response to drugs. Current 2D liver models often fail to capture responses seen in the clinic, as the cellular microenvironment does not accurately reflect what is found in vivo. Here we applied a vascularized human Liver-Chip model that contains primary human hepatocytes, sinusoidal endothelial cells, and Kupffer cells, cultured under physiological fluid flow in a spatial configuration that recapitulates their cytoarchitecture in the liver resulting in long-term viability and improved functionality. The Chip's fluidic structure allows for easy effluent sampling for the detection of various biomarkers and cytokine release, and the optical clarity of the Chip allows for various morphological analyses throughout the culture duration. This provides a platform to investigate mechanistic insights of various DILI and demonstrates the value of the human Liver-Chips for predicting human metabolism, safety testing, risk assessment, and drug discovery and development.

Liver-Chip



Liver-Chip Culture. Primary human hepatocytes were cultured in the upper channel and on the top of the ECM-coated membrane, and primary liver sinusoidal cells (LSECs) were cultured in the lower channel and on the opposite side of the membrane. Cells were allowed to acclimate for approximately 1 week prior to compound treatment using flow rates of 10 to 30 uL/hour

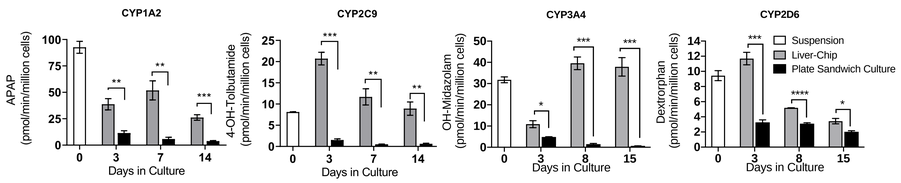
Morphology & Liver Function



Liver-Chip Morphology and Function
Hepatocytes: healthy morphology, MRP2 transporter activity via 5 (and 6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) and visualization of the bile canalicular network, albumin secretion and LDH level from hepatocytes after 2 weeks in culture
LSECs: Healthy morphology and CD31 expression after 2 weeks in culture

Results

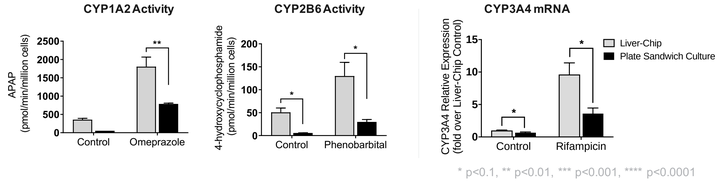
CYP450 Enzyme Activity



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

Cytochrome P450 enzyme activity
CYP450 drug metabolizing enzyme activity from the Liver-Chip compared to conventional plate sandwich culture and day 0 hepatocyte suspension over 2 weeks

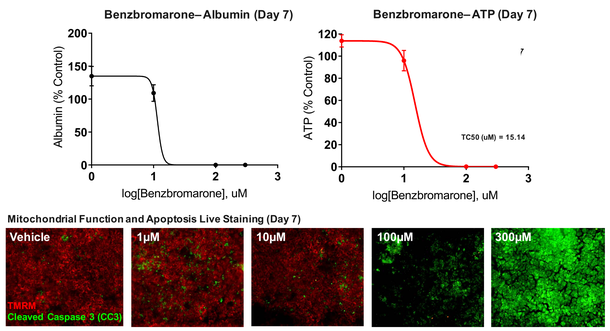
CYP450 Enzyme Induction



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

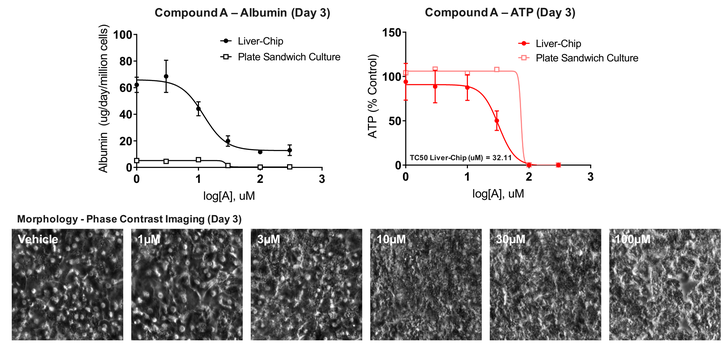
CYP450 enzyme induction
Use of prototypical inducers to evaluate the induction potential by measuring CYP450 enzyme activity and gene expression

Benzbromarone Toxicity in Co-Culture Human Liver-Chip



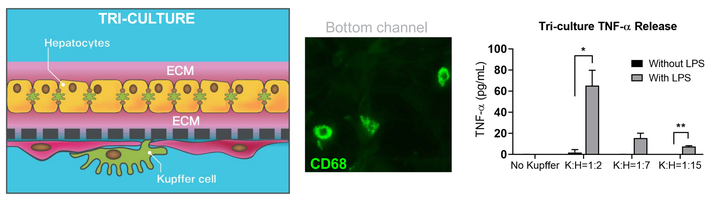
Benzbromarone Toxicity in Human Liver-Chips
Benzbromarone-induced hepatotoxicity was evaluated by albumin secretion, ATP level, mitochondrial function, and apoptosis after 7 days of treatment in the Liver-Chip.

Sanofi Compound A Toxicity in Co-Culture Human Liver-Chip



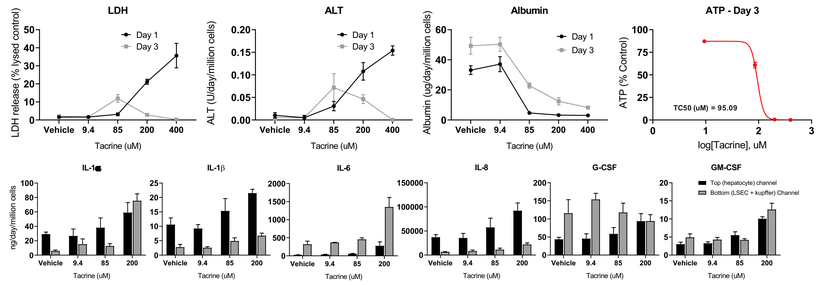
Sanofi Compound A Toxicity in Human Liver-Chips
Sanofi Compound A-induced hepatotoxicity was evaluated by albumin secretion, ATP level, and hepatocyte morphology after 3 days of treatment in the Liver-Chip.

Tri-Culture Human Liver-Chip



Tri-culture Human Liver-Chip
CD68 Kupffer cell marker expression in the Liver-Chip and TNF-α release by LPS stimulation in proportion to Kupffer cell ratio in tri-culture human Liver-Chip

Tacrine Toxicity in Tri-Culture Human Liver-Chip



Tacrine Toxicity in Human Liver-Chip
Tacrine-induced hepatotoxicity and immune response were evaluated by LDH, ALT, Albumin, and cytokine releases and ATP level after 1 and/or 3 days of treatment in the Liver-Chip.

Summary/Conclusions

- The human Liver-Chip maintained physiologically relevant activity of drug metabolizing enzymes over 2 weeks in culture.
- Successfully demonstrated utility of the Liver-Chip for liver safety assessment by measuring various markers/targets such as LDH, albumin, ATP, mitochondrial function, and apoptosis from benzbromarone and Sanofi Compound studies
- Showed the Liver-Chip is superior to conventional plate sandwich culture in hepatocyte function and sensitivity in toxicity response.
- Tri-culture Liver-Chip demonstrated additional functionality mediated by Kupffer cells which includes detecting immune response in DILI response.