Real-Time Monitoring of Immune-Mediated DILI in Human iPSC-Derived Isogenic Liver Co-Culture

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ABSTRACT

Background and Purpose
Activation of an innate immune response in the liver can amplify tissue damage in Drug-Induced Liver Injury (DILI). This mechanism of action cannot be detected in vitro with hepatocyte mono-culture systems as it requires the presence of innate immune cells, such as macrophages. Human iPSC-derived hepatocytes together with human iPSC-derived macrophages may be an ideal solution able to provide a biologically relevant co-culture system that is able to identify immune-mediated drug reactions.

Methods:
Human isogenic iCell Hepatocytes 2.0 and iCell Macrophages 2.0 (FUJIFILM CDI, both from donor 01279) were cultured on the AtlaZ System (Nanion Technologies) for real-time monitoring of cell adhesion and toxicity in response to compounds known to induce immune-mediated hepatotoxicity, such as Chlorpromazine and Diclofenac. In 3D co-culture, spheroids were assessed for immune-mediated mitochondrial disruption by Seahorse XF assay (Agilent).

Results:
A ratio of 2:1 Hep:Mac in co-culture improved the functional maturity of the iPSC-derived hepatocytes, as determined by albumin secretion, urea production, and CYP3A4 activity. Cells were assayed over a 7-day period to determine the toxic effect over time in the AtlaZ system. Compounds were tested as a dose response in the culture system, with a range of 3-300 µM for Diclofenac and 0.3-30 µM for Aflatoxin B1 to determine the IC_{50}. The Seahorse assay revealed metabolic disruption in an immune cell dependent manner. Hepatic functionality paired with isogenic immune cells provides a more biologically relevant assay system with low baseline hepatotoxicity for determining the effects of immune-mediated DILI.

Conclusions:
Determining the immune response to drug candidates is becoming a key factor in evaluating DILI risk. The AtlaZ provides quantitative label-free live-cell analysis of cell adhesion and cytotoxicity in response to test compounds in real-time. Human isogenic co-culture of iCell Hepatocytes 2.0 with iCell Macrophages 2.0 provides a system to assess immune-mediated DILI with a large dynamic range across multiple formats.

MATERIALS and METHODS

iCell Hepatocytes 2.0 Culture. iCell Hepatocytes 2.0 were thawed and plated overnight. Media was changed every day until Day 5. At Day 5, cells were treated with compound for 2 or 7 days for 2D studies. For 3D studies, cells were harvested and used to form 3D spheroids.

iCell Hepatocytes 2.0 Drug Exposure. iCell Hepatocytes 2.0 maintained for 5 days were treated with compounds, then assayed after 2 days or 7 days. Cell viability was measured using CellTiter-Glo® (Promega).

iCell Macrophages 2.0 Culture. iCell Macrophages 2.0 were thawed and plated for imaging and marker analysis. For 3D studies, cells were thawed and used to form 3D spheroids.

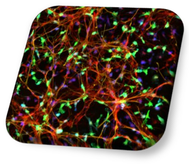
Macrophage Polarization. Macrophages were maintained for 3 days after thaw, then stimulated for 24 hours and the supernatants collected. Cytokine release was quantified using the Luminex multiplex system. LPS: 1 µg/mL; IFNγ: 50 ng/mL; IL-4: 50 ng/mL; IL-13: 50 ng/mL; TGFβ: 25 ng/mL; IL-10: 50 ng/mL.

Spheroid Formation. iCell Hepatocytes 2.0 were harvested 5 days after plating and transferred to a ULA plate at 10,000 cells/spheroid. For co-culture spheroids, isogenic iCell Macrophages 2.0 were thawed and added to the ULA plate at 5,000 cells/spheroid (in addition to the hepatocytes).

Secreted ALB/AFP. Supernatant was collected from monoculture or isogenic co-culture spheroids at multiple time points after spheroid formation. Concentrations of Albumin (ALB) and Alpha-fetoprotein (AFP) were determined by ELISA.

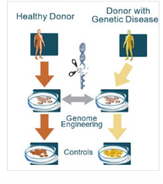
FUJIFILM Cellular Dynamics, Inc is the leading provider of iPSC-derived cell types and disease models. We are helping to advance the field with 3D and co-culture systems to better replicate human tissue and biology. FCDI continues to be a leader in new products, manufacturing capacity and quality, and custom services.

Human iPSC-derived Cells

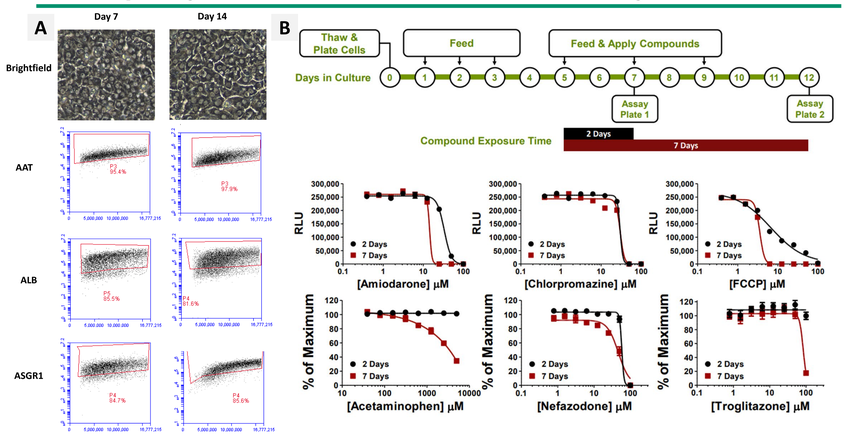


Paired Media & Kits

Custom Services

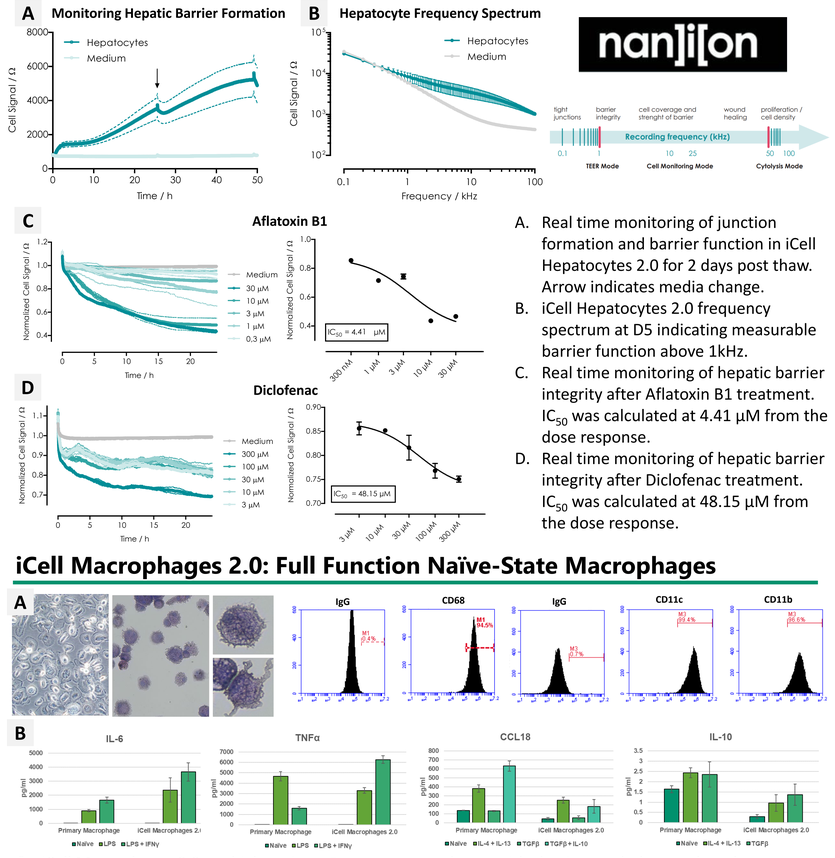


iCell Hepatocytes 2.0: Consistent Function for Toxicity Studies



A. iCell Hepatocytes 2.0 have consistent expression of the hepatic markers Albumin (ALB), α-1-Antitrypsin (AAT), and Asialoglycoprotein Receptor 1 (ASGR1) and can be stably cultured for more than 2 weeks. **B.** iCell Hepatocytes 2.0 were used for evaluation of liver toxicity *in vitro* over short- and longer-term chronic drug exposure times. Cells were treated with compounds, then assayed at earlier (day 7) or later (day 12) time points.

AtlaZ: Real Time Monitoring of Barrier Function and Hepatotoxicity

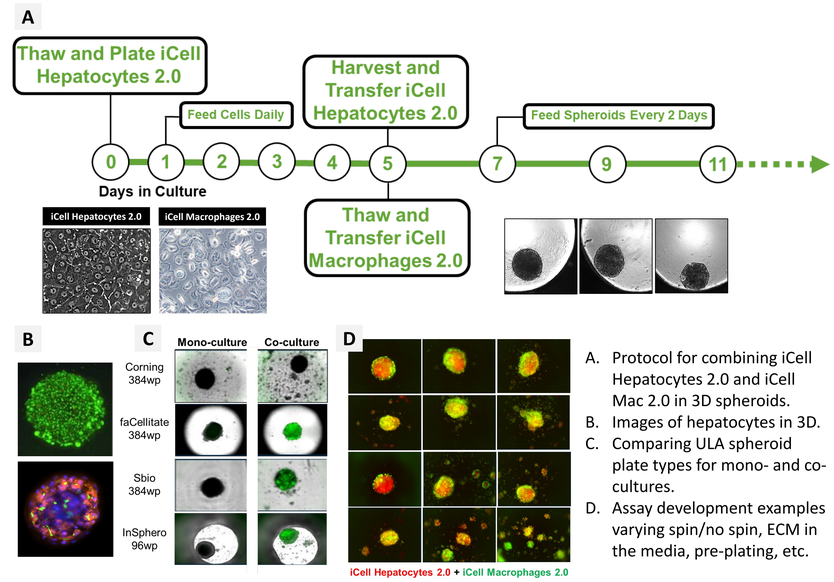


A. Real time monitoring of junction formation and barrier function in iCell Hepatocytes 2.0 for 2 days post thaw. Arrow indicates media change.
B. iCell Hepatocytes 2.0 frequency spectrum at D5 indicating measurable barrier function above 1kHz.
C. Real time monitoring of hepatic barrier integrity after Aflatoxin B1 treatment. IC_{50} was calculated at 4.41 µM from the dose response.
D. Real time monitoring of hepatic barrier integrity after Diclofenac treatment. IC_{50} was calculated at 48.15 µM from the dose response.

iCell Macrophages 2.0: Full Function Naïve-State Macrophages

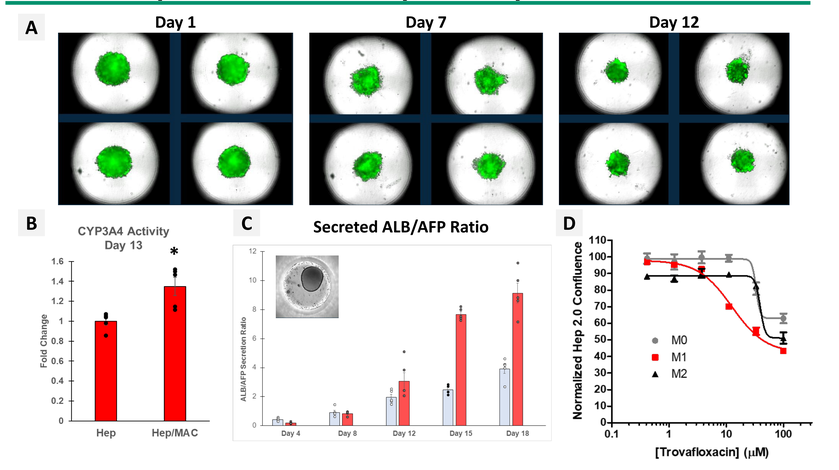
A. iCell Macrophages 2.0 are high purity naïve state macrophages with classic morphology and marker expression. **B.** Naïve state iCell Macrophages 2.0 secrete cytokines in response to pro- and anti-inflammatory stimuli with a larger dynamic range than primary monocyte-derived macrophages.

Isogenic Spheroids of iCell Macrophages 2.0 and iCell Hepatocytes 2.0



A. Protocol for combining iCell Hepatocytes 2.0 and iCell Mac 2.0 in 3D spheroids.
B. Images of hepatocytes in 3D.
C. Comparing ULA spheroid plate types for mono- and co-cultures.
D. Assay development examples varying spin/no spin, ECM in the media, pre-plating, etc.

Immune Component Co-Culture Improves Hepatic Function



A. Isogenic Co-culture spheroids with 10k hepatocytes and 5k macrophages at 1, 7, and 12 days of maintenance. iCell Macrophages 2.0 are auto-fluorescent green.
B. CYP3A4 activity at Day 13 of spheroid maintenance. *p<0.01
C. Co-culture improves hepatic function as shown by ratio of secreted ALB/AFP Isogenic Co-culture spheroids at Day 1 and Day 7. Gray bars: hep-only spheroids; Red bars: hep/mac co-culture spheroids.
D. Hepatocytes co-cultured with macrophages in transwell plates were treated with trovafloxacin after 7 days in culture. Macrophage polarization state affects hepatic drug response.

Summary and Conclusions

- iCell Hepatocytes 2.0 have consistent, reproducible expression of hepatic markers and maintain function over time in 2D monoculture.
- The AtlaZ system offers real-time monitoring of barrier function and hepatotoxicity.
- iCell Macrophages 2.0 are full function naïve macrophages that can be used across workflows for a dye-free trackable immune component co-culture solution.
- Co-culture of isogenic iCell Hepatocytes 2.0 and iCell Macrophages 2.0 improves hepatic function and allows *in vitro* interrogation of immune-mediated DILI.