Incorporating iPSC-Derived Macrophages into Co-Culture Systems to Assess Immune-Mediated Toxicity Across Organ Systems

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ABSTRACT

Macrophages are key players in regulating organ homeostasis, tissue repair, and waste removal. In response to acute or chronic tissue injury, macrophages can contribute to tissue injury with diverse outcomes such cytotoxicity, inflammation, and fibrosis. Immune component co-culture is critical to understanding the mechanisms of immune-mediated toxicity. Human iPSC-derived iCell® Macrophages 2.0 ("Mac 2.0") can be used to model drug-induced livery injury, cardiac fibrosis, and peripheral neuropathy through co-culture with other iPSC-derived cell types, including iCell Hepatocytes 2.0, iCell Cardiac Fibroblasts, iCell Cardiomyocytes^2 and iCell Sensory Neurons. In 3D spheroid culture with iCell Hepatocytes 2.0, the ratio of secreted ALB to AFP is increased 4-fold compared to hepatocyte monoculture after 2 weeks, with a concomitant increase in basal CYP3A4 activity. iCell Macrophage 2.0 are also able to sensitize hepatocytes to Trovafloxacin, with LPS treatment allowing detectable hepatotoxicity at the 10 µM clinical Cmax of TVX compared to untreated cells. Co-culture of iCell Macrophage 2.0 with iCell Cardiomyocytes^2 leads to 2-fold longer beat periods and a significant increase in spike amplitude with LPS stimulation. While the practical application of incorporating macrophages into toxicology workflows can be technically and scientifically challenging, iCell Macrophages 2.0 provide an easy-to-use alternative to immortalized cell lines, without the inherent variability of sourcing and deriving primary macrophages. Human iPSC-derived macrophages offer a consistent, renewable source of functional macrophages to interrogate acute and chronic tissue injury.

MATERIALS and METHODS

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. 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.

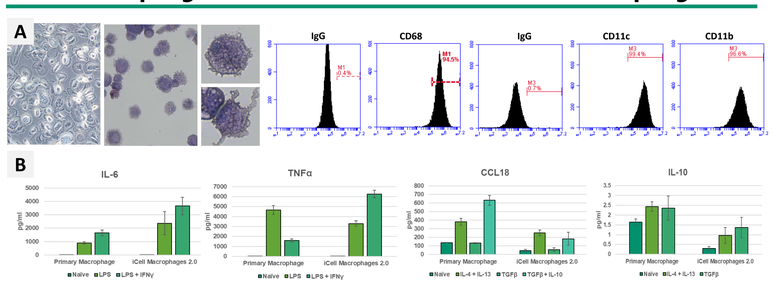
iCell Hepatocytes 2.0 Culture. iCell Hepatocytes 2.0 were thawed and plated overnight. Media was changed every day until Day 5. At Day 5, cells were treated with compound for 2 or 7 days for 2D studies. For 3D studies, iCell Hepatocytes 2.0 were harvested 5 days after plating and transferred to a ULA plate at 10,000 cells/spheroid. Isogenic iCell Macrophages 2.0 were thawed and added to the ULA plate at 5,000 cells/spheroid (in addition to the hepatocytes). Supernatant was collected from monoculture or isogenic co-culture spheroids at multiple time points after spheroid formation. ALB and AFP concentrations were determined by ELISA.

Trovafloxacin Treatment. iCell Hepatocytes 2.0 were maintained in 96-well plates until Day 5. At the same time, iCell Macrophages 2.0 were plated into 96-well transwells and then treated with either 100 ng/ml LPS and 50 ng/ml IFNγ together (M1), 50 ng/ml of both IL-4 and IL-13 (M2) or left untreated for 18 hours. Hepatocytes were dosed with trovafloxacin (0→100 µM) and then the plates were combined to expose the macrophage transwell culture system to the hepatocytes. Cell viability of the hepatocytes was measured by confluence (Incucyte) and CellTiter-Glo® 2.0 (Promega).

Sensory Neurons Culture. iCell Sensory Neurons, 01279 were cultured (62K cells/cm^2) in Complete Sensory Neurons Medium following the users guide. iCell Macrophages 2.0 were thawed and added directly into sensory neuron cultures at day 4 of culture at a 3:2 macrophage to sensory neuron ratio.

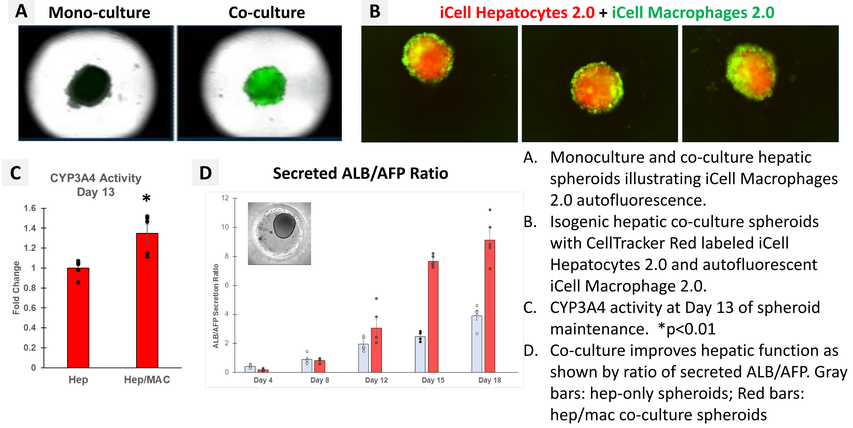
Cardiomyocytes Culture. iCell Cardiomyocytes^2 were cultured on a 96w Cytoview MEA plate in iCell Cardiomyocytes Maintenance Medium (iCMM) until Day 4 and then increasing amounts of iCell Mac 2.0 were added in different media, including 50:50 iCMM and IMDM+1%FBS. On Day 7, wells were treated +/- LPS (100 ng/ml) and incubated for 3 days. MEA recordings were taken on Day 10.

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.

Immune Component Co-Culture Improves Hepatic Function

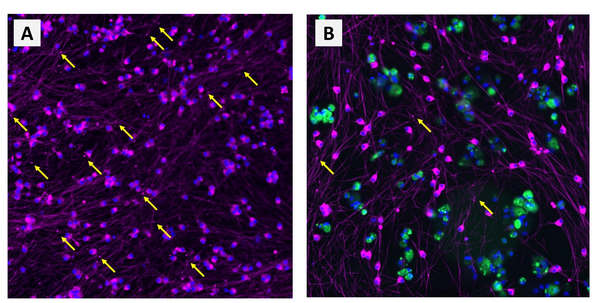


A. Monoculture and co-culture hepatic spheroids illustrating iCell Macrophages 2.0 autofluorescence.
B. Isogenic hepatic co-culture spheroids with CellTracker Red labeled iCell Hepatocytes 2.0 and autofluorescent iCell Macrophage 2.0.
C. CYP3A4 activity at Day 13 of spheroid maintenance. *p<0.01
D. Co-culture improves hepatic function as shown by ratio of secreted ALB/AFP. Gray bars: hep-only spheroids; Red bars: hep/mac co-culture spheroids

Polarized Macrophages Increase the Sensitivity of TVX Hepatotoxicity

A. Images of iCell Macrophages 2.0 and iCell Hepatocytes 2.0 used for TVX dose response.
B. Hepatocyte confluence in a TVX dose response with different stimulation states of iCell Macrophages 2.0.
C. Images of iCell Hepatocytes 2.0 labeled with Hoechst and Calcein AM. Pro-inflammatory macrophages (M1 (LPS/IFN-gamma) sensitize hepatocytes to TVX toxicity, as illustrated by cell confluence.

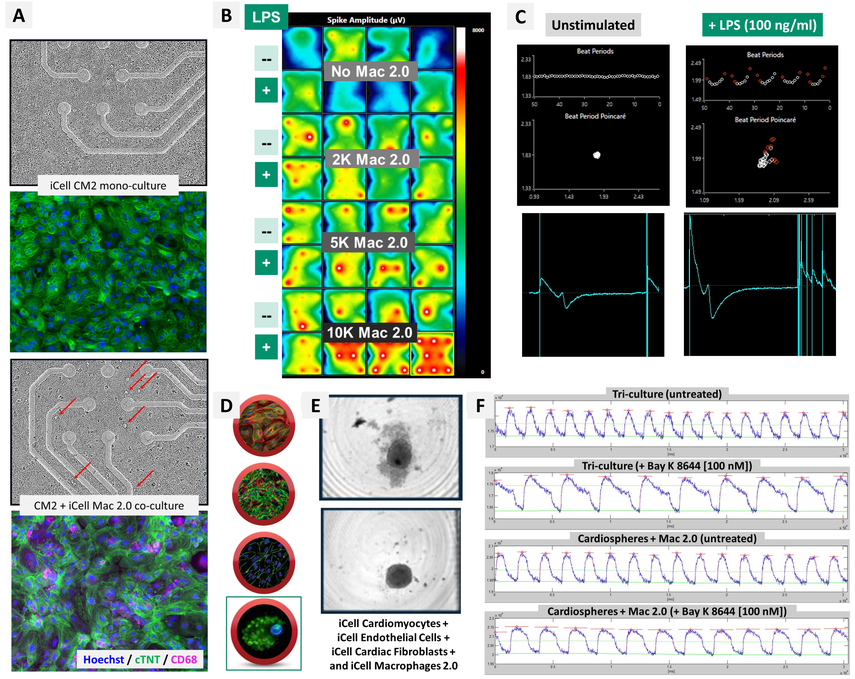
Co-Culture of iCell Macrophages 2.0 with iCell Sensory Neurons



Hoechst / (Autofluorescent) iCell Macrophages 2.0 / β3-Tubulin

A. iCell Sensory Neurons (magenta; β3-Tubulin) have a long branching axon system in 2D culture. Some cellular debris is observable (yellow arrows).
B. iCell Macrophages 2.0 (green) are compatible in culture with iCell Sensory Neurons. Macrophages appear to clean up the culture, reducing cellular debris.

Polarized Macrophage Co-culture Alters Cardiac Activity



A. iCell Macrophages 2.0 are compatible in co-culture with iCell Cardiomyocytes^2 (CM2). Mono-culture pictured on top and co-culture with iCell Mac 2.0 on bottom. Macrophages are readily observable on the MEA (red arrows).
A. Heat map from cardiac MEA assay. Increasing number of iCell Macrophage 2.0 in culture leads to higher sodium spike amplitude (mV) recorded in iCell Cardiomyocytes^2.
B. LPS stimulation of cardiomyocyte/macrophage co-culture results in dramatic changes in cardiac activity, including longer beat period, altered depolarization and repolarization, as well as detection of arrhythmias. CM2/Mac 2.0 co-culture was established for 2 days prior to treatment with LPS for 3 days. MEA recordings are from DIV 10 overall.
C. Four different iCell products were used to make 3D spheroids, incl. cardiomyocytes, fibroblasts, endothelial cells, and macrophages.
D. iCell Cardiospheres (3 cell types; top) and 3D spheroids in co-culture with Mac 2.0 (bottom). Notice debris removal.
E. Spontaneous Ca^{2+} oscillations measured from 3D spheroids. Untreated wells have similar baseline activity, but cardiac traces are altered (quad- vs. tri-culture) when cells are exposed to BayK8644 (L-type Ca^{2+} channel activator).

Summary and Conclusions

- 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-mediate DILI.
- iCell Sensory Neurons are compatible in co-culture with iCell Macrophages 2.0, with macrophages appearing to clear cellular debris. This co-culture enables *in vitro* models of neuroinflammatory pain.
- Inflammatory macrophages alter cardiac beating activity. This affects iCell Cardiomyocytes^2 spike amplitude, beat rate, beat period, and calcium handling (as shown in 3D cell culture)

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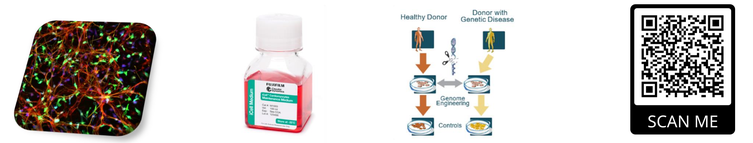

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FUJIFILM Cellular Dynamics, Inc is the leading provider of iPSC-derived cell types and disease models. We are helping to advance the field with 3D and co-culture systems to better replicate human tissue and biology. FCDI continues to be a leader in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

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