# Enabling High-throughput 3D Cell-based Assays with Commercially-available Sources of Human iPSC-derived Cell Types

Coby Carlson, Rebecca Fiene, Michelle Curtis, David Majewski, Ravi Vaidyanathan, Scott Schachtele, Kirk Twaroski, and Simon Hilcove

FUJIFILM Cellular Dynamics, Inc., Madison, WI USA

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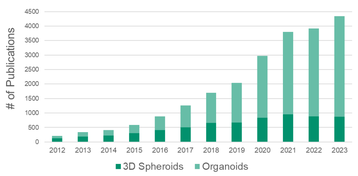
## Overview

- Increased assay complexity can be achieved with 3D culture systems.
- Human iPSC technology has helped to enhance physiological relevance by building a bridge between animal testing and human diseases.
- Like “organoids”, 3D spheroid products can be created by mixing individual cell types at defined # and ratios and allowing them to self-assemble in co-culture.
- This approach enables a more flexible method (modular incorporation of defined cell types, including disease-specific lines) while also allowing more control over variability (individual components are well-defined and highly reproducible), both of which are critical to the success of incorporating such technologies into cell-based assay workflows.
- Here we present examples of enabling 3D cell-based assays with human iPSC-derived cell types in 384-well format:
- “Cardiospheres” from cardiomyocytes, cardiac fibroblasts, and endothelial cells show improved myocardial maturity via positive inotropic response (inc. $Ca^{2+}$ waveform amplitude) to compounds like isoproterenol and dobutamine.
- A diverse range of “neurospheres” can be created using healthy or diseased neurons and astrocytes to model neurodegenerative disease, with the option of incorporating microglia to study neuroinflammation.
- Heps and Macs together in 3D yield a more complex liver co-culture system
- Newly released iCell Hepatic Stellate Cells (HSC) are truly quiescent out of thaw and play a critical role in hepatic homeostasis.
- Importantly, all these systems described above are isogenic, meaning the cells are derived from the same iPSC donor background.
- Commercially available iPSC-derived cell types from FCDI are manufactured at scale, quality controlled, and cryopreserved, so that they are ready-to-use with confidence at any point in time.
- Implementing these cells into modular 3D assay workflows is novel approach to complement current organoid research and enhance the biological complexity required for drug discovery, toxicity screening, & disease modeling.

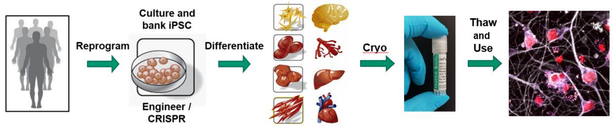
PubMed result: 3D Spheroids and Organoids



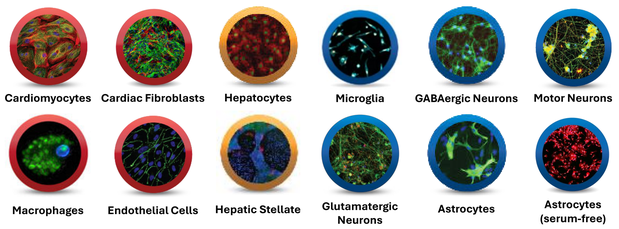

Trending Topics:
- 3D cell culture
- Organoids
- Spheroids
- Advanced models
- Organ-on-a-chip

## Materials, Methods, and Instruments

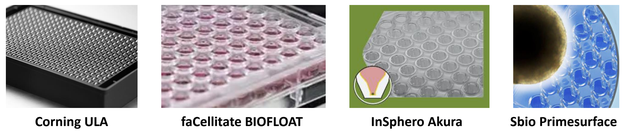
### ► Induced Pluripotent Stem Cell Technology



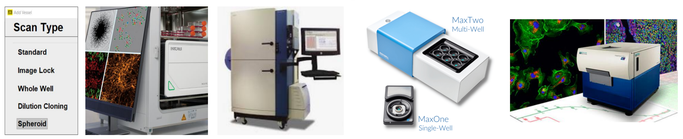

### ► Individual Human iPSC-derived Cell Types = iCell Products




### ► Ultra Low Attachment (ULA) 3D Cell Culture Spheroid Plates



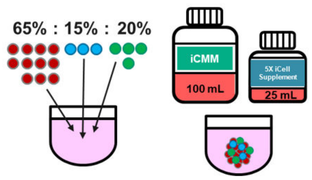

### ► Platform Technologies to Monitor, Image, Characterize 3D Spheroids



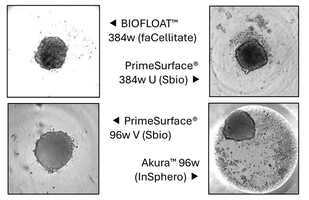

## iCell Cardiospheres: Human, isogenic, tri-culture 3D microtissues for advanced cardiac assays

**A Cardiospheres protocol**

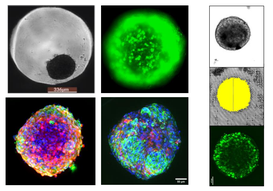
- iCell Cardiomyocytes
- iCell Cardiac Fibroblasts
- iCell Endothelial Cells
- [Donors 01434 or 11713]



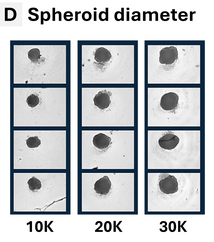

**B ULA spheroid-forming plates**

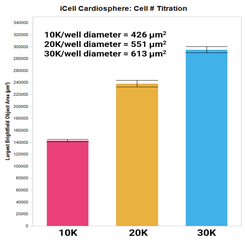



**C Imaging of cardiac spheroids**

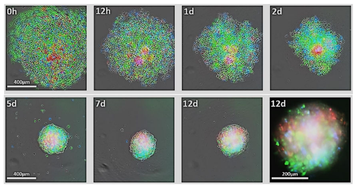


**D Spheroid diameter**

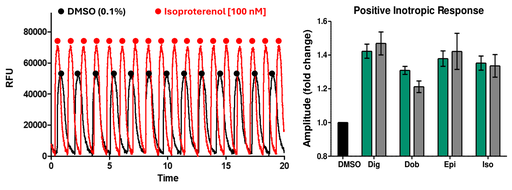


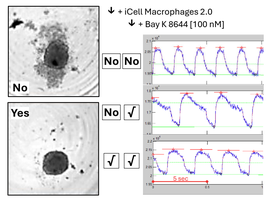

**E Forming spheroids w/ cell tracker dyes**




**F Spontaneous $Ca^{2+}$ waveforms and positive inotropy**



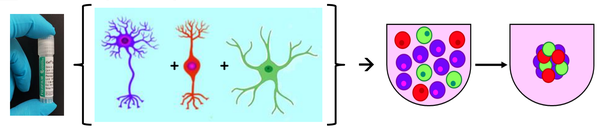

**G Addition of iCell Mac 2.0**




A. Cardiospheres can be made following the Application Protocol.
B. Numerous Ultra Low Attachment (ULA) plates have been tested.
C. Cardiospheres can be imaged, stained, used for cardiotoxicity testing, or loaded with Calcium 6 dye for functional assays.
D. Diameter is impacted by cell #, ratios, plate type, and day in vitro.
E. Uniform sphere formation (1 per well) and survival of each cell type can be visualized over 10-14 days with cell tracker dyes.
F. Dosing with digoxin, dobutamine, epinephrine, or isoproterenol results in a positive inotropic response (↑ peak amplitude).
G. Macrophages clean up debris and modify $Ca^{2+}$ handling properties.

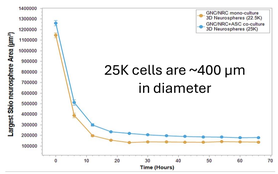
## iCell Neurospheres: Create your own 3D human iPSC-derived “brain-in-a-dish”

**A Neurospheres protocol**

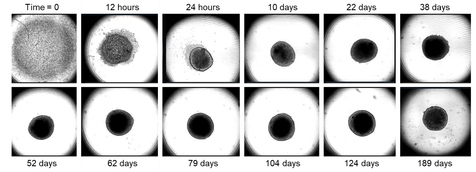


● iCell GlutaNeurons ● iCell GABANeurons ● iCell Astrocytes 2.0, 01279

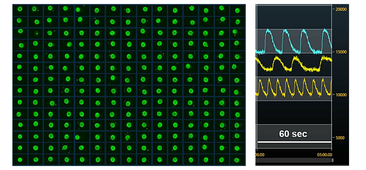
**B Rapid spheroid formation**



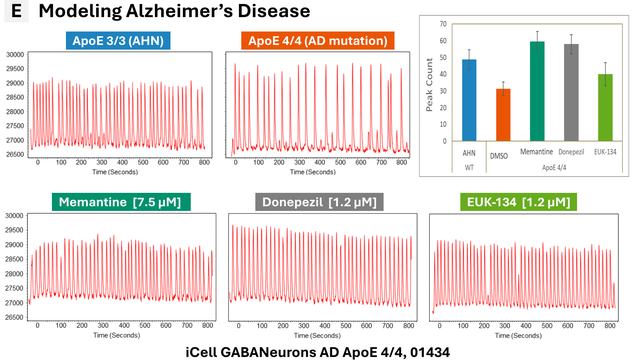

**C Long-term survival of 3D spheroids**




**D Functional $Ca^{2+}$ Oscillation Assay**

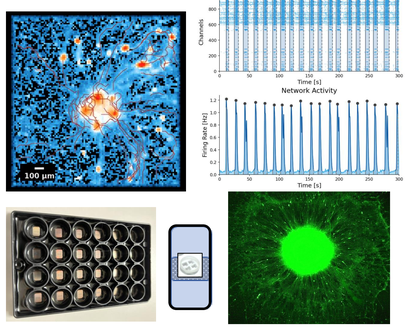



**E Modeling Alzheimer’s Disease**




iCell GABANeurons AD ApoE 4/4, 01434

**F HD-MEA in 3D**

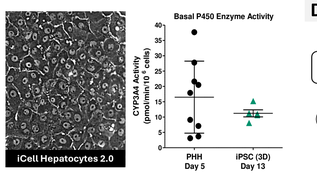



Three-dimensional (3D) spheroids featuring human iPSC-derived cell types (such as glutamatergic + GABAergic neurons, astrocytes, etc.) have emerged as a promising *in vitro* model system for drug screening and disease modeling. Recent publications have demonstrated that organoids/spheroids can acquire more “brain-like” complexity and are amenable to HTS assays.

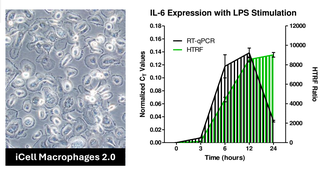
A. Schematic for designing your own iCell Neurospheres for “brain-in-a-dish”.
B. Cells self-assemble in ULA plates to form 3D structures within 24-28 hours.
C. Images of Neurospheres over time showcase long-term survival in culture.
D. Calcium oscillation assay was developed to demonstrate neural function.
E. AD disease model with ApoE 4/4 cells yields $Ca^{2+}$ traces with lower peak count and higher amplitude; treatment with known drugs can reverse the phenotype.
F. 3D spheroids are formed and then transferred to an HD-MEA plate (Maxwell) with PDMS well dividers (Ibidi) to contain the structures; robust electrophysiological measurements showing activity and network connectivity were collected.

## iCell Liverspheres: Hepatocytes, Hepatic Stellate Cells, and Macrophages together in 3D

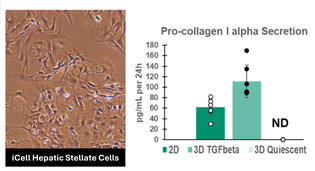
**A**



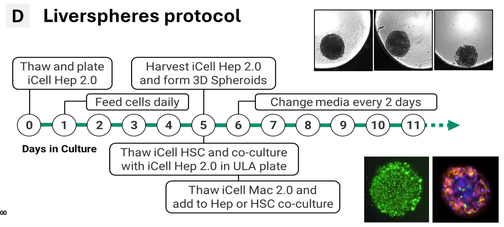

**B**



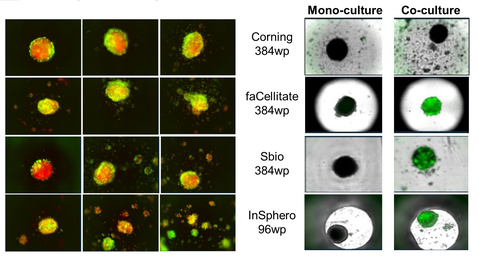

**C**




**D Liverspheres protocol**

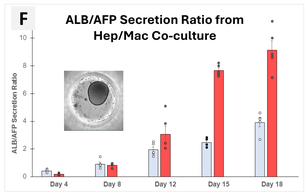



**E Assay Development**

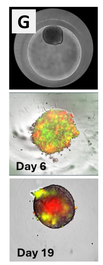



A. iCell Hepatocytes 2.0: morphology in 2D + CYP activity
B. iCell Macrophages 2.0: morphology + cytokine release
C. iCell Hepatic Stellate Cells: 2D culture + pro-collagen secretion; truly quiescent in 3D culture.
D. Protocol workflow to generate iCell Liverspheres: pre-plate Heps, harvest, and then add Mac +/- HSC in 3D
E. Examples of assay development varying spin/no spin, ECM in media, pre-plating, etc. Plus, comparison of ULA spheroid-forming plate types for mono- and co-cultures.
F. Hep/Mac co-culture improves hepatic function as shown by Albumin/Alpha FetoProtein secreted ratios.
G. Hep/Mac/HSC is possible for long-term tri-culture (HSC red, Mac green, Hep no color). 3D spheroids made on Day 5.

**F**




**G**




## Summary and Future Directions

There is a great deal of excitement and promise around 3D cell culture. Research with iPSC-based organoids and spheroids is focused on creating a more complex and biologically relevant model system to bridge the translational gap, better our understanding of human disease, and facilitate the discovery of new drugs. The advantage of the modular approach presented here with commercially available, cryopreserved, ready-to-use cells is that you can vary cell type numbers and ratios to have exquisite control over the spheroid size and composition. Importantly, this method further enables disease modeling studies and the incorporation of iPSC-derived microglia into neurospheres, for example, is something that is not easily accomplished with organoids. FUJIFILM CDI will continue to develop new applications with iCell products and establish advanced co-culture systems that are amenable to standard workflows.



Cellular Dynamics