Enabling High-throughput 3D Cell-based Assays with

Commercially-available Sources of Human iPSC-derived Cell Types

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

FUJIFILM Cellular Dynamics, Inc. / Madison, WI USA



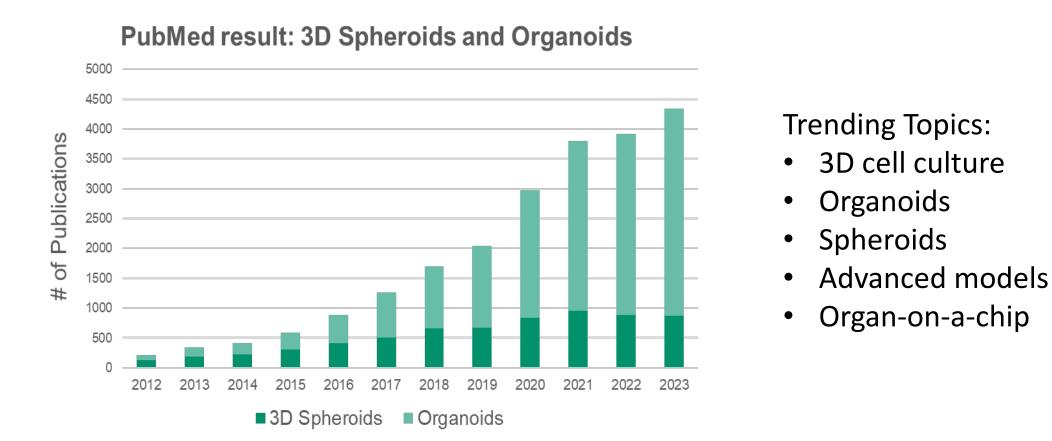
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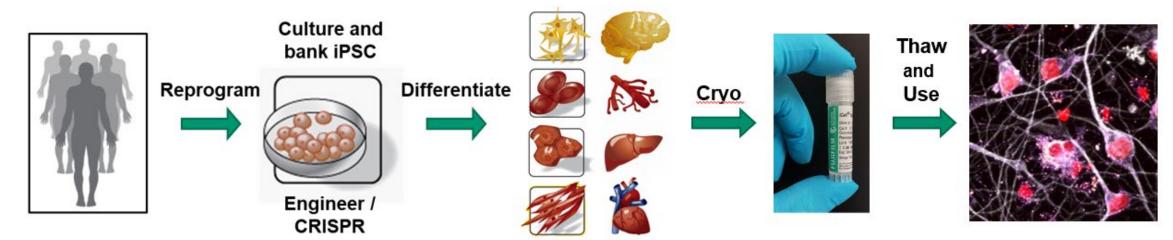
Overview

- Increased assay complexity can be achieved with three-dimensional (3D) culture systems.
- Human induced pluripotent stem cell (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 numbers 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 (increased Ca²⁺ 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.
- Hepatocytes and macrophages together in 3D yield a more complex liver co-culture system
- An isogenic blood-brain barrier (BBB) model system composed of astrocytes, pericytes, and brain microvascular endothelial cells. Importantly, all of these systems described above are isogenic, meaning the cells are derived from the same iPSC donor background.
- These commercially available iPSC-derived cell types from FUJIFILM CDI are manufactured at scale, quality controlled, and cryopreserved, so that they are ready-to-use with confidence at any point in time.
- Implementation of these cells into modular 3D assay workflows is novel approach to complement current organoid research and enhance the biological complexity required in cell-based assays for drug discovery, toxicity screening, and disease modeling.

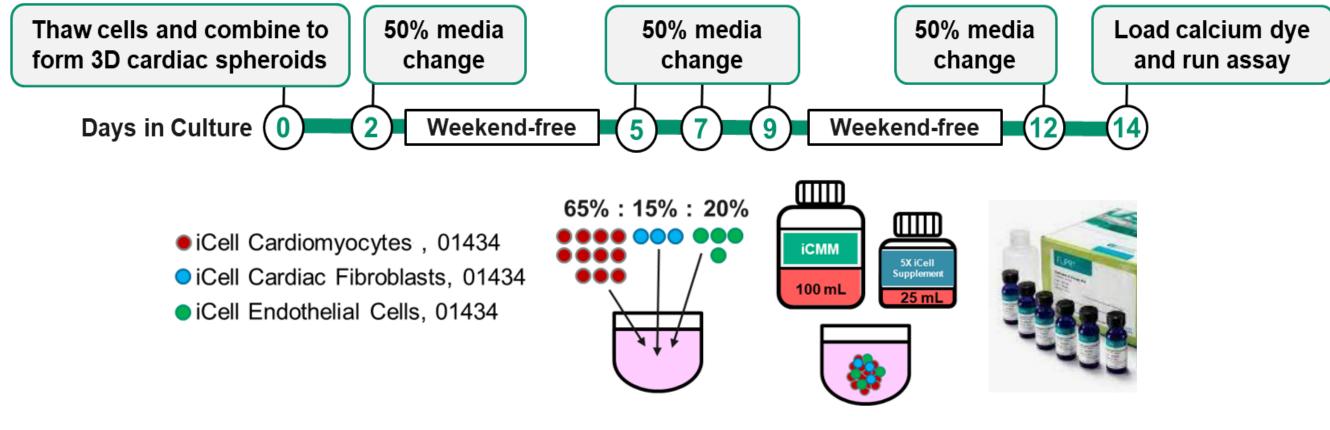


Methods

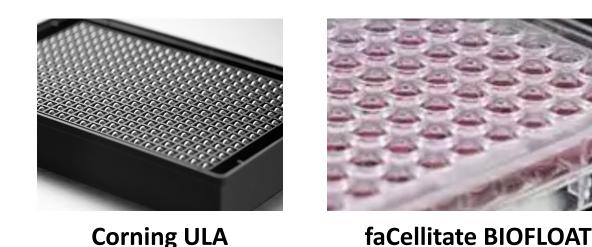
► Induced Pluripotent Stem Cell Technology



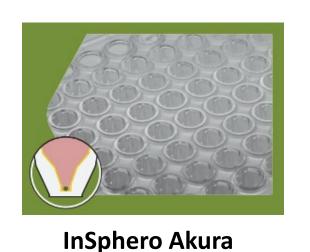
► Application Protocol to make 3D cardiac tri-culture spheroids



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

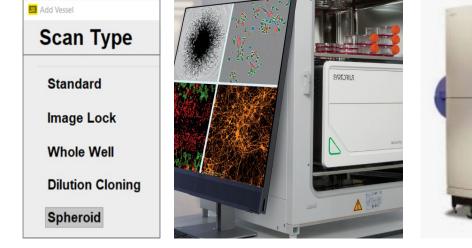






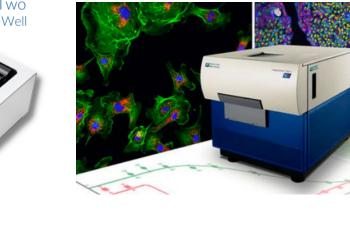


► Platform Technologies to Monitor, Image, Characterize 3D Spheroids

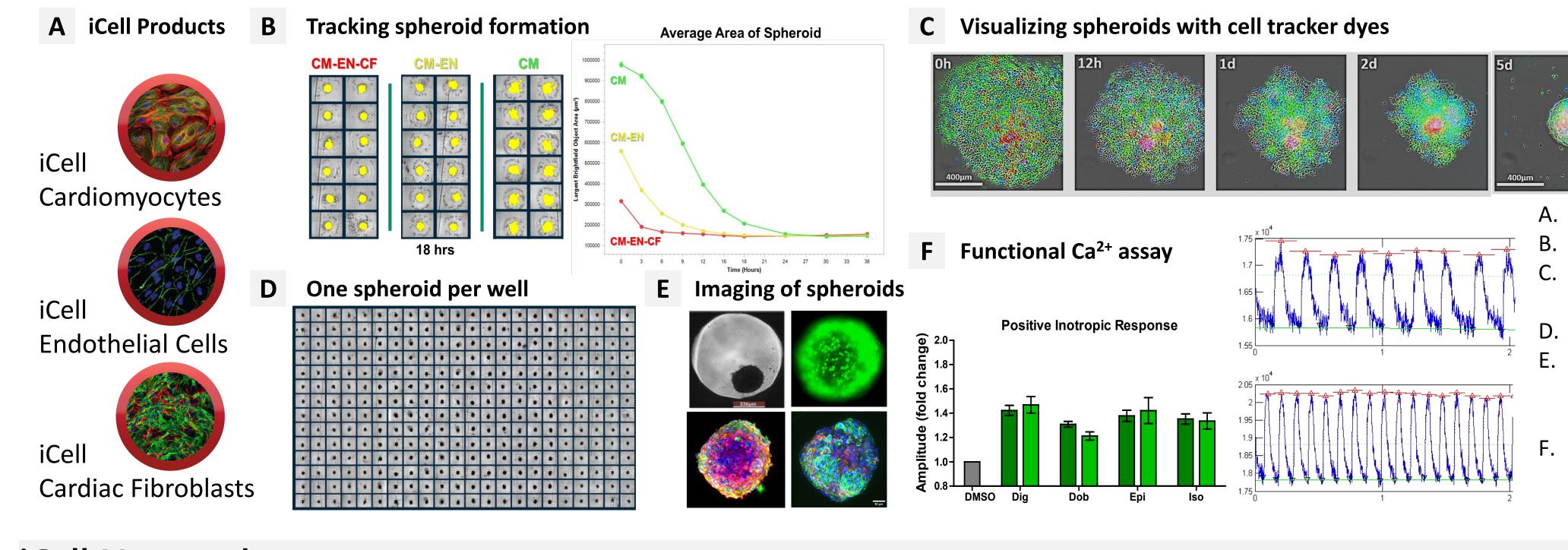


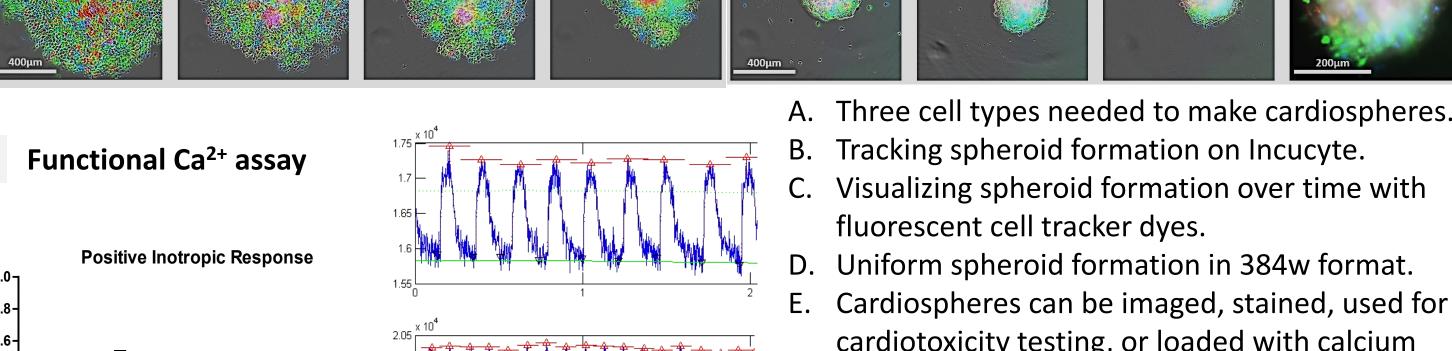






iCell Cardiospheres: human, isogenic, tri-culture microtissues for advanced cardiac assays





Characterization of spheroid diameter

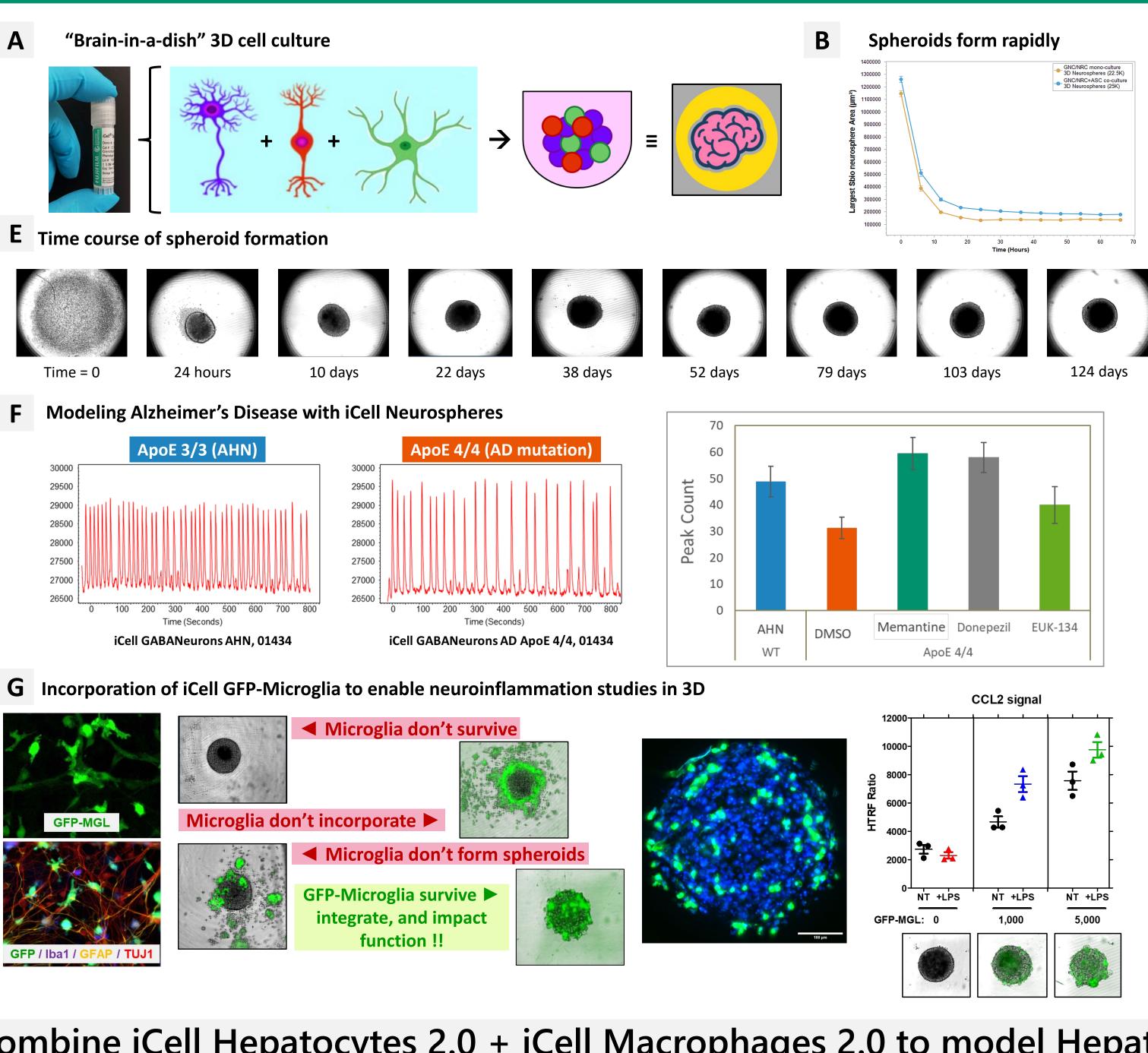
fluorescent cell tracker dyes. Uniform spheroid formation in 384w format.

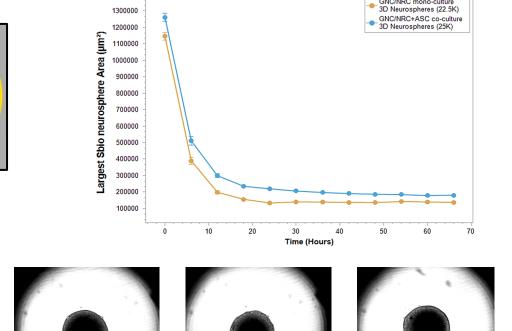
Cardiospheres can be imaged, stained, used for cardiotoxicity testing, or loaded with calcium dye for functional assays.

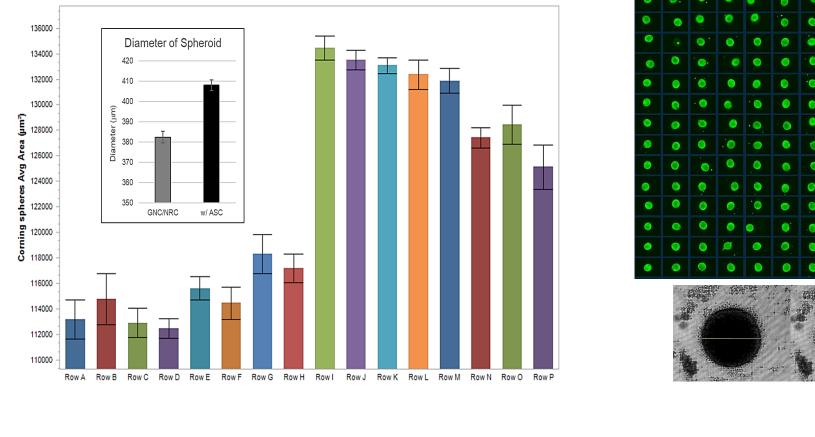
F. Tri-culture spheroids respond (↑ peak amplitude) to positive inotropic compounds such as digoxin, dobutamine, epinephrine, and isoproterenol.

D Imaging of Neurospheres

iCell Neurospheres







Donepezil 1.2 μM EUK-134 1.2 μM Memantine 7.5 μM iCell GABANeurons AD ApoE 4/4, 01434

Three-dimensional (3D) spheroids featuring human iPSC-derived cell types have emerged as a promising in vitro model system for drug screening and disease modeling. Recent publications have demonstrated that iPSC-derived cortical spheroids acquire more "brain-like" complexity and are a robust assay platform for HTS. A. Schematic for designing your own iCell Neurospheres for "brain-in-a-dish" studies;

B. Cells self-assemble in ULA plates to form 3D structures within 24-28 hours;

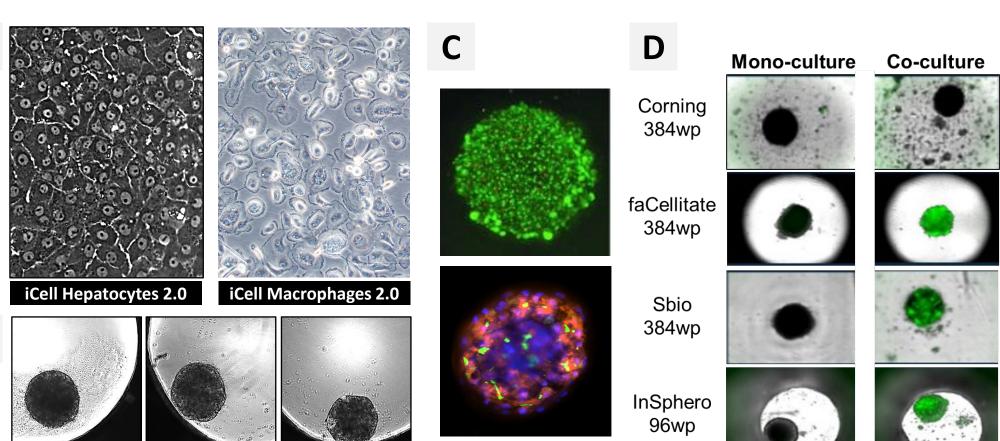
Neurospheres from an input of 25K cells are ~400 μm in diameter.

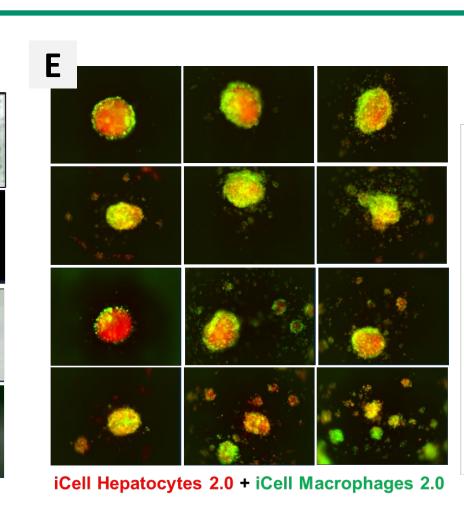
D. Images in 384w, on Incucyte, with mask for analysis (yellow), or with Cal6 dye;

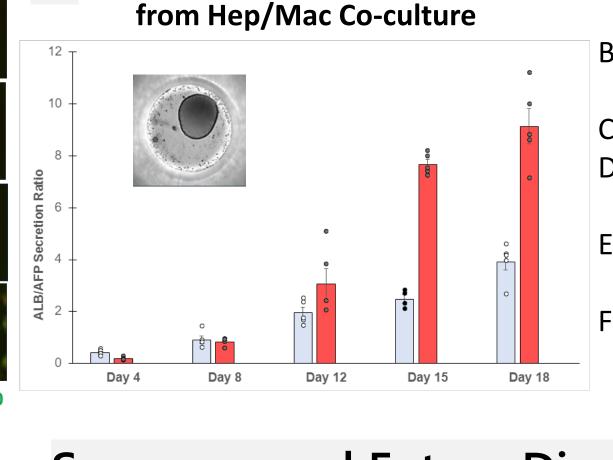
E. AD disease model with ApoE 4/4 cells yields Ca2+ traces with lower peak count and higher amplitude; treatment with known drugs can reverse the phenotype.

GFP-microglia are useful tool to optimize co-cultures; not all conditions support incorporation of MGL though; Once in, the model is useful for neuroinflammation.

Combine iCell Hepatocytes 2.0 + iCell Macrophages 2.0 to model Hepato/Immuno Interactions in vitro







ALB/AFP Secretion Ratio

- A. iCell Hepatocytes 2.0 and iCell Mac 2.0 in 2D cell culture.
- B. Robust protocols to generate 3D cultures of iCell Hepatocytes 2.0. Images of hepatocytes in 3D.
- Comparing ULA spheroid plate types for mono- and co-cultures.
- E. Examples of assay development varying spin/no spin, ECM in media, pre-plating. Co-culture improves hepatic function as shown by Albumin/Alpha FetoProtein secreted ratios.

Blood-Brain Barrier: 3D spheroid model for permeability studies

iCell Blood-Brain Barrier Kit C Tri-culture BBB spheroids iCell Astrocytes 2.0 iCell Pericytes iCell BMEC **B** Various approaches to model the BBB are part of the iCell BBB Kit.

Model the BBB with iPSC-derived cell types; three new ones featured here

B. Various approaches to model the BBB. C. Combining three BBB cell types in 3D cell culture plates allows them to selfassemble and be used to study

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.

Matrix / Hydrogel

permeability of molecules.