Advanced Human iPSC-derived Models for Translational Drug Discovery: 3D Spheroids and Microphysiological Systems

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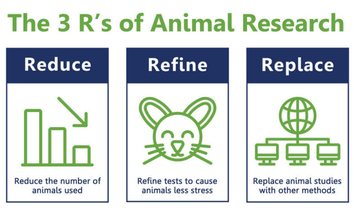
FUJIFILM Cellular Dynamics

OVERVIEW: Drug Development should be Human-centered

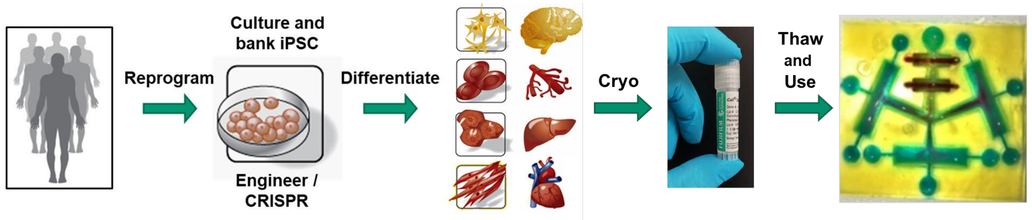
Too many drug failures in past 20+ years



Many reasons to reduce animal testing

FDA Modernization Act 2.0

In 2022, the **FDA Modernization Act 2.0** ended the long-standing mandate that required drugs to be tested on animals before they are tested in humans. This change also authorized the use of alternative technologies to reduce the reliance on animal testing. These accepted options can be grouped into four different categories: (1) cell-based assays, which include human induced pluripotent stem cells (iPSC); (2) 3D organoids & spheroids; (3) "organ chips"; and (4) computer models, which covers AI, machine learning, and bio-simulation. This poster will focus on the combination of iPSC-derived cell types as 3D spheroids or in **organ-on-a-chip** (OoC) systems.



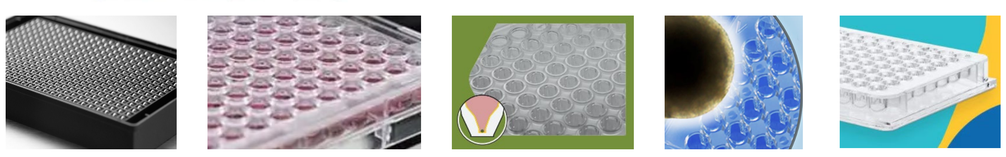
Human iPSC-derived cell types are a promising new alternative to animal models since they offer more physiological relevance, meaningful drug responses, and opportunities for "disease-in-a-dish" studies. When highly specialized cell types generated via human iPSC technology (such as **iCell® products**) are paired with cutting-edge organ-on-a-chip technologies, this approach is gaining increased traction as the best hope for improving the pre-clinical pipeline for new therapeutics.

Combine with Numerous Commercially Available Platforms

4Dcell EXPLORE BETTER. · aimbiotech · AlveoliX In-vitro models inspired by nature · Jananda™ *In vitro solutions for in vivo results* · AxoSim Human Data, Faster.

BiomimX THE BEATING ORGANS-ON-CHIP · CN-BIO · Curiochips® · emulate

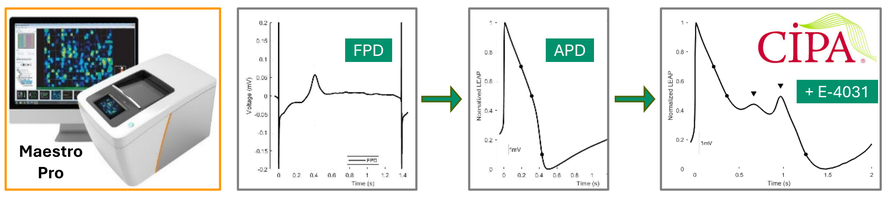
MIMETAS the organ-on-a-chip company · NETRI PROVIDING ORGANS-ON-CHIP FOR THE INDUSTRY · SYNVIVO



Corning ULA · faCellitate BIOFLOAT · InSphero Akura · Sbio PrimeSurface · Sigma MilliCell

The development of **3D spheroids/organoids** and **OoC systems** has revolutionized in vitro research, offering dynamic, physiologically-relevant platforms that enable the creation of multi-cellular models which closely mimic the structure and function of native human organs. A critical requirement of such systems as they evolve and improve the scalability, complexity, and throughput, is that they maintain consistency. There are now many providers with commercially available options for organ-on-a-chip device technology and spheroid-forming ultra-low attachment (ULA) plates. Our mission is to support the integration of iPSC-derived cell types into these different platforms to reshape research across neuroscience and broader biomedical fields with the goal of bridging the gap between results from pre-clinical studies and positive clinical outcomes.

Assess Cardiovascular Risk and Immune-related Toxicity on MEA



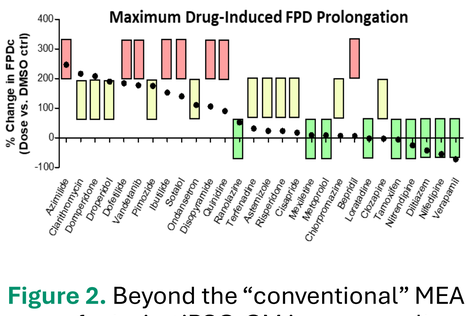
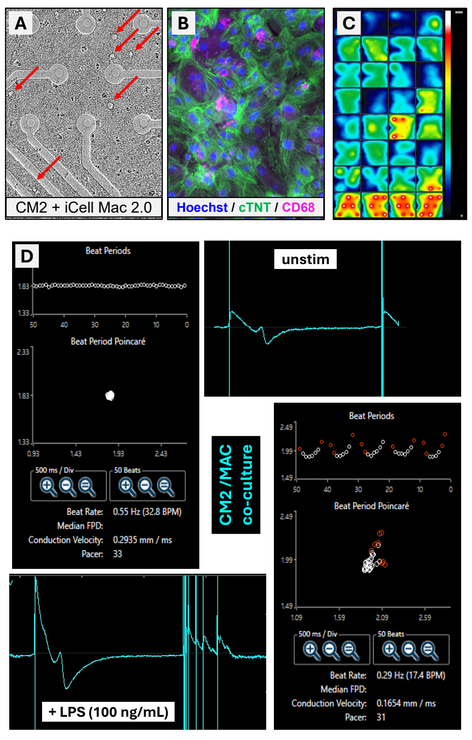


Figure 1. iCell Cardiomyocytes² were the featured cell type used in the seminal CiPA study from 2016-2018 to evaluate and validate drug effects from 28 different compounds, assess their cardiac risk, and improve safety testing guidelines overall. The Maestro Pro MEA (Axion Biosystems) and 48-well plates are the most widely accepted multi-electrode array system in this field.

Figure 2. Beyond the "conventional" MEA assay featuring iPSC-CM in mono-culture, which has been accepted recently by the FDA into the ISTAND program, the NAMs concept pushes the idea of creating more complex & biologically relevant models. To this end, **iCell Macrophages 2.0** were co-cultured with iCell CM2 on MEA to evaluate the impact of an immune response on cardiac activity.
(A) Brightfield image of CM2 on an MEA with red arrows pointing to iCell Mac 2.0;
(B) fluorescent image with CM2 marker cTNT staining in green and Mac marker CD68 in magenta;
(C) Bottom row of heat map (8th of 8) shows presence of Mac results in increased Sodium (Na) Spike Amplitude.
(D) After LPS stim for >24 h, CM2/Mac co-cultures have longer and more irregular Beat Periods, altered repolarization, and detection of arrhythmias;
Similar responses are not seen in CM2 mono-culture or unstim co-culture control wells (data not shown). Studies are ongoing to further understand the mechanism of action leading to these observations.



Interrogate Blood-Brain Barrier Dynamics with 96-well TEER Assays

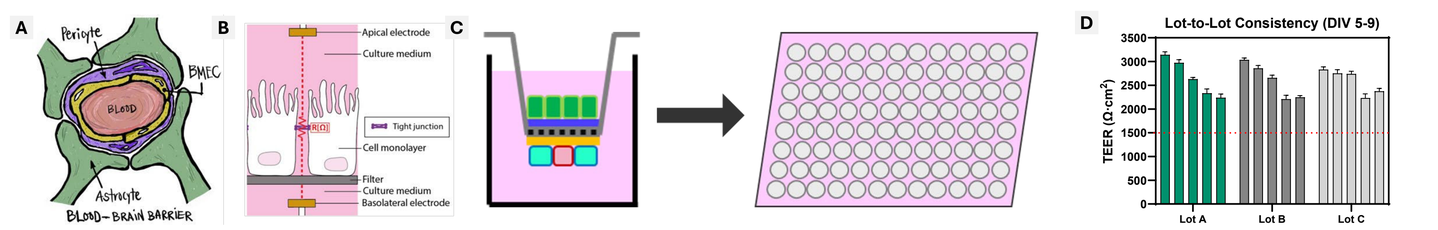


Figure 3. iCell Blood-Brain Barrier (BBB) Isogenic Kit is composed of human iPSC-derived cell types from male iPSC donor 01279, including **(A)** iCell Brain Microvascular Endothelial Cells (BMEC), iCell Pericytes (PERI), and iCell Astrocytes 2.0 (ASC).
(B) Schematic for Trans-Endothelial Electrical Resistance (TEER) measurements across a cell monolayer with electrodes on the apical and basolateral sides. TEER is used to evaluate the integrity of tight junction formation in a barrier.
(C) ASC (red) and PERI (blue) are co-cultured on the basolateral side of the membrane with BMEC (green) on the apical side. This protocol was miniaturized to Transwell-96 Permeable Supports in a critical step for the advancement of this NAM.
(D) Lot-to-lot consistency testing was performed under the same assay conditions with three different lots of iCell BMEC but the same lots of iCell Astrocytes 2.0 and iCell Pericytes. TEER values exceeded 2000 ohms·cm² and were sustained over the 5 days of measurement. The red dotted line at 1500 ohms·cm² indicates the expected threshold for in vitro BBB models. Error bars indicate SEM with n=12 wells per data point. One iCell BBB Kit is enough to fill all wells in this assay format.

Enhance Neuronal Phenotypes on Organ Chips

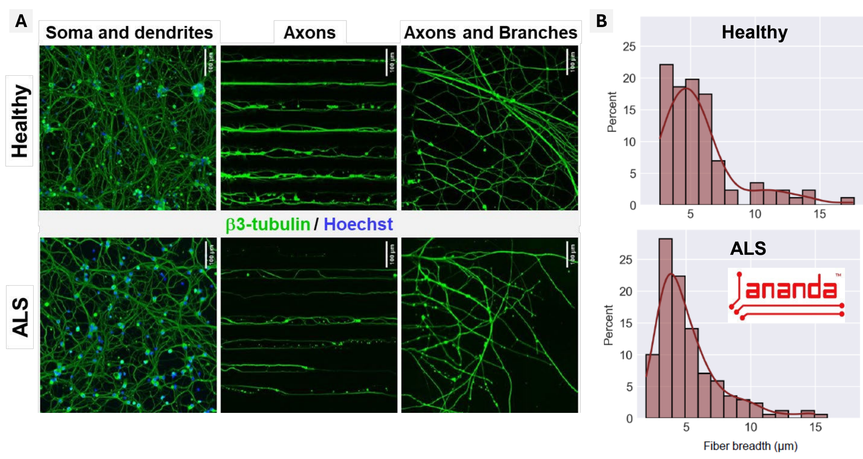


Figure 4. iCell Motor Neurons are used for ALS disease modeling by comparing cells derived from a Healthy iPSC donor to those from an iPSC line harboring TDP43 M337V. NeuroHTS™ plates (Ananda Devices) were used to screen for neuronal features, with specific focus on neurite morphology, axonal structures/branching, and network formation.
(A) Healthy and ALS iCell Motor Neurons were cultured until DIV 21. Cells were fixed, stained, imaged, and analyzed with a 7+ factor profile analysis algorithm.
(B) Significant decrease in fiber thickness (breadth in µm) was observed for ALS vs. control, indicating higher axonal bundling in the healthy neurons.

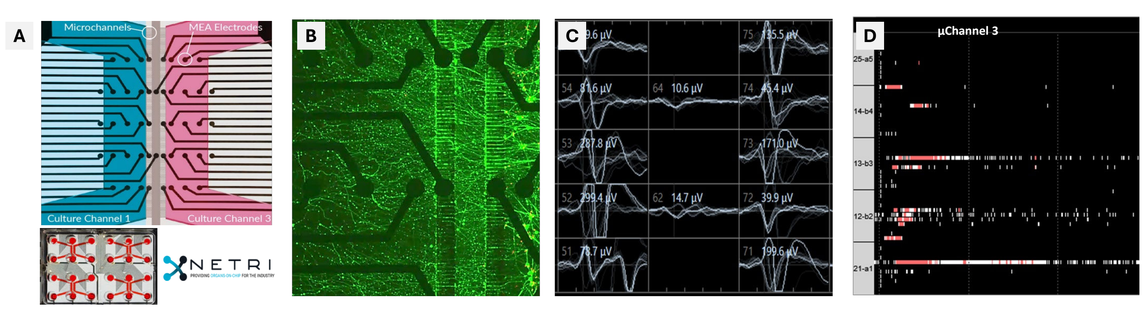
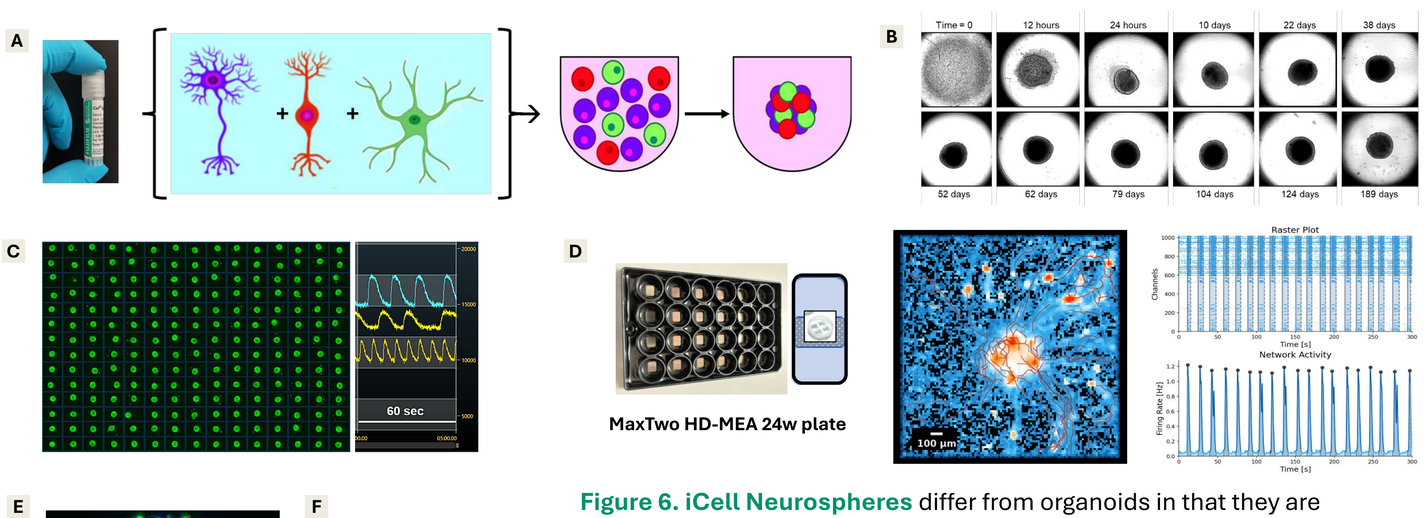


Figure 5. iCell Sensory Neurons exhibit lower levels of spontaneous activity and longer timeframes for onset in standard culture compared to other iPSC-derived neurons.
(A) DuaLink™ MEA (NETRI) is a compartmentalized microfluidic device compatible with the Maestro MEA system.
(B) Neurons were seeded into Ch3 and readily project neurites across the chip and thru microchannels (Ch1 & Ch2). Staining with Calcein AM (green) was performed on DIV 29 (*image courtesy of NETRI*).
(C) Action potentials with spike amplitudes ~100 µV appeared after only 2 weeks in culture.
(D) Cells doses with Capsaicin (2 µM) in Ch1 , Ch2, and Ch3 sequentially. Raster plot of spike activity (~90 sec) is shown.
(E) Number of spikes recorded from microchannel 1µ2 in response to the dose location is graphed. Blue is pre-dose levels. Data suggest that the capsaicin response was strongest at the soma (Ch3).

Assemble your own iCell Neurospheres… with Microglia



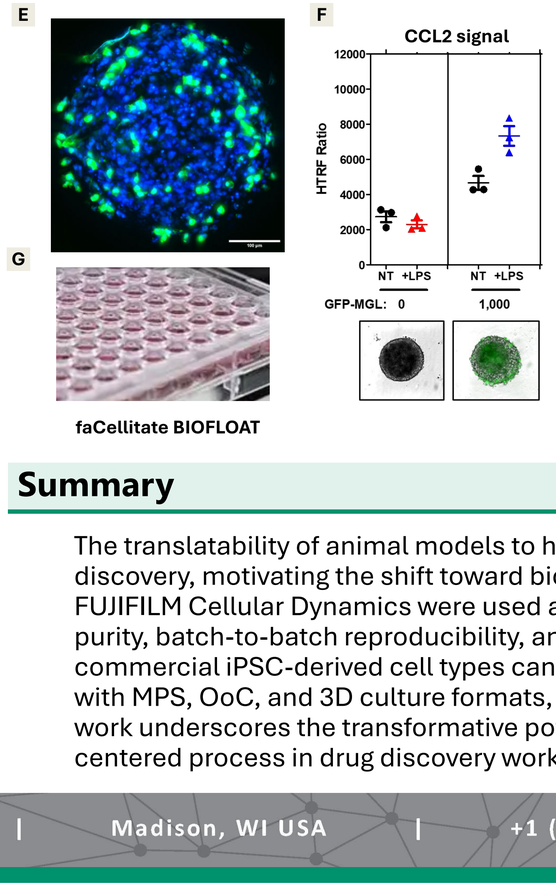


Figure 6. iCell Neurospheres differ from organoids in that they are generated using a modular approach to assemble 3D spheroids from:
(A) human iPSC-derived neuronal cell types, including iCell GlutaNeurons and iCell GABANeurons, iCell Astrocytes 2.0 and iCell Microglia.
(B) Ultra-low attachment (ULA) spheroid-forming plates, such as BIOFLOAT from faCellitate (see **G**), have kept neurospheres in culture for >6 months.
(C) Calcium oscillation assays also show functionality of 3D neurospheres.
(D) Wide-bore tip transfer of 3D spheroids to an HD-MEA system (Mxwbio) for robust electrophysiological measurements of spike activity and network connectivity.
(E) Upgrading "brain-in-a-dish" NAMs to incl. iPSC-derived GFP-microglia requires testing of numerous parameters, which is enabled by fluorescent cells to investigate cell ratios, cell survival, integration, etc. in 3D culture.
(F) iCell Neurospheres can be used for neuroinflammation studies and measurement of LPS-stimulated cytokines (like CCL2).

Summary

The translatability of animal models to human clinical outcomes remains a significant challenge in drug discovery, motivating the shift toward biologically relevant human cell-based platforms. iCell products from FUJIFILM Cellular Dynamics were used across diverse NAM platforms, highlighting their scalability, high purity, batch-to-batch reproducibility, and consistent in vitro phenotypes. Our results confirm that commercial iPSC-derived cell types can successfully generate complex, human-relevant tissues compatible with MPS, OoC, and 3D culture formats, improving the physiological relevance of in vitro drug testing. This work underscores the transformative potential of iPSC-based models and NAMs to craft a more human-centered process in drug discovery workflow pipelines.