From 2D to 3D: Identifying the Optimal Human iPSC-based Model for Neurotoxicity

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ABSTRACT

Background and Purpose: Human induced pluripotent stem cell (hiPSC)-derived neurons offer a relevant and accessible cell source for neurotoxicity testing. Indeed, hiPSC-derived neurons are actively being used across biopharma to evaluate off-target compound toxicity and as a part of pre-clinical target validation. While 2D monocultures are classically employed for such assays, it offers a singular evaluation of neurotoxicity, potentially overlooking the greater context and influence of brain complexity. Next-generation multicellular and 3D neural spheroid models, built using iPSC-derived neurons and glial cells, have been shown to produce advanced functions (i.e., neural network activity, improved maturation) that may provide a more faithful and predictive model for neurotoxic evaluation. However, complexity often comes with an increase in experiment timelines, more complex data sets, and challenges in reproducibility and accessibility. In this poster, we present a series of experiments across a variety of platforms designed to evaluate the cell viability, morphology, or functional network activity of hiPSC-derived neurons in 2D or 3D culture after being exposed to neuromodulatory or neurotoxic compounds.

Methods: All human iPSC-derived cell types used in this study were from FUJIFILM CDI. Neuronal culture media and supplements are described for each particular assay format. Multiple kits for cell viability were tested, including CellTiter-Glo (Promega) and PrestoBlue (Thermo Fisher). CLARIOstar Plus (BMG Labtech) multi-mode plate reader was used for all fluorescent and luminescent detection. Multi-electrode array (MEA) assays were performed on a Maestro Pro system (Axion Biosciences) using 48-well CytoView MEA plates. Imaging of neuronal cultures and tracking of neurite outgrowth took place on an Incucyte SX5 live-cell imaging system (Sartorius) or an ImageXpress micro (Molecular Devices) high-content imager. Calcium assays were done with a FLIPR-Penta (Mol Dev) or the FDSS/uCell (Hamamatsu). Compounds used in this study include: excitotoxic agents like glutamate, kainic acid, AMPA, and NMDA; seizurogenic drugs like 4-AP, bicuculline, picrotoxin, and GABAzine; and AMPA/NMDA receptor antagonists such as AP5, DNQX, and MK-801. Controls such as staurosporine and rotenone were used for maximum and indiscriminate toxicity.

Results: We demonstrated the effects of timing, cell density, and neuronal cell type on cell viability. Monocultures of hiPSC glutamatergic, GABAergic, and dopaminergic neurons demonstrate different dose-response kinetics to excitotoxic exposure (glutamate) and known toxic agents (Staurosporine).We then showed that combining hiPSC neurons and hiPSC astrocytes in co-culture results in the emergence of neural network activity, as recorded by multielectrode arrays (Axion). Exposure of neuron-astrocyte co-cultures to neuromodulators (i.e., picrotoxin, gabazine, bicuculline) results in changes to network activity, providing a sensitive model for subtoxic effects, such as induction of seizurogenic activity. Last, we show that hiPSC-derived "neurospheres", formed by combining fully differentiated iPSC-derived neurons and astrocytes at defined ratios and cell numbers to mimic specific regions of the brain, develop spontaneous functional activity which can be measured as calcium wave oscillations. Perturbations in the oscillation patterns occur upon exposure to neuromodulatory compounds, mimicking our MEA observations in 2D co-cultures.

Conclusions: These data confirm hiPSC-derived neuronal cells as a useful model for neurotoxicity and demonstrate their ability to provide continuity and flexibility in neurotoxicity model choice, while retaining toxicity predictability.

Glutamate-induced Excitotoxicity with hiPSC-derived Neurons

iCell GlutaNeurons

Assay	IC_{50} (µM)
CellTiter-Glo 2.0	62
CytoTox-ONE	60
CyQUANT Direct	31
PrestoBlue	32

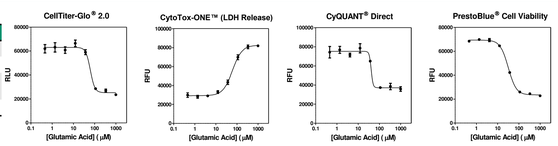
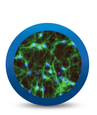
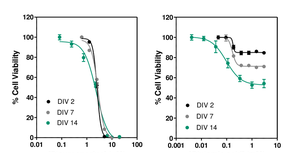


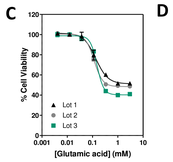
Figure 1. (A) iCell GlutaNeurons can be used in a variety of assays to assess neurotoxicity. iCell GlutaNeurons are a highly pure population of hiPSC-derived cortical glutamatergic neurons. In monoculture, these cells are a useful model for neurotoxic compound testing. **(B)** A variety of commercial fluorescent and luminescent cell viability assays were used to test 48-hour glutamic acid cytotoxicity in iCell GlutaNeurons cultured for 7 days in a 384- or 96-well plate. Imaging was performed using a BMG Labtech CLARIOstar plate reader. Glutamic acid induced an excitotoxic response in all assays shown, with IC_{50} values varying across cytotoxicity assays.



iCell GABANeurons



IC_{50} value (µM)	DIV 2	DIV 7	DIV 14
Glutamic acid	183	145	83
Staurosporine	2.55	2.75	2.36



IC_{50} value (µM)	Lot 1	Lot 2	Lot 3
Glutamic acid	135	115	150

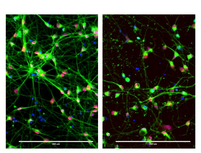


Figure 2. (A) iCell GABANeurons (40K/well) were cultured in PLO/laminin-coated 96-well plates for 2, 7, or 14 days. Cells were then dosed with either staurosporine or glutamic acid for 48 hours and tested for viability with a CellTiter-Glo® assay. **(B)** Data illustrate that staurosporine kills cells at any time point and the viability is reduced to zero; however, neurons require ≥2 weeks of culture to establish proper synaptic networks to model glutamate-induced excitotoxicity (which only drops assay signal to ~50%). **(C)** Three different lots of iCell GABANeurons were tested after 14 days in culture for glutamate-induced toxicity. **(D)** Fluorescent images of iCell GABANeurons on day 15 either untreated or dosed with 1 mM glutamic acid for 48 h. Cells were stained with antibodies for TUJ1 (green), PSD95 (red), and nuclear dye Hoechst 33342 (blue). Synaptically interconnected neurons are disrupted and dying in the presence of the excitotoxic compound glutamic acid. Scale bar represents 200 µm.

Evaluating Compound Pharmacology on Neural Networks using MEA

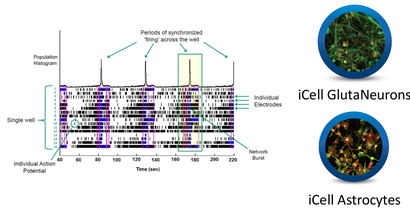


Figure 3. Architecture of a spike raster plot. Electrophysiological spiking activity recorded from cultured neurons in a single well on an MEA can be depicted in a time-sequenced raster plot. A black tick mark is drawn each time a neuron fires an action potential. They are colored blue if a burst of spikes are recorded on an individual electrode. Pink boxes outline a network burst when the individual bursts align across all 16 electrodes in the well. Population histograms indicate periods of synchronized firing. Spike raster plots provide a simple method to visualize neuronal network activity.

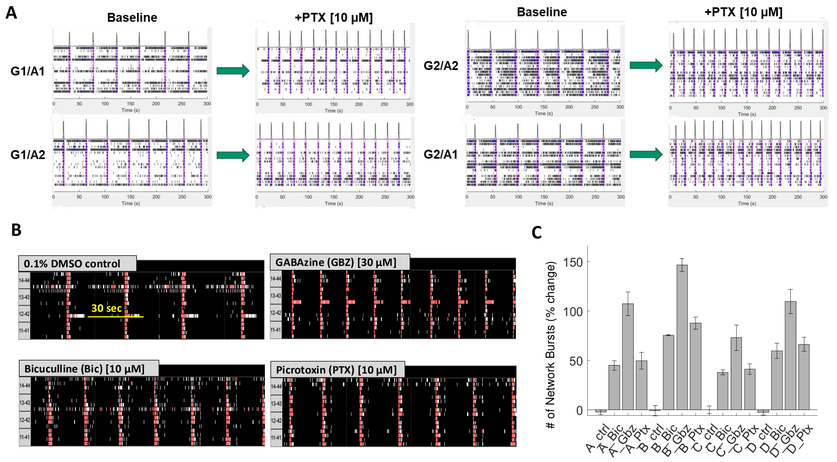


Figure 4. Consistent MEA Network Development and Responses to GABA-A Antagonists. Two lots of iCell GlutaNeurons and iCell Astrocytes were thawed and placed in various combinations to test the effects of manufacturing lot variation on MEA network development. **(A)** Raster plots of the various lot combos (G1/A1, G1/A2, G2/A1, G2/A2) in a 48-well CytoView MEA recorded on Day 21 before & after exposure to Picrotoxin (PTX). **(B)** Raw data traces from co-cultures post-dose with the GABA-A antagonists (Bic + GBZ). A pro-seizurogenic response is observed visually and quantified in panel **(C)** where the "# of Network Bursts" increased significantly as compared to DMSO control and similarly across all lots (G1/A1, G1/A2, G2/A1, G2/A2); n=3 wells per dose).

Chemotherapy-induced Neurotoxicity using iCell Sensory Neurons

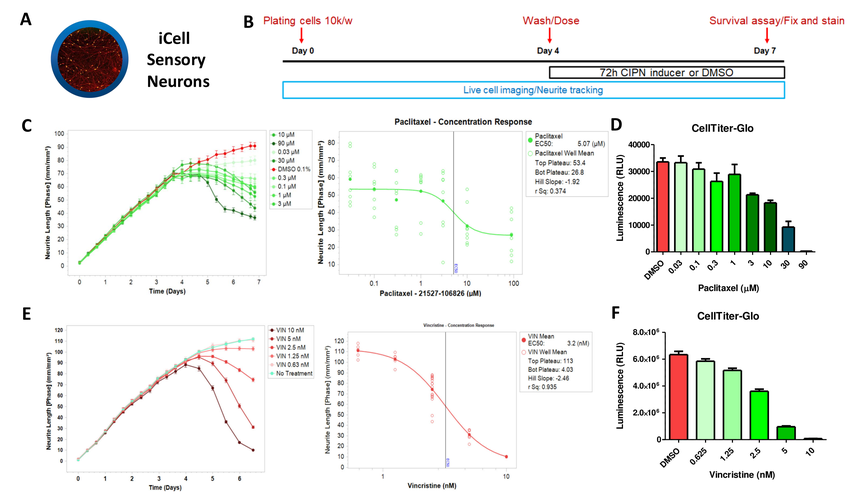
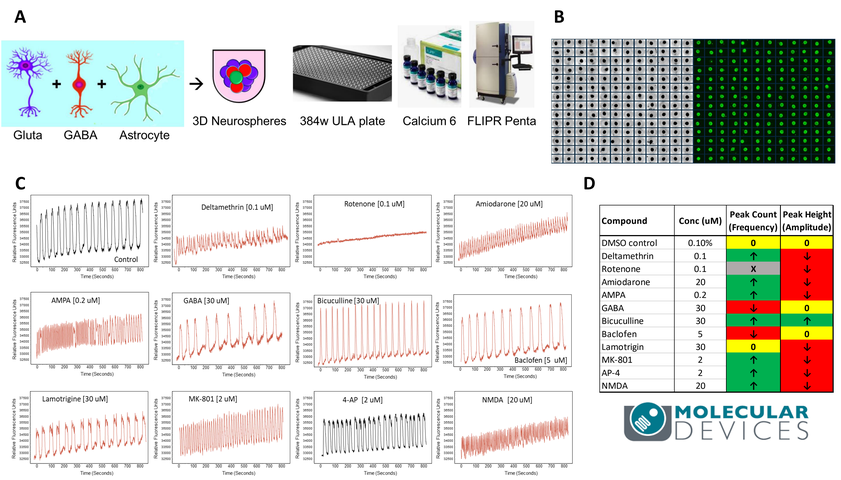


Figure 5. iCell Sensory Neurons Demonstrate CIPN-mediated Toxicity. (A) iCell Sensory Neurons are a pure population of hiPSC-derived peripheral sensory neurons. **(B)** Schematic of workflow where iCell Sensory Neurons were cultured for 7 days and chemotherapeutic agents were applied on day 4; neurite outgrowth and toxicity was monitored out to day 7. Incucyte assay with Paclitaxel shows **(C)** increasing doses results in truncation of neurite outgrowth or neurite degeneration (EC_{50}=5 µM). **(D)** Dose-dependent cell death from Paclitaxel measured by CellTiter-Glo. Incucyte analysis of Vincristine treatment shows **(E)** increasing doses results in decreased neurite length (EC_{50}=3.2 nM). **(F)** Dose-dependent cell death in response to Vincristine (CellTiter-Glo).

Exploring Compound Pharmacology in 3D Neurospheres



Compound	Conc (uM)	Peak Count (Frequency)	Peak Height (Amplitude)
DMSO control	0.10%	0	0
Deltamethrin	0.1	↑	↓
Rotenone	0.1	X	↓
Amiodarone	20	↑	↓
AMPA	0.2	↑	↓
GABA	30	↓	0
Bicuculline	30	↑	↑
Baclofen	5	↓	0
Lamotrigin	30	0	↓
MK-801	2	↑	↓
AP-4	2	↑	↓
NMDA	20	↑	↓

MOLECULAR DEVICES

Figure 6. iCell NeuroSpheres Respond to Neuromodulatory Compounds. (A) Cryopreserved iCell products are thawed to a cell density of 0.5 million/ml and combined in defined ratios to generate iCell NeuroSpheres. **(B)** Approximately 20 mL of cells are needed to fill a 384-well plate (25K in 50 µl per well). Cells are maintained in BrainPhys media until day 21 for calcium assay. **(C)** Pharmacological testing with iCell NeuroSpheres was performed in collaboration with Molecular Devices. Shown here are results for 11 compounds. **(D)** Summary of compound effects on calcium oscillation Peak Count and Peak Height. Data was analyzed w/ PeakPro2 software.

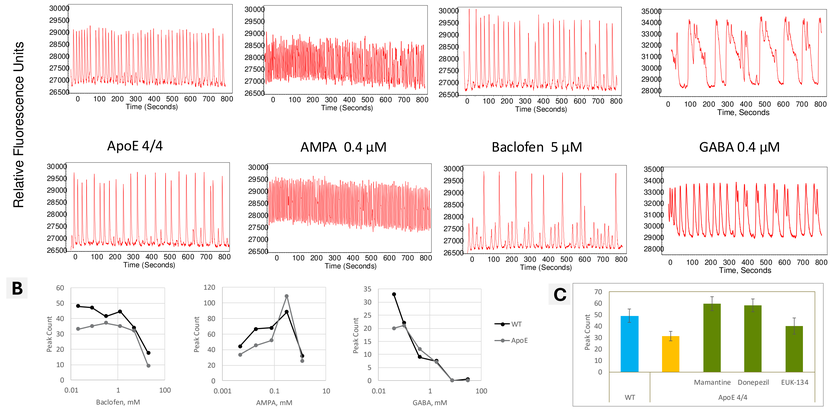


Figure 7. Comparing Effects of Neuromodulatory Compounds on Alzheimer's Disease (AD) iCell NeuroSpheres. (A) Calcium oscillations of iCell GABANeurons (ApoE E3/3) and genetically engineered iCell GABANeurons ApoE4/4 neurospheres were determined by kinetic calcium imaging using the FLIPR instrument and analyzed using PeakPro2 software. Neurospheres were treated with compounds for 60 minutes at different concentrations. **(B)** Both disease and control neurospheres showed similar responses to neuro-active compounds (AMPA, GABA, Baclofen) and which were in the expected concentration-response range. **(C)** Clinically-approved compounds (Memantine, Donepazil, EUK-124) have been used to treat AD symptoms, so these compounds were incubated with disease and control (WT) neurospheres for 45 minutes. Select compounds showed reversal of AD calcium waveform phenotypes to a similar phenotype as control neurospheres.

Summary and Conclusions

These data confirm hiPSC-derived neuronal cells as a useful model for neurotoxicity and demonstrate their ability to provide continuity and flexibility in neurotoxicity model choice, while retaining toxicity predictability.

- iCell neural cell viability can be monitored using a variety of commercial fluorescent and luminescent cytotoxicity assays.
- MEA is a sensitive assay for detecting neurotoxic phenotypes in neurons prior to cytotoxic events.
- iCell Sensory Neurons model chemotherapy-induced neurotoxicity using cell death and neurite outgrowth assays.
- Measuring calcium oscillations in iCell NeuroSpheres enables high throughput neurotoxicity testing in 3D.s

SCAN ME