Miniaturized Isogenic iPSC-derived 3D NeuroImmune Assembloids for High-throughput Drug Screening

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Abstract

NeuCyte, Inc. (NeuCyte) is a provider of cryopreserved, ready-to-use, induced NGN2 excitatory neurons and ASCL1/DLX2 GABAergic neurons. The SynFire® platform has been utilized to demonstrate efficacy in assessing drug seizurgenic risk and to study epilepsy and neurodevelopmental disorders due to inherited or de novo mutations. Recently, our cells were incorporated into a 3D Microphysiological System (MPS) to interrogate neuroinflammation and the blood-brain barrier within the Emulate Organ-on-a-Chip platform. Microphysiological systems (MPS) with defined cellular compositions provide scalable and reproducible brain models that better recapitulate the in vivo environment, in which preclinical drug discovery efforts can translate to a higher success rate for identified targets and compounds. Robust differentiation methods to generate neurons, astrocytes, and microglia from any genetic background enables generation of SynFire® isogenic NeuroImmune Assembloids (NIA) in which the 3D microenvironment recapitulates salient ex vivo brain phenotypes enabling improved translatable high-throughput preclinical drug discovery. NIAs use very few cells and yield a defined reproducible ratio of mature cells making them scalable and assay-ready. Here, we describe our isogenic platform for high-throughput drug-screening. Because the platform is also modular, the impact of a mutation can be studied in a cell-type specific manner to model non-cell autonomous phenotypes mimicking the cellular complexity of the human brain. Our platform can be adapted for high-throughput drug-screening yielding a cost-effective CNS-relevant drug discovery platform.

Isogenic NeuroImmune Assembloids for Drug Discovery

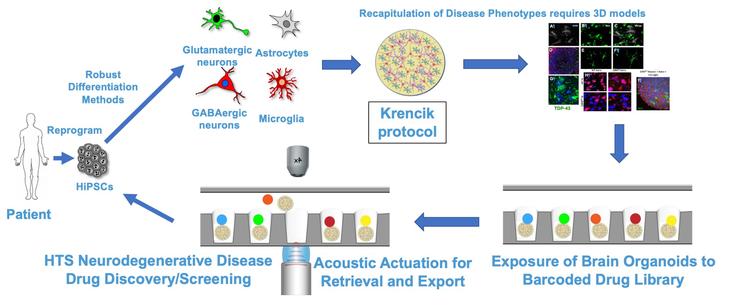


Figure 1. Isogenic iPSC-derived NeuroImmune Assembloid (NIA) Drug Screening Platforms. Patient cells are reprogrammed to hiPSCs. Robust differentiation methods developed at NeuCyte enable the development of 3D brain models with defined cellular ratios enabling pre-clinical assay development for Drug Discovery.

Acoustic Bio Organ-on-a-Chip (OoC) Platform

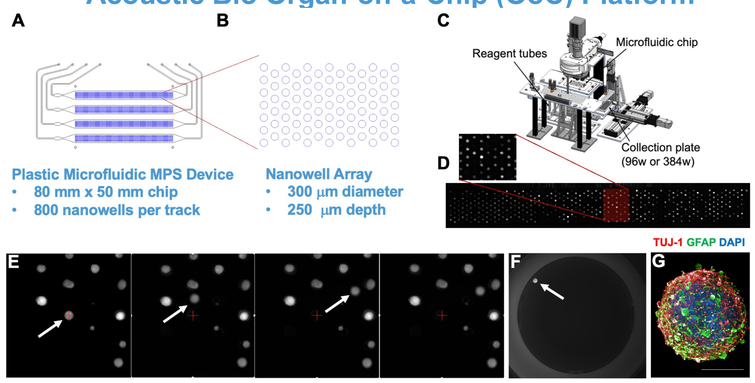


Figure 2. Acoustic Bio OoC platform. **(A)** Prototype microfluidic chip enables organoid culturing. **(B)** Expanded view of microwells within the OoC. **(C)** MACH1 platform enables acoustic ejection of organoids. **(D)** Representative image demonstrates approximately 80% loading of a single track which equates to ~7 plates (384w) in an hour without the need for automation. **(E)** Time-lapse images showing acoustic ejection of a single assembloid (**white arrows**) which is collected in a well (96w) for further analysis **(F, G)**.

Robust Methods for Generation of iPSC-derived Neural Cells

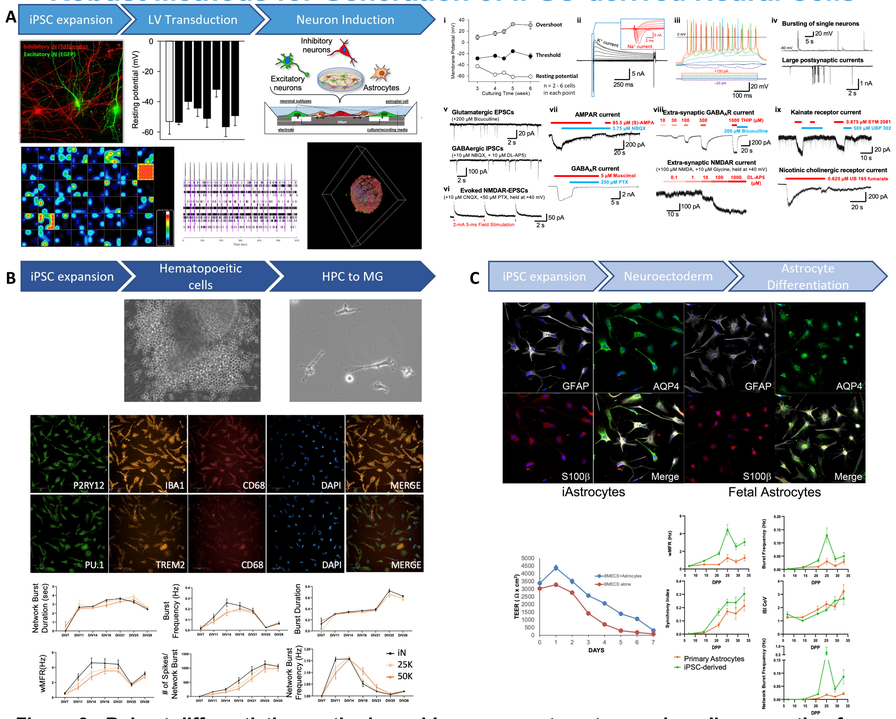


Figure 3. Robust differentiation methods enable neuron, astrocyte, or microglia generation from any iPSC. (A) SynFire neurons include excitatory glutamatergic and inhibitory GABAergic iNs. **(i)** SynFire neurons mature rapidly(5 wks; resting membrane potential ~–60 mV; stable excitability (action potential threshold and overshoot)). SynFire cultures show intrinsic and extrinsic properties, including **(ii)** voltage-dependent K^+ and Na^+ currents, **(iii)** evoked action potential, **(iv, top)** single neuron bursting (**top**), and large postsynaptic currents **(iv, bottom)**. **(v)**SynFire iNs, either excitatory iNs **(top)** or inhibitory iNs **(bottom)** show either glutamate-mediated excitatory postsynaptic currents (EPSCs) or inhibitory postsynaptic currents (IPSCs), respectively. **(vi)** iNs exhibit robust NMDA currents. **(vii–ix)** Ionic receptor expression was confirmed by micro perfusion of either **agonists** or **antagonists**, including **(vii, top)** AMPA-, **(vii, bottom)** $GABA_A$-, **(viii, top)** extra-synaptic $GABA_A$-, **(viii, bottom)** extra-synaptic NMDA-, **(ix, top)** kainate, and **(ix, bottom)** nicotinic cholinergic receptors. **(B)** Microglia generated from any iPSC can be co-cultured with SynFire neurons. **(C)** Serum-free generated iPSC-derived astrocytes cultured with SynFire neurons exhibit functional characteristics like primary astrocytes.

Microfluidic OoC Enables Image-based HTS of NIAs

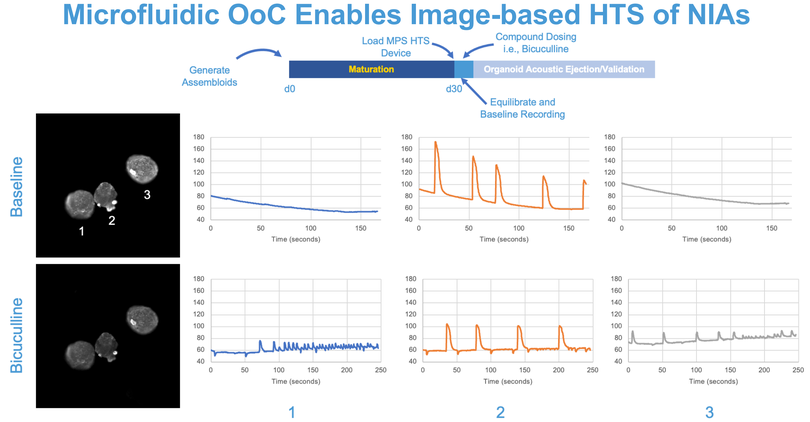
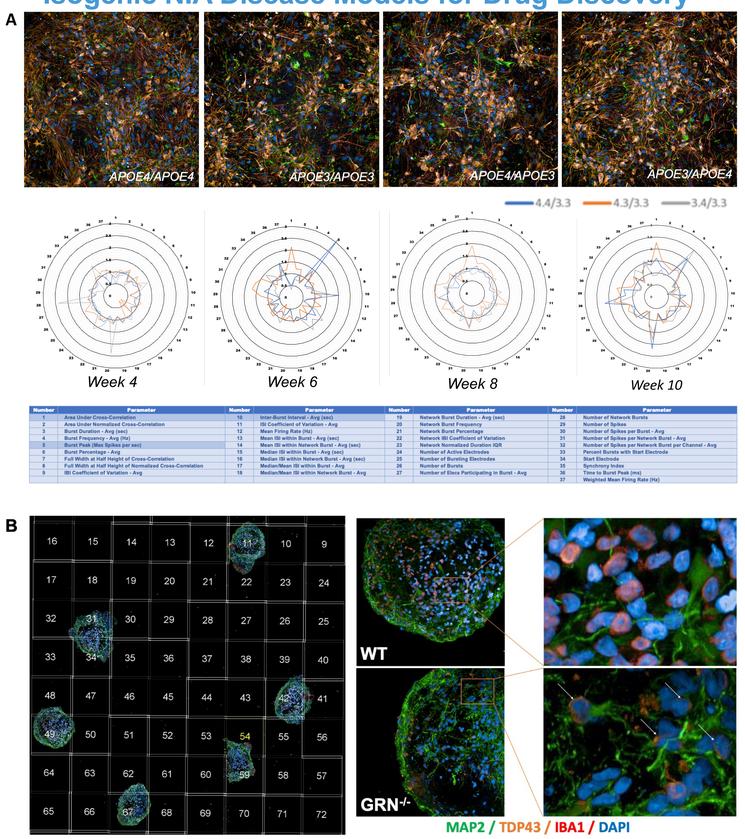


Figure 4. 3D Assembloids exhibit spontaneous calcium transients that can be detected by the Acoustic Bio MACH1. Human iPSC-derived NGN2 neurons, GABAergic neurons, and primary astrocytes are assembled into a 3D organoid and after 30 DIV, exhibit calcium transients. Representative images showing baseline calcium transients of three assembloids at Baseline and following treatment with Bicuculline.

Isogenic NIA Disease Models for Drug Discovery



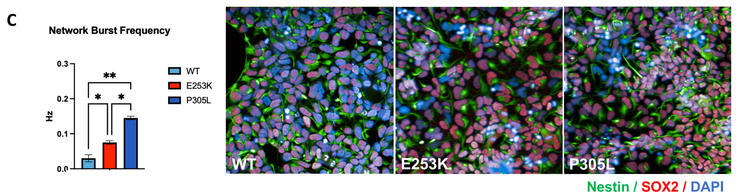


Figure 5. Development of NIA disease models for drug screening. (A) Isogenic APOE4 and APOE3 iPSC-derived Glutamatergic and GABAergic neurons facilitate identification of electrophysiological AD phenotypes uncovered by Multi-Electrode Arrays (MEA) for drug screening. **(B)** NIAs assembled from $GRN^{-/-}$ and isogenic WT iPSC-derived neurons, astrocytes and microglia reveal robust TDP-43 mis-localization enabling drug screening for ALS/FTD. **(C)** KAND isogenic NIAs that will include KAND astrocytes generated from NSCs will enhance detection of non-cell autonomous phenotypes that lead to seizures.

Future Directions

- Develop iPSC-derived NIAs from AD, ALS/FTD, and KAND patient lines to capture phenotypes that can enable high-throughput drug screening
- Optimization of Cytokine Release Assays for NIAs in OoCs
- Develop DNA-encoded Bead-based Libraries enabling screening within the Acoustic Bio OoC
- Build out Organoid-based drug screening platforms