Integration of Microglia into 3D Human iPSC-derived Brain Microphysiological Systems for Disease Modeling

Angela Murchison[1], Nicolas Butelet[1], Martin Nicholson[1], Peng Zhou[1], Dominique V. Lessard[1], Wayne W. Poon[1]
[1]NeuCyte, Inc., 319 North Bernardo Avenue, Mountain View, CA 94043

Abstract

Microglia have been implicated in Alzheimer Disease Genome-wide Association Studies focused on late-onset Alzheimer Disease risk (LOAD) in which a number of human-specific genes are connected to modifying disease etiology. Because of this link between microglia and human disease, there is a growing need for a human source of microglia to model aspects of Alzheimer disease and to be utilized to identify AD/ADRD therapeutics. While human primary sources of microglia are difficult to obtain, induced pluripotent stem cell (iPSC)-derived microglia protocols have been developed to generate a renewable human microglia source, accelerating mechanistic studies on neuroinflammation and AD. These in vitro models recapitulate many of the salient features of *in vivo* microglia. Outside the context of a brain environment, microglia rapidly undergo transcriptomic changes and de-differentiation. Thus, recent chimera models have been developed to study microglia in a homeostatic CNS environment. While these models better recapitulate relevant disease-specific phenotypes, they are not amenable for high-throughput screening and drug discovery. Here, we describe our development of a 3D Microphysiological Systems (MPS) platform incorporating isogenic neurons, astrocytes, and microglia to recapitulate AD/ADRD neuropathological phenotypes. The ability of NeuCyte to generate these iPSC-derived cells from any genetic background enables identification of non-cell autonomous phenotypes and guides therapeutic drug discovery for AD. Importantly, this platform is scalable and translatable to high-throughput drug screening for AD/ADRD. Lastly, because this platform is modular, brain microvascular endothelial cells can be incorporated to recapitulate the CNS/BBB interface in order to study the role of BBB dysfunction in disease, model ARIA (Amyloid-Related Imaging Abnormalities), and improve CNS drug delivery.

Isogenic iPSC-derived MPS Model Development for Drug Discovery

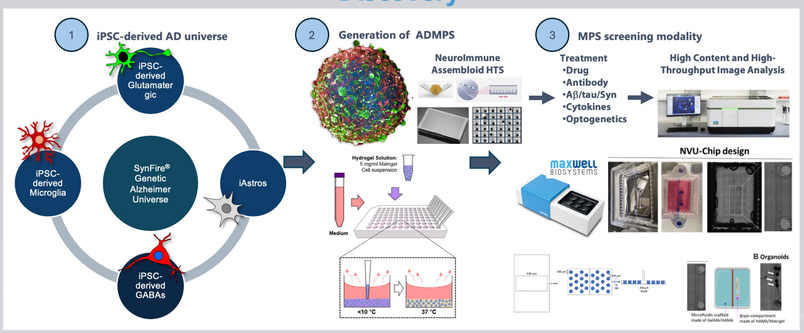
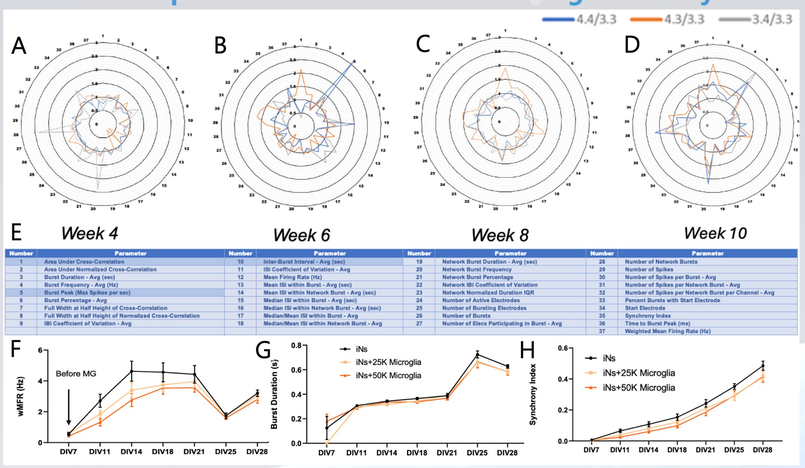


Figure 1. Development of an AD/ADRD MPS-NVU platform. (1) The SynFire® Alzheimer Genetic Universe is composed of *NGN2* glutamatergic neurons, ASCL1/DLX2 GABAergic neurons, iPSC-derived astrocytes, and iPSC-derived microglia. (2) 3D MPS can be generated in the form of 3D hydrogels or spheroids from iPSC-derived cells containing either isogenic gene-edited or patient-derived iPSCs with known AD/ADRD mutations. (3) NIA MPSs can be integrated into an AD/ADRD MPS-NVU (Neurovascular unit) amenable for drug screening.

Development of an NIA Model for Drug Discovery



Number	Parameter	Number	Parameter	Number	Parameter	Number	Parameter
1	Area Under Cross-Correlation	10	Inter-Burst Interval - Avg (sec)	19	Network Burst Duration - Avg (sec)	28	Number of Network Bursts
2	Area Under Normalized Cross-Correlation	11	ISI Coefficient of Variation - Avg	20	Network Burst Frequency	29	Number of Spikes
3	Burst Duration - Avg (sec)	12	Mean Firing Rate (Hz)	21	Network Burst Percentage	30	Number of Spikes per Burst - Avg
4	Burst Frequency - Avg (Hz)	13	Mean ISI within Burst - Avg (sec)	22	Network ISI Coefficient of Variation	31	Number of Spikes per Network Burst - Avg
5	Burst Peak (Max Spikes per sec)	14	Mean ISI within Network Burst - Avg (sec)	23	Network Normalized Duration IQR	32	Number of Spikes per Network Burst per Channel - Avg
6	Burst Percentage - Avg	15	Median ISI within Burst - Avg (sec)	24	Number of Active Electrodes	33	Percent Bursts with Start Electrode
7	Full Width at Half Height of Cross-Correlation	16	Median ISI within Network Burst - Avg (sec)	25	Number of Bursting Electrodes	34	Start Electrode
8	Full Width at Half Height of Normalized Cross-Correlation	17	Median/Mean ISI within Burst - Avg	26	Number of Bursts	35	Synchrony Index
9	ISI Coefficient of Variation - Avg	18	Median/Mean ISI within Network Burst - Avg	27	Number of Elecs Participating in Burst - Avg	36	Time to Burst Peak (ms)
						37	Weighted Mean Firing Rate (Hz)

Figure 2. Development of an NIA for drug screening. (A-D) 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. (E) Neuronal phenotypes and differences between disease and healthy controls detected over a 10-week culture window. Significant phenotypes over multiple timepoints are highlighted in E. Isogenic microglia added to the electrophysiologic system do not affect baseline ontogeny (F-G).

Generation of Functional Microglia for Disease Modeling

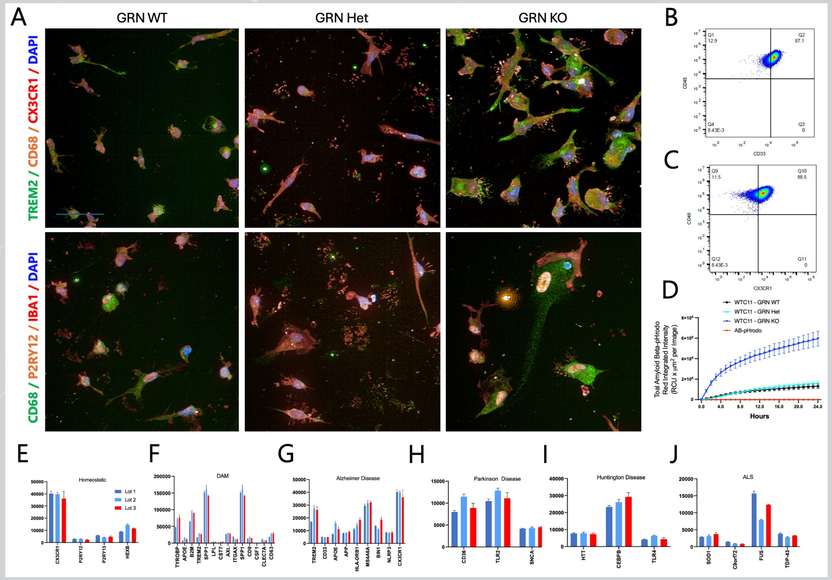


Figure 3. iPSC derived ADRD Microglia exhibit canonical markers as well as disease related gene expression and functionality. (A) Representative images of WT, GRN$^{+/-}$, GRN$^{-/-}$ iMicroglia. (B,C) Microglia are highly pure when assayed by flow cytometry for CD68, CX3CR1, and CD33. Phagocytic activity of the microglia reveal a cell autonomous phenotype of increased phagocytosis by the GRN$^{-/-}$ microglia (D). Gene expression analysis demonstrates the Microglia consistently express genes regulating homeostasis (E), as well as those related to neurodegenerative diseases (F-J).

Neurodegenerative MPS Plaque Models

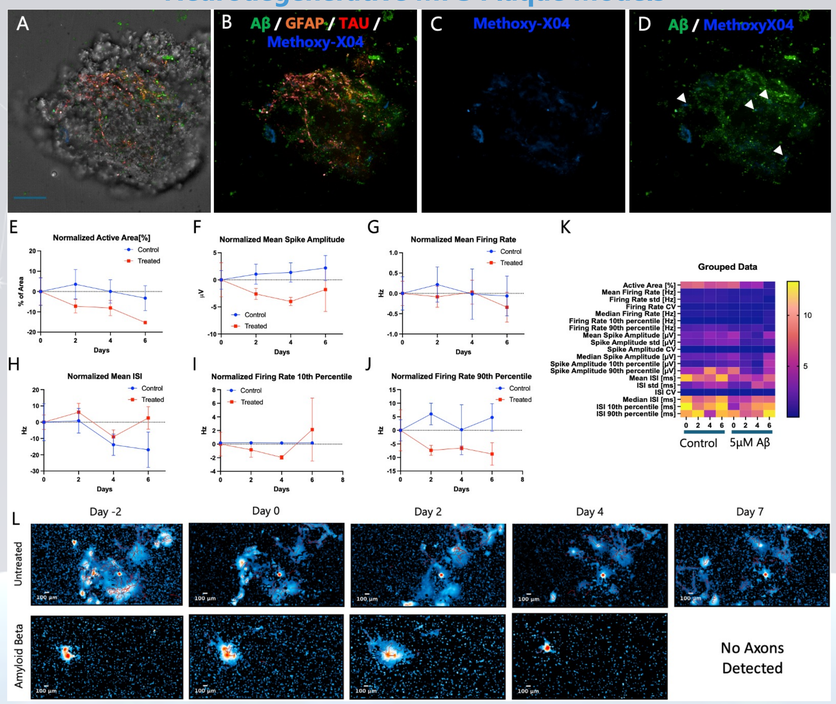


Figure 4. Identification of electrophysiological phenotypes in an Aβ-treated 3D MPS. Aβ plaques are visualized with Methoxy-X04 (blue) after incubation with Aβ (5µm, 7d). (A-D) Aβ aggregates, labeled with 6E10 Aβ antibody, co-localize with Methoxy-X04 labeled plaques. (E-K) Neuronal network parameters assessed by HD-MEA are altered by Aβ plaques. (E) Normalized active area, (F) spike amplitude, (G) mean firing rate, (H) normalized mean interspike interval (ISI), (I) the 10th percentile normalized firing rate, and (J) the 90th percentile normalized firing rate are shown. (F) Representative neuronal axon tracing similarly shows a decreased propagation across the 7 days of Aβ treatment (scale = 100µm).

Integration of Microglia within Isogenic NeuroImmune 3D Models

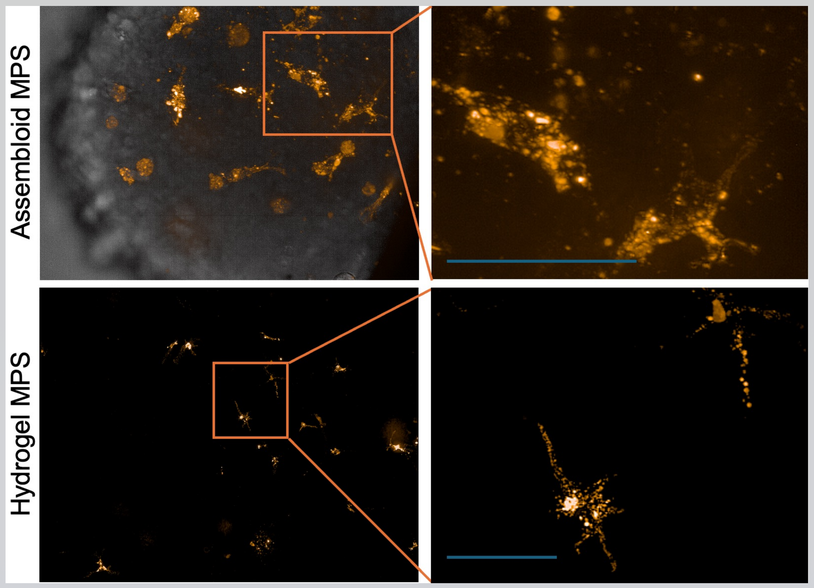


Figure 5. Isogenic microglia rapidly integrate into ADRD MPS. Microglia, labeled with DiI, integrate within the first week of addition to isogenic ADRD astrocytes and neurons. Ramified processed are seen in the Assembloid and hydrogel MPS models (scale = 50 µm).

Interrogating Neuroinflammatory Mechanism with Isogenic AD/ADRD MPS

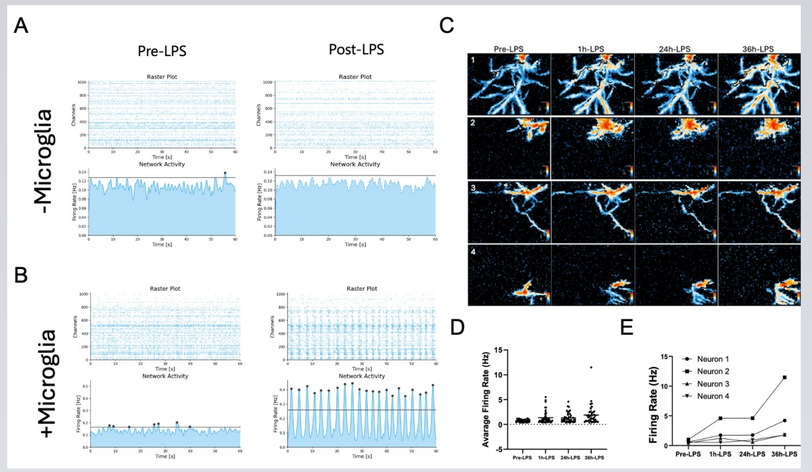


Figure 6. Microglia integration within a 3D MPS enables the study of neuroinflammatory mechanisms. GRN$^{+/+}$ NGN2 neurons, ASCL1/DLX2 GABAergic neurons, and isogenic iAstrocytes were cultured in either 2D or 3D. After 30d of maturation, microglia were added (1:10 microglia: neuronal ratio), cultured for 7d, and then treated with LPS (100 ng/mL). (A) Representative raster plots and firing rate of neuron/astrocytes co-cultured alone or (B) co-cultured with microglia prior to (Pre-LPS) and after 1h of LPS treatment (Post-LPS). (C) Representative axon traces of individual neurons co-cultured with iAstrocytes and microglia (Pre-LPS) and after 1h following LPS treatment (1h-LPS), after 24h (24h-LPS), and after 36h (36h-LPS). (D) The average firing rates increase over time within individual wells. (E) Individual neuron firing rate highlights the heterogeneity of neuronal responses to LPS.

Future Directions

1. Integrate disease ADRD microglia into neuroimmune MPS from additional AD and ADRD disease backgrounds to elucidate disease relevant phenotypes.
2. Test ASOs and compounds in reversing discovered phenotypes.

Acknowledgements

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