Development of iPSC-derived Astrocytes for Disease Modeling

Nicolas Butelet[1], Angela Murchison[1], Peng Zhou[1], Martin Nicholson[1], Dominique V. Lessard[1] Wayne W. Poon[1]

[1]NeuCyte, Inc., 319 North Bernardo Avenue, Mountain View, CA 94043

Abstract

Emerging studies highlight the transcriptomic diversity of regionally-specified human astrocytes and the contribution of reactive astrocytes to neurological disease. Thus, there is a growing need for human astrocytes to model human disease and to be utilized in drug discovery efforts. While human primary sources of astrocytes are difficult to obtain, induced pluripotent stem cell (iPSC)-based technologies have enabled the development of protocols to generate hiPSC astrocytes. Many of the described methods for iPSC-derived astrocytes employ the use of serum, which leads to reactive astrocytes, limiting their use to study the role of astrocytes contributing to neuroinflammatory mechanisms of disease. Additional methods involve organoid-based differentiation that requires extended culturing that is not amenable to commercial scaling. Alternative transcription factor-based methods yield astrocytes quickly, but these astrocytes often do not recapitulate all the functional astrocyte properties. Providing a solution to the above mentioned methods, here–we describe a robust and rapid, serum-free protocol for the generation of cryopreserved iPSC-derived astrocytes from different disease backgrounds. These iPSC-derived astrocytes consistently display functional properties similar to primary astrocytes and can be combined together with iPSC-derived neurons and microglia from the same genetic background yielding isogenic co-cultures facilitating the study of human astrocytes and their contribution to neurological and neurodegenerative disease pathogenic mechanisms.

Development of iPSC-derived MPS Models for Drug Discovery

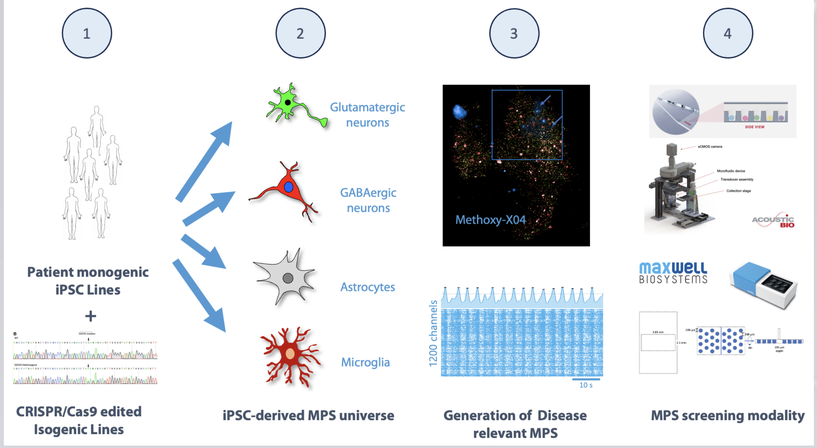


Figure 1. Overview of iPSC-derived MPS modeling for drug discovery. (1) CRISPR isogenic iPSC lines are generated from Patient iPSCs. (2) Patient and isogenic lines are differentiated to disease relevant neuronal cell types. (3) The different cell types are assembled into a 3D MPS to interrogate the effect of therapeutics using readouts including immunocytochemical or electrophysiologic readouts. (4) Phenotypic assay readouts are adapted to high-throughput screening modalities.

Differentiation and Cryopreservation of iPSC derived Astrocytes

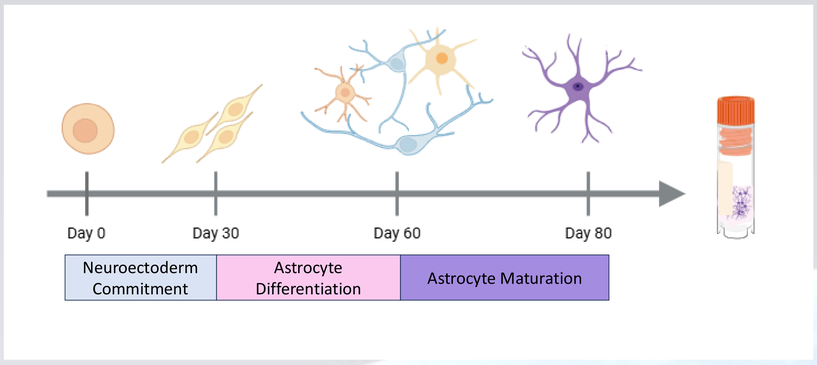


Figure 2. Differentiation and Cryopreservation of iPSC derived Astrocytes. 80-day differentiation scheme of iPSC derived astrocytes (iAstros) following 3 critical steps: 1. Neural ectoderm commitment 2. Differentiation of early neural ectoderm to neurons 3. Addition of pro-astrocytic molecules to enhance astrocyte maturation. iAstros are cryopreserved on day 80 after start of differentiation from iPSCs

Cryopreserved iPSC Derived Astrocytes Express Canonical Astrocyte Markers

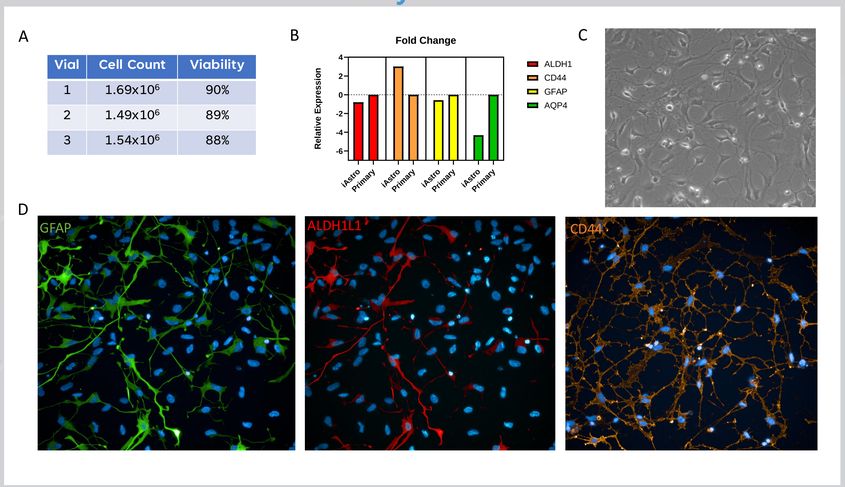


Figure 3. Cryopreserved iPSC-derived Astrocytes express canonical astrocyte markers. A. Cryopreserved iAstrocytes (1 x 10^6/vial) are highly viable (~90%). B. Brightfield and immunocytochemistry reveal canonical astrocyte morphology, as well as protein expression of classic astrocyte markers: GFAP, ALDH1L1 and CD44. C. RTqPCR relative expression of iAstrocytes compared to primary fetal derived astrocytes. Elevated expression of the astrocyte precursor marker CD44 combined with the decreased expression of the mature astrocyte marker AQP4 suggest cryopreserved iAstrocytes exhibit an early astrocyte developmental signature 7 days post-thaw.

Incorporation of iPSC-derived Astrocytes in a 3D Brain-in-a-Dish

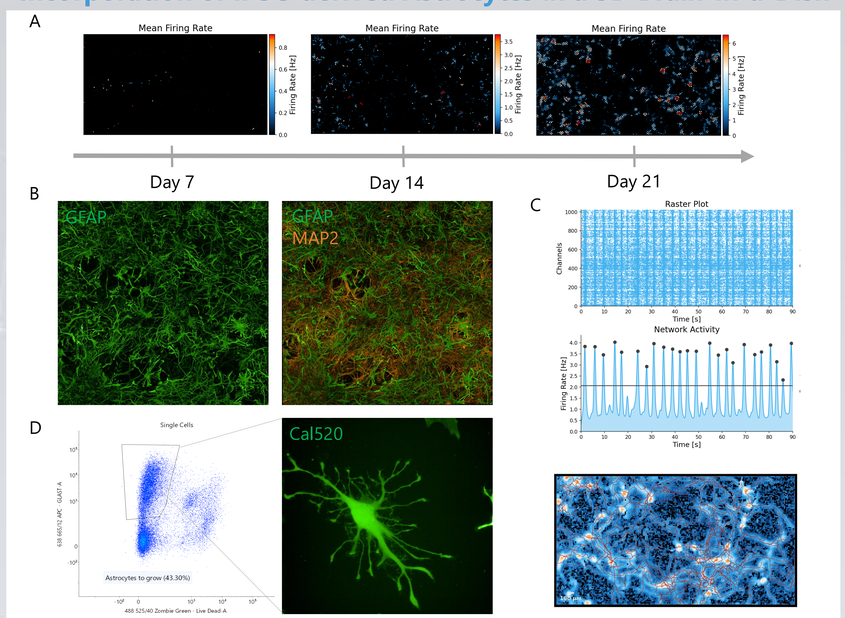


Figure 4. Incorporation of iPSC-derived Astrocytes within 3D "Brain-in-a-Dish" Co-Cultures. isogenic iPSC-derived excitatory neurons (iN), inhibitory neurons (iG), and astrocytes (iA) plated either iwithn a 96-well format for high-content imaging or 6-well high-density MEA to interrogate electrophysiology. A. MEA activity scans depicting the functional development of the neuronal networks from day 7 to day 21. B. Representative immunocytochemical image of the 3D "Brain-in-a-dish" on day 21. Neurons are visualized with MAP2 (red) and astrocytes visualized with GFAP (Green). C. Representative rastor plot depicting synchronous bursting of the 3D tri-culture. Representative visualization of axon tracking demonstrates neuronal network complexity. D. Florescent activated cell sorting (FACS) analysis of dissociated tri-cultures (d21) demonstrated that the GLAST-positive astrocyte population represented 43% of the total cells, which was comparable to the initial starting seeding density of 41% (left). After sorting, replated iAstrocytes demonstrated calcium transients when imaged following incubation with Cal520 (right).

Incorporation of isogenic iMicroglia into a 3D " Brain-in-a-dish" to study neuroinflammatory mechanisms

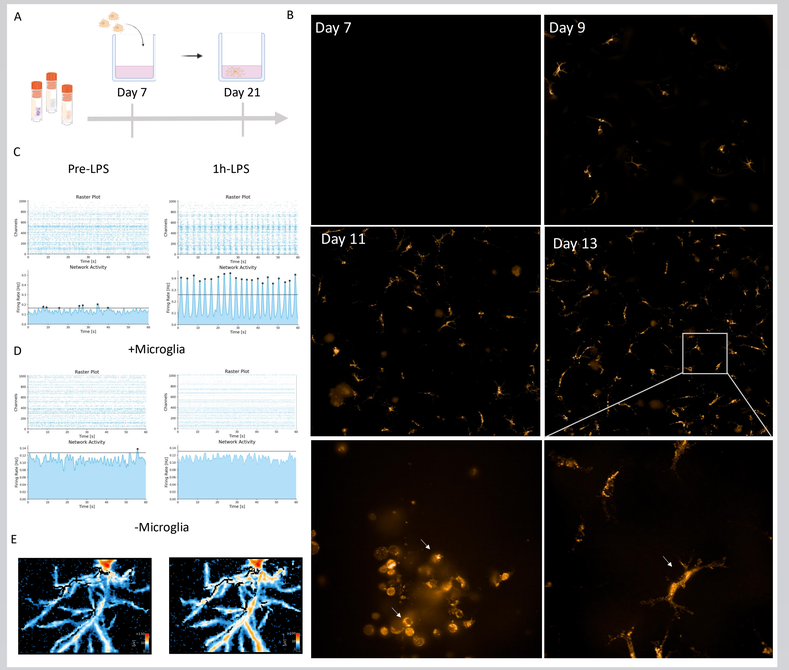
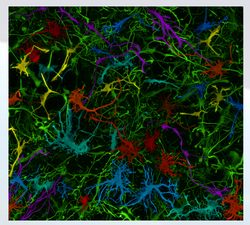


Figure 5. Incorporation of isogenic iMicroglia into a 3D " Brain-in-a-dish" to study neuroinflammatory mechanisms . A. Microglia are thawed (Day 0) and separately cultured from the neuron/astrocyte tri-cultures and subsequently combined into the 3D "Brain-in-a-dish" model on Day 7. These cultures are maintained until day 21 and subsequently processed for downstream analysis. B. Microglia, pre-stained with the membrane-permeable DiI fluorescent marker, can be monitored for 3D "Brain-in-a-dish" incorporation using live fluorescent microscopy. Images captured every other day demonstrate increased integration of microglia over time. There were no significant differences in microglia integration after day 13. C. Live imaging demonstrates clear morphological differences between non-integrated microglia (left) compared to microglia that have integrated into the 3D tri-culture (right). D. Representative raster plots of neuron/astrocytes co-cultured alone or following the addition of microglia (Pre-LPS). Then, the co-cultures were examined after 1h of LPS treatment (Post-LPS). (E) Representative axon traces of individual neurons co-cultured with iAstros and microglia (Pre-LPS) and after 1h following LPS treatment (1h-LPS)

Future Directions

- Explore the crosstalk between astrocytes and microglia within our model to take steps towards replicating neuroinflammation in a dish
- Replicate data with either APOE33 or APOE44 iPSC lines to investigate the role of the APOE4 genetic risk factor on astrocyte biology and how it impacts the trajectory and progression of Alzheimer's in APOE4 carriers.
- Integrate iPSC derived brain microvascular endothelial cells into the model to recapitulate the blood brain barrier and interrogate the functional role of astrocytes in BBB breakdown.



Acknowledgements

Funding Source: AG068992 and AG084421 (W.W.P.). We thank Drs. Edsel Abud and Ricardo Ramirez for all their insightful discussions on microglia biology and bioinformatics analysis, respectively.

Alzheimer's Disease Research Center · UCI MIND

UCI University of California, Irvine

NeuCyte