# Modeling Parkinson's Disease in 2D + 3D: Evaluating α-Synuclein Aggregation, Autophagy, and Neural Network Activity

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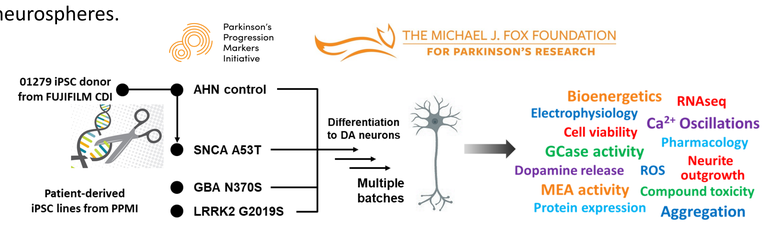
## Abstract

Discovery and validation of new treatments for Parkinson's Disease (PD) can greatly benefit from the creation of advanced, biologically relevant human cellular models. In partnership with the Parkinson's Progression Markers Initiative (PPMI) and The Michael J. Fox Foundation (MJFF), FUJIFILM Cellular Dynamics generated iPSC lines from clinically symptomatic PD patients carrying known risk-associated gene mutations. These human iPSC lines were differentiated into midbrain dopaminergic neurons (iCell ® DopaNeurons). The etiology of dopaminergic neuron cell death in PD is complex involving multiple factors that include mitochondrial dysfunction, impaired endosomal/lysosomal protein degradation, α-synuclein and tau aggregation, and neuroinflammation. In this study, we utilized dopaminergic neurons generated from apparently healthy normal (AHN) and PD donor-derived iPSCs harboring either the LRRK2 G2019S or GBA N370S mutations to investigate multiple mechanisms thought to underly PD. Dopaminergic neurons were cultured either in 2D (as a mono-culture or in co-culture with iCell Astrocytes) or as 3D spheroids (iCell NeuroSpheres). Mono-cultures of iPSC-derived dopaminergic neurons were evaluated for multiple cell functions related to neurodegeneration, including cell metabolism, neurite outgrowth and degeneration, α-synuclein aggregation, and lysosomal activation. Using α-synuclein preformed fibrils to seed the cultures, elevated α-synuclein aggregation was observed in LRRK2 G2019S and GBA N370S dopaminergic neurons compared to AHN controls. Interestingly, no striking differences were observed in any of the cells, with only a small change in puncta area for the GBA N370S mutant, when assessing engagement of autophagic pathways via LAMP-1 expression and puncta formation. We also evaluated neural network activity in both 2D, using multi-electrode array (MEA), and in 3D using calcium oscillation assays of dopaminergic neuron-containing iCell NeuroSpheres. Functional assays revealed diverse phenotypes for the PD patient-derived cells in both 2D and 3D systems compared to AHN controls. This study expands the characterization of LRRK2 G2019S and GBA N370S PD dopaminergic neurons and shows proof-of-concept data for 3D disease modeling of PD using multi-cellular neurospheres.




## Characterization of PD iCell DopaNeurons

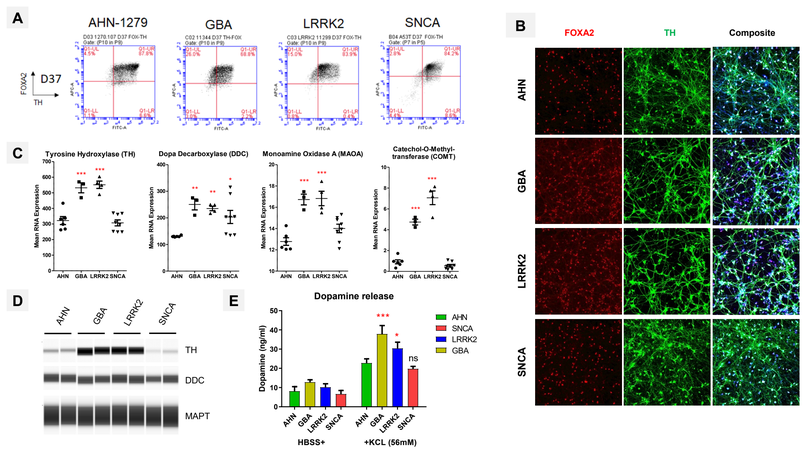



**Figure 1. Characterization of iPSC-derived Dopaminergic Neurons.** (A) Flow cytometry plots FOXA2 vs. TH to show DA purity; (B) ICC staining for FOXA2, TH, & LMX1 markers at D14; (C) Gene expression analysis for TH, DDC, MAOA, and COMT at D14; (D) Western blot analysis of TH & DDC protein expression relative to MAPT; (E) Dopamine release assay on D21 shows that KCl stimulation results in increased dopamine levels.

## Neurite Outgrowth Analysis of PD iCell DopaNeurons

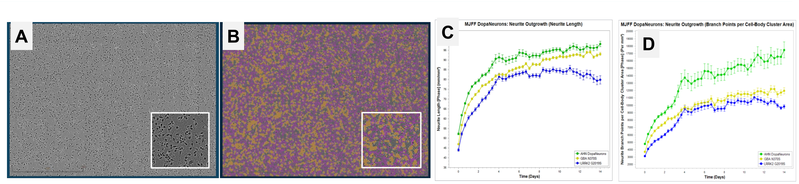



**Figure 2. Tracking Neurite Outgrowth** (A) Phase image (10X) from Incucyte SX5 of AHN iCell DopaNeurons at Day 14 and (B) Neurite (**magenta**) + cell-body cluster (**orange**) analysis masks from the neurite outgrowth module. Images from AHN (**green**), GBA (**yellow**), and LRRK2 (**blue**) [no SNCA A53T data here] were analyzed to provide metrics for (C) neurite length and (D) branch points over time. Cells were plated at a density of ~70K cells/well on PLO/laminin-coated 96-well plate and cultured in iCell DopaNeurons maintenance medium.

## Bioenergetic Analysis of PD iCell DopaNeurons

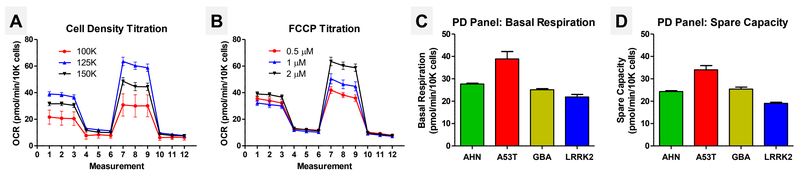



**Figure 3: Assay Optimization.** (A) Cell density titration of iCell DopaNeurons showed changes in oxygen consumption rate (OCR) assay signal on Day 21 on the Seahorse XF Pro Analyzer. Cell density of 125,000 cells/well is recommended. (B) Titration of the uncoupler FCCP on Day 21 resulted in the highest maximum respiration at 2 µM. PD panel of iCell DopaNeurons were then assayed with 1 µM Oligomycin, 2 µM FCCP and 0.5 µM Rot/Ant A on Day 14. SNCA A53T (red) showed the highest (C) basal respiration and the largest (D) spare capacity. Both GBA and LRRK2 came in lower for these metabolic metrics.

## α-Synuclein Aggregation in PD iCell DopaNeurons

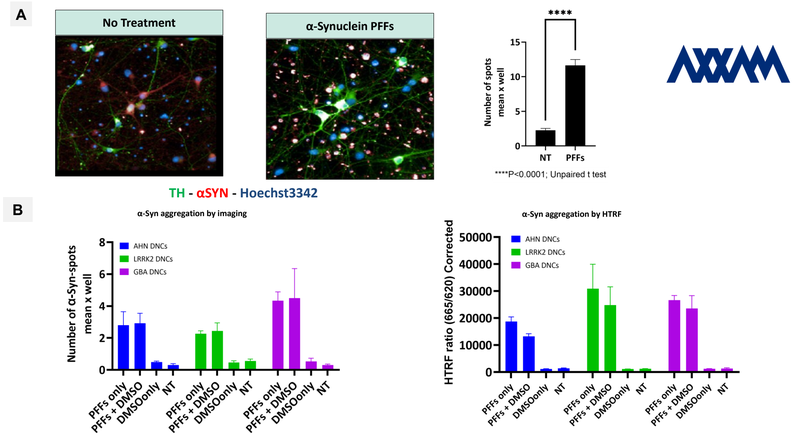



**Figure 4: Evaluation of α-Synuclein Aggregation following Treatment with Pre-formed Fibrils (PFF).** AHN DopaNeurons showed accumulation of total α-synuclein aggregates after seeding with α-synuclein PFFs, measured by high content image analysis. PD-derived cells showed higher α-synuclen aggregates compared to AHN control when treated with PFFs, as observed by imaging and TR-FRET readouts.

## LAMP-1 Expression & Puncta Formation in PD iCell DopaNeurons

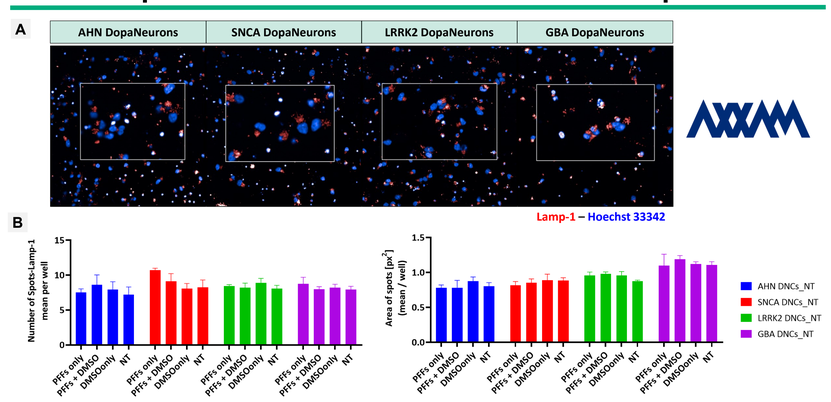



**Figure 5. LAMP-1 Expression in LRRK2, GBA, SNCA A53T and AHN cells.** iCell DopaNeurons were left untreated (NT) or treated with α-synuclein PFF prior to staining for LAMP-1, lysosomal associated membrane protein 1, to measure autophagy. The GBA-mutant cells (purple) show a slight increase in Lamp-1 positive puncta (by means of spot area image analysis).

## PD-relevant iCell DopaNeurons are Highly Active on MEA

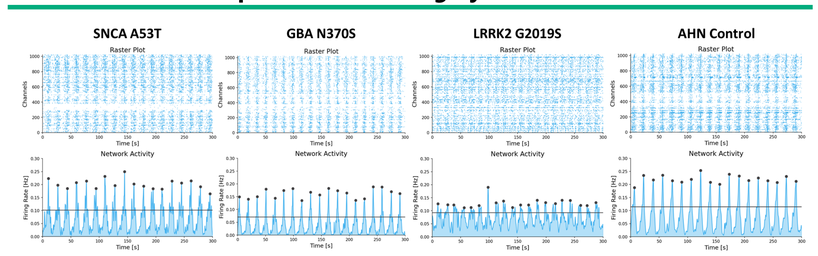



**Figure 6. Neuronal Network Activity on HD-MEA.** PD-relevant iCell DopaNeurons were cultured on high-density multi-electrode array (HD-MEA) plates from MaxWell Biosystems. Recordings were conducted on a MaxTwo system, and raster plots show the synchronous network activity detected by 1020 electrodes on Day 14. Approximately 400,000 iCell DopaNeurons were plated on PEI/laminin-coated surface. These data show human iPSC-derived cells are highly functional and show spontaneous electrical activity on MEA. This platform technology can be used to further investigate PD-relevant disease phenotypes.

## Delayed Network Bursting in LRRK2 G2019S iCell DopaNeurons

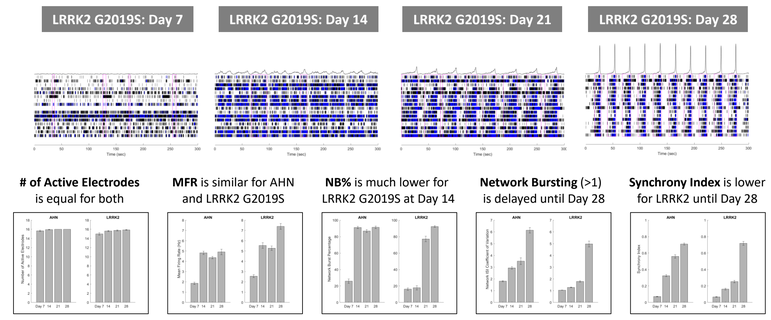



**Figure 7. Development of Network Activity Over Time.** Raster plots taken at different time points in culture (Day 7, 14, 21, & 28) show evolution of network activity for LRRK2 G2019S iCell DopaNeurons maintained on a CytoView MEA plate. Compared with AHN cells as control, the # of active electrodes and mean firing rate (MFR) are similar, but time to organized and synchronous network bursts is delayed for LRRK2. Data were obtained on a Maestro Pro MEA system using n=16 wells per condition. Expts are ongoing to track cultures out even longer.

## Calcium Oscillation Assay with 3D Neurospheres

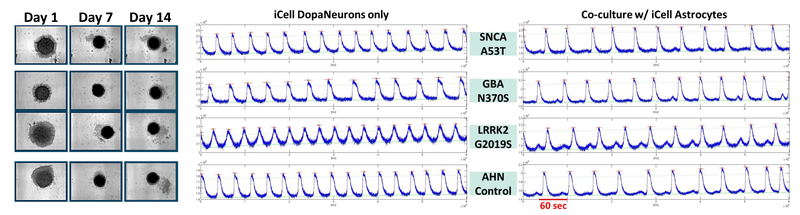



**Figure 8. Spontaneous Activity from Cells in 3D Cell Culture.** PD panel of iCell DopaNeurons were cultured in 384-well ULA spheroid plates (Sbio) with or without iCell Astrocytes (20K-25K cells per spheroid). These data examine the different $Ca^{2+}$ oscillation phenotypes at Day 14 and the impact of co-culture vs. mono-culture. The advantage of this modular approach to build your own "iCell NeuroSpheres" is that you can tweak cell type ratios and have control over the spheroid composition.

## LRRK2 G2019S Mutation-corrected Isogenic Control

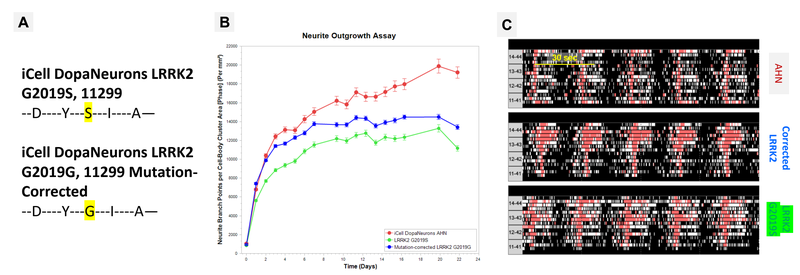



**Figure 9. Engineering and Characterization of LRRK2 G2019S Mutation-corrected Isogenic Control.** (A) Amino acid sequences for patient derived iCell DopaNeurons LRRK2 G2019S and LRRK2 G2019G Mutation-corrected isogenic cell line, both from donor 11299. Mutation correction was performed using CRISPR engineering. Characterization of (B) neurite outgrowth kinetics and (C) MEA activity shows an intermediate phenotype for LRRK2 G2019G mutation corrected iCell DopaNeurons compared to LRRK2 G2019S and AHN iCell DopaNeurons.

## Summary

| Product Name | Genotype | iPSC Source | Unit Size | Kit Number | Donor |
|---|---|---|---|---|---|
| iCell DopaNeurons | Apparently Healthy Normal (AHN) | FCDI iPSC line | 1M | R1088 | 01279 |
| iCell DopaNeurons PD SNCA A53T HZ | SNCA A53T (engineered line) | FCDI iPSC line | 1M, 5M | R1109, R1101 | 01279 |
| iCell DopaNeurons PD LRRK2 G2019S | LRRK2 G2019S (donor-derived) | PPMI Collection | 1M, 5M | R1229, R1231 | 11299 |
| **iCell DopaNeurons PD LRRK2 G2019G** | **Isogenic mutation-corrected control for LRRK2 G2019S** | **Mutation-corrected** | **1M, 5M** | **R1244, R1243** | **11299** |
| iCell DopaNeurons PD GBA N370S | GBA N370S (donor-derived) | PPMI Collection | 1M, 5M | R1232, R1234 | 11344 |

These data demonstrate the utility of donor-derived LRRK2, GBA, and engineered SNCA A53T dopaminergic neurons across multiple assays utilized for HTS assays.

The Parkinson's Disease Panel of iCell DopaNeurons display:

- Lot-to-lot consistency in purity and performance across multiple assays
- Altered α-synuclein aggregation using pre-formed fibril seeding protocol
- Increased LAMP-1 puncta size in GBA N370S iCell DopaNeurons
- Altered baseline MEA activity and calcium signaling

Lastly, the LRRK2 G2019G mutation-corrected isogenic cell line was successfully engineered offering an isogenic control for the patient-derived LRRK2 G2019S iCell DopaNeurons.

***Note: iPSC lines for the LRRK2 and GBA mutations are part of the Parkinson's Progression Markers Initiative (PPMI) iPS cell bank.***