Modelling Alpha-Synuclein Aggregation and Mitochondrial Dysfunction in a Human iPSC-Derived Dopaminergic Neuron Culture System

PSTR263.09

Matteo Zanella[1], Xin-Xuan Lin[1], Cindy J.M. Loomans[1], Fleur Stevenhagen[1], Nicole K. Polinski[2], Malika Bsibsi[1] and Anne-Marie Zuurmond[1]

[1] IQVIA Laboratories, Leiden, NL
[2] The Michael J. Fox Foundation for Parkinson's Research

Introduction

Human iPSC-derived models provide scalable, human-relevant platforms for neurodegenerative drug discovery and may improve translation beyond traditional cell and animal models. We explored and developed complementary iPSC-derived dopaminergic neuron models addressing key features of Parkinson's disease: α-synuclein (α-Syn) aggregation and phosphorylation, and mitochondrial dysfunction assessed by TOM20-based mitochondrial profiling

Methods

iPSC-derived (dopaminergic) neuronal lines were evaluated and selected based on cell health, morphology, and endogenous expression levels of total α-Syn and tyrosine hydroxylase (TH), a dopaminergic neuron marker, using high-content imaging and ELISA read-outs at multiple timepoints in culture (day 7,14, 21 and 28). Disease-relevant triggers were applied to promote the accumulation of pathological forms of α-synuclein (α-Syn). PFF uptake and the subsequent increase in phosphorylated α-Syn were quantitatively assessed as disease-associated readouts. Mitochondrial stress was induced by exposure to reference compounds and quantitatively assessed by high-content analysis using TOM20-based mitochondrial profiling.

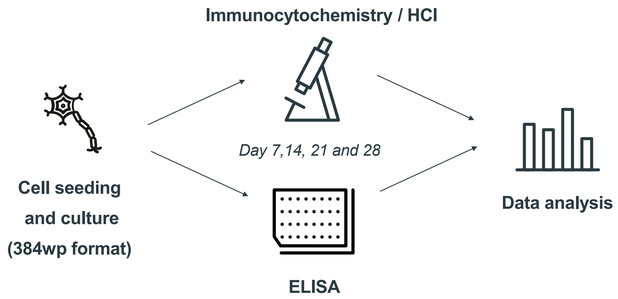


Figure 1. Experimental workflow

Results

Morphological characterization and marker assessment

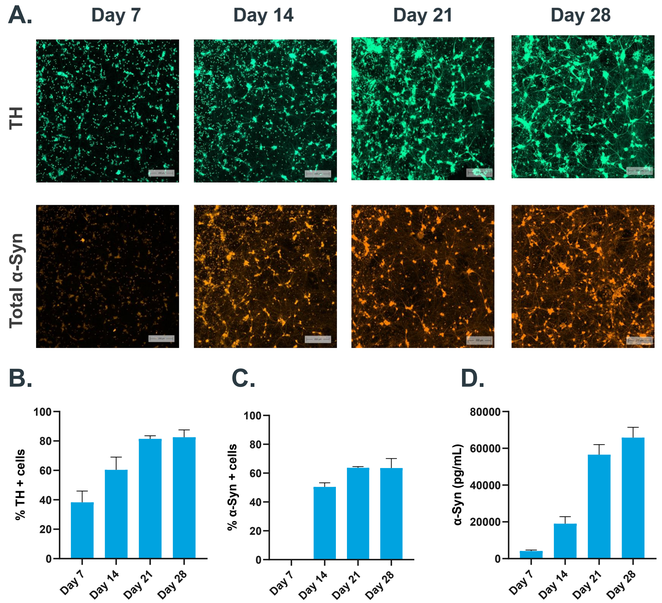


Figure 2. iPSC-derived dopaminergic neurons show robust TH and total α-synuclein (α-Syn) expression from 2 weeks in culture, with a progressive increase in expression over time. **A.** Cells seeded in 384-well plates were stained for TH (green) and total α-Syn (orange) after 7, 14, 21 and 28 days in culture. Images were acquired using Yokogawa CV8000. **B, C.** The percentages of TH- and α-Syn-positive cells were quantified using high-content analysis. **D.** Cells seeded in 384-well plates were lysed after 7, 14, 21 and 28 days in culture **D**. α-Syn concentration was quantified by ELISA.

α-Syn PFF-Induced Seeding

This work was partially funded by MJFF

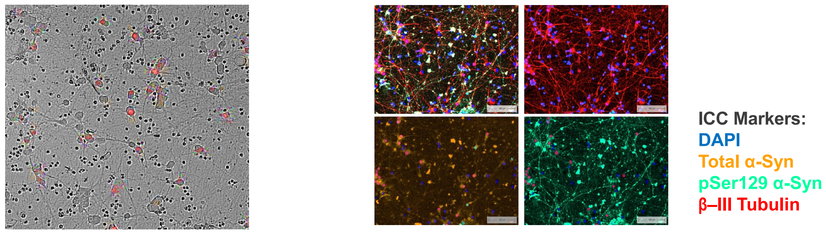


Figure 3. iPSC-derived dopaminergic neurons efficiently internalize α-synuclein preformed fibrils (PFFs). A. iPSC-derived dopaminergic neurons were treated with pHrodo-labeled α-Syn PFFs. Live-cell PFF internalization (orange/red) was monitored and quantified by high-content analysis for up to 48 h post-treatment. Representative image at 48 h is shown. **B.** Neurons were exposed to α-Syn PFFs for 14 days and stained for pSer129 α-Syn (green), total α-Syn (orange), and βIII-tubulin (red). An increased staining was observed for pSer129 when compared to non-treated control cells. pSer129 α-Syn staining and analysis requires further optimization for use as α-Syn aggregation model.

Mitochondrial stress induction

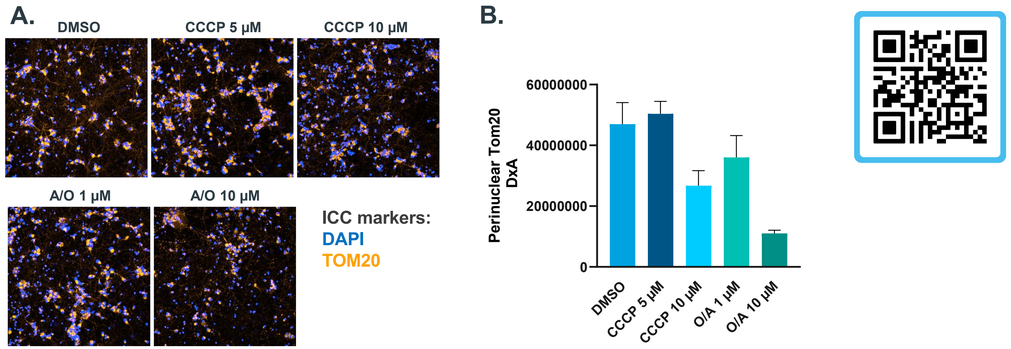


Figure 4. CCCP and Antimycin/Oligomycin (A/O) induce mitochondrial stress in iPSC-derived dopaminergic neurons. Neurons were exposed to CCCP (5 or 10 µM) or A/O (1 or 10 µM) for 24 h. A. Representative images of TOM20 (orange) and DAPI (blue). B. Quantification of perinuclear TOM20 DxA (area × intensity) by high-content analysis. Treatment resulted in a less compact and more fragmented/punctate TOM20 signal compared with DMSO control, suggesting mitochondrial dysfunction.

Conclusions

Collectively, these iPSC-derived dopaminergic neuron models provide robust and physiologically relevant platforms for investigating key features of PD pathology. Induction of a PD-associated phenotype, characterized by increased pSer129 α-Syn following PFF exposure, is currently under investigation and further optimization. The models are suitable for high-throughput screening and mechanistic studies, supporting therapeutic development for PD and other synucleinopathies while reducing reliance on animal studies in line with the 3Rs principle

© 2026. All rights reserved. IQVIA® is a registered trademark of IQVIA Inc. in the United States, the European Union, and various other countries.