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Case Study

Parkinson's Disease Modeling – Real-Time Insights with Live-Cell Analysis

Neurodegenerative diseases, such as Parkinson's Disease (PD), are chronic and debilitating disorders that progressively cause degeneration and | or death of neuronal cells. Improved *in vitro* models that recapitulate the chronic nature of the disease, in combination with advanced cell models such as primary cells or iPSCs in mono- or co-culture, may increase our insight into disease pathology and contribute to drug discovery. However, traditional approaches employed to study these dynamic biological changes are challenging. The study of these complex and sensitive models requires advanced technologies, such as live-cell analysis, that can follow and robustly quantify dynamic cellular processes under non-perturbing conditions.

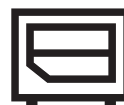
Case Profile

This case study explores how live-cell analysis can be used to monitor *in vitro* models of Parkinson's disease (PD) and quantify changes in morphology, cell health, and function.

Keywords:
Parkinson's Disease, Live-Cell Analysis, Neuroscience, Neurodegeneration, Disease Modeling, Neurotoxicity, Neurons, Glial Cells

Challenges

- Studying complex and disease-relevant neuronal models over long-time periods at a high-throughput
- Methods to quantify neuronal morphology or toxicity may be perturbing, use a single end-point, or are not compatible with sensitive models
- Techniques to assess phagocytosis may have laborious or destructive protocols and not enable monitoring of peptide engulfment in real-time



Solutions

- Live-cell analysis enables real-time visualization and quantification of *in vitro* models in a physiologically relevant environment in up to 96/384-well microplate formats
- The Incucyte® Neurite Outgrowth Assay is non-perturbing and compatible with sensitive cell types, including iPSC-derived or primary cells, in mono- or co-culture
- The Incucyte® Phagocytosis Assay provides a simple-mix-and read approach to kinetically visualize and quantify phagocytosis and assess disease-associated peptides

Quantitative Pharmacology in PD Co-culture Models

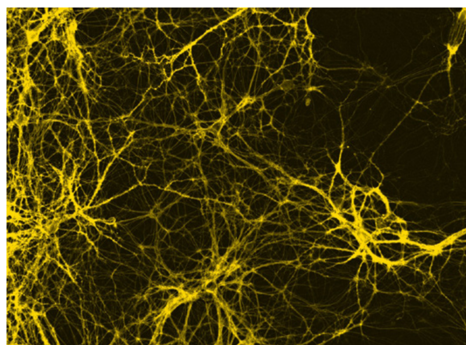
Monitoring neurite dynamics in long-term *in vitro* cell cultures is critical for the characterization and evaluation of neurodegenerative disease models and the assessment of pharmacological neurotoxic effects. Using the Incucyte® Live-Cell Analysis System in conjunction with the Incucyte® Neurotrack Analysis Software Module we can kinetically quantify neuronal outgrowth. This assay permits the analysis of neurons in mono-culture (label-free) or in co-culture with astrocytes, using a non-perturbing neuron-specific Incucyte® Neurolight Orange Lentivirus, for continuous analysis of neurite length and branch points.

To develop a model of PD, we created a co-culture system of rat primary striatal neurons and astrocytes in a 96-well microplate (Figure 1). Following infection of neurons with neurolight orange and a period of neurite development (10 days), the dopaminergic-specific neurotoxin 6-hydroxydopamine (6-OHDA) was applied to induce diseased-relevant neuronal damage, which was monitored using live-cell analysis. A time- and concentration-dependent effect on neurite disruption was observed, yielding an IC_{50} value for 6-OHDA of 70.5 µM for neurite length.

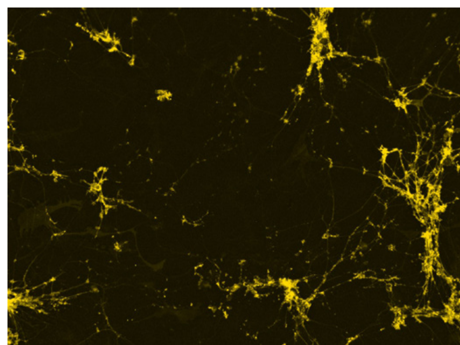
This model can also be used to assess neurotoxic compound specificity across different brain regions (Figure 2). To examine this, co-cultures of rat primary striatal, substantia nigra, or cortical neurons and astrocytes expressing neurolight orange were treated with 6-OHDA after 10 days. Bar graphs and drug-response curves show the selective profile of the drug to neurite length in different brain regions, with 6-OHDA selectively effecting the striatal and nigra neurons. The vehicle data highlights differential development of neurite formation across the brain regions.

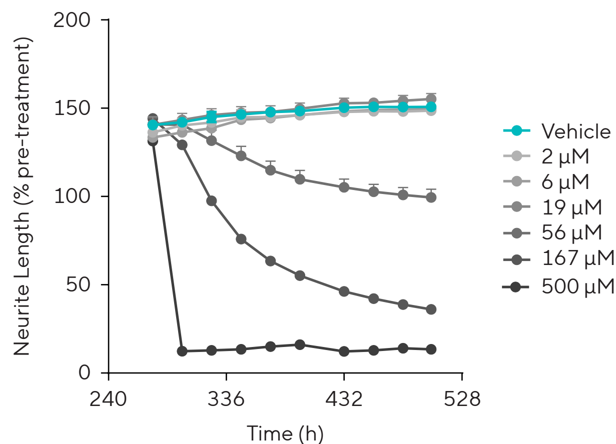
Live-cell analysis in combination with the neurite outgrowth assay, enable the monitoring of complex *in vitro* models in a non-perturbing manner with flexibility across different cell types, including primary, iPSC-derived, or immortalized neurons in mono- or co-culture. This approach provides a robust high-throughput assay to detect pharmacological effects on neurite dynamics and aids in the screening of neuroprotective agents.

Vehicle



6-OHDA 100 µM





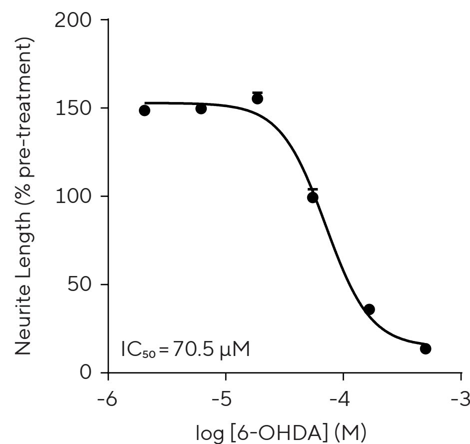


Figure 1: 6-OHDA-induced Neurite Disruption in a PD Model. Concentration-dependent neurotoxic effects of 6-OHDA on neurite length measured using live-cell analysis in rat primary co-culture models.

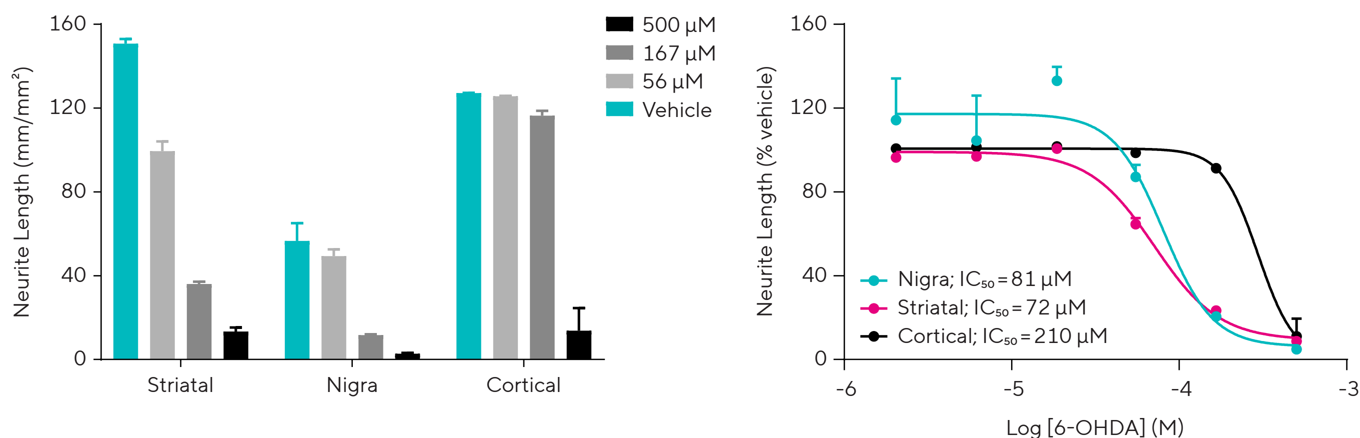


Figure 2: 6-OHDA selectively effects neurons from different brain regions. Rat primary neurons co-cultured with astrocytes respond differently to 6-OHDA, with live-cell analysis showing selective effects on substantia nigra and striatal neurite outgrowth compared to cortical regions.

Phagocytosis of Disease-Associated Peptides

Microglia are the resident macrophage of the brain, and as such play a pivotal role in the neuro-inflammatory response. Aggregated peptides, known to induce neuropathology, are rapidly phagocytosed by microglia. Here we explore how live-cell analysis can also be used to assess phagocytosis of peptides associated with PD (Figure 3). iPSC microglia precursor cells were differentiated in a 96-well plate to mature microglia over 2 weeks. Aggregated α-synuclein peptides were labeled using the pHrodo® Orange Cell Labeling Kit for Incucyte® and then added to the iPSC-derived microglia at a range of concentrations and phagocytosis was quantified via an increase in fluorescence.

The results show iPSC-derived microglia rapidly engulf aggregated α-synuclein and uptake was observed in a time- and peptide concentration-dependent manner.

The phagocytosis assay provides a simple yet powerful solution to visualize iPSC-derived microglia and kinetically quantify phagocytosis of disease-relevant peptides in a high-throughput format. This approach has the potential to facilitate the development of novel neuro-therapeutics that target microglial function.

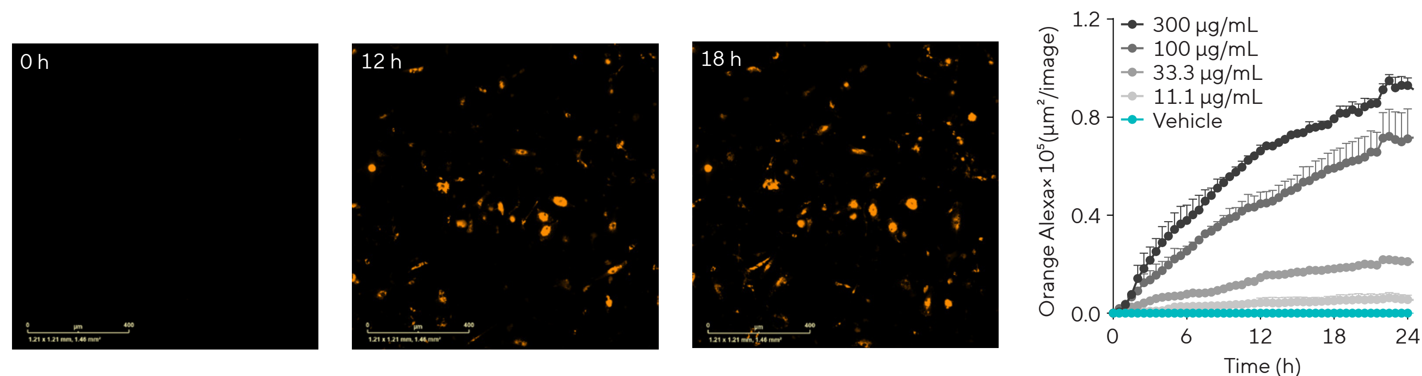
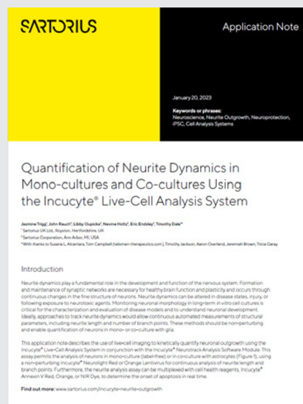


Figure 3: Phagocytosis of α-synuclein in PD neuro-inflammatory model. iPSC-derived microglia engulf aggregated α-synuclein in a time- and concentration-dependent manner, as shown by an increase in orange fluorescence area following engulfment.

Summary

This case study discusses the use of live-cell analysis to enhance the study of PD models. It highlights the challenges of traditional methods in studying complex neuronal models and presents live-cell analysis as a solution. This technology allows real-time visualization and quantification of cellular processes in a non-perturbing manner, using tools like the Incucyte® Neurite Outgrowth and Phagocytosis Assays. These assays enable the monitoring of neurite dynamics and phagocytosis in PD models, providing insights into neurodegeneration and neurotoxicity. The approach supports high-throughput screening and development of neuroprotective agents against disease-associated toxicity and neuroinflammation.

Explore More Resources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Application Note

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Keywords or phrases:
Neuroscience, Neurite Outgrowth, Neuroprotection, iPSC, Cell Analysis Systems

Quantification of Neurite Dynamics in Mono-cultures and Co-cultures Using the Incucyte® Live-Cell Analysis System

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Introduction

Neurite dynamics play a fundamental role in the development and function of the nervous system. Formation and maintenance of synaptic networks are necessary for healthy brain function and plasticity and occurs through continuous changes in the fine structure of neurons. Neurite dynamics can be altered in disease states, injury, or following exposure to neurotoxic agents. Monitoring neuronal morphology in long-term in vitro cell cultures is critical for the characterization and evaluation of disease models and to understand neuronal development. Ideally, approaches to track neurite dynamics would allow continuous automated measurements of structural parameters, including neurite length and number of branch points. These methods should be non-perturbing and enable quantification of neurons in mono- or co-culture with glia.

This application note describes the use of live-cell imaging to kinetically quantify neuronal outgrowth using the Incucyte® Live-Cell Analysis System in conjunction with the Incucyte® Neurotrack Analysis Software Module. This assay permits the analysis of neurons in mono-culture (label-free) or in co-culture with astrocytes (Figure 1), using a non-perturbing Incucyte® Neurolight Red or Orange Lentivirus for continuous analysis of neurite length and branch points. Furthermore, the neurite analysis assay can be multiplexed with cell health reagents, Incucyte® Annexin V Red, Orange, or NIR Dye, to determine the onset of apoptosis in real time.

Find out more: www.sartorius.com/incucyte-neurite-outgrowth

Application Note

Quantification of Neurite Dynamics in Mono-cultures and Co-cultures Using the Incucyte® Live-Cell Analysis System

Monitoring neuronal morphology in long-term *in vitro* cell cultures is critical for characterization and evaluation of disease models and to understand neuronal development.

Read More

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