SARTORIUS

Simplifying Progress

Quantitative, Live-Cell Kinetic Analysis of Microglial Function and Morphology

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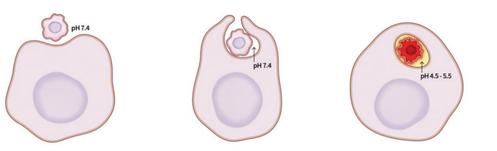
Summary & Impact

- Microglia are the resident immune cells of the central nervous system and play significant roles in the regulation of homeostasis and the management of tissue response to inflammatory or pathological insults.
- Microglia impact synaptic remodeling and turnover of dendritic spines through the removal of damaged or unnecessary neurons or synapses.
- Limited tools and in vitro model systems exist to enable optimizing, monitoring, and analyzing functional and morphological changes of these cells.
- We characterize Rat Primary, iPSC-derived, and immortalized microglia and present data evaluating the ability of these cells to phagocytose pHrodo® labeled *E. coli* bioparticles and apoptotic Neuro-2A (N2A) cells using a quantitative, live-cell imaging approach with the Incucyte® S3 Live-Cell Analysis System for Neuroscience.

Quick Guide

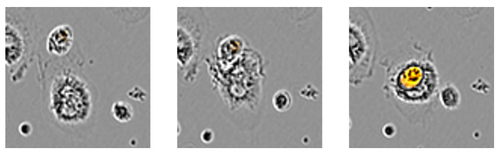
Phagocytosis | Efferocytosis



pHrodo® Labeled Cells Added to Phagocytes
Little or no pHrodo® fluorescence while apotoic cells remain in the pH 7.4 extracellular environment.

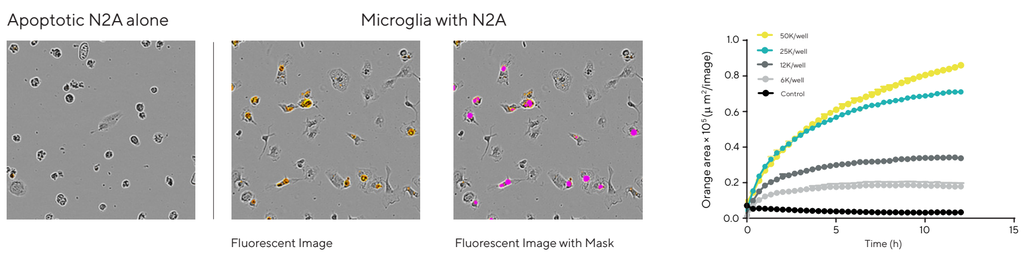
Phagocytosis Initiated Following Receptor Activation
Formation of the phagocytic cup.

Formation of the Phagosome
Engulfment of pHrodo® labeled cells by pinching off. The acidic environment of the phagosome (pH 4.5 - 5.5) leads to increased pHrodo® fluorescence.



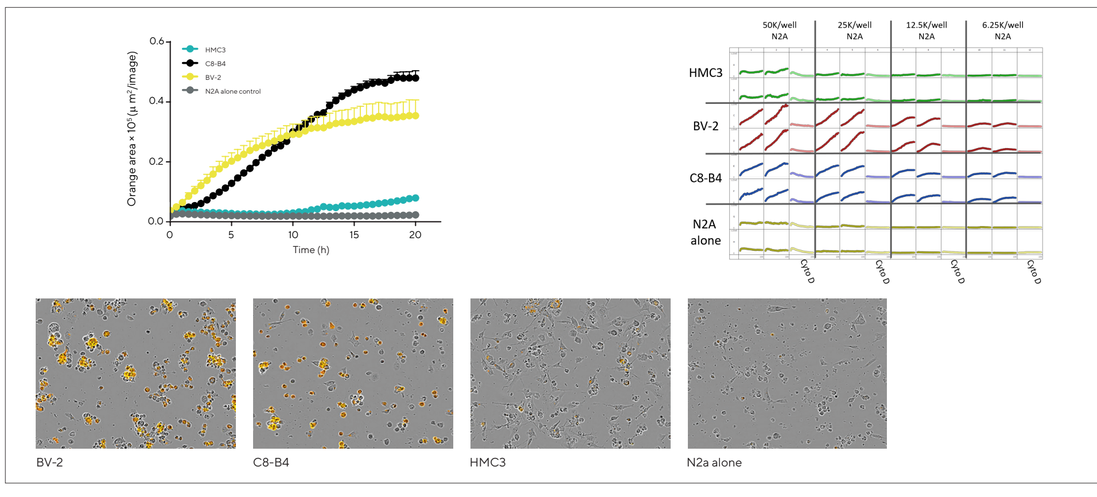
Time-lapse visualization of iPSC-derived microglia (Axol BioSciences) engulfing pHrodo® Orange-labeled apoptotic N2A cells. Images verify the entry of an apoptotic target cell into the cytoplasm of microglia.

Primary Microglial Efferocytosis of Apoptotic Neuro-2a Cells



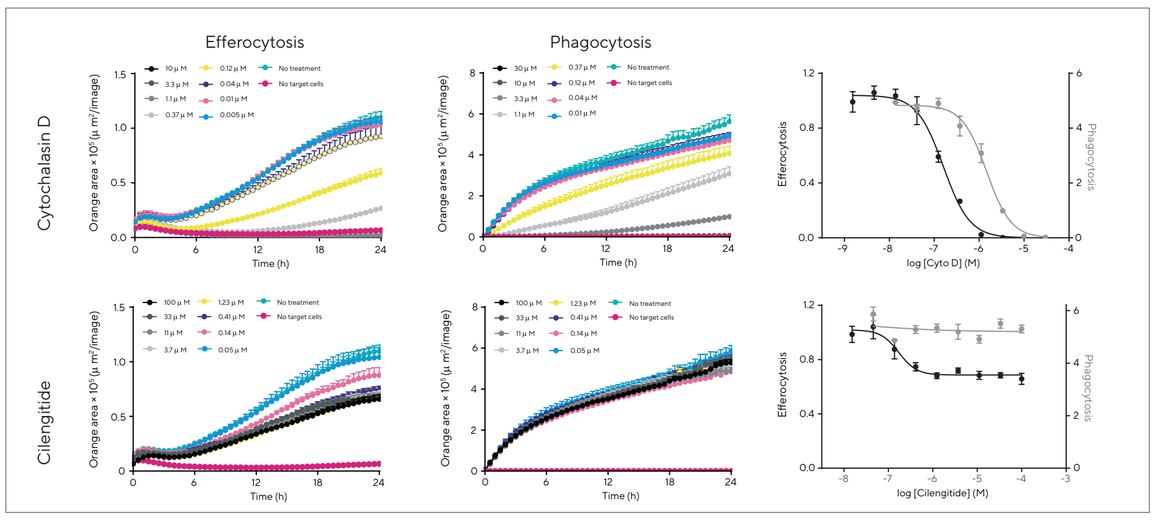
N2A cells were pre-treated with staurosporine (24 hrs), labeled with the Incucyte® pHrodo® Orange Cell Labeling Kit, and added to pre-plated primary rat microglia (Brain Bits, 20,000 K/well). N2A cells alone have minimal fluorescence (left image). Engulfment of labeled apoptotic N2A cells by microglia causes an increase in orange fluorescence (florescent image) that is segmented using automated Incucyte® Live-Cell Analysis Software (fluorescent image with mask). A kinetic N2A density-dependent response is quantified over time (right).

Characterization of Immortalized Microglia Efferocytosis



- Kinetic efferocytosis response of N2A cell engulfment for three different immortalized microglial cell lines (HMC3, BV2, and C8-B4). BV-2 and C8-B4 cells showed an increasing signal over time while HMC3 cells displayed minimal efferocytosis.
- Microplate graph of the kinetic efferocytosis response from a full 96-well plate. HMC3, BV2, and C8-B4 cells were seeded on the same plate at 5K cells/well. pHrodo®-labeled apoptotic N2A cells were added at decreasing densities from left to right. Cytochalasin D was added at each N2A density to inhibit efferocytosis.
- Images taken at 20 hrs show the fluorescent signal from BV-2 and C8-B4 cells and lack of signal from HMC3 cells over control apoptotic N2A cells lone.

Effect of Cytochalasin D & Cilengitide on Microglial Function



BV-2 effector cells (20,000 K/well) efferocytose apoptotic N2A cells (left-hand panels) or E. coli bioparticles (middle panels). Cytochalsin D (top panels) elicits a concentration-dependent inhibition of both efferocytosis and phagocytosis, yielding IC_{50} values of 0.16 µM and 1.5 µM, respectively. Cilengitide, an inhibitor of αVβ3 and αVβ5 integrins, selectively attenuates efferocytosis (IC_{50} value 0.17 µM), with minimal effect on phagocytosis at the highest concentration tested (100 µM). These data support the role of integrins in the cell interactions required for efferocytosis, but not in the phagocytosis of bacteria-based bioparticles.

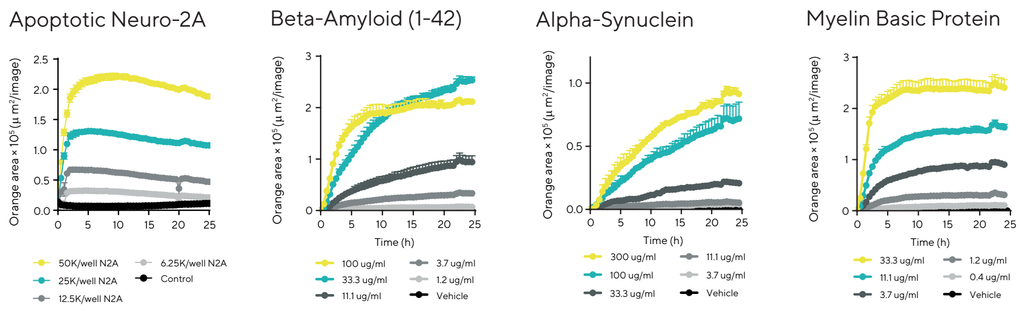
Incucyte® S3 Live-Cell Analysis System for Neuroscience

Incucyte® S3 Live-Cell Analysis System
A fully automated phase contrast and two-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

Incucyte® Software
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

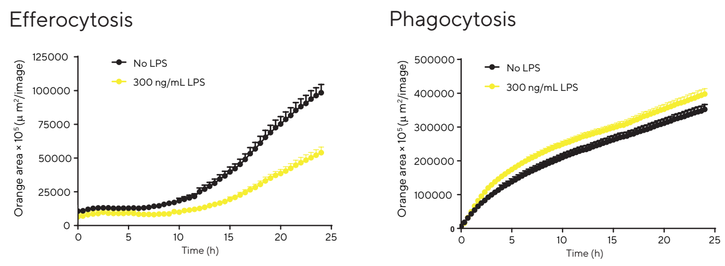
Incucyte® Reagents and Consumables
A suite of non-perturbing cell labeling and reporter reagents.

iPSC-derived Microglial Phagocytosis of Relevant Target Material



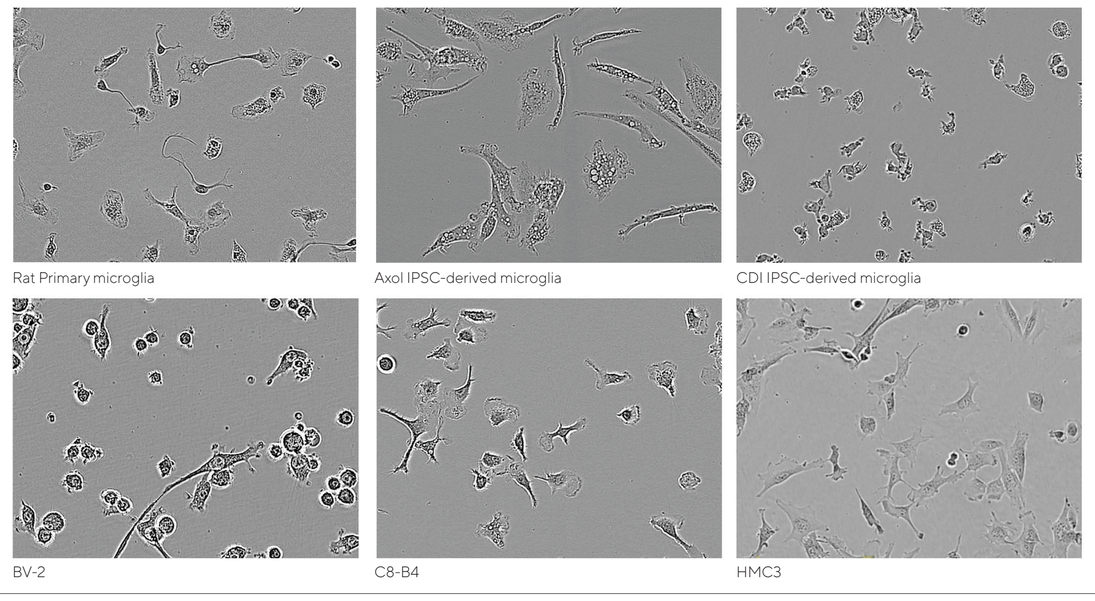
iPSC-derived microglia (Axol BioSciences, 30,000 K/well) phagocytose physiologically relevant target material. Kinetic graphs display the concentration-dependent response to pHrodo® labeled apoptotic Neuro-2A cells, Beta-Amyloid(1-42), Alpha-Synuclein, and Myelin Basic Protein.

Effect of LPS Activation on Microglial Cell Line Function



- BV-2 cells were incubated with 300 ng/mL LPS for 24 hrs prior to the addition of either Incucyte® pHrodo® labeled N2A cells or bioparticles to induce microglial activation.
- LPS treatment decreased the efferocytosis response to apoptotic N2A cells and minimally increased phagocytosis of bioparticles.

Differential Morphology of Microglial Sources



Microglia from a variety of sources were characterized for morphological phenotype (20× zoomed to same scale). Representative images from: Rat Primary microglia (Brain Bits-top left), iPSC-derived microglia (Axol BioSciences-top middle), iPSC-derived microglia (Cellular Dynamics International-top right), immortalized murine microglia cell lines BV-2 (bottom left) and C8-B4 (bottom middle), and HMC3 human immortalized microglia (bottom right). Differential morphology was observed depending on species and source.

Conclusions

- Microglia in vitro can have vastly different morphology depending on the species and source of the cells.
- Microglia can phagocytose a variety of targets including E. coli bioparticles, apoptotic N2A cells, and physiologically relevant protein targets.
- Immortalized microglia have varying degrees of phagocytic potential, and this can be modified by activation with LPS.
- Phagocytosis and efferocytosis are distinct processes that can be selectively inhibited by pharmacological means.
- The Incucyte® S3 Live-Cell Analysis system for Neuroscience can be used with the pHrodo® Orange Cell Labeling Kit to characterize morphology and quantify functional phagocytic and efferocytic activity in microglia.