Live-cell analysis of uptake of neuropathology-associated peptides by human iPSC-derived microglia

G. Lovell, M. Bowe, S. Lopez Alcantara, J. Rauch, L. Oupicka, A. Overland, C. Schramm, T. Dale, D. Trezise
Essen BioScience, Welwyn Garden City, AL7 3AX UK & Ann Arbor, MI, 48108, USA

Summary & Impact

- The presence of aggregated peptides such as Amyloid β (1-42) and α-Synuclein are associated with disease phenotypes (Alzheimer's disease and Parkinson's disease, respectively).
- Microglia, as the resident macrophage of the brain, have several functions including clearance of peptide aggregates (phagocytosis) and apoptotic cells (efferocytosis).
- Here we describe characterisation of the activation and function of microglia using IncuCyte® live-cell analysis.
- Phase and fluorescence images were captured with IncuCyte® and segmented fluorescence was quantified.
- In all cases robust, time-dependent signal changes were observed, consistent with known microglia function.
- We conclude that live-cell analysis is a flexible and powerful method for analysing microglia activity, where morphological and functional parameters can be readily quantified and integrated over time.

IncuCyte® System for continuous live-cell analysis: Methodology

IncuCyte® 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.

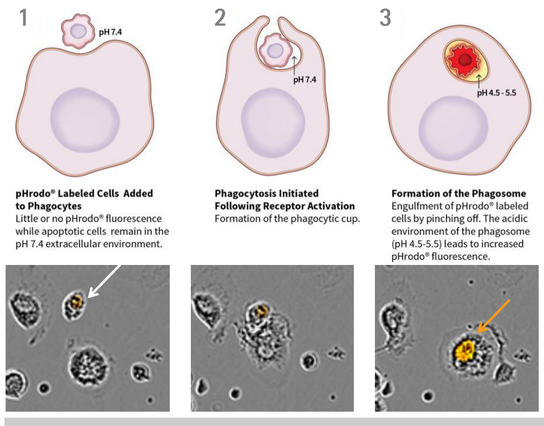
IncuCyte® Software

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

Kinetic quantification of phagocytosis by Microglia



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

Phagocytosis Initiated Following Receptor Activation
Formation of the phagocytic cup.

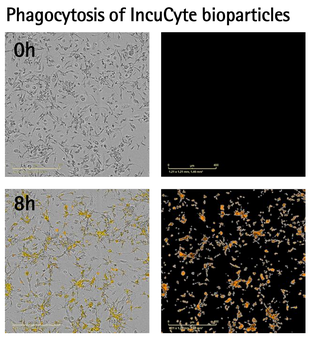
Formation of the Phagosome
Engulfment of pHrodo® labeled cells by pinching off. The acidic environment of the phagosome (pH 4.5-5.5) leads to increased pHrodo® fluorescence.

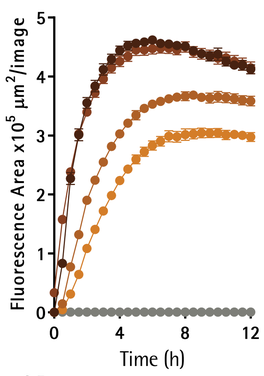
Phagocytosis Assay Principle

- Microglial precursors (Axol Bioscience) were seeded into a 96-well plate (30K/well) & differentiated to mature microglia for 2 weeks before assay
- Target apoptotic cells were labelled with a pH-sensitive fluorophore (IncuCyte® pHrodo® Orange Cell Labelling Kit). Non-engulfed labelled target cells (white arrow) have low fluorescence.
- Labelled target cells were added to mature microglia (30K/well) and placed into the IncuCyte system where phase and fluorescence images were acquired every 30 minutes for 24h.
- Target cells were engulfed into the acidic phagolysosome of the microglia (cytoplasmic, orange arrow) where the fluorescence increases in intensity.
- The response was quantified as an increase in fluorescence area and shows a rapid phagocytosis of the target cells.

Live-cell analysis and quantification of Microglia function

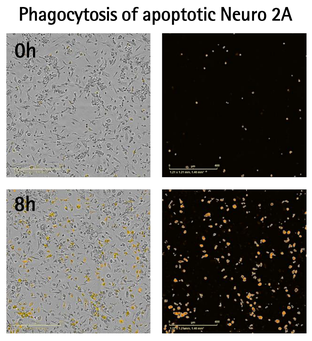
Phagocytosis of IncuCyte bioparticles

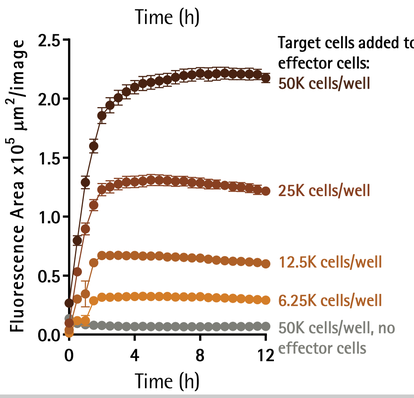




- IncuCyte® pHrodo® E. coli Bioparticles were added to iPSC microglia (Axol Bioscience).
- Phase and fluorescence images show an increase in cytoplasmic fluorescence (grey mask indicates segmented fluorescence) within the microglia between 0 and 8h.
- Bioparticles were engulfed within 8 h with a density-dependent increase in rate and total fluorescence area until 10 µg/ml.

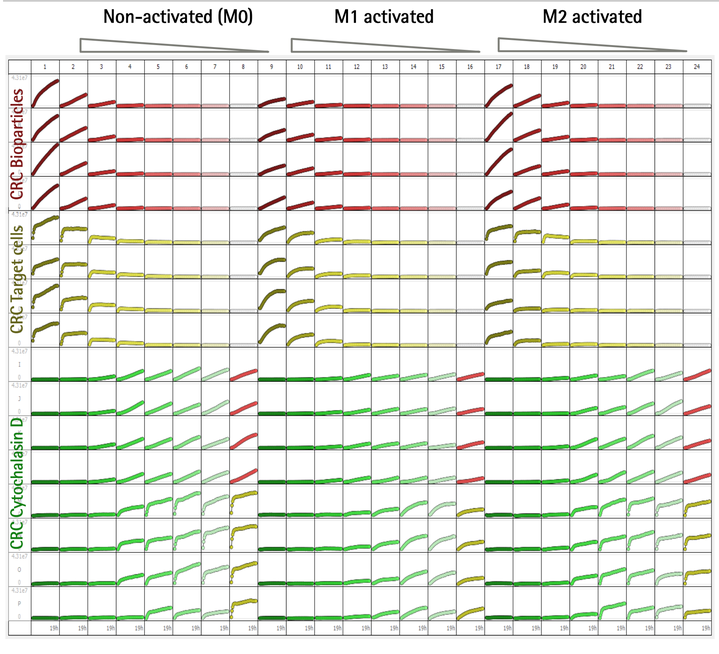
Phagocytosis of apoptotic Neuro 2A

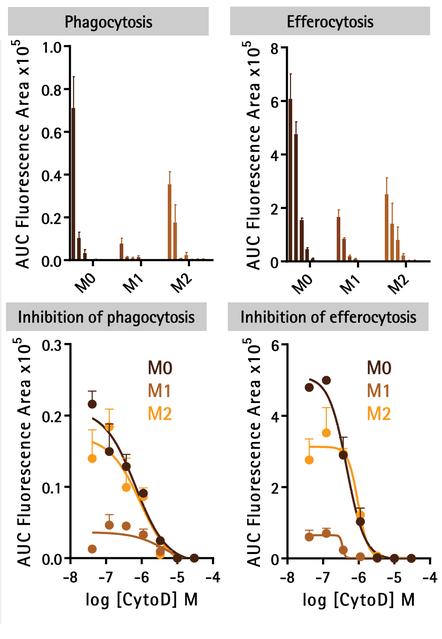




- Apoptotic target cells labelled with IncuCyte® pHrodo® Orange Cell Labelling kit were added to iPSC microglia (Axol Bioscience).
- Both cell types are visible in Phase HD images, however at 8h the number of target cells has reduced and fluorescence within the cytoplasm of microglia has increased.
- Target cells were rapidly engulfed within 2h in a density-dependent manner.

Activation state of Microglia affects phagocytic function

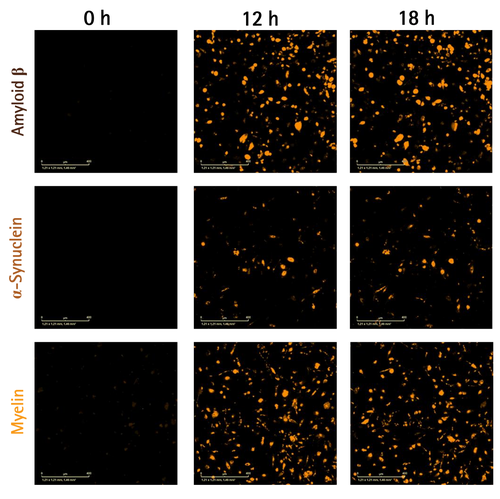




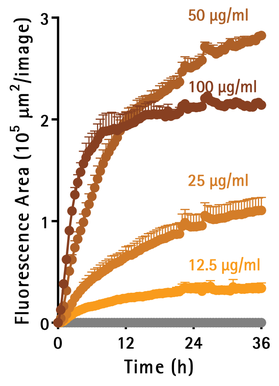
- iPSC Microglia (Axol Bioscience) were left unactivated (M0, col 1-8) or treated with LPS and IFN γ (M1, col 9-16) or IL-4 and IL-13 (M2, col 17-24) to induce polarisation of the cells. Half of these cells (rows I – P) were then treated with a concentration range of Cytochalasin D, an inhibitor of phagocytosis.
- Target material (IncuCyte® pHrodo® E. coli Bioparticles or apoptotic Neuro2A labelled with IncuCyte® pHrodo® Orange Cell Labelling Kit) was added at increasing concentrations (rows A-H) or at a single density (rows I-P).
- The plate view shows an overview of phagocytosis, plotting fluorescence area over time per well of the 384-well plate.
- In both cases microglia activated to M1 phenotype had the lowest phagocytic function with unactivated cells (M0) showing the highest rate of both phagocytosis and efferocytosis. CytoD inhibits both phagocytosis and efferocytosis in a concentration dependent manner.

iPSC Microglia engulf aggregated Amyloid β, Myelin and α-synuclein

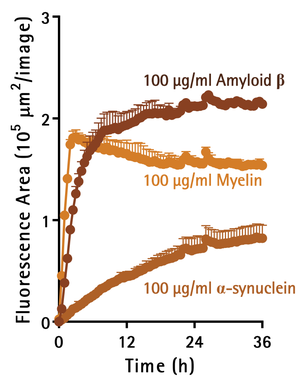
Phagocytosis of aggregated peptides (100 µg/ml)



Uptake of Amyloid β

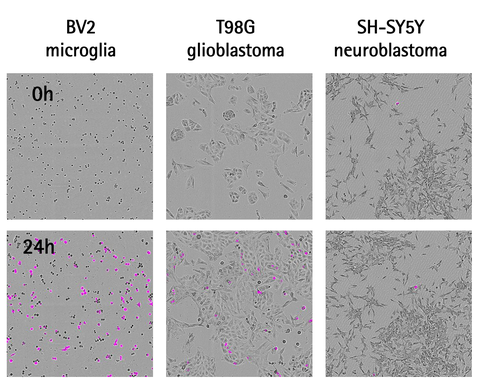


Uptake of 100 µg/ml peptides

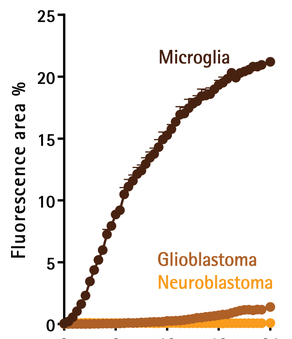


- Axol iPSC microglial precursor cells were seeded into a 96-well plate at 30k cells/well and differentiated to mature microglia for 2 weeks. Peptides were labelled using IncuCyte® pHrodo® Orange Cell Labelling Kit and aggregates were formed at 37 ˚C for 48h prior to assay. The peptides were added to cells at 0.4 – 300 µg/ml and phase and fluorescent images were acquired in IncuCyte for 36h.
- Uptake of Amyloid β is concentration-dependent. 0.4 µg/ml is engulfed at a low rate, with fluorescence reaching a plateau at 0.3 x10^5 µm^2/image at 24h; 100 µg/ml, shows the highest rate of engulfment rate, with fluorescence area reaching plateau at 2.0 x10^5 µm^2/image by 12h.
- Rate of phagocytosis varies between peptides. Myelin is taken up most rapidly by microglia (fully engulfed within 2h), while phagocytosis of Amyloid β is less rapid, begins to slow after 12h. α-synuclein uptake is more gradual and at 24h, the fluorescence area is approximately 30% that of Amyloid β.
- This variance may be due to differential size or aggregation form of each peptide.

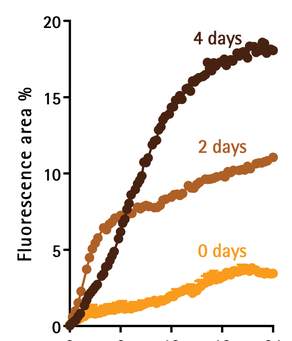
Engulfment of Aβ is dependent on cell type and peptide aggregation



Microglia engulf Amyloid β



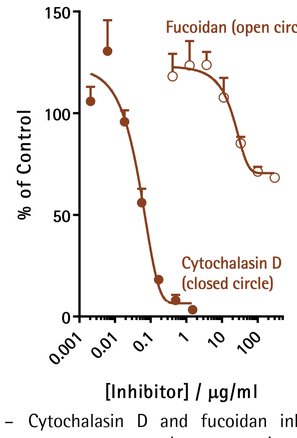
Aggregation of Amyloid β



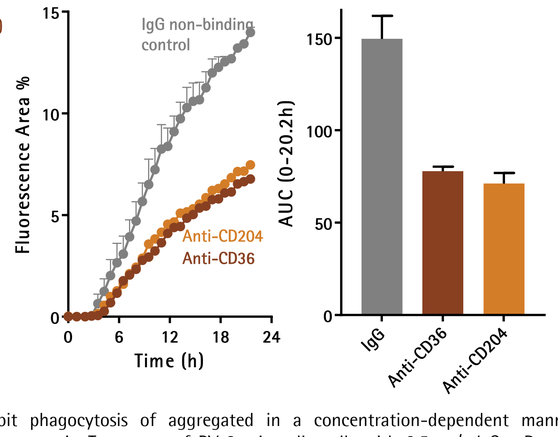
- Internalisation of aggregated labelled Amyloid β by three cell lines was compared. Phase HD images show differences in morphology between cell lines and the increase in fluorescence area (pink mask) at 24h. Data indicates the change in normalised fluorescence area over time (fluorescence area was normalised to phase area per well to account for proliferation and variations in cell morphology).
- BV-2 microglia cells are phagocytic and rapidly engulf aggregated peptide with concomitant increase in fluorescence area over 24h, while glioblastoma and neuroblastoma cells show minimal peptide internalisation.
- The phagocytosis of labelled Amyloid β which was aggregated for 0, 2 or 4 days was compared. Increasing the aggregation of the peptide changes the kinetic profile of uptake by BV-2 cells, and longer aggregation times resulted in greater engulfment over a 24h period.

Blockade of scavenger receptors inhibits engulfment of Aβ

Inhibition of phagocytosis



Blockade of scavenger receptors using monoclonal Ab



Inhibitor	Target
Fucoidan	Scavenger receptors - nonspecific
Cyto D	Actin (inhibits polymerisation)
Anti-CD204	Scavenger receptor A
Anti-CD36	Scavenger receptor B

- Cytochalasin D and fucoidan inhibit phagocytosis of aggregated in a concentration-dependent manner. CytoD inhibits cytoskeletal rearrangement, a key process in phagocytosis. Treatment of BV-2 microglia cells with 0.5 µg/ml CytoD completely inhibits engulfment of Amyloid β aggregates in a concentration-dependent manner. Fucoidan is a polysaccharide known to bind scavenger receptors. Treatment of the cells with 300 µg/ml fucoidan achieves partial (~35%) inhibition.
- Binding scavenger receptors A and B with monoclonal antibodies (anti-CD204 and anti-CD36 respectively) prevents the cell from engaging with target material. Inhibition of phagocytosis is observed in the presence of 10 µg/ml of both antibodies achieving approximately 50% inhibition compared to IgG (non-binding antibody control).