Characterization of human iPSC-derived Microglia using live-cell imaging reveals hallmarks of morphology and function

Key points

- Microglia are the resident macrophage of the central nervous system and play a critical role in the immune response of the brain. Inflammation caused by neurological damage leads to activation of microglia, which is thought to play a key role in the progression of neurodegenerative diseases.
- This study characterized human iPSC-derived microglia (Axol Bioscience) using IncuCyte® Live Cell assays (Essen BioScience, part of the Sartorius Group) to validate their morphological and functional features, offering insights into their suitability for immune and neurodegenerative disease research.
- Differentiation was monitored using non-perturbing live-cell imaging, and confirmed the expected morphologies and the presence of known Microglia markers.
- Live-cell functional assays quantified the ability of human iPSC-derived microglia to phagocytose apoptotic neuronal cells (efferocytosis) and neurodegenerative disease associated peptides.
- Therefore, hiPSC-derived Microglia may represent a physiologically-relevant, functionally-validated cellular model for studying microglia function, and facilitate the advancement of neurodegeneration research.

Background

As the macrophage of the central nervous system, microglia have important roles in synaptogenesis and pathogen control, surveying the central nervous system for neuronal damage and infectious agents. Upon damage to the brain, microglia become activated and proliferate, migrate, and phagocytose damaged neurons and infectious agents.

Recent advances in stem cell technologies have the potential to generate patient-specific microglia from human induced pluripotent stem cells (hiPSCs). Modelling the interface between the nervous and immune system can enable the development of novel drug discovery targets in the fields of neurodegeneration and neuroinflammation. These hiPSC-derived Microglia can therefore offer several advantages over traditional immortalised cell lines (e.g. BV-2) and primary sources, and an enhanced physiologically-relevant cellular model to advance disease research, facilitating the discovery of treatments modulating inflammatory pathways.

Objectives

- To describe the morphological, marker expression and functional characteristics of hiPSC-derived microglia (Axol Bioscience).
- Characterization of the hiPSC-derived Microglia was conducted using assays measuring typical microglia functions: phagocytosis of *E. coli* bioparticles and apoptotic neurons (efferocytosis); engulfment of aggregated neurodegenerative disease associated peptides (Amyloid β, α-Synuclein, Myelin basic protein).

Materials and Methods

1. Monitor differentiation of iPSC-derived Microglia

Axol hiPSC-derived Microglia were seeded at a range of densities from 10,000 to 30,000 cells/well in 96-well ImageLock plates (Essen BioScience). On Days 2 and 3, a 50% media change was performed using Microglia Maintenance Medium (Axol Bioscience), and again on every other day until day 14 when full microglial maturity had been reached. Throughout the 14 day differentiation period images were acquired using an IncuCyte® S3 Neuroscience live-cell imaging system at 20x magnification in HD-Phase to monitor morphological changes.

2. Immunocytochemistry of iPSC-derived Microglia

Following differentiation iPSC-derived microglia were fixed using 4% paraformaldehyde and immunostaining with for the presence of known microglia markers TMEM119 and TREM2 conducted. Primary antibodies were added at 1 µg/mL in blocking buffer (PBST + 1% BSA + 10% NGS), followed by addition of an Alexa-conjugated secondary antibody (PBS only). The level of expression was quantified by capturing and analyzing images with an IncuCyte S3 to determine the fluorescent area. Data was normalized to the phase area measurement to mitigate differences in cell number per well.

3. Target material preparation

Apoptotic Neuro-2A cells or disease related peptides were labelled using the IncuCyte® pHrodo® Orange cell labelling kit (Essen BioScience). To induce apoptosis, Neuro-2A cells were treated with 600 nM staurosporine for 24 hours, washed to remove the compound, and incubated IncuCyte® pHrodo® Orange for 1 hour. After labeling, the cells were spun down and washed to remove excess dye.

Disease related peptides were reconstituted in a 1% NH_4OH/PBS solution at a concentration of 2 mg/mL. Prior to labeling, peptides were incubated for 48 hours at 37 ˚C in order to form protein aggregates. To label the aggregates, solutions were centrifuged at 13,000 RPM for 5 minutes, resuspended in IncuCyte® pHrodo® labeling buffer, and allowed to label for one hour at 37 ˚C with the IncuCyte® pHrodo® Orange reagent. After labeling, the labeled peptide aggregates were spun down and washed to remove excess dye.

4. Live-cell analysis of phagocytic function

After 14 days of hiPSC-derived microglia differentiation, target material was added and phase and fluorescent images acquired every 30 minutes for 24 hours at 10x magnification using an IncuCyte® S3 Neuroscience instrument. Increasing concentrations of either IncuCyte® pHrodo® labelled *E. coli* bioparticles® (1.25 - 10 µg/well) or apoptotic Neuro2A (6,250 – 50,000 cells/well) to measure phagocytic and efferocytic activity, respectively. To study the engulfment of disease relevant peptides, protein aggregates (0.41 – 300 µg/mL) were added to Axol hiPSC-derived Microglia (30,000 cells/well).

Target material labelled with the pH-sensitive fluorophore IncuCyte pHrodo® reagent undergoes fluorescence enhancement upon engulfment, when encountering the acidic microenvironment of the phagolysosome. Non-engulfed objects have low intensity used to set the threshold for fluorescence change. Phagocytosis and efferocytosis were quantified using IncuCyte® software, applying background subtraction and segmentation enabling accurate quantification of the fluorescence image. Data was expressed as the fluorescent area observed per image.

Results

Live-cell monitoring of cells over the 14 day differentiation period showed a change in morphology from small, round shaped monocytes to the larger, elongated structures of the fully mature microglia cells. Cells appeared healthy throughout the time-course at all seeding densities tested (10,000 – 30,000 cells/well) and media changes appeared to be non-perturbing. At full maturation a mixture of ramified and amoeboid morphologies were observed (Figure 1), and immunostaining confirmed the presence of microglia-specific markers TMEM119 and TREM2 (Figure 2).

Axol hiPSC-derived Microglia demonstrated efficient phagocytosis of *E. coli* bioparticles and efferocytosis of apoptotic Neuro2A cells. Engulfment events are visible in the time-course of images (Figure 3) which show microglia moving towards, and finally engulfing, an apoptotic target cell over a two hour period (Figure 3). Axol hiPSC-derived Microglia were observed to phagocytose apoptotic Neuro-2A cells in a density dependent manner (Figure 3). Axol hiPSC-derived Microglia also displayed a density-dependent increase in phagocytic activity at higher concentrations of Bioparticles® compared to cultures treated with lower concentrations of bioparticles (Figure 4). In addition, microglia phagocytosed aggregated peptides (Amyloid β 1-42, α-Synuclein, Myelin) with differential kinetics. This function enables modeling of microglia function in neurodegenerative diseases.

Conclusions

The data presented here strongly demonstrate that human iPSC-derived Microglia display the morphological, surface marker expression and functional characteristics expected from microglia. This includes normal microglia phagocytic uptake of apoptotic neuronal cells and bacterial Bioparticles®. Microglia also phagocytosed aggregated neurodegenerative disease associated peptides, displaying differential kinetics of engulfment.

Axol human iPSC-derived Microglia therefore provide an ideal physiologically-relevant, functionally-validated cellular model for studying microglia function in vitro, as well as being a practical model for advancing neurodegenerative research.

Figures

Figure 1: Visualize morphological changes during differentiation. Monocytes at day in vitro (DIV) 0 appear as small rounded structures. As differentiation occurs over a 14-day period, the cells become larger and more elongated, indicative of microglia. Note the characteristic ramified (red arrow) and ameboid (yellow) morphologies at day 14.

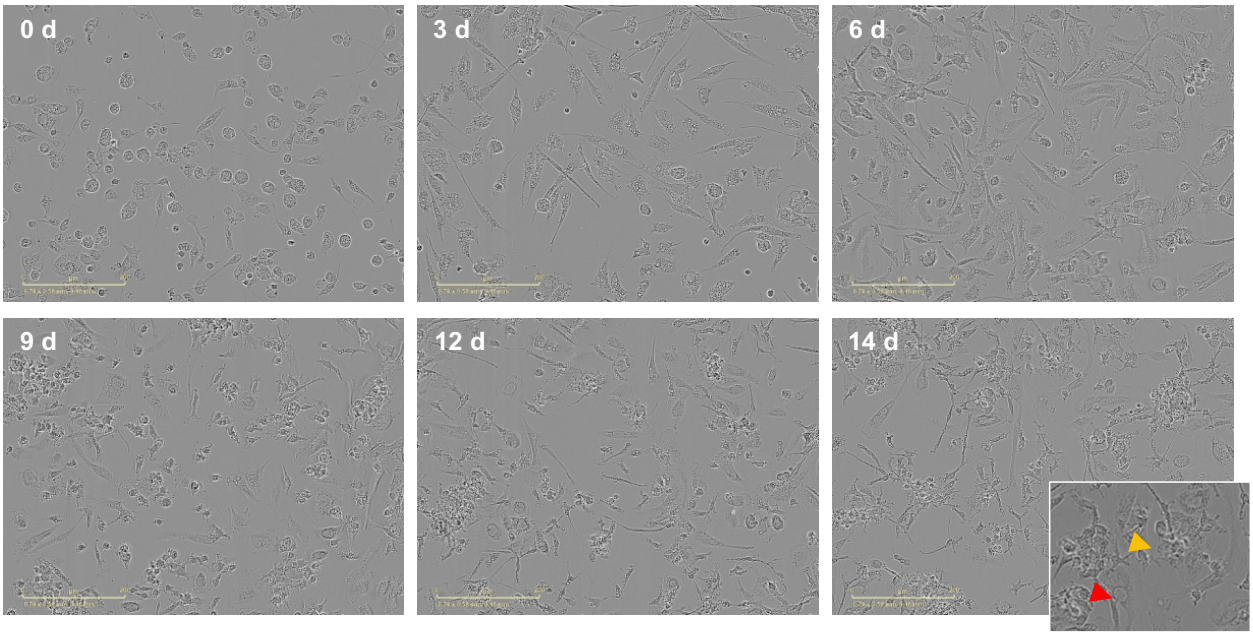


Figure 2: Cells display microglia specific markers TMEM119 and TREM2. After 14 days differentiation cells were fixed and immunostaining was performed using primary antibodies (TREM2, TMEM119 and IgG1 isotype control; mouse anti-human) followed by an anti-Mouse-Alexa conjugated secondary antibody. The plate was replaced into the IncuCyte and Phase and fluorescence images were acquired. Quantification was achieved by determining the masked fluorescent area (blue) and normalizing to the phase area to mitigate differences in cell number; data is expressed as % area, mean ± SEM from 3 replicates.

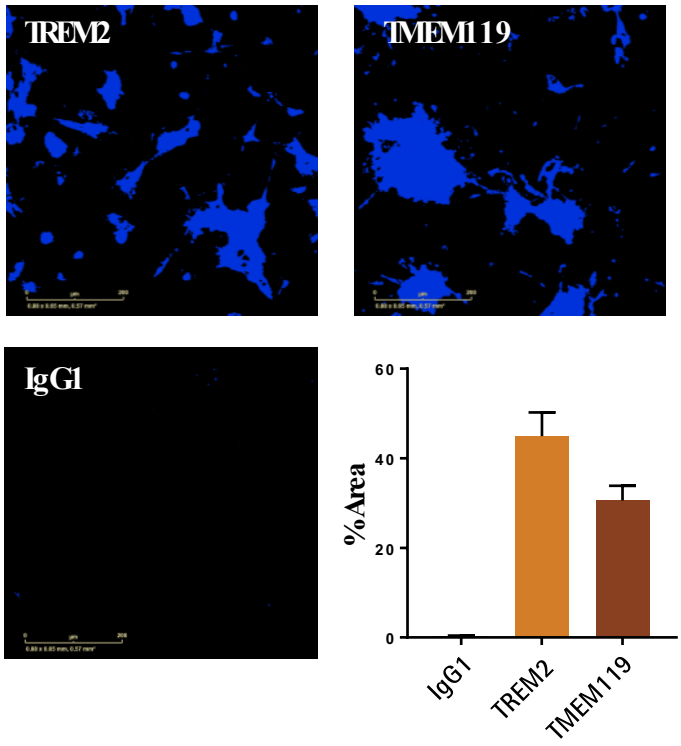


Figure 3: Microglia engulfment of apoptotic Neuro2A. Representative image series taken over two hours of iPSC-derived microglia (Axol BioSciences) engulfing IncuCyte® pHrodo Orange labelled apoptotic Neuro2A cells. Images verify the entry of an apoptotic target cell into the cytoplasm of the microglia. Neuro2A target cells were labelled with the IncuCyte pHrodo Orange cell labelling kit and apoptosis induced with staurosporine.

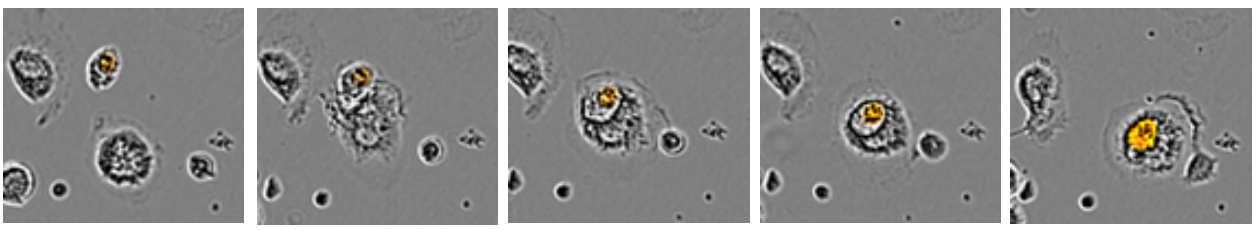
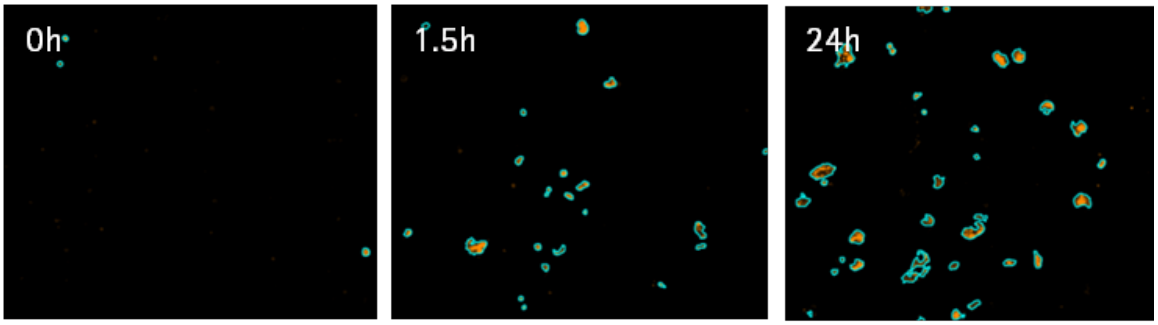


Figure 4. Efferocytosis of pHrodo labeled apoptotic Neuro2A cells and phagocytosis of pHrodo labeled *E. coli* bioparticles. 30K microglia/well **were** treated with increasing concentrations of either IncuCyte® pHrodo® red *E. coli* bioparticles® (1.25 - 10 µg/well), or pHrodo® Orange labeled apoptotic Neuro-2A cells (6,250 – 50,000 cells/well) to measure phagocytic and efferocytic activity, respectively. Images at 1, 1.5, and 24 hours show fluorescent image mask with the phase channel turned off. As target cells become engulfed by microglia, fluorescence increases as indicated by the fluorescence mask at increasing time-points. Microglia display a density dependent increase in phagocytic activity. (Left) kinetic graphs show microglia temporal response to target cells at increasing concentrations and (Right) 24-hour end point graphs display the concentration dependent response



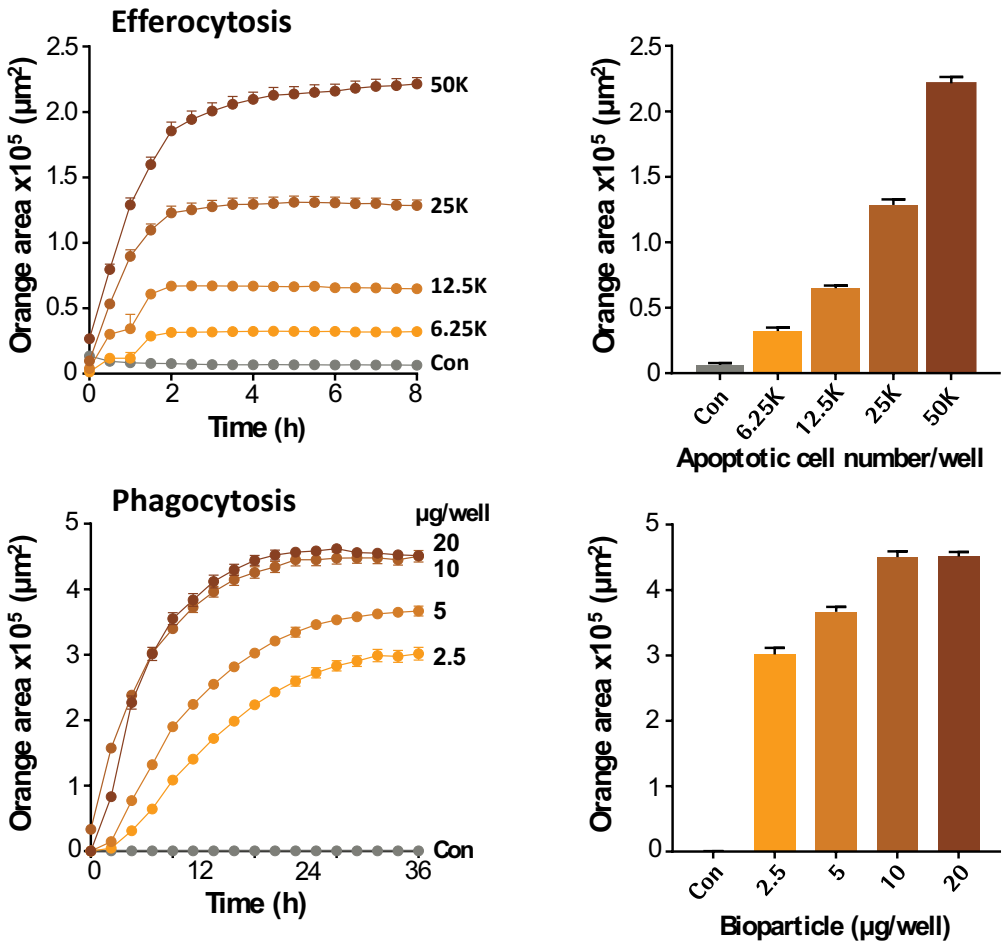
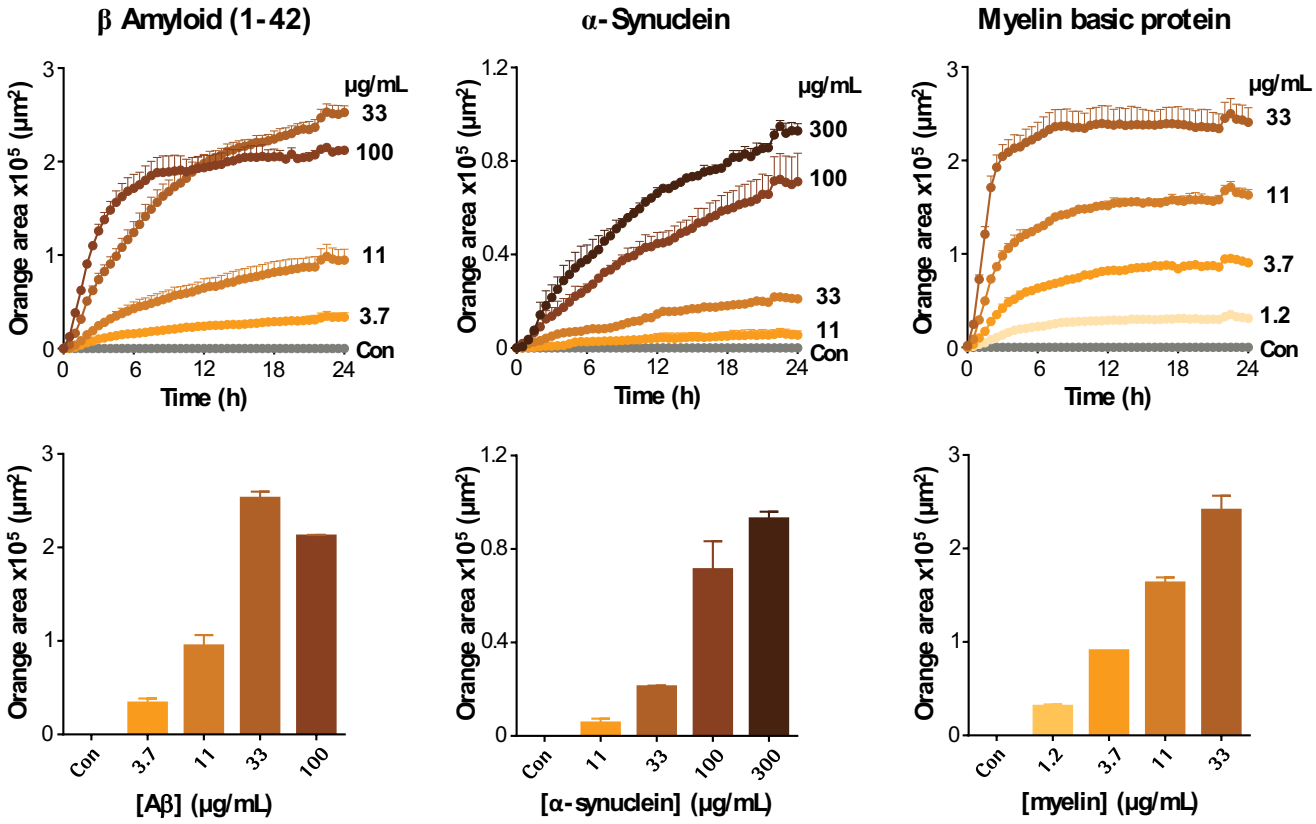


Figure 5: Phagocytosis of aggregated neurodegenerative disease-associated peptides.

30K microglia/well were exposed to a concentration range of labelled, aggregated peptides β Amyloid (3.7 – 100 µg/mL), α-Synuclein (11 – 300 µg/mL), Myelin basic protein (0.4 – 33 µg/ml). Phase and fluorescent images were acquired over 24 h and increasing fluorescence intensity was observed inside the cells. Differential rates of uptake were observed for the peptide aggregates.



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