

# Development of high content approaches for investigating microglia function in neurodegenerative disorders

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## 1 Introduction

The role of Central Nervous System (CNS) resident macrophages, termed microglia, in neurodegeneration have come to prominence in recent years, being important in brain homeostasis and the clearance of pathogenic material. Microglial function has been implicated in a wide variety of CNS disease<sup>(1)</sup> including Amyloid Lateral Sclerosis (ALS), Alzheimer's, Huntington's and Parkinson's Disease (AD, HD & PD respectively) and Multiple Sclerosis (MS).

Assessing primary human cells, whether monocyte, macrophage or microglia, in sufficient quantities for drug discovery is extremely challenging and so a number of immortalized human monocyte cell lines and primary material from murine and rodent sources are available to act as a surrogate of the phagocytic ability of human microglial cells. We have established robust high content assays in these cell types to assess phagocytic function with the use of various fluorescent substrates, these have been successfully multiplexed with quantification of target proteins and cell viability assessments. These assays are HTS compatible and are capable of being performed in 1,536 well plates, allowing conservation of precious primary material and expensive fluorescent reagents.

More recently, iPSC derived monocyte and macrophage cells have become commercially available for use within these assays. As these can be obtained from patients with underlying CNS diseases, they may provide information on therapeutics which can modify some of the phenotypic markers of CNS disease.

Here we describe a selection of the assays we have established to study phagocytic activity as a surrogate for microglial function.

## 2 Materials & Methods

THP-1 and Neuro2a cells were obtained from commercial vendors and cultured in the absence of antibiotics as described in protocols supplied with cells.

LPS (10 ng/ml) and Cytochalasin D (10 µM) are used as positive and negative controls to increase and decrease phagocytosis respectively.

A range of substrates have been used including red and green pHrodo<sup>®</sup> bioparticles, FluoSpheres<sup>®</sup> of various different sizes, coatings and fluophores, fluorescently labelled amyloid beta and fluorescently labelled apoptotic Neuro2a cells. The substrate concentration used is dependent upon cell type and assay format.

For all assays; test compounds and substrate are added simultaneously.

Cell staining has been performed with a range of different reagents including DiD, Dil, DiO and different fluophore CellMask<sup>™</sup> stains.

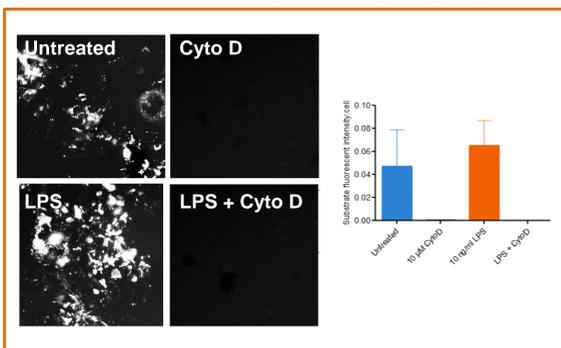
These assays can be performed in a "live cell" format but can also be fixed using paraformaldehyde and standard ICC staining performed.

iPSC derived macrophages were supplied by Axol Bioscience Ltd and were derived from a healthy donor using an episomal vector.

All assays are read using a GE Healthcare Life Sciences InCell 2200 Cell Imaging System.

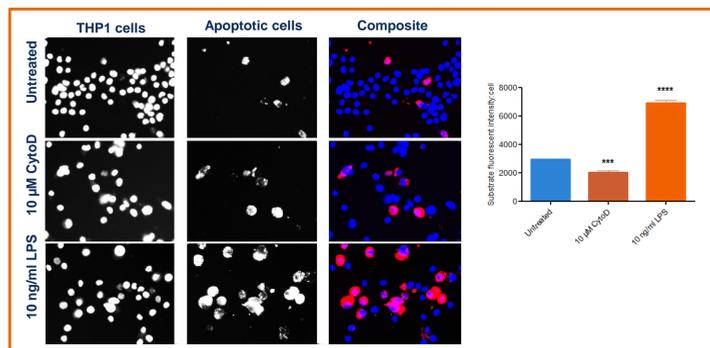
## 3 Results

### pHrodo<sup>®</sup> E. Coli Bioparticle phagocytosis



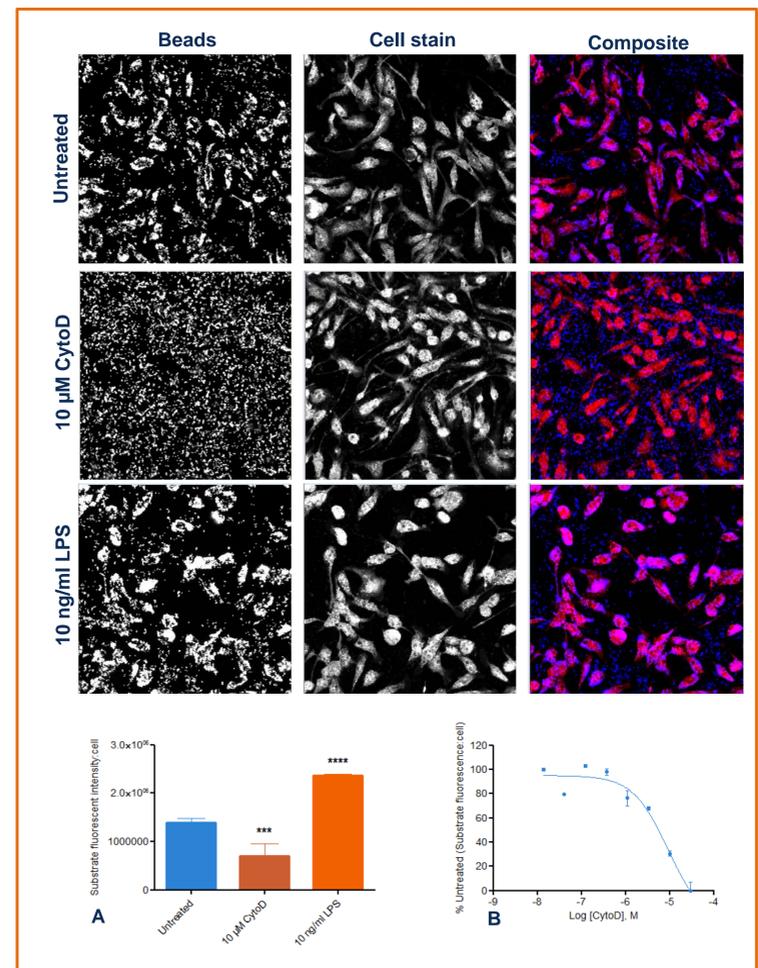
**Figure 1:** Phagocytosis of pHrodo<sup>®</sup> Red *E. Coli* bioparticles by naive THP1 cells. Briefly, THP1 cells were seeded onto 96 well plates and incubated overnight. 10 ng/ml LPS and/or 10 µM Cytochalasin D (CytoD) was then added to the cells in conjunction with the pHrodo<sup>®</sup> particles and incubated for 24 hours. Images were then taken and the fluorescence intensity of the pHrodo<sup>®</sup> particles was measured using the InCell 2200 and normalized to cell number. As can be observed, in untreated conditions, cells phagocytosed bioparticles which was inhibited by the addition of CytoD. LPS did not significantly increase uptake, likely due to the fact that the pathways stimulated by LPS were already activated by the *E. coli* particles.

### Apoptotic cell phagocytosis



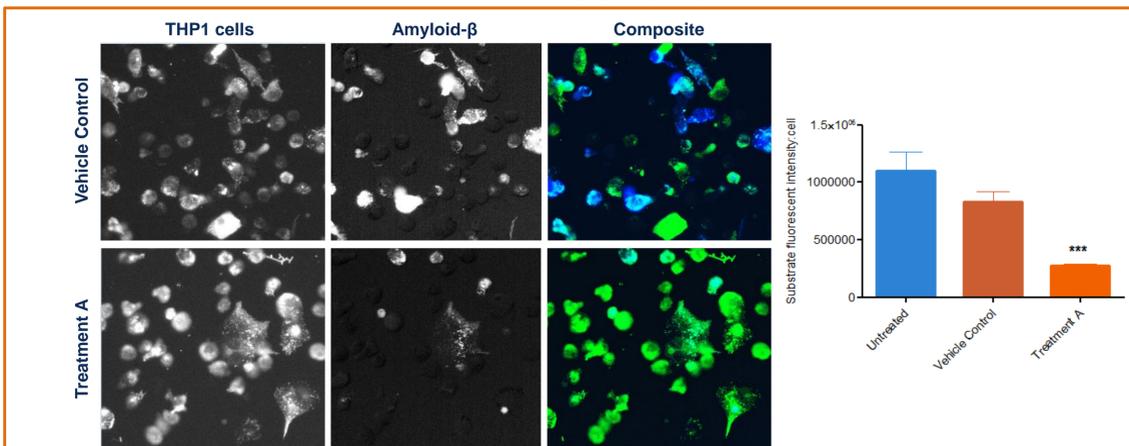
**Figure 2:** Phagocytosis of apoptotic Neuro 2A (N2A) cells by naive THP1 cells. Briefly, THP1 cells were loaded with DiD live cell stain (blue in image) and seeded onto 96 well plates and incubated overnight. Separately, N2A cells were loaded with a cell stain (red in image) and incubated with Staurosporine to induce apoptosis overnight. The following day, 10 ng/ml LPS and/or 10 µM CytoD was added to the cells in conjunction with the apoptotic N2A cells and incubated for 24 hours. Images were then taken and the fluorescence intensity of the N2A cell stain within THP1 mask was measured using the InCell 2200 and normalized to THP1 cell number. As can be observed in untreated conditions, cells phagocytosed low amounts of N2A cells, and so addition of CytoD did not have a large effect. LPS had a strong effect on uptake. \*\*\* p<0.001 compared to untreated; \*\*\*\* p<0.0001 compared to untreated

### FluoSpheres<sup>®</sup> phagocytosis



**Figure 4:** Phagocytosis of FluoSpheres<sup>®</sup> by iPSC derived macrophages. iPSC derived macrophages were differentiated and provided by Axol Bioscience in 96 well plates. These were then treated with LPS or varying concentrations of CytoD. As can be observed, CytoD and LPS have a significant and substantial effect on bead uptake (A) and CytoD treatment has a dose response relationship with FluoSpheres<sup>®</sup> phagocytosis (B). \*\*\* p<0.001 compared to untreated; \*\*\*\* p<0.0001 compared to untreated.

### Fluorescent Amyloid-β phagocytosis



**Figure 3:** Phagocytosis of fluorescently labelled amyloid-β. THP1 cells were seeded onto 96 well plates and incubated overnight. Cells were loaded with DiI live cell stain (green in image) and treated with either vehicle control or "treatment A" and incubated with fluorescently labelled Amyloid-β (Blue in image) for 24 hours. Wells were then imaged on the InCell 2200 and the fluorescence intensity of Amyloid-β within cells was measured and normalized to THP1 cell number. As can be observed, untreated cells uptake amyloid-β, a process which can be modulated by targeted treatment. \*\*\* p<0.001 compared to untreated.

## 4 Conclusions

Charles River Discovery have established a suite of assays to study the phagocytic activity of various cell types as a surrogate for microglial function. These assays have been shown to be:

- Fully HTS compatible;** have been successfully miniaturized to 1,536w format and are able to be multiplexed with quantification of proteins of interest and cell viability assessments.
- Robust** with low intra- and inter-plate variance observed and reproducible pharmacology across multiple days.
- Highly flexible,** amenable to the study of many different phagocytosis and disease processes simply by fluorescently tagging the substrate of interest, including amyloid beta, apoptotic cells, bacterial components and carboxylate FluoSpheres<sup>®</sup>.

The recent availability of monocyte lineage iPSCs allows access to cells obtained from patients suffering from CNS diseases which may provide more relevant therapeutics. We have been able to substitute these cells into our suite of assays with minimal modifications and have used these to assess a putative therapeutic to modulate phagocytosis.

## 5 References

- Cartier N, Lewis C-A, Zhang R, Rossi FMV. The role of microglia in human disease: therapeutic tool or target? *Acta Neuropathologica*. 2014;128(3):363-380. doi:10.1007/s00401-014-1330-y.
- Ribes S, Ebert S, Regen T, et al. Toll-Like Receptor Stimulation Enhances Phagocytosis and Intracellular Killing of Nonencapsulated and Encapsulated *Streptococcus pneumoniae* by Murine Microglia. *Infection and Immunity*. 2010;78(2):865-871. doi:10.1128/IAI.01110-09.

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