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


1 Discovery from Charles River, Chesterfield Research Park, Saffron Walden, Essex, CB10 1XL, UK
2 Discovery from Charles River, Darwinweg 24, 2333 CR Leiden, Netherlands
3 Neurodegeneration Consortium, MD Anderson Cancer Center, Institute for Applied Cancer Science, Houston, Texas, USA
4 Axol Bioscience, Chesterfield Research Park, Saffron Walden, Essex, CB10 1XL, UK

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 including Amyloid Lateral Sclerosis (ALS), Alzheimer’s, Huntington’s and Parkinson’s Disease (AD, HD & PD respectively) and Multiple Sclerosis (MS).

Accessing 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 Neurona 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® bioparticles, FluoSpheres® of various different sizes, coatings and fluorophores, fluorescently labelled amyloid beta and fluorescently labelled apoptotic Neumoa 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 DIL, Dil, DIO and different fluorophore CellMask® 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® E. Coli Bioparticle phagocytosis

Apoptotic cell phagocytosis

FluoSpheres® bioparticle phagocytosis

Fluorescent Amyloid-β phagocytosis

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:

1) Fully HTS compatible; have been successfully miniaturized to 1,536-well format and are able to be multiplexed with quantification of proteins of interest and cell viability assessments.
2) Robust with low intra- and inter-plate variance observed and reproducible pharmacology across multiple days.
3) 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®.

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

pHrodo® and FluoSpheres® are registered trademarks of Thermo Fisher Scientific.
CellMask® is a trademark of Thermo Fisher Scientific.