Human iPSC Derived Microglia Cell Assays

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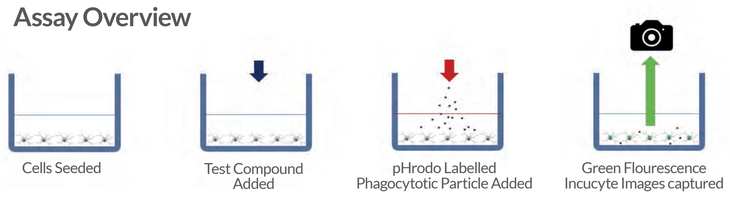
Introduction

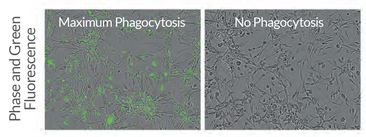
- Microglia are the resident immune cell of the central nervous system (CNS) and pivotal to the neuroinflammatory response and protection of neuronal function
- The cells make up 10-20% of the cells of the CNS and in a resting state survey their microenvironment for threats to homeostasis within the neuronal microenvironment that would interfere with neuronal function. This homeostasis can be disrupted by bodies such as protein aggregates and apoptotic neurons, leading to both acute and chronic neuroinflammation
- Microglia are activated following the detection of a threat to this homeostasis and undergo a number of phenotypic responses (cell migration, proliferation, cytokine release and phagocytosis), preventing change to the homeostasis within the neuronal microenvironment and associated detrimental effects on neuronal functional
- The ability of microglial to detect and respond to aggregated proteins (E.g. ß-Amyloid plaques, Lewy bodies/α-synuclein) or apoptotic neurons is important in the pathogenesis of a range of neurodegenerative diseases like Alzheimer's Disease, Motor Neurone Disease and Parkinson's Disease
- This work describes the use of iPSC derived microglia to develop cell models to functionally characterise a range of phenotypic activities (cytokine release, phagocytotic activity and cell migration) *in-vitro*
- The ability to measure these functions in a lab will allow us to expedite the development of more clinically predictive disease relevant human cell models for a range of neurodegenerative diseases with the aim of identifying more effective treatments any improved model will bring

Phagocytosis Assay

Assay Overview



- iPSC derived microglia cells were seeded into transparent bottomed 96 or 384 well plates and incubated at 37°C/ 5% CO_2
- Test compounds (either DMSO or aqueous solution) were acoustically dispensed using an Echo555 and pre-incubated with the cells for 0.5 – 1 hour
- The appropriate pHrodo labelled particle was dispensed into the well containing cells and compound and the plate placed on an IncuCyte® S3 Live Cell Analysis System
- Phase and either green or red fluorescent images were captured at intervals for later analysis

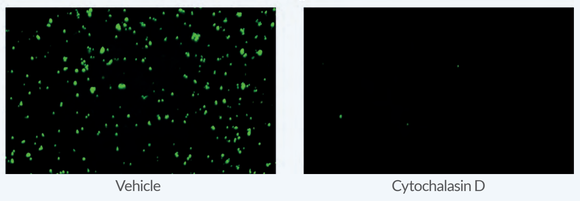


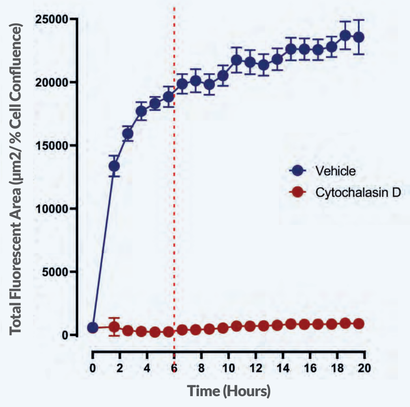
- An algorithm was constructed to generate a mask defining regions of high fluorescence
- The total fluorescent area was calculated per image and used as a measure of the level of phagocytosis

Phagocytosis of E. coli Bioparticles by Human iPSC Microglia

- iPSC derived microglia cells phagocytose E.coli bioparticles with the signal plateauing about 6 hours after addition of the pHrodo labelled particle
- Phagocytosis is inhibited by the cytochalasin D - an inhibitor of actin polymerisation

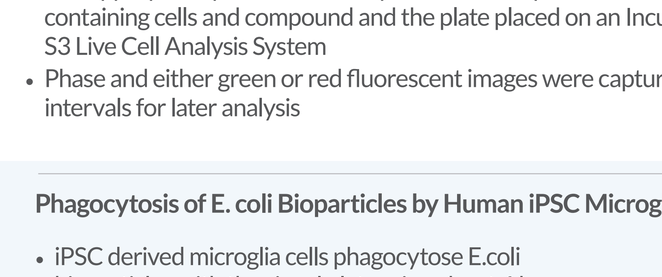
Green Fluorescent Image at 6 hours





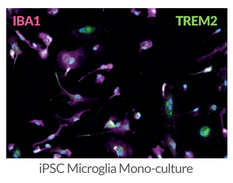
Phagocytosis of a Diverse Panel of Particles

Green/ Red Fluorescent image captured at 10 hours post addition of particle

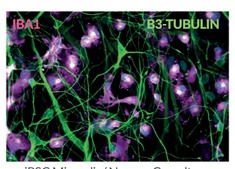


- Human iPSC derived microglia can additionally be assayed for phagocytotic activity with a variety of pHrodo labelled particles:
  - E. coli bioparticles, ß-Amyloid peptide and α-synuclein peptide was labelled with pHrodo Green
  - Zymosan bioparticles or apoptotic M059J cells (Glioblastoma cell line treated with doxorubicin) were labelled with pHrodo Red
- Phagocytosis was inhibited using the actin polymerisation inhibitor, Cytochalasin D, to ensure the specificity of the signal for phagocytosis activity

Phagocytosis in a Co-culture of Human iPSC Microglia and Neurons

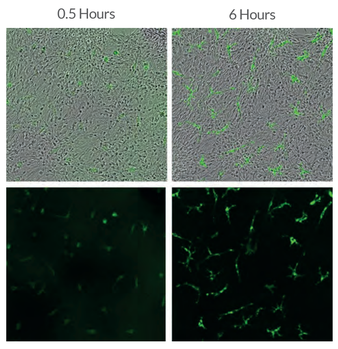


iPSC Microglia Mono-culture



iPSC Microglia/ Neuron Co-culture

- iPSC derived microglia cells were cultured with iPSC derived neurons and the individual cell types identified with immunocytochemistry using Iba1 and TREM2 (microglia) and ß3-tubulin (neurons)
- A phagocytosis assay performed on the co-culture using pHrodo labelled ß-Amyloid 1-42 peptide demonstrated that iPSC microglia in co-culture with the iPSC neurons, whilst morphologically different can still functionally phagocytose

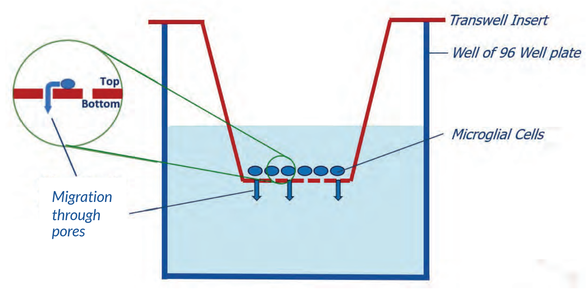


Phagocytosis of ß-Amyloid by iPSC Microglia in co-culture

Cell Migration Assay

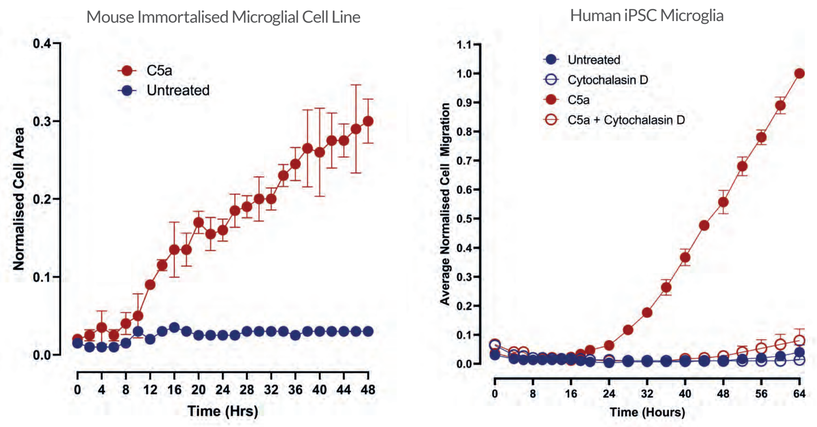
Assay Overview

- Cell migration of iPSC derived microglia cells was examined using the Incucyte® Chemotaxis System from Sartorius
- In response to the addition of stimuli the cells migrate from the top of a membrane through an 8µm pore through to the bottom of the membrane. Any increase in cell area on the bottom of the insert signifies cell migration
- Cell migration in response to the inflammatory mediator, complement component 5a (C5a) was examined in human iPSC derived microglia and compares with a mouse immortalized cell line (C8-B4)



C5a Induced Cell Migration

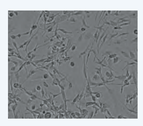
- Cell migration is induced in both the mouse immortalized and human iPSC derived microglia cell models, albeit on different timescales



Cytokine Release Assay and Cell Profiling

Assay Overview

- The release of cytokines from human iPSC microglia challenged with a variety of molecules (LPS or LPS/ATP, ß-Amyloid, α-Synuclein, Interferon-γ) have been profiled using several pioneering technologies available at the Medicines Discovery Catapult
- Both the supernatant media surrounding the treated cells in addition to the cells themselves can be profiled



iPSC Microglia

Media: Cytokine Ab Array
Luminex
Quanterix Simoa

Cells: Nanostring
Mass Spec Imaging

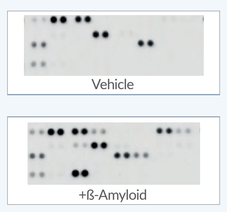
Cell media was removed and analysed using several technologies:

- **Cytokine Antibody Array**- detection of up to 105 cytokines
- **Luminex Technology/Quanterix Simoa**- Quantification of specific cytokines from small volumes of cell media

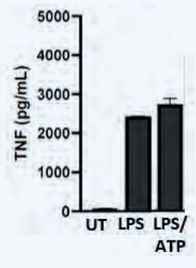
Cells can be analysed using the following technologies:

- **Nanostring nCounter**- Expression profile of up to 800 genes
- **Mass Spec Imaging**- Phospho-lipid analysis

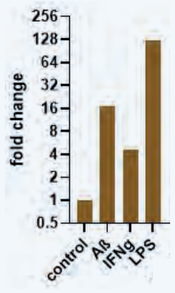
Cytokine Antibody Array



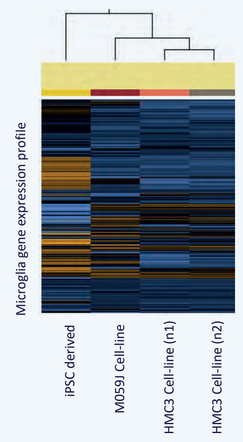
Luminex



Quanterix Simoa



Nanostring nCounter Transcriptome Profiler



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Through collaborative programmes of R&D we are tackling the most challenging issues in drug discovery, addressing systemic problems and bottlenecks and using innovative technologies to enable "fast-to-patient" medicines discovery.

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