An iPSC-derived Neuroinflammation/Neurotoxicity *In Vitro* Model of Neurons and Glial Cells

Malika Bsibsi[1], A. Popalzij[1],Matteo Zanella[1], Lieke Geerts[1], Mark Musters[1], Inês Ferreira[2], Stefan Kostense[1], David F. Fischer[3] and Marijn Vlaming[1]
[1] Charles River, Leiden, NL, [2] bit.bio, Babraham Research Campus, Cambridge, UK, [3] Charles River, Chesterford Research Park, UK

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1 INTRODUCTION

Neuroinflammation occurs in most, if not all, neurodegenerative and inflammatory diseases. Mono *in vitro* cultures of astrocytes and microglia are powerful tools to study specific molecular pathways involved in neuroinflammation. However, more complex neuronal *in vitro* models are required to capture the effects of cellular communication on neuroinflammation and neurodegeneration in a human-based model which reduces animal testing in the early stages of drug discovery.

The Goal: to set up a complex *in vitro* culture model of iPSC derived-, neurons (N), astrocyte (A), microglia (M) and oligodendrocytes (O) to study neuroinflammation and neurodegeneration.

2 METHODS

The different iPSC derived cell types were cultured according to the manufacturer's protocols (ioGlutamatergic Neurons, iOligodendrocyte-like cells, ioMicroglia from Bit.bio and iPSC derived astrocytes from FujiFillm). Neuroinflammation and neurotoxicity were triggered in this model with LPS, nigericin and beta amyloid fibrils, followed by electrochemiluminescence-based detection to measure Neurofilament light chain (NfL); an established biomarker associated with neurotoxicity and neurodegeneration.

To assess the health of the cultures upon treatment immunocytochemistry (ICC) was performed for DAPI, MBP, beta-III tubulin and Neurofilament H (NFH). High content imaging was performed using the Yokogawa CV8000 and Arivis was used to create an algorithm to quantify live nuclei and MBP production

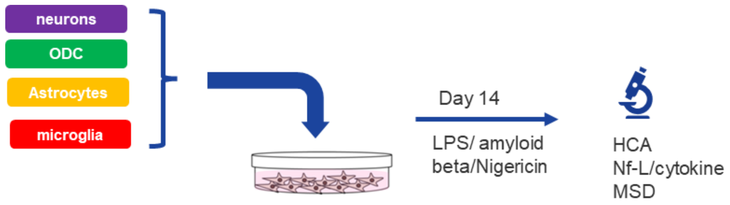

Figure 1. : Schematic workflow of multi-cellular co-culture using iPSC-derived CNS cell types.
Neurons, astrocytes, oligodendrocytes and microglia were thawed and seeded at day 0. Treatment with several neuroinflammatory triggers was started at day 14 and performed for 48 hours up to day 16. at day 16 supernatants were harvested, and plates were fixed.

3 RESULTS

Phenotypic characterization by immunostaining

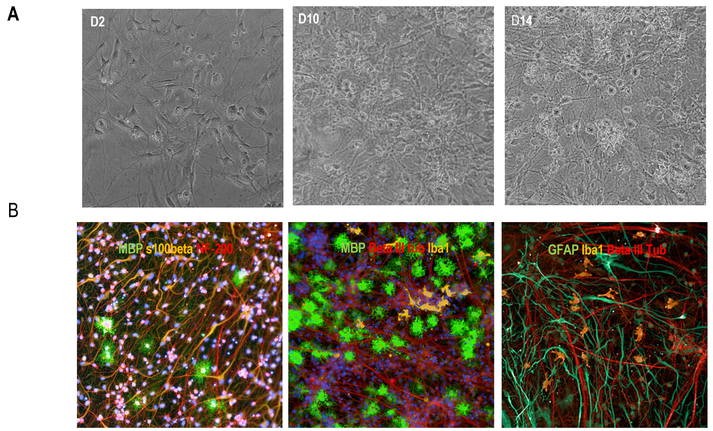

Figure 2: characterization of the multi-cellular co-culture model
A) Brightfield live images (200x total magnification) at different culture time points post-co-culture. B) Representative images of multi-cellular co-cultures. Cells were stained for DAPI, MBP as myelin/oligodendrocyte marker, beta-III tubulin or NF-200 to stain neuronal axons, GFAP as astrocyte marker and Iba1 microglia marker.

Live nuclei and MBP Quantification in multi-cellular co-cultures upon treatment with different insults

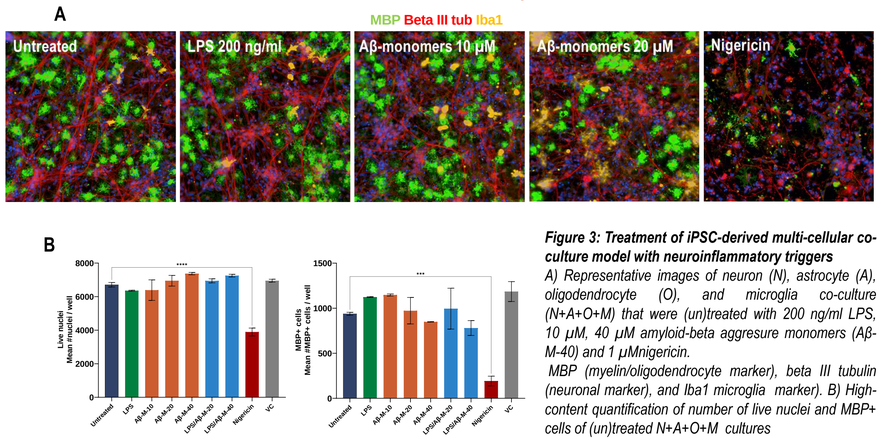

Figure 3: Treatment of iPSC-derived multi-cellular co-culture model with neuroinflammatory triggers
A) Representative images of neuron (N), astrocyte (A), oligodendrocyte (O), and microglia co-culture (N+A+O+M) that were (un)treated with 200 ng/ml LPS, 10 µM, 40 µM amyloid-beta aggresure monomers (Aβ-M-40) and 1 µMnigericin.
MBP (myelin/oligodendrocyte marker), beta III tubulin (neuronal marker), and Iba1 microglia marker). B) High-content quantification of number of live nuclei and MBP+ cells of (un)treated N+A+O+M cultures

Neurofilament light chain (Nf-L) measurement

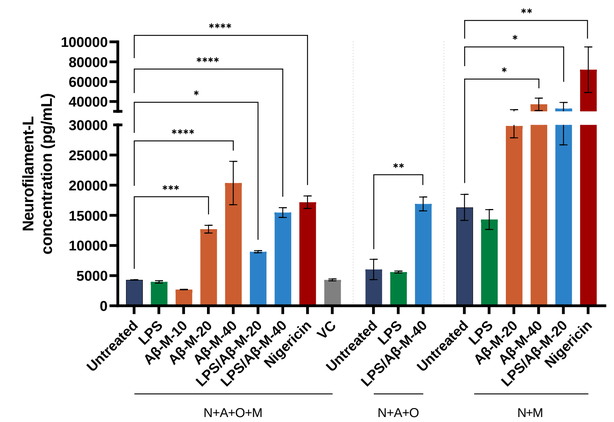

Figure 4: Neurofilament-L (Nf-L) release upon treatment with neuroinflammatory triggers
NF-L release in supernatant harvested after treatment of iPSC-derived multi-cellular co-cultures with different triggers including LPS and amyloid-beta aggresure monomers (Aβ-M-40) for 48 hours. Bars represent mean values of duplicate wells with error bars indicating standard deviation.
*[One-way ANOVA with Tukey's multiple comparison vs untreated of respective co-culture (N+A+O+M, N+A+O, or N+M) or unpaired T-test ; *p<0.05; **p<0.005; ****p<0.0001; not-significant not indicated].*

4 HEADER

In conclusion, Charles River successfully implemented a multicellular co-culture model using iPSC-derived glutamatergic neurons, oligodendrocyte-like cells, astrocytes and microglia that can be used in the early stages of drug discovery.
Inducing acute neuroinflammation with the tested triggers caused neuronal and oligodendrocyte damage and microglia activation and clustering, specially with a high concentration of Aβ monomers. Hin addition, nigericin induced excessive toxicity.
Next to high imaging, Nf-L detection on culture supernatant was used as a measure for neurotoxicity and degeneration upon treatment with the different triggers. Remarkably, in neuron-microglia cultures (N+M) higher NF-L release was measured compared to muti-cellular cultures N+A+O+M and N+A+O.