Establishment and Validation of an *In Vitro* Co-Culture Model to Study Myelination Using Human iPSC-derived Glutamatergic Neurons and Oligodendrocytes

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

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1 INTRODUCTION

Oligodendrocytes, the myelinating cells of the central nervous system (CNS), wrap their cell membrane around axons to support rapid nerve impulse conduction. They develop from bipotential oligodendrocyte-type-2-astrocyte progenitors (O2A), which can differentiate *in vitro* into either oligodendrocytes or astrocytes. Oligodendrocyte progenitor cells (OPC) react in human adult CNS to injury by proliferation and migration. Oligodendrocyte dysfunction and disrupted myelin is involved in the pathogenesis of neurodegenerative disease such as multiple sclerosis (MS) and Alzheimer's disease (AD). Although microglia and astrocytes have been extensively characterized in neurodegeneration, oligodendrocytes have received less attention due to the complexity of primary oligodendrocyte isolation and culturing. Induced pluripotent stem cells (iPSCs)-derived oligodendrocytes can provide a suitable solution to study differentiation and maturation of oligodendrocytes as well as myelination.

Goals: 1) to characterise commercially available iPSC-derived oligodendrocytes (ioOligodendrocyte-like cells, bit.bio) and 2) to develop a co-culture *in vitro* model with iPSC-derived glutamatergic neurons (ioGlutamatergic Neurons, bit.bio) to evaluate the myelination processes and oligodendrocyte maturation

2 METHODS

ioGlutamergic neurons and ioOligodendrocyte-like cells from bit.bio were cultured according to the manufacturer's protocols.
At day 0 ioGlutamergic neurons and ioOligodendrocyte were cultures separately and at day 4 ioOligodendrocyte were detached and resuspended in co-culture media consisting of BrainPhys (Stemcell) with oligodendrocyte and neuronal supporting supplements. Upon thawing, ioOligodendrocyte like cells resemble a pre-myelinating oligodendrocyte state and they then rapidly mature and acquire a typical oligodendrocyte-like morphology with multiple branched processes, from day 6 of culture onwards. Cell were fixed at multiple time points for immunocytochemistry. Immunocytochemistry 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 analyse total MBP production and myelination.

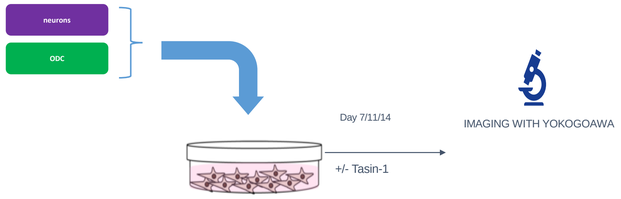

Figure 2. Schematic workflow of ioGlutamatergic neurons and ioOligodendrocyte-like cells co-culture
Neurons and oligodendrocytes were first cultured separately up to day 4. Oligodendrocytes were detached and added to neurons (Day 0 post-co-culture). Co-culture was maintained for up to day 7, day 11, or day 14 post-co-culture.

3 RESULTS

MBP expression in ioOligodendrocytes-like cells monocultures

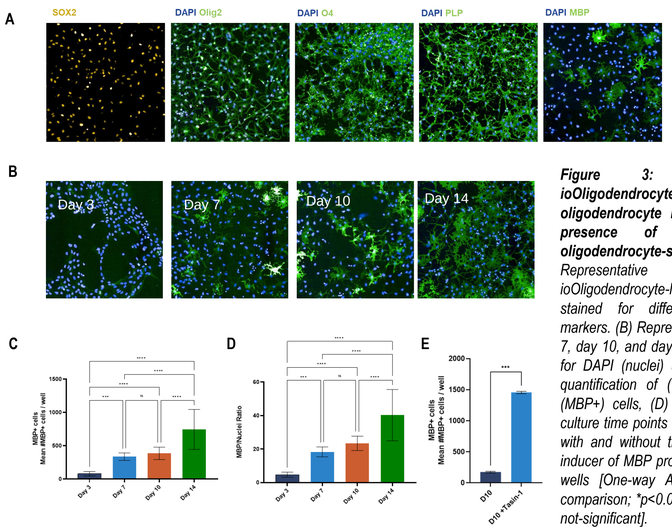

***Figure 3: Characterization of ioOligodendrocyte-like cells show oligodendrocyte morphology in culture and presence of early and late-stage oligodendrocyte-specific markers** . (A) Representative images of day 10 ioOligodendrocyte-like cells (20x objective) stained for different oligodendrocyte-specific markers. (B) Representative images of day 3, day 7, day 10, and day 14 post-thaw cultured stained for DAPI (nuclei) and MBP. (C-E) High-content quantification of (C) number of MBP positive (MBP+) cells, (D) MBP/Nuclei ratio at different culture time points and (E) number of MBP+ cells with and without treatment with Tasin-1 (known inducer of MBP production) at day 10. (C-E) N=2 wells [One-way ANOVA with Tukey's multiple comparison; *p<0.05; **p<0.005; ****p<0.0001; ns not-significant].*

MBP expression in ioOligodendrocyte + ioGlutamatergic neurons co-cultures

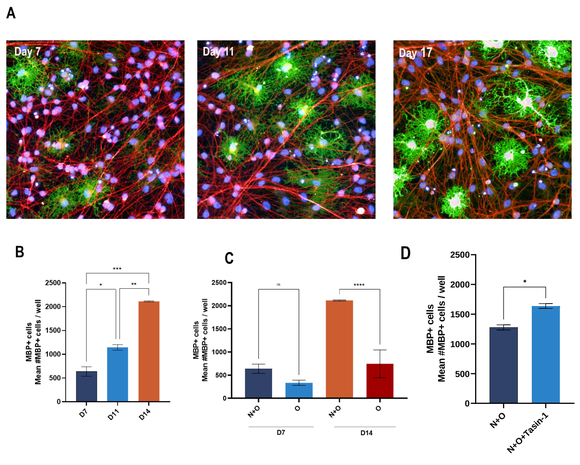

***Figure 4: Myelin production in ioGlutamatergic Neuron and ioOligodendrocyte co-culture.** (A) Representative images of ioOligodendrocytes and ioGlutamatergic neurons co-culture at different co-culture time points. Cells were stained for DAPI, MBP as oligodendrocyte marker, and beta-III tubulin as neuronal marker. (B) High-content quantification of number of MBP+ cells at different co-culture time points (D) number of MBP+ cells comparing neuron-oligodendrocyte co-culture (N+O) versus mono-culture of oligodendrocytes (O) at day 7 and day 14 post co-culture (N+O) or post-thaw (O) and (E) number of MBP+ cells of day 14 N+O co-culture in untreated and Tasin-1 treated cells (C-E) N=2 wells [One-way ANOVA with Tukey's multiple comparison or unpaired T-test; *p<0.05; **p<0.005; ****p<0.0001; not-significant not indicated].*

Axonal wrapping in ioOligodendrocyte + ioGlutamatergic neurons co-cultures

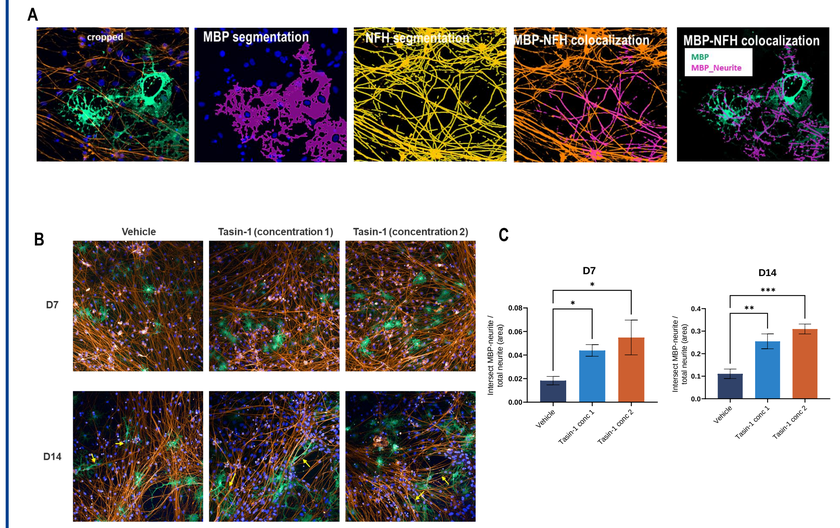

Figure 5: Neurite/axonal wrapping wrapping in ioGlutamatergic Neuron and ioOligodendrocyte co-culture.
*(A) Segmentation of MBP and Neurofilament H (NFH) signals in a representative image of N+O co-culture. (B) Representative images of day 7 (upper row) and day 14 (lower row) N+O co-cultures stained for DAPI (nuclei), MBP (oligodendrocytes) and NFH (neurites). (C) High-content quantification of the intersection of MBP and NFH signals in day 7 (left graph) and day 14 (right graph) N+O co-culture. N=2 wells [One-way ANOVA with Tukey's multiple comparison; *p<0.05; **p<0.005].*

4 CONCLUSIONS

In conclusion, Charles River has successfully established a relevant *in vitro* mono- and co-culture myelination model using iPSC-derived ioOligodendrocyte cells and ioGlutamatergic Neurons. Immunofluorescent staining of ioOligodendrocyte cells at different time points showed positive staining for key oligodendrocyte lineage markers including Olig2, O4, and SOX2 and myelin markers, including myelin-binding protein (MBP) and myelin proteolipid protein (PLP). At day 3 post seeding the O4+ cells displayed a typical OPC-like morphology. They mature into oligodendrocyte-like cells with characteristic multiple branched processes. Co-culture of ioGlutamatergic Neurons and ioOligodendrocyte cells resulted in increased number of MBP+ cells compared to monocultures of oligodendrocytes in a time-dependent manner. Importantly, high content imaging showed that MBP+ cells surrounded neurites in the co-culture, indicating myelination of neuronal axons.