A Functional *In Vitro* Model of iPSC-Derived Oligodendrocytes for Studying Myelination and Demyelinating Diseases

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Introduction

Oligodendrocytes are glial cells of the central nervous system responsible for forming the myelin sheath around neuronal axons, thereby facilitating rapid nerve impulse conduction and protecting axons from degeneration. Demyelination and oligodendrocyte dysfunction are implicated in various neurodegenerative disorders, including multiple sclerosis, Alzheimer's disease, and Parkinson's disease. To better investigate the role of oligodendrocytes in these conditions, induced pluripotent stem cell (iPSC)-derived oligodendrocytes have been increasingly utilized. However, many in vitro models struggle to generate functionally myelinating oligodendrocytes. In this study, we aimed to develop functional iPSC-derived oligodendrocyte-like cells using the Quick-Glia™ technology. Our results showed that the iPSC-derived oligodendrocytes expressed mature myelinating markers including CLDN11, GALC, CNP, MAG, PLP, and MBP. After three days *in vitro* culture on the micropillar plates, the cells successfully ensheathed the micropillars, with 83% of the population co-expressing O4 and MBP, indicating a high degree of functional differentiation. This platform demonstrates robust myelination capacity and offers a reliable in vitro model for studying oligodendrocyte biology, disease mechanisms, and drug screening. Furthermore, it enables the generation of patient-specific oligodendrocytes from iPSCs derived from individuals with demyelinating diseases such as multiple sclerosis, facilitating the evaluation of potential remyelinating therapies and improving our understanding of oligodendrocyte contributions to neurodegeneration.

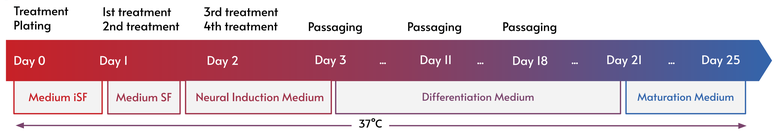
Methods

Human iPSCs were differentiated with our Quick-Tissue™ technology. Oligodendrocyte differentiation and maturation were assessed by RNA sequencing and quantitative real-time PCR, targeting key oligodendrocyte markers. To evaluate the cells' ability to wrap axon-like structures and form myelin, we employed a 96-well micropillar assay combined with immunofluorescence microscopy (Mei *et al.*, 2014).

Source hiPSC line:

- California Institute for Regenerative Medicine (CIRM) human induced pluripotent stem cell (hiPSC) line: CW50065; healthy donor, Caucasian Female, 74 years old

A



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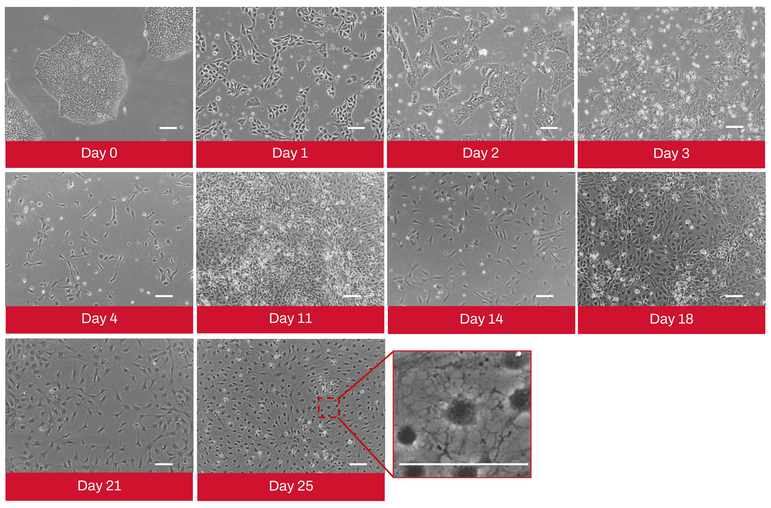
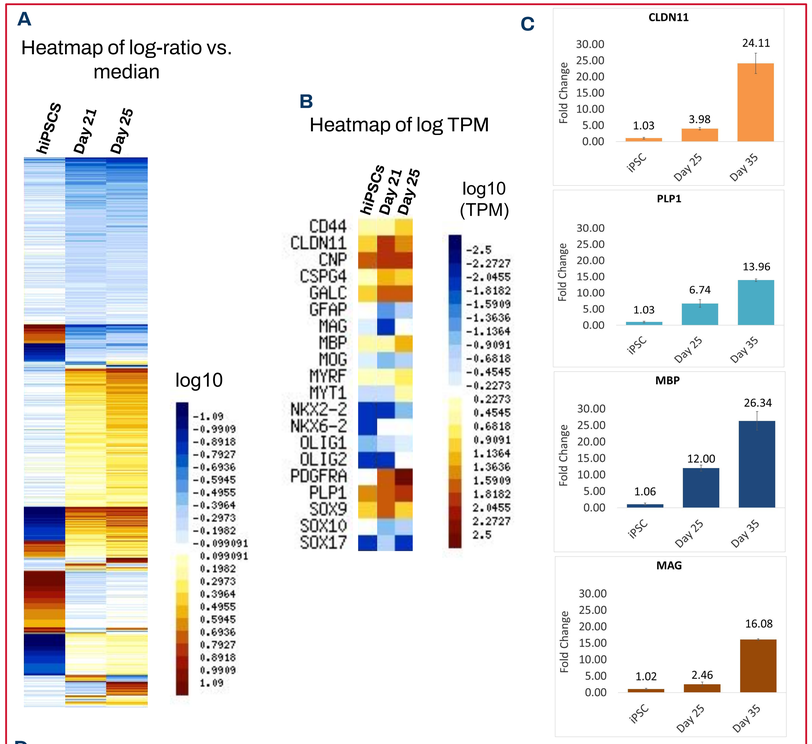
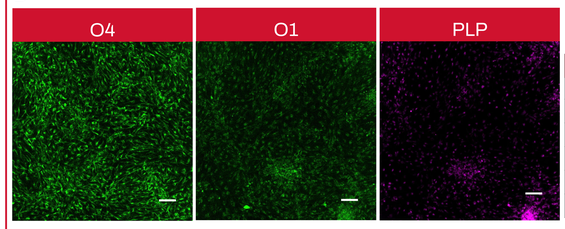


Differentiation of Oligodendrocyte-like cells from hiPSCs using Quick-Glia™ Oligodendrocytes protocol: (**A**) Schematic workflow illustrating the differentiation of oligodendrocyte-like cells from hiPSCs over a 25-day period. (**B**) Representative phase-contrast images showing the morphological changes occurring throughout the 25-day differentiation process (scale bars = 100 µm).

Characterization of marker expression: RNA-seq, RT-qPCR, and IF



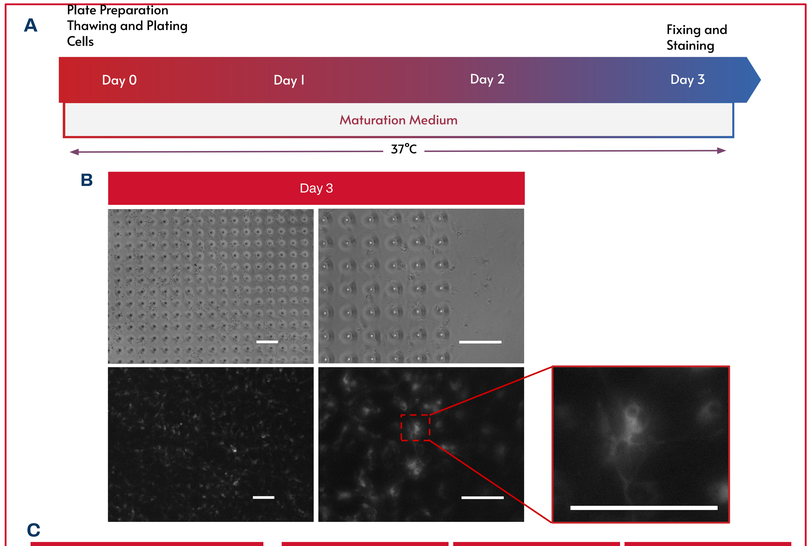
D



hiPSC-derived oligodendrocytes - Day 31		
	O4	O1/PLP1
DAPI count	20401	27602
O4/O1 positive	27852	18186
PLP1 positive	1067	6752
% of O4/O1+	97%	66%
% of PLP1+		37%

Characterization of hiPSC-derived oligodendrocyte-like cells differentiated by the Quick-Glia™ Oligodendrocytes protocol: (**A**) A heat map of total transcripts was generated based on RNA-seq data. (**B**) A heat map with selected transcripts of interest was generated based on RNA-seq data. (**C**) Real-time quantitative PCR analysis of expression levels of Oligodendrocytes-associated genes (PLP1, CLDN11, MBP, and MAG). The relative gene expression is normalized to phosphoglycerate kinase 1 (PGK1), and then calculated as a fold induction relative to hiPSCs as a control. Error bars show standard deviation. (**D**) Immunofluorescence staining of hiPSC-derived Oligodendrocytes culture shows the expression of O4 and O1 (green) and PLP (magenta) on day 31 (scale bars = 100 µm).

Functional assay using micropillar plate



hiPSC-derived oligodendrocytes - Day 3	
Hoechst count	6684
O4 positive	5742
MBP positive	4738
% of O4+	86%
% of MBP+	83%

Imaging of Quick-Glia™ Oligodendrocytes culture during a myelination assay (Mei *et al.*, 2014): Thirty-one days after differentiation induction using Quick-Glia™ Oligodendrocytes, the culture was frozen and stored in liquid nitrogen. On day 0, hiPSC-derived oligodendrocyte like-cells were thawed and plated on a micropillar plate, then incubated at 37 °C with 5% CO_2 for three days. (**A**) The workflow of the myelination assay performed with frozen hiPSC-derived oligodendrocyte like-cells. (**B**) Images taken on day 3 after the assay started are shown. After thawing, hiPSC-derived oligodendrocyte like-cells were transduced with an EGFP-expressing lentivirus on day 0. (**C**) Immunofluorescent staining of Quick-Oligodendrocytes™ culture shows the expression of O4 (green) and MBP (magenta) on day 3 post-thaw (scale bars = 100 µm).

Reference: Mei, F., Fancy, S.P., Shen, Y.A.A., Niu, J., Zhao, C., Presley, B., Miao, E., Lee, S., Mayoral, S.R., Redmond, S.A. and Etxeberria, A., 2014. Micropillar arrays as a high-throughput screening platform for therapeutics in multiple sclerosis. *Nature medicine*, **20**(8), pp.954-960.

Future Directions

- Differentiate oligodendrocytes from various human induced pluripotent stem cell (hiPSC) lines derived from healthy donors and patients with neurodegenerative diseases.
- Evaluate candidate drugs that promote remyelination *in vitro* and *in vivo*.
- Assess compounds that induce or contribute to demyelination to better understand disease mechanisms.

Acknowledgements

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- Please visit us at booth #300, go to www.biosciences.ricoh.com or scan the QR code for more information on our services and products.
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