RAPID AND CONSISTENT GENERATION OF HUMAN IPSC-DERIVED OLIGODENDROCYTE-LIKE CELLS USING OPTI-OX TECHNOLOGY

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Abstract

Oligodendrocytes (OLs) are the myelinating cells in the central nervous system. By ensheathing axons, OLs enhance the action potential conduction velocity. OLs arise from oligodendrocyte precursor cells (OPCs) during pre- and postnatal development. The death of OLs and the impairment of differentiation of OPCs into OLs are a major pathological characteristic in demyelinating diseases.

The development of therapies that promote myelination in neurological conditions, particularly demyelinating diseases, is hampered by the limited translatability of existing preclinical animal models, and the lack of reliable in vitro models. Human induced pluripotent stem cells (hiPSCs) can be used to generate OLs for in vitro applications, however, current differentiation protocols are often lengthy, challenging to reproduce, and are difficult to scale.
Our proprietary opti-ox* (optimised inducible overexpression) technology enables highly controlled expression of transcription factors, deterministically programming hiPSCs into specific cell types of interest, to provide a robust, consistent, and reliable source of human cells for in vitro applications.

We have used opti-ox to rapidly program hiPSCs into oligodendrocyte-like cells (ioOligodendrocyte-like cells), a population of oligodendroglial cells resembling a pre-myelinating oligodendrocyte state.
By day 1, the cells present an OPC-like morphology and are positive for oligodendroglial lineage markers OLIG2, SOX10 and O4. By day 8, the cells show increased complexity with an OL-like morphology, and increased expression of other mature oligodendrocyte markers such as *MBP*, *MAL*, *CNP* and *MYRF*, seen by qRT-PCR, bulk RNA and scRNAseq. Furthermore, whole transcriptome analysis demonstrates equivalent expression profiles between three different manufactured lots indicating consistency and experimental reproducibility.

With over 35% of cells being MBP positive at day 8 (shown by scRNAseq), ioOligodendrocyte-like cells provided a relevant, consistent, and scalable source of human cells that can be used for investigations into novel therapeutics and molecular mechanisms that regulate this critical glial cell type that is implicated in various human diseases.

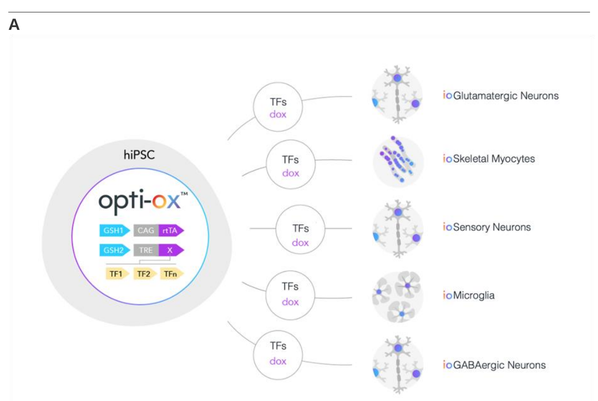
1. Deterministic cell programming of iPSCs into defined human cell types

opti-ox technology for the deterministic cell programming of hiPSCs into defined human cell types.

opti-ox is a dual cassette Tet-ON system that ensures tightly controlled and homogeneous expression of programming TFs by preventing silencing of the inducible expression cassette after genetic engineering of hiPSCs.

TF expression through opti-ox has been demonstrated to generate cell types from all three germ layers in a robust, scalable manner.

Additionally, we have developed a Discovery Platform that allows for the identification of core TF networks that drive cell fate acquisition from pluripotent stem cells.

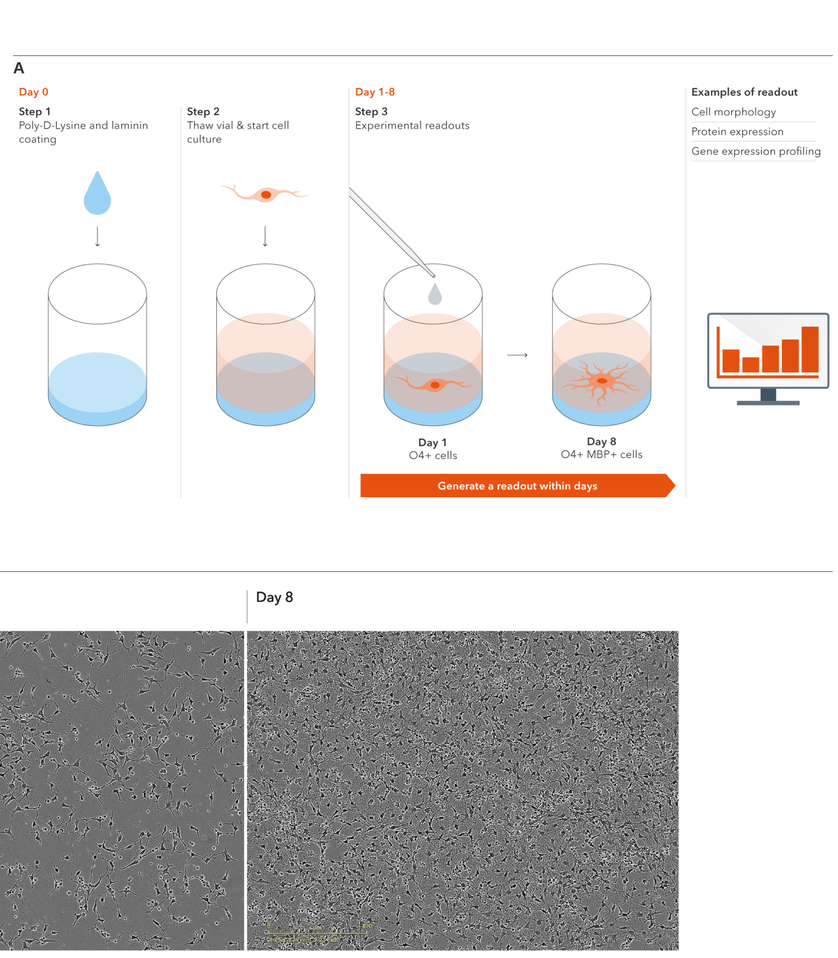


2. Development of an oligodendroglia model for rapid readouts acquisition

ioOligodendrocyte-like cells resemble a pre-myelinating oligodendrocyte state.

(A) ioOligodendrocyte-like cells are programmed to rapidly mature when in a monolayer culture, by following a simple protocol. Upon thawing, cells are ready for experimentation and results can be generated through the recommended culturing period of 8 days.

(B) Upon deterministic programming, cells show rapid morphological changes, acquiring an OPC-like morphology by day 1 post-revival. By day 8, cells have matured and display an oligodendrocyte-like morphology with multiple branched processes.



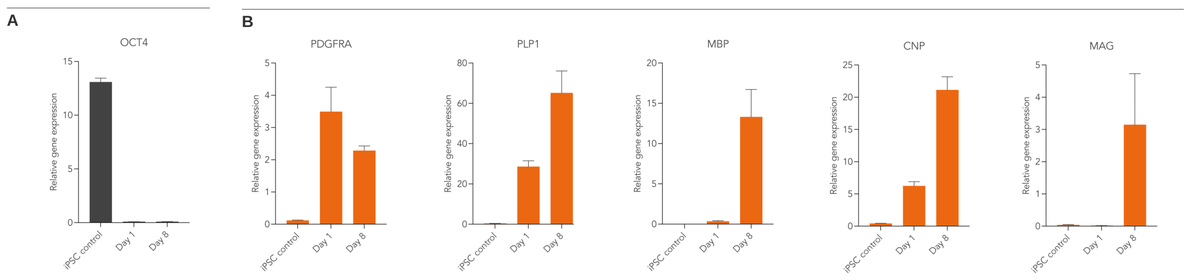
3. qRT-PCR shows that ioOligodendrocyte-like cells express relevant markers and present a maturing oligodendroglial population

ioOligodendrocyte-like cells do not express pluripotent genes and express key oligodendroglial genes showing an increased maturation in the recommended culturing time.

(A) Gene expression of *OCT4*, a key pluripotency gene, is rapidly downregulated and it is not expressed in ioOligodendrocyte-like cells at either day 1 or 8.

(B) The cells express key oligodendroglial markers by day 1, with increased expression of the mature markers *PLP1*, *MBP*, *CNP* and *MAG* by day 8.

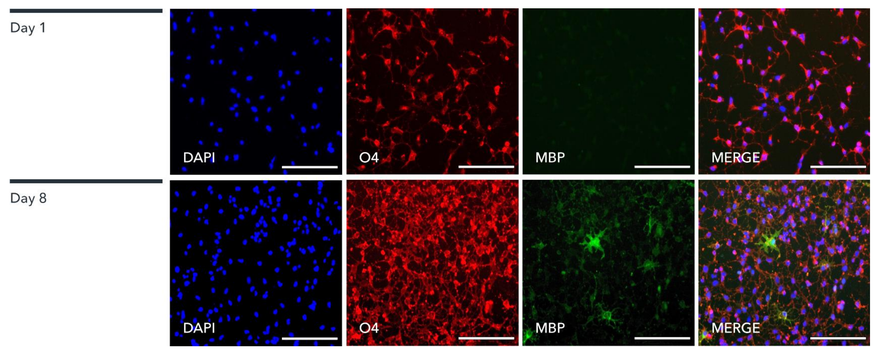
Data is expression relative to the housekeeping gene *HMBS*. n=3 technical replicates.



4. ioOligodendrocyte-like cells homogeneously express key markers and mature over time

Immunocytochemistry demonstrates the homogenous expression of key markers.

Immunocytochemistry on day 1 demonstrates that the cells are positive for O4 and show an OPC-like morphology. On day 8, ioOligodendrocyte-like cells are positive for O4 and MBP, and an increased morphology complexity, showing an increase in the population maturity. DAPI was used as counterstain (blue). Scale bar is 170 µm.



5. scRNAseq shows a pure population of oligodendroglial lineage cells

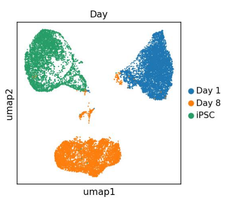
scRNAseq analysis was performed with ioOligodendrocyte-like cells at three specific timepoints: iPSCs, day 1 and 8.

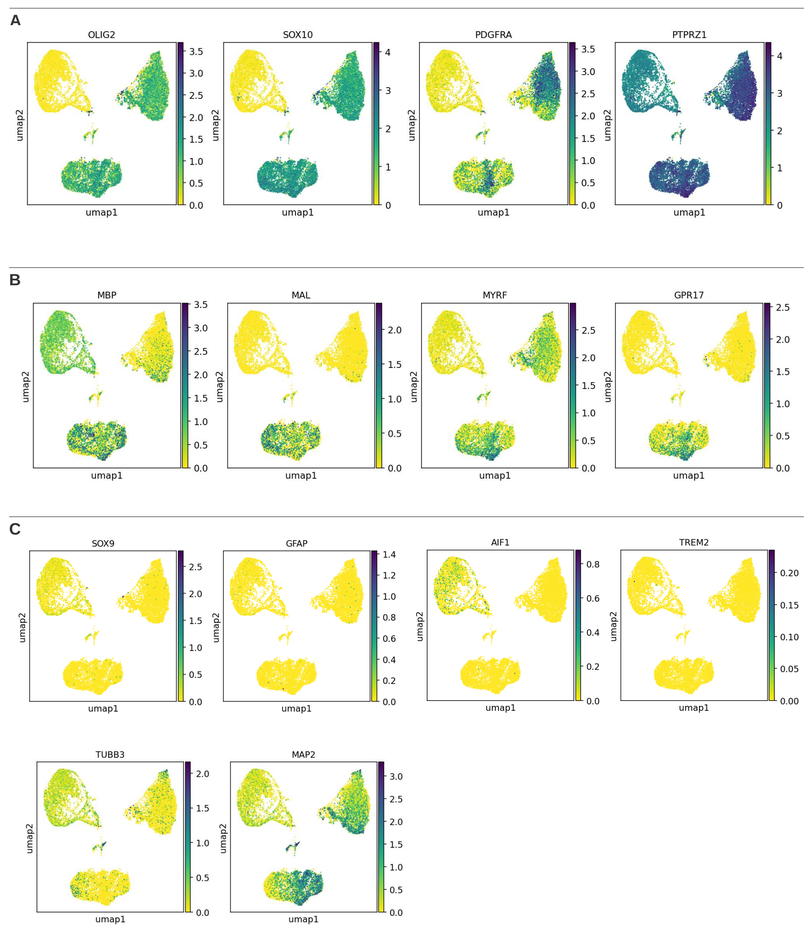
(A) By day 1, the population consists of OPC-like cells that express distinct oligodendroglial lineage genes such as *OLIG2* and *SOX10* and progenitor genes such as *PDGFRA*, and *PTPRZ1*.

(B) By day 8, there is an increase in the expression of genes associated with mature OLs, like *MBP*, *MAL*, *MYRF* and *GPR17*; where *MBP* is expressed in over 35% of cells demonstrating increased maturity of the population.

(C) ioOligodendrocyte-like cells do not show expression of astrocyte markers *SOX9* and *GFAP*, nor of the microglia markers *AIF1* and *TREM2*.
Also, there is no co-expression of the neuronal markers *MAP2* and *TUBB3*, suggesting a pure population of oligodendroglia cells.

Gene expression was assessed by Parse Biosciences single cell RNA-sequencing.



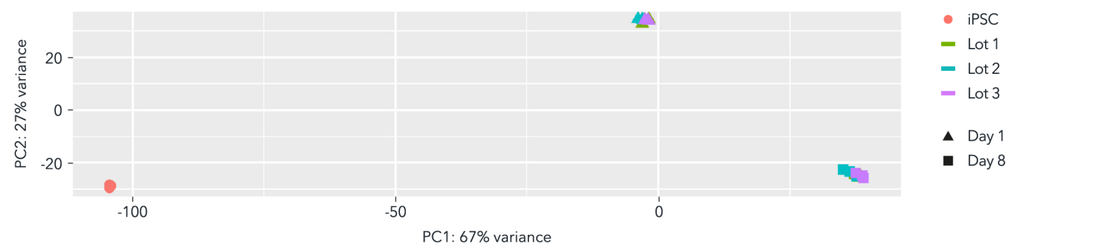


6. Whole transcriptome analysis demonstrates high lot-to-lot consistency of ioOligodendrocyte-like cells

Ready for experimentation from day 1, pure populations of oligodendroglial cells with equivalent expression profiles can be generated consistently from every vial, allowing confidence in experimental reproducibility.

Bulk RNAseq analysis was performed on three different manufactured lots of ioOligodendrocyte-like cells at different time points: iPSCs, day 1 and 8.

Principal component analysis represents the variance in gene expression between samples of ioOligodendrocyte-like cells. This analysis shows high consistency between each programming experiment of ioOligodendrocyte-like cells at each given timepoint.



Summary & conclusions

- We have developed hiPSC-derived oligodendrocyte-like cells, which **resemble a pre-myelinating oligodendrocyte state**.
- The cells can be used as a **valuable tool to study neurodegenerative and demyelinating diseases**, where oligodendroglial cells are known to play a critical role.
- Upon deterministic programming, cells rapidly acquire an OPC-like **morphology** and mature towards an oligodendrocyte-like morphology with multiple branched processes.
- ioOligodendrocyte-like cells express **key oligodendroglia markers** and show signs of maturation by day 8, as seen by ICC, qRT-PCR, and/or scRNAseq.
- By day 8, scRNAseq analysis shows that **35% of the cells are *MBP* positive**.
- **High lot-to-lot consistency** of ioOligodendrocyte-like cells provides confidence in experimental reproducibility.