Human Induced Pluripotent Stem Cell-Derived Oligodendrocyte Progenitors: A Cellular Tool for In Vitro Myelination Studies

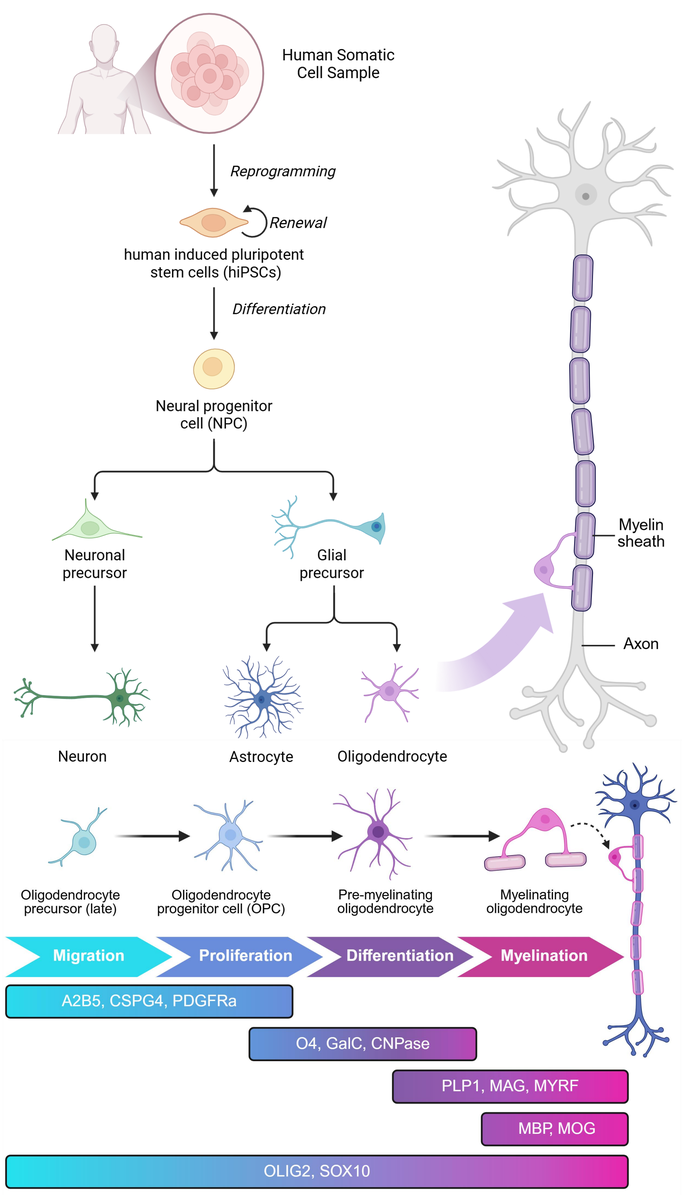
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Abstract

Oligodendrocyte maturation and CNS myelination are essential for functional neural circuitry, and disruption of these processes contributes to numerous neurological diseases. Human induced pluripotent stem cell (iPSC)-derived oligodendrocyte progenitor cells (OPCs) provide a valuable platform for modeling human oligodendrogenesis and myelination in vitro. Here, we developed an optimized differentiation workflow capable of generating highly enriched O4-positive OPC populations in under 30 days. Generated OPCs displayed characteristic progenitor morphology and robust oligodendrocyte lineage identity, confirmed by flow cytometry, immunocytochemistry, and progressive expression of oligodendrocyte and myelin-associated markers including MBP, PLP1, MAG, MOG, CNP, and CLDN11. To investigate the role of multicellular interactions in oligodendrocyte maturation, OPCs were incorporated into both 2D and 3D neural culture systems. In co-culture with human iPSC-derived excitatory neurons, oligodendrocytes developed complex branching morphologies and extended MBP-positive processes closely associated with neuronal networks, consistent with early axonal engagement. Tri-culture systems containing neurons, astrocytes, and OPCs further promoted extensive cellular integration and enhanced oligodendrocyte maturation within a more physiologically relevant neural microenvironment. To model human myelination in three dimensions, we generated human iPSC-derived myelinoids composed of neurons, astrocytes, and oligodendrocytes and maintained them for 8 weeks in 3D culture. Immunostaining confirmed successful incorporation of all three neural cell populations, while transmission electron microscopy revealed both myelinated and unmyelinated fibers throughout the myelinoids. Higher magnification ultrastructural analysis demonstrated varying stages of membrane wrapping and compaction, ranging from loose lamellae to tightly compacted multilamellar structures, consistent with an active and dynamic myelination process. Together, these findings demonstrate that multicellular and three-dimensional environments promote oligodendrocyte maturation and support formation of myelin-like structures in vitro, establishing a scalable human iPSC-derived platform for studying myelination and advancing remyelination therapeutic discovery.

iPSC-Derived OPC Lineage Progression



Human iPSC-derived oligodendrocyte lineage progression and maturation. Schematic overview of human iPSC-derived oligodendrocyte differentiation from somatic cell reprogramming through neural and glial lineage specification to mature myelinating oligodendrocytes. The diagram highlights key developmental stages, including neural progenitor cells (NPCs), oligodendrocyte progenitor cells (OPCs), pre-myelinating oligodendrocytes, and mature myelinating oligodendrocytes, alongside representative lineage markers associated with migration, proliferation, differentiation, and myelination. Mature oligodendrocytes extend processes that wrap axons to form compact myelin sheaths.

Oligodendrocyte Progenitor Characterization

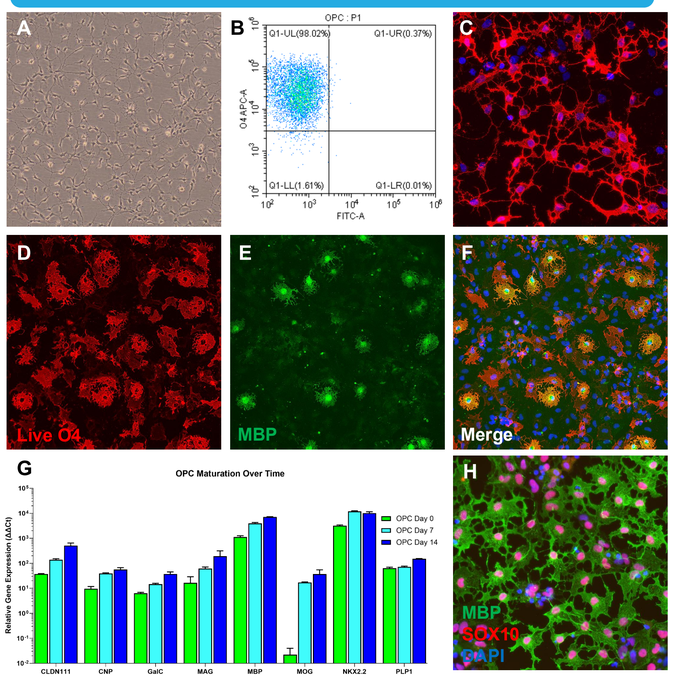


Figure 1. Oligodendrocyte progenitor characterization and maturation. (A) Brightfield image of human iPSC-derived OPCs after plating. (B) Flow cytometry showing high O4 expression following thaw. (C) Representative live O4 staining confirming OPC identity and morphology. (D–F) Immunostaining for O4 (red), MBP (green), and DAPI (blue) demonstrating oligodendrocyte maturation and myelin-associated protein expression. (G) Relative gene expression of oligodendrocyte and myelin-associated markers during maturation. (H) MBP (green) and SOX10 (red) staining with DAPI (blue) confirming oligodendrocyte lineage identity and maturation.

Co-culture of OPCs with Excitatory Neurons

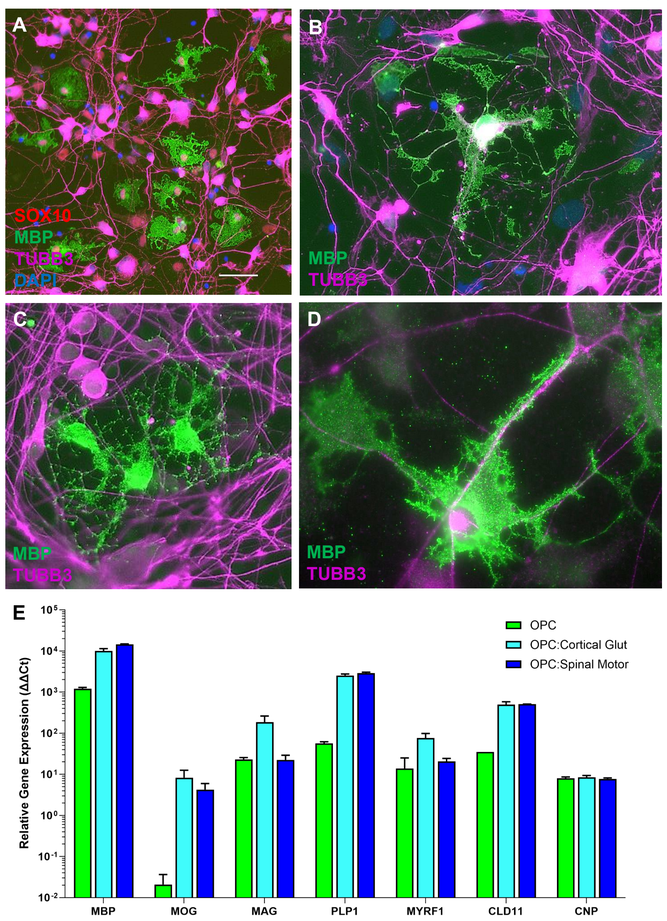


Figure 2. Neuron-OPC co-culture promotes axonal engagement. (A) SOX10 and MBP staining confirm oligodendrocyte lineage identity in the presence of βIII-tubulin-positive neurons. (B-D) Following one week of co-culture, MBP-positive cells acquire complex branched morphology and extend processes that align with neuronal fibers, demonstrating the cell-cell interactions required for axonal engagement. (E) Multiple myelin-associated genes are upregulated in neuronal co-culture conditions relative to OPC monoculture.

Conclusions

- **Human iPSC-derived OPCs demonstrated robust oligodendrocyte lineage identity and maturation capacity.**
- **Neuron co-culture promoted oligodendrocyte branching and axonal engagement.**
- **Tri-culture systems supported extensive integration between neurons, astrocytes and oligodendrocytes.**
- **Human myelinoids exhibited both myelinated and unmyelinated fibers after 8 weeks of 3D culture.**
- **TEM analysis revealed dynamic stages of membrane wrapping and myelin compaction.**
- **These results support human myelinoids as promising platforms for studying myelination and therapeutic discovery.**

Tri-culture of OPCs with Neurons and Astrocytes

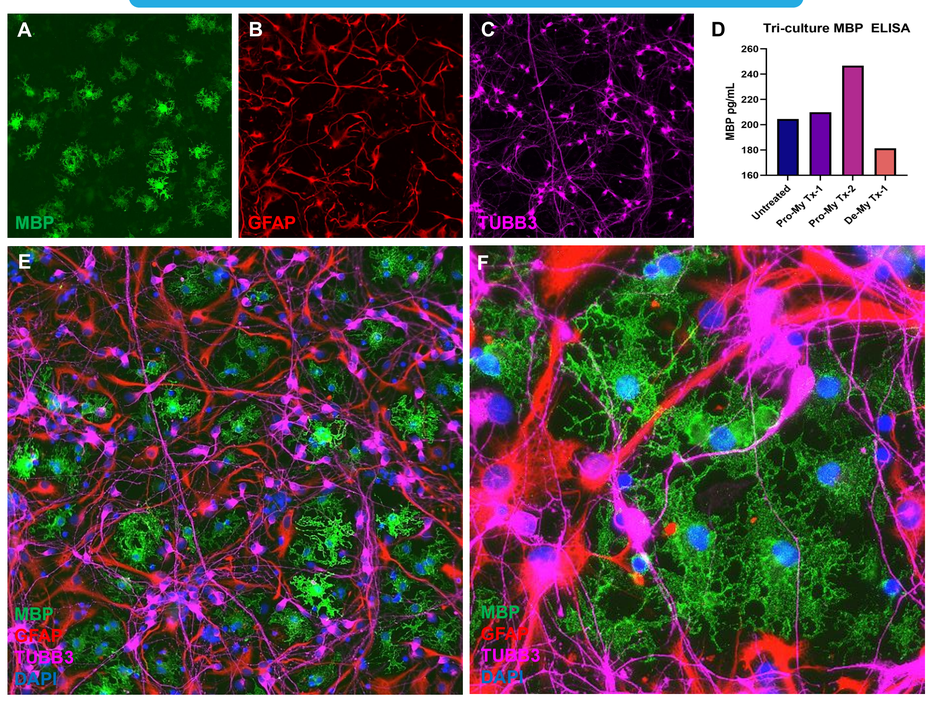


Figure 3. Two-Dimensional Tri-culture of Oligodendrocyte Progenitor Cells with Neurons and Astrocytes. (A–C) Representative immunostaining of neuron-astrocyte-oligodendrocyte tri-cultures showing MBP-positive oligodendrocytes (green), GFAP-positive astrocytes (red), and TUBB3-positive neurons (magenta). (D) ELISA quantification of MBP from tri-culture conditions following treatment with pro-myelinating or de-myelinating compounds, demonstrating assay responsiveness to modulation of myelination-related biology. (E) Merged images demonstrate successful integration of all three cell types within the culture system. (F) Higher magnification highlights extensive cellular interactions between oligodendrocytes, neurons, and astrocytes, including complex branching and close association of MBP-positive processes with neuronal networks.

Dynamic Myelination in 3D Tri-culture

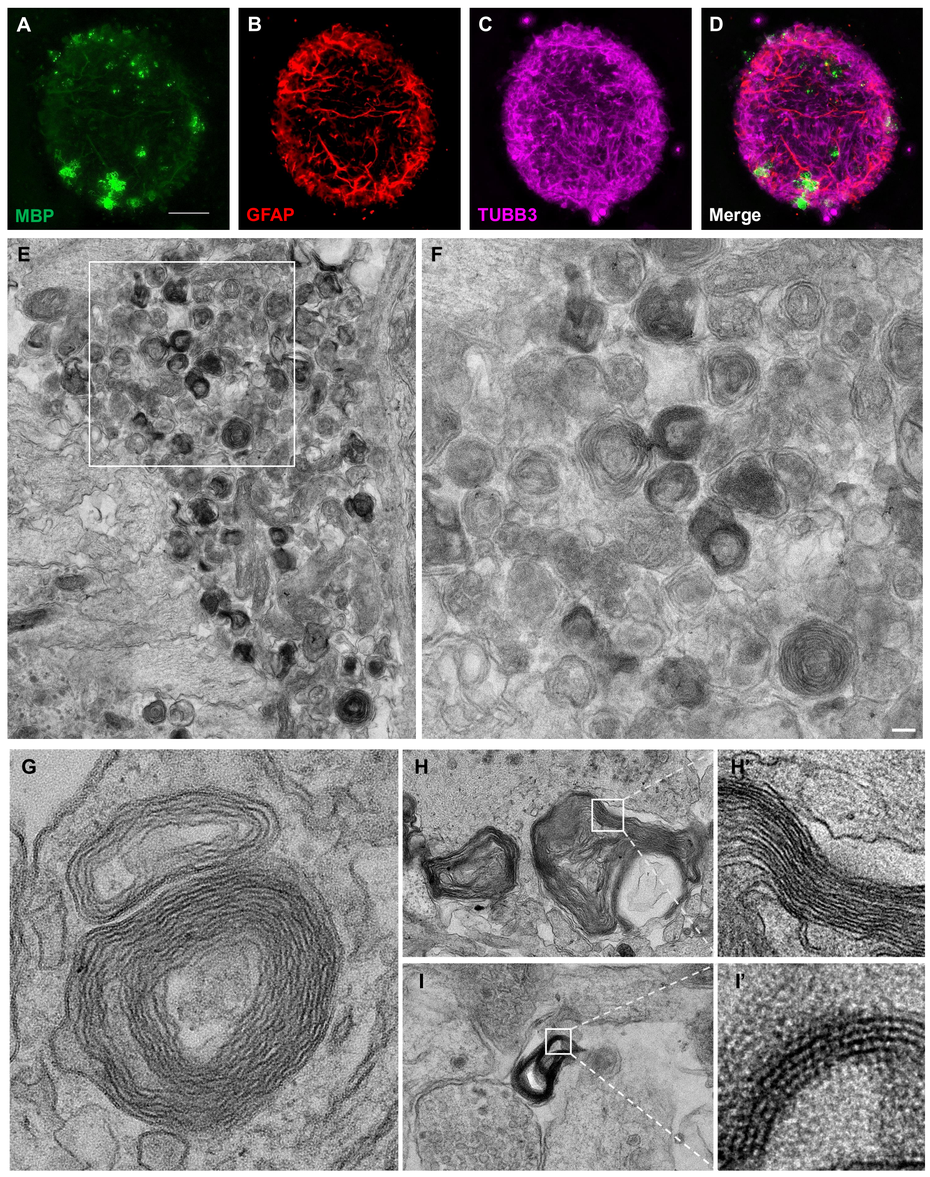


Figure 4. Human 3D myelinoid characterization and ultrastructural analysis of myelin formation. (A–D) Representative immunostaining of human myelinoids showing MBP-positive oligodendrocytes (green), GFAP-positive astrocytes (red), and TUBB3-positive neurons (magenta). Merged images demonstrate successful integration of all three neural cell types within the 3D spheroid culture. (E–F) Transmission electron microscopy (TEM) images showing the distribution of both myelinated and unmyelinated fibers throughout the myelinoids following 8 weeks of 3D culture. (G–I) Higher magnification TEM images highlighting varying degrees of membrane compaction and concentric wrapping, ranging from loose to tightly compacted lamellae, consistent with an ongoing and dynamic myelination process. Insets show enlarged views of representative myelin-like structures.

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