Modulation of Glial Differentiation in a 3D iPSC-derived CNS Model

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Poster #100

AxoSim Human Data, Faster.

BrainSim® Myelinating Organoids

Overview

A crucial aspect of accurately modeling the central nervous system involves the concurrent differentiation of neurons and glial cells in a 3D organoid organization, including astrocytes and the oligodendrocyte lineage pathway, which consists of oligodendrocyte precursor cells (OPCs), oligodendrocytes (OLs), and myelinating OLs. AxoSim's 3D BrainSim® model addresses this need by differentiating neuron and glial cell types from iPSCs over a 12-week culture period. In addition to baseline media, AxoSim has tested a modified media as well as media supplemented with the pro-myelinating drug clemastine to assess the modulability of the OPC/OL lineage.

Methods

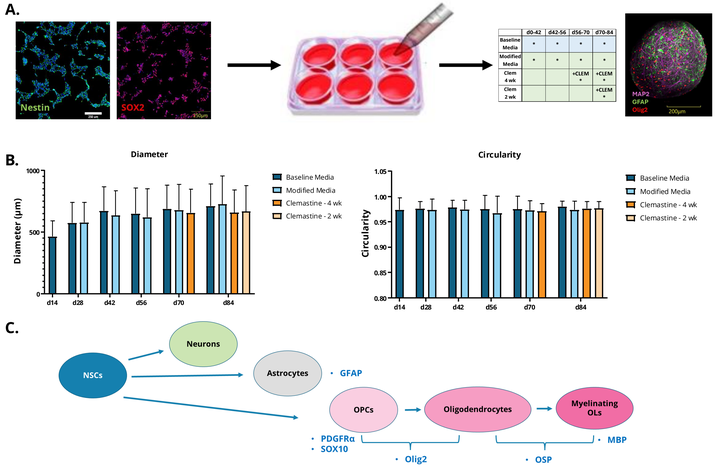
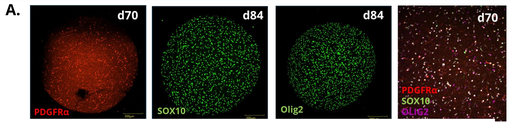
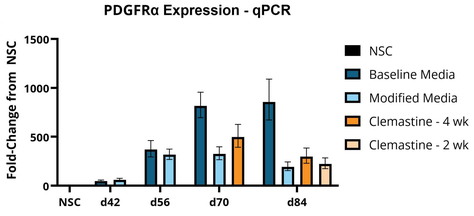
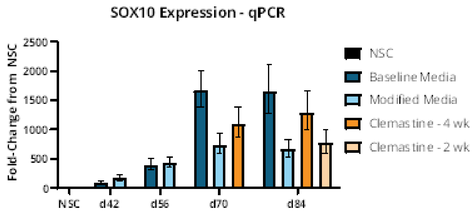


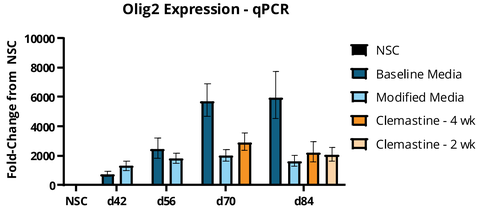
Figure 1. (**A**) iPSC-derived NSCs are plated in 6-well plates and cultured as spheroids over 12 weeks. Spheroids were cultured in baseline media, modified media, or modified media supplemented by 2- or 4-week clemastine treatments. (**B**) Spheroid diameter and circularity were consistent among the four test groups. (**C**) Markers for glial differentiation pathways were measured at d42, d56, d70, and d84.

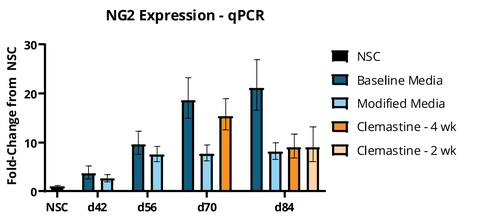
OPC/OL Differentiation











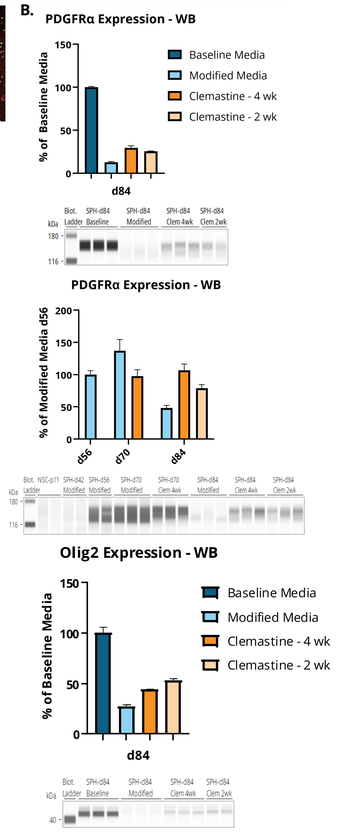
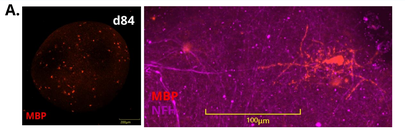
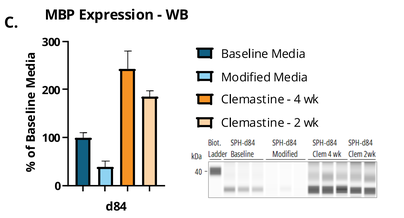


Figure 2. (**A**) IHC exhibits expression of colocalized OPC/OL markers in baseline media. (**B**) Western blot analysis shows peak OPC/OL marker expression in baseline media, with increased expression the clemastine-treated groups at d84 over modified media. (**C**) qPCR confirms peak OPC/OL transcript expression in baseline media, with an increase in clemastine-treated media over modified media alone.

Myelinating OL Differentiation





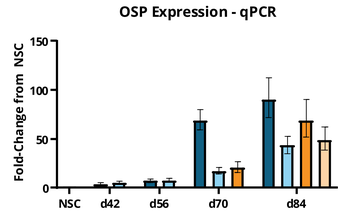


Figure 3. (**A**) IHC showing expression in baseline (left) and 4-week clemastine treated modified media (right). (**B**) qPCR analysis exhibits a clemastine-dependent increase in mature OL expression at d84 in myelin basic protein (MBP) and oligodendrocyte specific protein (OSP). (**C**) Western blot analysis confirms the effect of clemastine treatment at d84 on MBP expression.

Astrocyte Differentiation

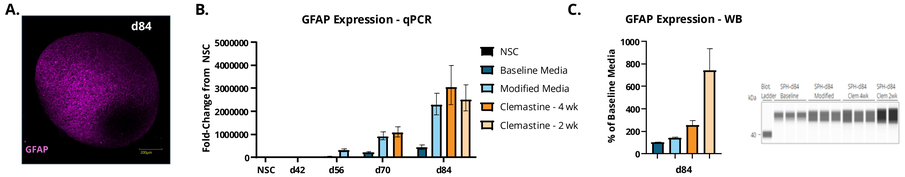


Figure 4. (**A**) IHC expression of GFAP in baseline media at d84. (**B**) Modified media and clemastine increased GFAP expression via qPCR. (**C**) Clemastine further increased GFAP expression via western blot.

Conclusions

- While all conditions tested successfully differentiated glial cells, the baseline media proved to be superior to the modified media in the differentiation of OPCs and OLS.
- By d84, the 4-week clemastine treatment had a robust effect on various OPC and OL markers, most notably MBP.
- The successful modulation of the OPC/OL differentiation pathway by clemastine demonstrates the potential for BrainSim® to assess drugs targeting this pathway, such as those aiming to treat demyelinating disorders like multiple sclerosis.