

Differences Between Oligodendrocyte Precursor Cells and Astrocytes in

Neuronal Organoid Culture

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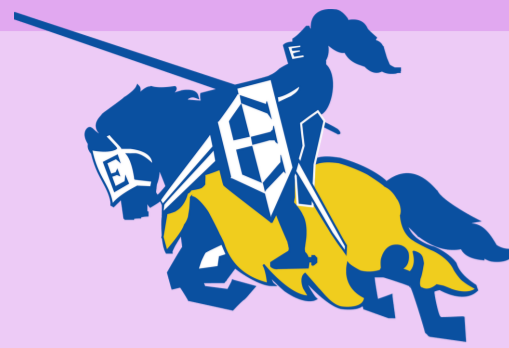
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Abstract

This study investigates how astrocytes and oligodendrocyte precursor cells (OPCs) influence neuronal branching in organoid cultures while addressing the limited understanding of how different glial cell types influence neural development. During the experiment, neurons were cultured with either astrocytes or OPCs using stem cell-derived organoids. The branching patterns were analyzed through a microscope. Results indicated differences in neuronal connectivity between the two conditions, suggesting that glial cell type plays a role in shaping neuron structure. These findings support the idea that astrocytes and OPCs contribute differently to neural development and may impact recovery processes following spinal cord injury.

Methodology

Pluripotent stem cells were first cultured in neural media and differentiated into neural progenitor cells using Dual SMAD inhibition (SB431542 and LDN-193189).

Astrocyte Differentiation

EGF, LIF, and CNTF supported early cell growth and astrocyte maturation

OPC Differentiation

IGF, SHH, PDGF-AA, and NAC were used to induce differentiation

Neuronal Differentiation

Neurogenin-2 (Ngn2) was activated using doxycycline

Organoid Formation and Analysis

Differentiated cells were combined into 3D models to study interactions.

Cells were aggregated using AggreWell plates

Two conditions were created: Neurons + Astrocytes and Neurons + OPCs

Organoids were stained and imaged using fluorescence microscopy

Blue marker represents nuclei (DAPI)

Green marker represents Neurons

Red marker represents Astrocytes

Magenta represents OPCs

Findings

- Organoids formed successfully in all conditions
- The astrocyte condition showed the most organized and dense structure
- The OPC condition showed reduced organization and more irregular morphology
- Differences in structure suggest that glial cell type influences development
- Staining appeared dispersed rather than clearly defined
- Neuronal branching could not be clearly identified

Introduction/ Summary

Spinal cord injury disrupts communication between neurons and glial cells in the central nervous system. Glial cells, such as astrocytes and oligodendrocyte precursor cells (OPCs), play important roles in supporting neuron development and synapse formation. Astrocytes, one of the most abundant glial cell types in the CNS, contribute significantly to neuron development and synapse formation. OPCs represent another glial population that can transform into oligodendrocytes, which are responsible for myelinating axons. Beyond their role in myelination, OPCs can form synaptic connections with neurons and increase signal efficiency.

This study compares organoids containing neurons with astrocytes versus neurons with OPCs to determine how each cell type affects neuronal organization and branching. Understanding these differences may provide insight into neural development and potential recovery after spinal cord injury.

Results

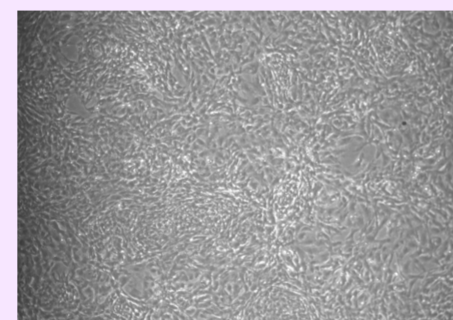


Figure 1. Undifferentiated cells (Astrocyte condition)

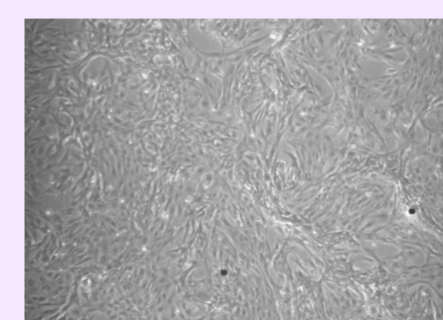


Figure 2. Undifferentiated cells (OPC condition)

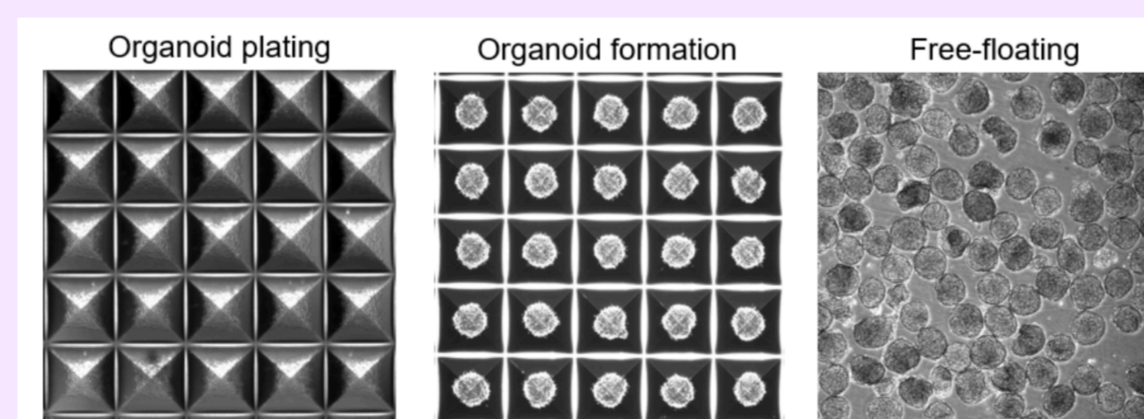


Figure 3. Organoid formation process

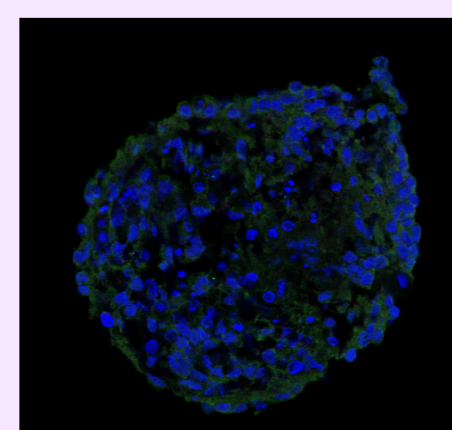


Figure 4. Astrocyte organoid

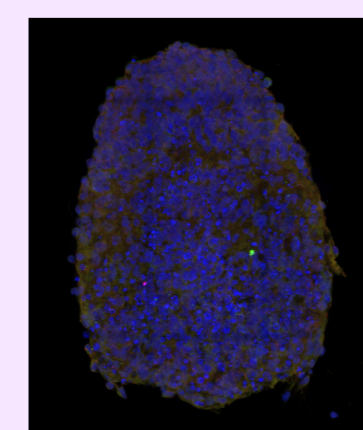


Figure 5. OPC organoid

Discussion

Astrocytes appear to support stronger structural organization in neuronal organoids compared to OPCs, likely due to their role in cell communication and synapse support. However, neuronal branching could not be observed because the staining was not clearly defined. OPCs may also require more time or optimized conditions to fully differentiate, which could explain the weaker structural organization. These limitations are important when relating the results to spinal cord injury, where astrocytes and OPCs both play key roles in repair and regeneration. Future studies should improve staining clarity and allow more time for cell development to better evaluate neuronal branching patterns.

References

1. Chung, Won-Suk, et al. "Astrocytes Control Synapse Formation, Function, and Elimination." *Cold Spring Harbor Perspectives in Biology*, vol. 7, no. 9, 1 Sept. 2015, www.ncbi.nlm.nih.gov/pmc/articles/PMC4527946/. Body.
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