Investigating the Effect of Oxygen Availability on Neurodevelopment in Organoids

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OBJECTIVE

- Cortical organoids are three-dimensional cell cultures derived from induced pluripotent stem cells (iPSCs) to provide a unique platform for studying neurodevelopment *in vitro.*

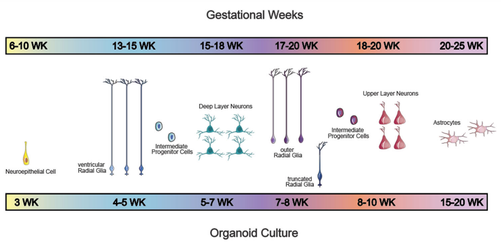
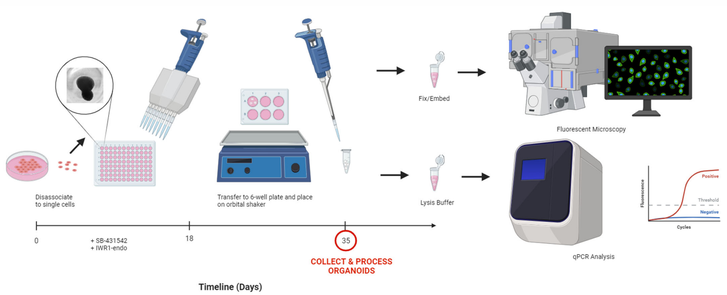


Figure 1. Timeline of cell types involved in neurodevelopment in organoids and in human embryo.

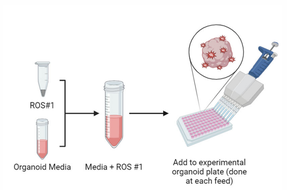
- Current cortical organoids are limited in their ability to recreate complex brain physiology and neural circuitry, impacting their accuracy as disease and injury models [1].
- The tissue of the human brain stays at a more hypoxic state than the standard culturing protocols [2]
- Growing cortical organoids at oxygen levels like the brain could be helpful at making them better disease models

EXPERIMENTAL DESIGN

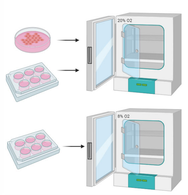
Organoid Differentiation



APPROACH 1: Inhibiting Reactive Oxygen species



APPROACH 2: Higher vs Lower Oxygen Availability in Environment



FINDINGS

Inhibition of Reactive Oxygen Species in Organoids

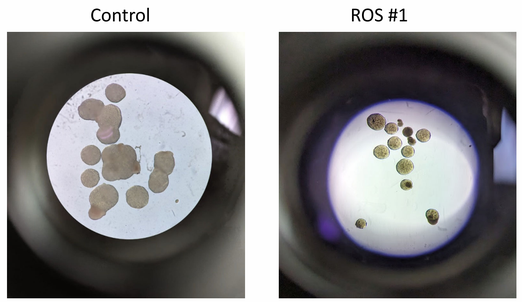


Figure 2. Morphological changes within the organoids from ROS experiment where organoids with ROS inhibitor were much smaller with less dense cell populations.

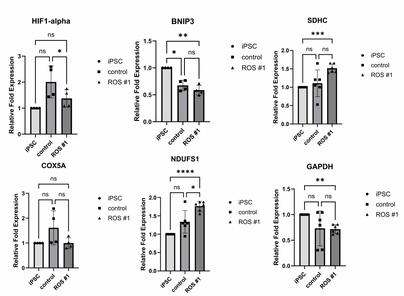


Figure 3. Gene expression profiles of iPSCs, control organoids, and ROS #1 organoids . ROS #1 organoids had significantly higher expression of NDUFS1 but significantly lower expression of HIF1-alpha.

Incubating Organoids at Lower O2 levels

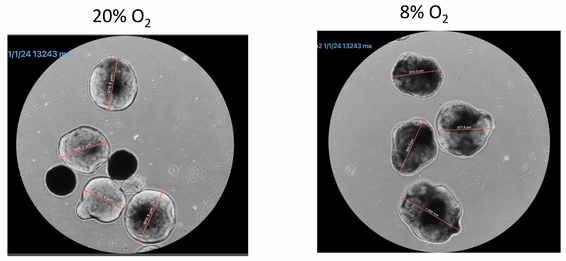


Figure 4. **Morphological changes within incubation experiment** where organoids grown at lower oxygen levels appeared to have a larger diameter, but not significantly than normoxic 20% O_2 organoids.

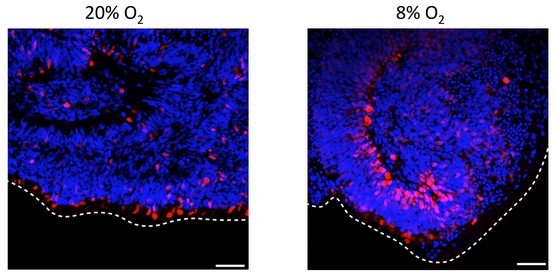


Figure 5. Immunohistochemical staining and confocal imaging of organoid sections show there was a significant increase in proliferating cells under lower oxygen conditions.

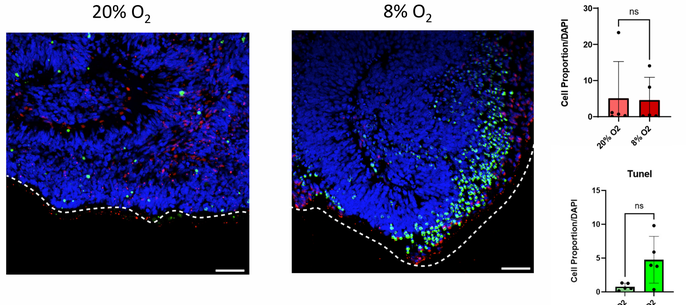


Figure 6. Immunohistochemical staining and confocal imaging of organoid sections show there was no significant change in neurons when placed at lower oxygen conditions. There was higher cell death present in 8% O_2 organoids localized under the deep layer neurons.

CONCLUSIONS

- Inhibiting reactive oxygen species was not a favorable method as the ROS #1 organoids were very small and depleted and were difficult to analyze and quantify.
- The ROS inhibited organoids showed upregulation of TCA cycles but lower levels of hypoxia genes indicating that they are stuck in the OXPHOS/TCA cycle
- Having more hypoxic environmental conditions, like the brain environment, showed quicker development in size, rosette structures, and proliferation
- Lower O_2 conditions visually appear to increase cell death, however not significantly.

FUTURE WORK

- Identification of dead cells by staining and quantifying intermediate progenitors and radial glia populations
- Repeating experiment using H1 cell line (biological replicate)

ACKNOWLEDGEMENTS

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REFERENCES

[1] Andrews MG, Kriegstein AR. Challenges of Organoid Research. Annual Review of Neuroscience. 2022;45. doi:https://doi.org/10.1146/annurev-neuro-111020-090812
[2] Gunnar Andreas Walaas, Gopalakrishnan S, Bakke I, et al. Physiological hypoxia improves growth and functional differentiation of human intestinal epithelial organoids. Frontiers in Immunology. 2023;14. doi:https://doi.org/10.3389/fimmu.2023.1095812

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