

## Storage Temperature Affects Müller Glia Susceptibility to Hypoxia

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**Background/Objective:** We conducted this project to differentiate cell susceptibility to hypoxia (low O<sub>2</sub> level) between immortalized Müller glia (MIO-M1) stored in -80 versus liquid nitrogen.

**Method:** MIO-M1 cells were cultured and seeded in 12 well plates at 40K/well and in 6 well plates at 75K/well until confluence. Cell culture conditions: centrifugation time and speed, neutralization, and storage temperature were varied. Cells were subjected to chemically induced hypoxia by treatment with 300 and 400uM CoCl<sub>2</sub> for 24 and 48hours. Oxygen level was measured in cells, cells were imaged and counted.

**Results:** Centrifugation and neutralization conditions did not affect cell survival. We found that storing MIO-M1 cell in -80 makes these cells more susceptible to death by hypoxia. First, we found that %live MIO-M1 cells stored in liquid nitrogen is near 100% compared to ~60% live cells when stored in -80. Second, cells stored in -80 showed significant susceptibility to chemically induced hypoxia compared to cells stored in liquid nitrogen that survived treated for 24 and 48 hours.

**Conclusion:** We concluded that storage of these cells in liquid nitrogen maintain survival during hypoxic events. Cells' susceptibility to hypoxia indicates that storage in -80 affect glycolysis efficiency in these cells to maintain survival. During hypoxia, mitochondrial is dysfunctional as they depend on O<sub>2</sub> for ATP production during oxidative phosphorylation. Cells stored in liquid nitrogen survived hypoxic conditions, which may indicate that MIO-M1 depends on glycolysis for ATP production. Further investigation will proceed to determine Müller glia metabolism preference.