HT-22 Hippocampal Neurons as a Model System to Study Oxidative Stress and the Nrf2 pathway

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by the formation of amyloid beta (Aβ) plaques in the brain and is the seventh leading cause of death in the United States. Chronic inflammation and oxidative stress associated with AD leads to neuronal cell death. A cellular protective mechanism against oxidative stress involves the Nuclear factor erythroid 2-related factor (Nrf2) pathway. Nrf2 is responsive to the reactive oxygen species (ROS) produced when the cell is under oxidative stress, leading to its translocation into the nucleus where it activates transcription of genes that produce antioxidant enzymes like heme oxygenase-1 (HO-1). To study this pathway in neurons, our lab chose to use the mouse hippocampal HT-22 neuronal cell line. Our previous attempts to grow these cells in culture proved difficult, leading us to hypothesize that providing a growth enhancing surface of collagen would provide a more stable surface in which to propagate these cells. Here we show that HT-22 cells grown on rat tail collagen provide a model system to investigate the Nrf2 pathway. We also demonstrate that HT-22 cells are viable on tissue culture plastics without the need for collagen.

Introduction

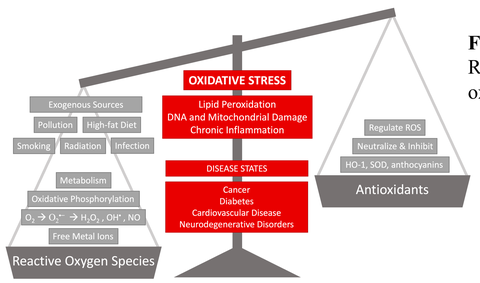

Figure 1. An imbalance between ROS and antioxidant defenses in oxidative stress.

- Oxidative stress occurs when cells make or are exposed to reactive oxygen species (ROS) at a greater level than they can regulate by their antioxidant defenses
- Oxidative stress is a component of many disease states, including Alzheimer's disease (AD) and AD related dementias (ADRD)
- HT22 cells are a mouse neuronal cell line isolated for the hippocampus, a region heavily impacted in AD
- Previous attempts to culture these cells in our lab have been met with difficulty maintaining sufficient cell proliferation and survival
- We hypothesize that providing a matrix of collagen on which the cells would be cultured might provide enhanced cell survival

Methods

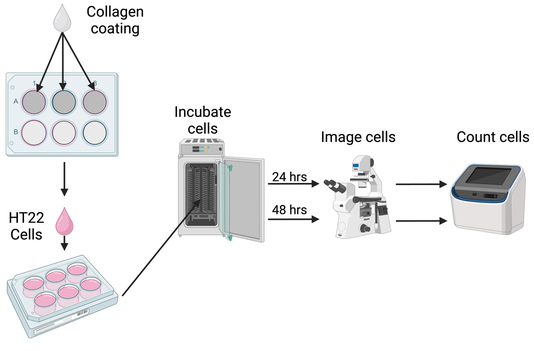

Figure 2. Graphical representation of the experimental design. Figure created with BioRender.com

Results

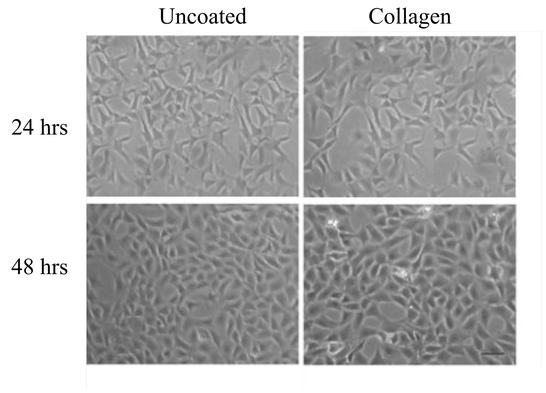

Figure 3. Representative images of HT22 cells cultured on uncoated or collagen coated wells at 24 and 48 hours.

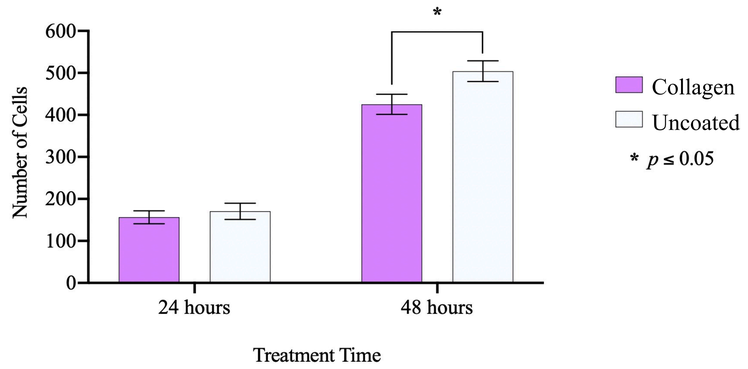

Figure 4. Total hemocytometer HT22 cell counts at 24 and 48 hours. Bars represent means and standard errors of 3 independent experiments in which culture conditions were performed in triplicate. An independent samples t-test did not reveal a significant effect of treatment (collagen coated plate vs. uncoated plate) on the number of cells following 24 hours of growth $t(10)=0.572$, $p=0.580$. An independent-samples t-test revealed a significant effect of treatment (collagen coated plate vs. uncoated plate) on the number of cells following 48 hours of growth, $t(10)=-2.283$, $p=0.046$.

Conclusions

- We did not see an HT22 survival benefit by culturing cells on collagen
- Only statistically significant finding at 48hr, with uncoated vessel containing more cells
- There may be a slight reduction in proliferation of HT22 cells when cultured on collagen.
- Collagen coating may slow proliferation of HT22 cells due to stronger interactions with the bottom of the well
- Likely inconsistencies in tissue culture products
- Previous attempts occurred during COVID-19 pandemic, hard to find proper tissue culture vessels
- Not necessary to coat vessels with collagen, saves time and money

Future Directions

- Conduct Cell Death assays to see if collagen coating caused an increase in cell death or just slowed proliferation of the cells
- Demonstrate glutamate treatment induces ROS and cell death of HT22
- Determine how glutamate affects Nrf2 pathway
- Determine how glutamate affects lipid oxidation and protein carbonylation
- Test compounds from Dr. Green on ability to block ROS and decrease cell death

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reSEaRCh Science and Engineering Research Center

NIH National Institute of General Medical Sciences

Resources

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