Examining in-vitro conditions for studying primary glial cell function and interaction

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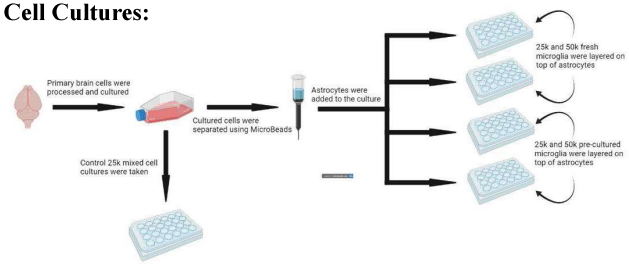
INTRODUCTION

Glial cells such as microglia and astrocytes are vital for neuronal health and proper brain function. Microglia are the immune cells located in the central nervous system. They have an important role in surveying the brain to respond to both injury and infection. Astrocytes are a glial cell also found in the central nervous system. Some functions of the astrocytes are to provide metabolic support, provide synaptic support and regulate toxins. These cells along with other glial cells provide important support functions in the central nervous system and their combined function and interactions are important to study. In vitro cultures of these cells are very difficult to examine. When cultured properly these cells can be used to study intercellular interactions and injury models such as mechanical scratch injuries and an immune response.

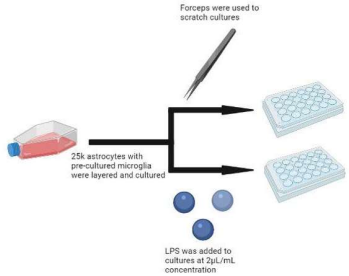
METHODS

Subjects: C57 Black 6 Mice (control colony mice)

Cell Cultures:



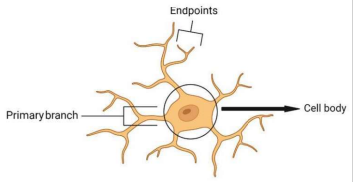
Injury Model:



Characterization methods: Immunohistochemistry (IHC) was used to stain certain target proteins using primary and secondary antibodies. qRT-PCR was used to quantify gene expression of target genes and establish fluorescence by isolating cells and using lysis buffer to access the RNA and then cDNA. Image J was used to measure the cell count in quadrant, branch length, primary branch number, cell body measurement, and endpoint number.

METHODS CONT.

Figure 3. Image J was used to measure the cell count in quadrant, branch length, primary branch number, cell body measurement, and endpoint number.



RESULTS

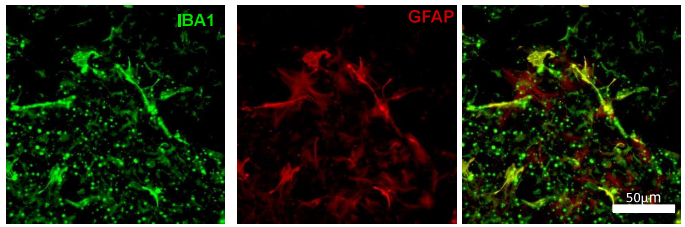
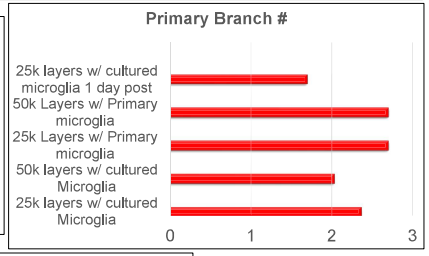


Figure 4. A 20x image of the 25k pre-cultured microglia and astrocytes. Red is GFAP, green is IBA 1.

Figure 5. A graph of the number of primary branches that were found on microglia in each culture type. The 25k pre-cultured microglia can be seen with the most average primary branches.



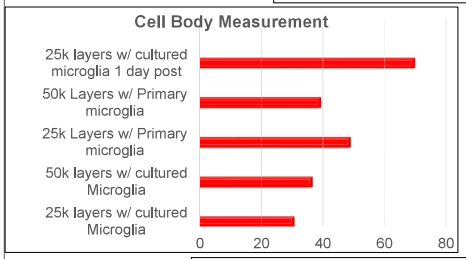
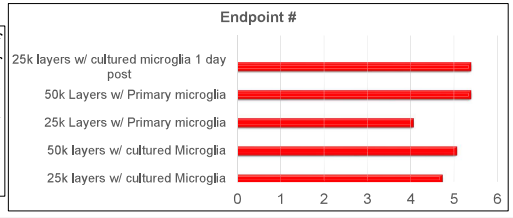


Figure 6. A graph of the area of cell body on microglia found in each culture type. The 25k pre-cultured microglia have smaller cell bodies.

Figure 7. A graph of the number of endpoints that were found on microglia in each culture type. Almost all the culture types are equivalent.



RESULTS CONT.

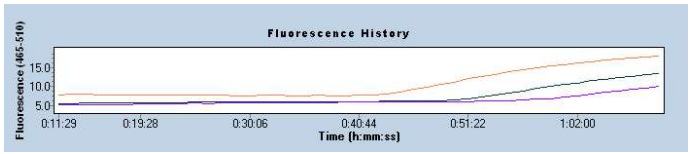


Figure 8. A graph displaying the reading that was displayed when the qRT-PCR was ran showing the fluorescent reading. Purple is a water control, green is mechanical scratch, and orange is LPS.

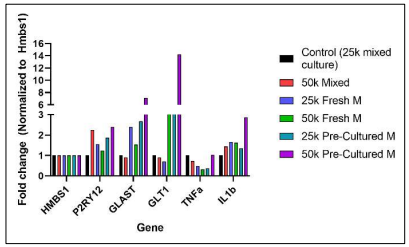
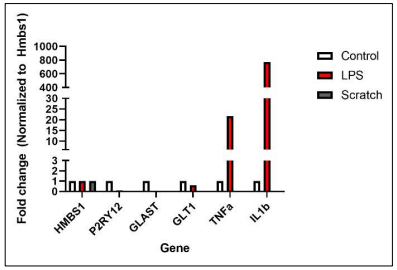


Figure 9. A bar graphs of the qRT-PCR data of the different types of cultures. Genes 2-4 are all expected to be seen in higher amounts while the last two should be lower. The 25k pre-cultured sample had the most optimal levels of gene expression

Figure 10. A bar graph of the qRT-PCR data of the 25k layered pre-cultured microglia cell cultures that were tested with mechanical scratch and LPS.



CONCLUSION/FUTURE DIR.

Conclusion: The 25k pre-cultured microglia model was found to be the optimal (not perfect) model for examining the function and interaction between glial cells. This culture model can be used to test various injury models to provide the best in vitro culture.

Future Directions: While running the qRT-PCR there could be more genes tested, the phagocytotic ability of the microglia could be tested, and this model could be used in TGFb signaling to set up more ideal cultures.

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