Isolation of Rodent Microglia to Assess Gene Silencing and Drug Targeting

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Introduction

- As the population ages, neurodegeneration is becoming more prominent
- As the resident immune cell of the brain, microglia monitor the CNS for pathogens and apoptotic signals
- Isolating microglia for culture will enable us to explore the effects of siRNA medicine on microglial states

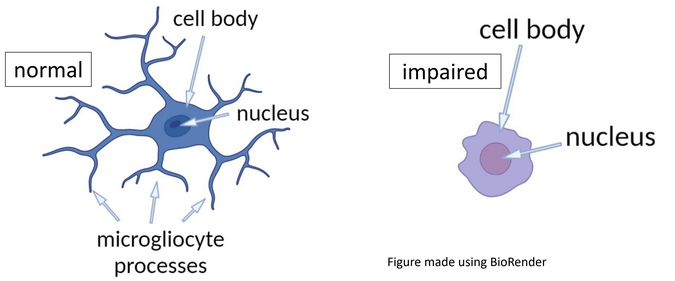

Figure 1. Microglia are important for our understanding of neurodegenerative disease. Key features of microglia include the central cell body containing the nucleus and branch-like microgliocyte processes (left), which provide defense from pathogens. Impaired microglial dysfunction is seen in age-associated neurodegenerative diseases (right).

Microglia Isolation Workflow

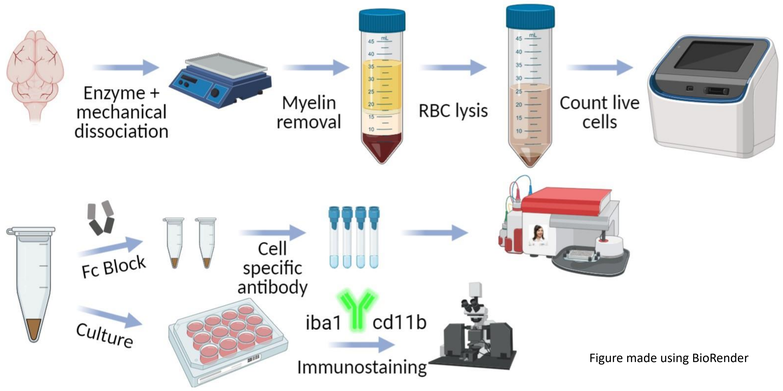

Figure 2. The process of isolating microglia starts with a brain dissociation using enzymatic and mechanical methods. Then, the cell sample is either prepared for flow cytometry analysis or cultured for several days then stained with microglia-specific fluorescent antibodies for an immunostaining.

Validation of Microglia Isolation through Flow Cytometry

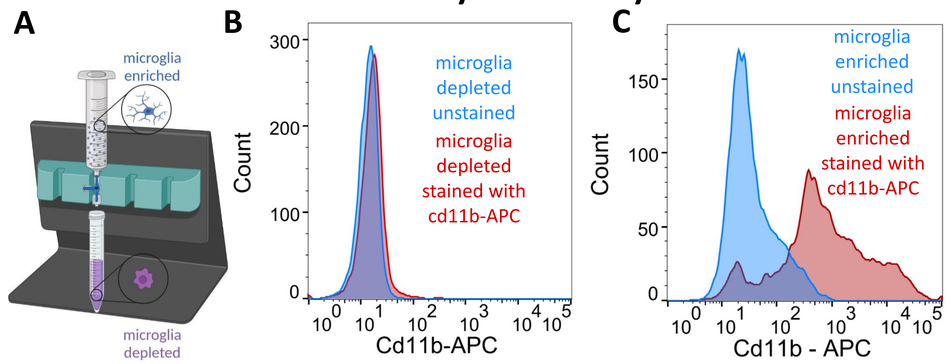

Figure 3. (a) A diagram showing the isolation of microglia, with the microglia attaching to the beads within the top tube, making what remains attached to the beads microglia enriched while the solution in the bottom tube is microglia depleted. (b) The results of flow cytometry run on the murine microglia depleted solution, confirming a negative test for microglia. (c) The results of flow cytometry run on the murine microglia enriched solution, confirming a positive test for microglia.

Confirmation of Microglia Isolation

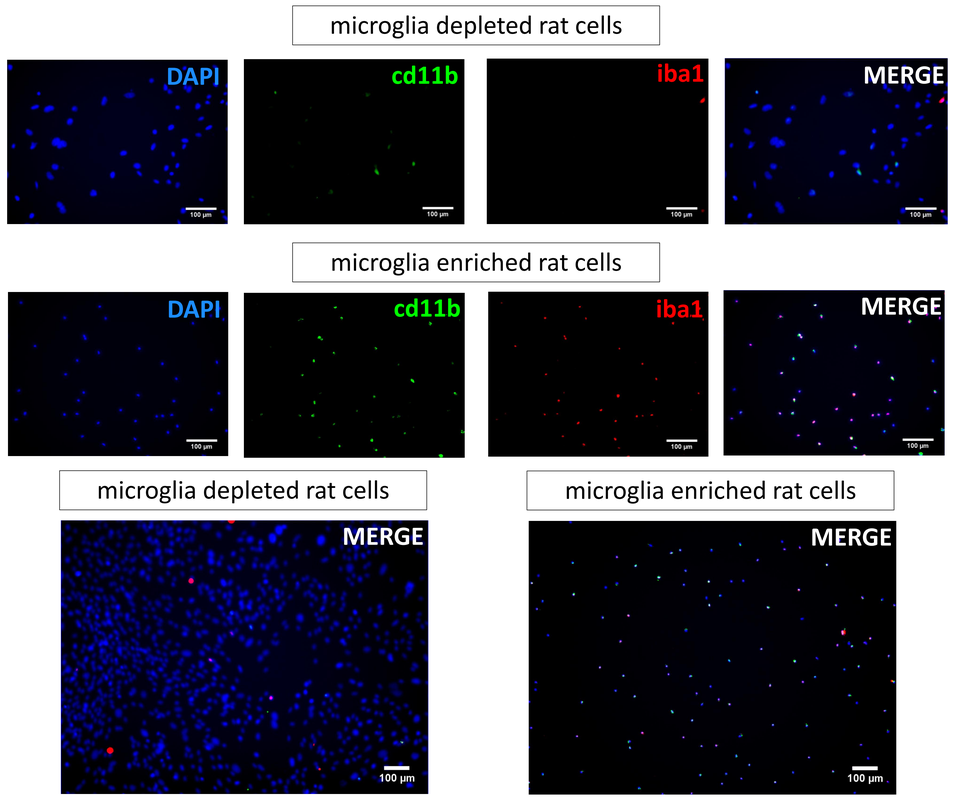

Figure 4. Immunostaining of microglia depleted and enriched rat cells showing DAPI in blue (nuclei), cd11b in green (microglia), iba1 in red (microglia), and merged.

Microglia Remain Viable in Culture

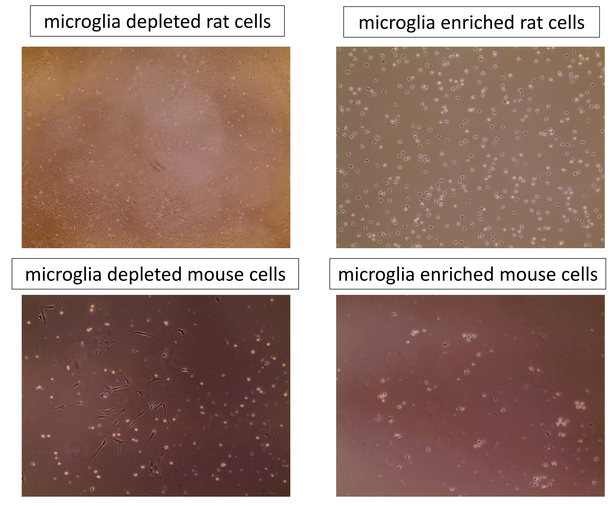

Figure 5. Pictures of cells cultured for several days taken using a 10x objective

Conclusion

- **Microglia can be isolated through magnetic sorting and validated with flow cytometry and immunostaining using the antibodies cd11b and iba1, microglia specific markers**
- Isolated microglia from rat and mouse brains remain viable in culture for several days

Future Work

- Observing phenotypic changes in microglia after siRNA-mediated gene silencing
- Applying therapeutics to diseased murine microglia

Acknowledgements

Thank you to the Vanderbilt Institute of Nanoscale Science and Engineering (VINSE), the Lippmann Lab, and the National Science Foundation (NSF) and the grant DMR 1852157 for making this research possible.

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