Primary isolation of vascular cells from murine brain for single cell sequencing

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Abstract

When studying primary tissues, the cell isolation protocol is of critical importance for assessing cellular behavior and characteristics. The vasculature poses an additional problem due to the tight association between mural cells and endothelial cells. Here we share a protocol for efficient, fast and clean isolation of vascular cells from mouse brain. In optimal conditions, a single cell brain solution can be obtained in 75 minutes. The last 30 minutes, the sample is at 4 °C. This protocols has been optimized for generation of pure single cells from the vasculature, but is also applicable for other cell types.

Introduction

The following protocol relies heavily on the Neural Tissue Dissociation kit from Myltenyi Biotec with some important changes. A summarizing flow diagram is displayed below.

Reagents

- Animals: Any adult C57Bl6 mouse, preferably with fluorescent reporter for FACS - Neural Tissue Dissociation kit \(P): Myltenyi Biotec: 130-092-628 - Myelin Removal kit II: Myltenyi Biotec: 130-096-733 - LS columns: Myltenyi Biotec 130-042-401 - Dulbecco's modified Eagle Serum \(DMEM) \(without phenol red): ThermoFisher Scientific: 31053-044 - Penicillin/Streptomycin \(P/S): ThermoFisher Scientific: 15140-122 - 100 mm plastic petri dishes of any provider - 70 µm mesh: Becton Dickinson \(BD): 352350 - MACS buffer: PBS with 0,5% FBS - 5 ml self-standing tubes from any provider - 1 ml syringe and 20g needle - Pasteur pipettes - FACS tubes compatible with the FACS used - PBS with calcium and magnesium added: ThermoFisher Scientific: 14040091 - Fetal Bovine Serum \(FBS): Any provider - FACS buffer: DMEM without phenol red with 2% FBS and 1% P/S.

Equipment

- Rotator capable of incubation at 37 ºC - Myltenyi QuadroMACS Separator \(130-090-976) and MACS MultiStand \(130-042-303) - Sharp scissors - Swing-bucket centrifuge capable of centrifuging 50 ml and 15 ml tubes at 4 ºC and 300g. - Tools for brain dissection

Procedure

1. Preparation: From the Neural Tissue Dissociation kit, mix 2850 µl of buffer 2 with 75 µl of buffer 1 for 600 mg of tissue \(a full adult brain weighs roughly 500-600 mg). This will yield enzyme mix 1. Warm the enzyme mix 1 for 10 minutes at 37 ºC. 2. Kill the mouse using an approved method of euthanasia \(terminal anesthesia followed by heart perfusion with HBSS, or cervical dislocation). Remove the brain from the skull. 3. Transfer the brain to a Petri dish and chop it with sharp scissors for about 30 seconds, or until no large pieces are visible. The pieces should be small enough to pass through a cut pipette tip \(see next step). 4. Transfer the brain pieces to a 5 ml tube and mix them with enzyme mix 1. Use a p1000

with cut tip and wash the petri dish. 5. Incubate for 17 minutes at 37 ºC with slow rotation \(around 20 rotations/minute). 6. During incubation: Prepare enzyme mix 2: Mix 30 µl of buffer 3 with 15 µl of buffer 4. 7. Take the suspension from the rotator and add enzyme mix 2 8. Mix the solution with a Pasteur pipette and a rubber bulb 10 times. When clogging occurs, remove the clog by tapping the tip of the Pasteur pipette on the bottom until the clog resolves. The solution should pass the Pasteur pipette without clogging before proceeding to the next step. 9. Incubate for 12 minutes at 37 ºC with slow rotation. 10. Pass the brain suspension firmly, but without creating bubbles or foam, through a 20 G needle \(use a 1 ml syringe). Do this a minimum of 10 times. The suspension should pass the needle without clogging, and no major tissue pieces should be visible after this step. 11. Incubate 10 minutes at 37 ºC. 12. Transfer the suspension to a 50 ml tube, add 20 ml of cold PBS \(with Mg and Ca) and pass through a 70 µm mesh. From this moment on, the cell suspension should stay on 4 ºC. 13. Spin down 300g for 5 minutes at 4 ºC. 14. Resuspend the pellet in 1800 µl of MACS buffer and 200 µl of Myelin removal beads. 15. Incubate at 4 ºC for 15 minutes, no agitation. 16. Add 18 ml of MACS buffer to a total of 20 ml. 17. Spin down at 300g for 5 minutes, 4 ºC. 18. Preparation: Place 3 LS columns on the Myltenyi QuadroMACS Separator. Rinse each LS column with 3 ml of MACS buffer. Discard eluent. 19. Resuspend the pellet in 3 ml of MACS buffer. 20. Add 1 ml of the cell suspension on each column. Collect the eluent containing the cells\! 21. After the suspension has run through, wash the column twice with 1 ml of MACS buffer. Collect the eluent\! 22. Spin down the eluent at 300g for 5 minutes. 23. Dissolve the pellet in 500 µl of FACS buffer \(volume depending on the size of the pellet). 24. Incubate with FACS antibodies or proceed straight with FACS.

Timing

Time from dissected brain to +4 degrees: Max 45 minutes. Myelin removal: 30 minutes

Troubleshooting

- Keep the times in between the incubation steps as short as possible. - Do not work with more than two brains at the same time, the protocol should be executed as fast as possible. - This protocol is specifically designed for adult mouse brain tissue. When using young mice, shorten the incubation times. - The protocol is designed to efficiently dissociate pericytes from endothelial cells, and this requires strong mechanical dissociation and slightly extended incubation times. These changes might not be optimal for other cell types.

Anticipated Results

This protocol provides a single cell suspension from adult mouse brain, with viable vascular cells. The cells are efficiently dissociated from each other, with an important focus on the separation between endothelial cells and pericytes.

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Figures

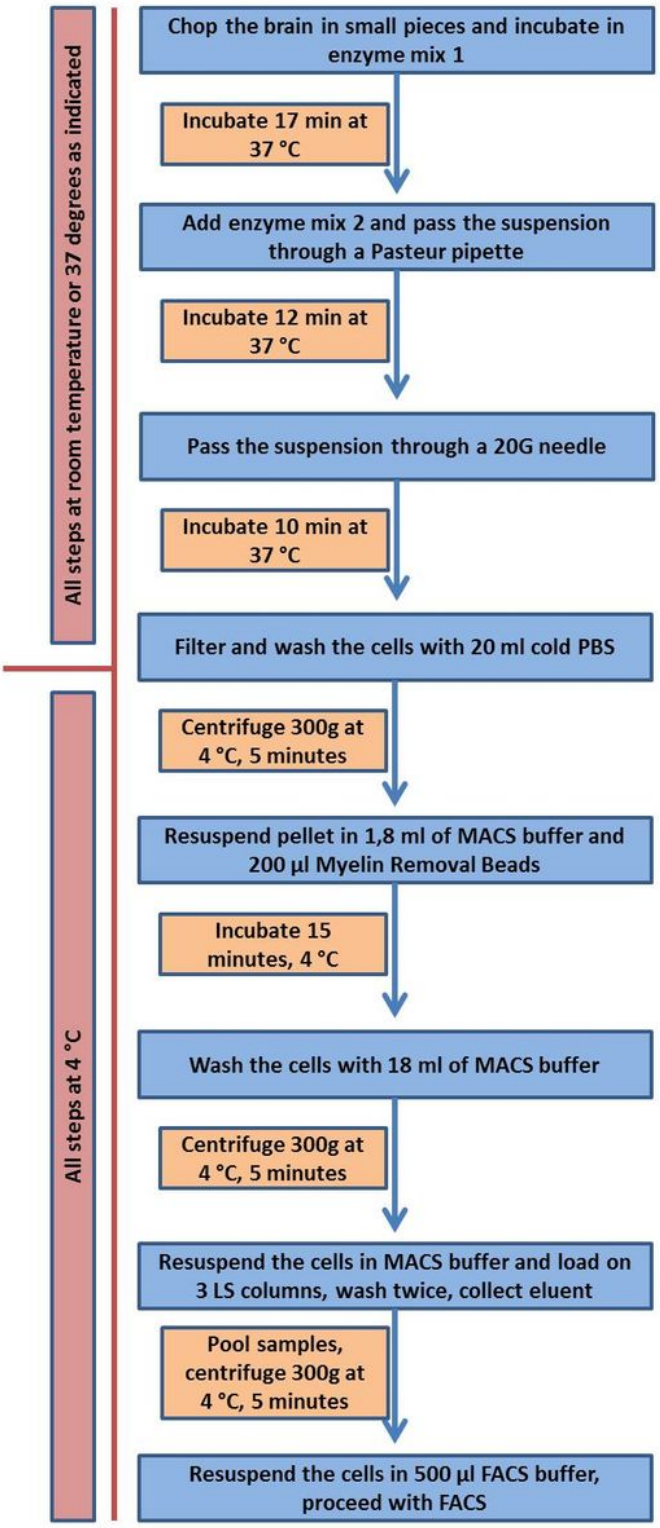

Figure 1

Flow chart Isolation of vascular single cells from mouse brain