Preparation of single cell suspensions from the adult mouse lung

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Method Article

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Abstract

Cell isolation protocols are of paramount importance for studying cells from a primary context. Long incubation times are likely to influence gene expression, which will confound interpretations on the obtained data. Every organ poses different problems when preparing single cell suspensions. The vasculature poses an addition problem due to the tight association between mural cells and endothelial cells. Here we share a protocol for quick and efficient isolation of vascular cells from mouse lungs. The entire protocol can be completed in 45 minutes. This protocols has been optimized for quick generation of pure single cells from the vasculature, but is also applicable for other cell types and organs .

Introduction

The following protocol relies on both Collagenase type 2 and the combination of enzyme 3 and 4 from the Neural Tissue dissociation kit for efficient isolation of primary cells from adult mouse lungs. A schematic representation of the involved steps is included below. 

Reagents

- Animals: Any adult C57Bl6 mouse, preferably with a fluorescent reporter for FACS sorting. - Collagenase type 2: ThermoFisher Scientific: C6885) - 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 - Fetal Bovine Serum \(FBS): Any provider - FACS buffer: DMEM without phenol red with 2% FBS and 1% P/S. - Neural Tissue Dissociation kit \(P): Myltenyi Biotec: 130-092-628 - Pasteur pipettes - 70 µm mesh: Becton Dickinson \(BD): 352350

Equipment

- Centrifuge capable of centrifuging 15 ml tubes at +4ºC and 300g. - Tools for lung dissection - Rotator capable of incubation at 37ºC - Sharp scissors

Procedure

1. Preparation. Weigh two aliquots of 10 mg of collagenase type 2 powder. Keep at -20°C until ready to be used 2. Kill the mouse using an approved method of euthanasia \(terminal anesthesia followed by heart perfusion with HBSS, or cervical dislocation). Remove the lungs. 3. Transfer the lungs 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. Dissolve 10 mg of collagenase powder in 2 ml of DMEM 5. Transfer the lung pieces to a 5 ml tube and mix them with the collagenase solution \(5 mg/ml). Use a p1000 with cut tip and wash the petri dish. 6. Mix the suspension well using the cut p1000 tip. The solution has to pass the tip freely with no blockage 7. Incubate the suspension for 10 minutes at 37 °C with fast rotation \(>20 rotations per minute). 8. During incubation: Prepare Neural

Tissue dissociation enzyme mix: Mix 30 µl of buffer 3 with 15 µl of buffer 4. 9. Take the suspension from the rotator and add the Neural Tissue dissociation enzyme mix. 10. 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. 11. Incubate for 5 minutes at 37 ºC with fast rotation 12. Pass the lung 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. 13. Incubate 10 minutes at 37 ºC 14. Transfer the suspension to a 15 ml tube, add 8 ml of cold FACS Buffer and pass through a 70 µm mesh. From this moment on, the cell suspension should stay on 4 ºC. 15. Centrifuge at 300g for 5 minutes at 4 ºC. 16. Resuspend the cells in 500 µl of FACS buffer and proceed with FACS.

Timing

From time of dissection to cells at +4 °C in FACS buffer: 45 minutes

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Figures

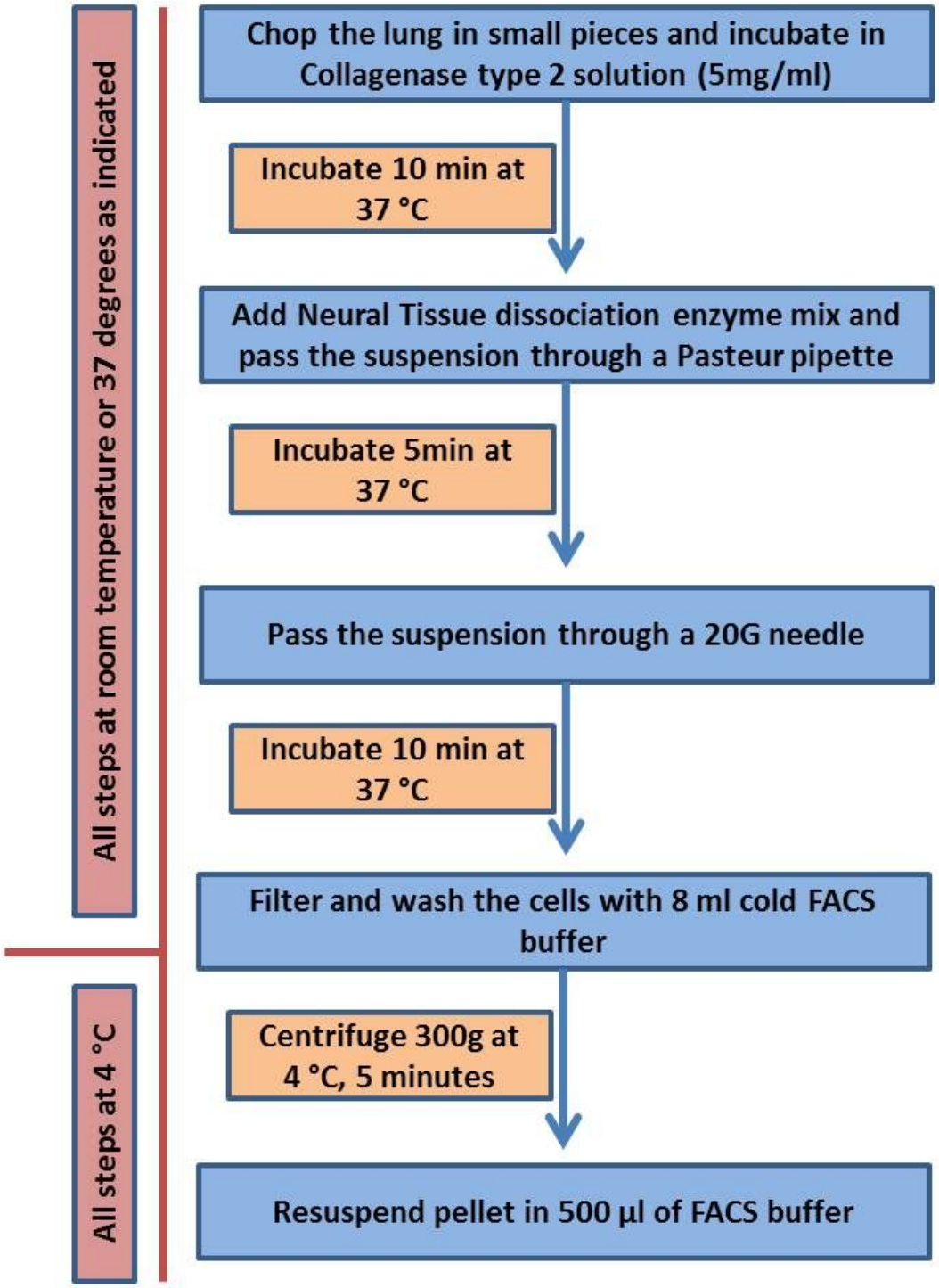

Figure 1

Flow chart Isolation of vascular single cells from mouse lung