Isolation of Lung Endothelial Cells

1. 2~3 week old mice were anesthetized with avertin and sacrificed by cervical dislocation.
2. Lungs were removed, diced as fine as possible, and digested in HBSS supplemented with 10mg/ml type II collagenase (Worthington; LS004202, 50mg) and 20ug/ml DNase I (Sigma) for 40min at 37 degree. Use 10ml of collagenase solution per 5 lungs and shake vigorously during the digestion.
3. The cells were then strained sequentially through a 100um and a 40um strainer and add equal volume of FBS for quenching collagenase activity. Centrifuge the cells at 1000rpm, 10min, 4 °C.
4. The cells then washed once with 10ml HBSS. Centrifuge at 1000rpm, 10min, 4 °C.
5. Resuspend the cells with 5ml HBSS.
6. The resuspension of cells was then laid over 5ml Histopaque 1077 (sigma) very carefully and centrifuged at 2000rpm (keep break "0"), 25min, 4 °C.
7. Take the cloudy interface and centrifuge the cells at 1000rpm, 10min, 4 °C.
8. Wash the cells once with 10ml HBSS/0.75% BSA and centrifuge the cells again.
9. Resuspend the cells with 1ml of sterile MACS buffer (PBS/0.75 BSA/2mM EDTA), transfer to e-tube and centrifuge again at 1200rpm, 10min, 4 °C.
10. The cell pellet was resuspended with 90ul MACS buffer and 10ul of CD31-conjugated microbeads (Miltenyi Biotec) was added to the cells and incubated for 15min , 4 °C.
11. Before application of the cells to the magnetic MACS columns, each column was equilibrated with MACS buffer.
12. 1ml MACS buffer was added to the cells and the entire volume was applied to the column.
13. The column was washed three times with 1ml MACS buffer and gentle positive pressure was applied with plunge before the last wash.
14. The columns were then removed from the magnet the cells were eluted with 1ml MASC buffer with positive pressure into 15ml conical tube.
15. The cells were then centrifuged at 1000rpm, 5min, 4 °C and resuspended with EC growth media (Advanced DMEM supplemented with 100ug/ml heparin, 100ug/ml ECGS, 20% FBS, 1Xglu-pen-strep, 25mM HEPES) and plated on 0.1% gelatin-coated plates.
16. The cells were allowed to grow to be confluent before being re-selected with ICAM2 or CD31 (critical for increasing the purity of EC). Use enzyme-free solution for trypsinizing cells (same process).

The purity of endothelial cells was determined with VEGFR2 or CD31 immunostaining.