Intima Isolation from Aorta for scRNAseq

Materials/Reagents

- Silicon coated 35mm dishes
    - Sylgard 184 (Fisher Scientific# 50-366-794)—to make coated dishes
- Pins for aorta (FST# 26002-20)
- Microscalpel (EMS#72046-30)
- Versene (room temp)
- 1X Trypsin (37C)
- 0.04% BSA in PBS-/- (room temp)
- 0.04% BSA + 5% FBS in PBS-/- (37C)
- 1X RBC lysis buffer (Fisher# 00-4333-57)

(*For maximum speed, two individuals are needed)

Protocol

1. Individual #1: Sacrifice mouse
2. Individual #1: Perfuse through left ventricle with 10mLs of Versene
3. Individual #1: Remove all internal organs to expose the aorta. Pass mouse off individual #2
   (While Individual #2 is dissecting, individual #1 start sacrificing and repeating steps 1-3 for a total of 6 mice)
4. Individual #2: Under a dissecting microscope, carefully remove adventitia with fine dissecting scissors
5. Individual #2: Dissect out the aorta and place in silicon coated dish filled with Versene
6. Restrain aorta with pin and finish cleaning off adventitia to the best of your capability
7. Filet open aorta by cutting the aorta longitudinally, exposing the endothelium
8. Pin aorta so the endothelium side is facing up
   (Individual #2, repeat until all 6 aortas are filet open and pinned onto *one* 35mm silicon coated dish)
9. Once pinned, remove Versene and add 2mL of 1X Trypsin
10. Incubate aortas with trypsin for 5min at 37C
11. After incubation, take aortas to dissecting microscope and use microscalpel to gently, scrape intima layer off of aorta
12. Collect all liquid in dish (which contains intima cells) with P1000 pipette and transfer into a 15mL conical tube
13. Add another 2mL of 1X Trypsin to aortas and incubate at 37C for 5 mins
14. After incubation, take aortas to dissecting microscope and use microscalpel to gently, scrape intima layer off of aorta
15. Collect all liquid with P1000 pipette and transfer into a 15mL conical tube
16. Wash aortas with 2mL of 0.04% BSA + 5% FBS in PBS-/- and transfer liquid to 15mL conical tube

17. Stop trypsin reaction by filling 15mL tube with an additional 6mLs of 0.04% BSA + 5% FBS in PBS-/-
18. Spin 15mL tube at RT for 5 min at 300g
19. Carefully aspirate out the supernatant (pellet will be very small, if visible at all)
20. Add 200uL of 1X RBC for 1 minute at RT
21. Stop reaction by adding 5mL of 0.04% BSA in PBS-/-
22. Spin at RT for 5min at 300g
23. Cells are ready for library prep (expect 2,000-10,000 cells)