Systems *Bioengineering* Laboratory

Mouse Primary Podocyte Isolation

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Materials

- 10X HBSS (Life Technologies, Cat: 14065-056)
  - Dilute to 1X with sterile ddH_2O
- Collagenase A (Roche Diagnostics GmbH, Cat: 10103586001)
  - Dissolve in HBSS to 1 mg/mL
- Iron Oxide Perfusate
  - 2.5mg/mL Fe_2O_3 (Sigma, Cat: 310050-500G), 1% BSA in 1X HBSS
- 100 µm cell strainer (BD Biosciences, Cat: 352360)
- 40 µm cell strainer (BD Biosciences, Cat: 352340)
- 25G butterfly phlebotomy needle set (BD Biosciences, Cat: 367298)
- Dissection instruments (sterile)
- Type I (rat tail) collagen (BD Biosciences, Cat: 354249)
  - 0.23mL of 17.4M acetic acid
  - 2.54mL of rat tail collagen (~4.3 mg/mL)
  - 197.23 mL of ddH_2O
  - Filter all 200 mL with a 0.22 µM filter
- 35 mm cell culture dishes
  - Coated with type I rat tail collagen
- Ketamine/Xylazine anesthetic
  - 1.17 µL Ketamine
  - 1 µL Xylazine
  - 7.83 µL 1X PBS
  - 10 µL/g of body mass
- RPMI cell culture medium
- Pharmacia LKB P-1 Pump (Pharmacia, Cat: 19-4611-XX)

Methods

1. Deeply anesthetize mouse with an IP injection of the ketamine/xylazine

   Note: This step requires proper training for animal handling and adequate experience to ensure animal welfare. All animal work must have prior approval by the local Institutional Animal Care and Use Committee.

   a. Weigh animal and prepare sterile injection of final solution for 10 µL/g of body mass
   b. Immobilize the animal by holding down from its scruff gently and inject full volume of the solution at a slow and steady pace.
   c. Return the animal to its cage and wait for 10 minutes checking its breathing intermittently.
   d. After the mouse becomes unresponsive to pinch/pull test, immobilize the animal on the surgical block by taping the paws open.
2. Spray the abdomen and chest with 70% ethanol, wait for it to dry
3. Perform a horizontal incision of the abdomen, followed by a left lateral thoracotomy, ensuring to cut away the diaphragm laterally
4. Cannulate the left ventricle with the 25G butterfly needle connected to the P-1 pump
   a. Perfuse for 1 minute with 1X 4ºC HBSS. Check kidneys for a pale color.
   b. Perfuse for 5 minutes with 4ºC iron oxide HBSS solution. Check kidneys for slightly pink hue.

Note: Pump flow rate should be at a maximum of 10 mL/minute. For ease, use pins to hold the butterfly needle in place.

5. Remove the kidneys and after decapsulating them, bilaterally dissect and mince them with a sterile, double-sided razor blade
6. Transfer minced kidneys to a 2 mL Eppendorf tube containing 1 mL Collagenase A in HBSS (1 mg/mL) and mix by gently shaking the tube
7. Incubate the 2mL centrifuge tube in a rotating carrousel at 37ºC for 30 minutes
8. Using a 100 µm cell strainer, filter the digested tissue into a 50mL centrifuge tube
    a. Gently use the top of the 2 mL Eppendorf tube to press the tissue into the filter
    b. Rinse the Eppendorf tube twice with 1 mL 1X HBSS
    c. Rinse the strainer five times with 1 mL 1X HBSS
9. Strain the eluent again using a new 100um strainer
    a. Wash the 50mL tube twice with 2 mL 1X HBSS and strain
10. Centrifuge the eluent at 200g for 5 minutes at 4ºC
11. The mixture should separate into three distinct layers
    a. On top is a semi-clear layer of supernatant; remove this by aspirating slowly using a 200 µL pipette tip
    b. Next is a thin layer of red, the non-glomerular fraction; remove this as well, ensuring not to disturb the bottom pellet
12. Using 1X HBSS, resuspend the pellet of glomeruli and transfer the contents to the original 2 mL centrifuge tube for washing using the Dynabead magnet system
13. Use the Dynabeads magnet system to wash glomeruli with 1.5 mL of 1X HBSS three times
    Note: Glomeruli will stick to the back wall of the centrifuge tube when the magnet is engaged. The 2 mL Eppendorf tubes have a straight side wall that works better with the Dynabead magnets.
14. Replace HBSS with 1.5mL of RPMI culture medium
15. Wash the wells of the collagen coated culture plate twice with 1X PBS before transferring the glomeruli to the wells
16. Transfer the contents of the 2mL centrifuge tube to the culture plate and store at 37°C
    Note: Do not touch for minimum of 3-4 days to allow glomeruli to settle with gravity and stick to the collagen-coated surface and for podocytes to move onto the culture plate. Podocytes may take up to 10 days to dedifferentiate and move onto the culture surface.