Systems *Bioengineering* Laboratory

Rat Glomerular Isolation

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If sacrificing via Perfusion:

Materials

- 300-400mL 4% PFA per rat (on ice)
- 3:1 mass ratio Ketamine / Xylazine preparation. We use 37.5mg/12.5mg Ketamine / Xylazine mixture.
- Isoflurane
- 3x50mL conical tubes per rat
- 12.5 mL of 20U heparin/mL PBS.
- 2x Hemostat
- 2x Surgical scissors
- Tweezers (large and small)
- Disposable razors
- Red biohazard bag
- Syringe with needle (26 or 27g)
- Pump with tubing
- Ventilated Hood with isolation chamber
- Blunted 18g needle

Methods

1. Weigh rat. Calculate dose of Ketamine/Xylazine mixture to be used. See Ketamine / Xylazine preparation for how to make this cocktail. We currently use 40mg ketamine per kg rat.

2. Administer Ketamine/Xylazine via IP injection.

3. Place rat in isolation chamber in ventilated hood. There is a 50mL conical tube in the chamber with holes in it. Place a few tissues inside along with a small amount (1-2mL) of isoflurane. Cover the chamber.

4. Observe the rat for sedation. After a few minutes, the rat should be unresponsive to stimulus.

5. Prepare and label a 50mL conical tube with approximately 15mL of PBS for the kidney that will be taken and undergo glomerular isolation.

6. Prepare and label a 50mL conical tube with approximately 15mL of PFA for the perfused kidney.

7. Prior to using the pump, prime it with PBS to clean it out and remove any air.

8. Without letting any air bubbles into the line, prime the line with approximately 12.5 mL of the PBS with heparin. After this, make sure the line feed is placed into the PFA. The PFA, heparin, and 50mL tubes containing PBS and PFA should all be cold and on ice.

9. Take the rat out of the chamber and place on workspace in the hood. With its stomach up, tape the arms and leg so that they are restrained.

10. Verify that the rat is sedated. The tail should be pale, the rat should not be moving, and if the paw is pinched the rat should not respond to pain stimulus. If necessary, wait a few more minutes. If the rat is still not completely sedated, inject another smaller dose of Ketamine / Xylazine.

11. Starting low in the abdomen, pinch the skin up and cut upwards to expose the abdominal cavity.

12. Locate the right (or left, does not matter) kidney and pinch off the vascular attaching it with a hemostat (should be located near the middle of the kidney). Make sure the hemostat is attached firmly.

13. Being careful not to puncture any internal organs, use a scissor to sever the kidney from the body. Place this kidney in the 50mL tube with ice cold PBS. Someone else should transfer this to the cold room and put in an ice try. After a few minutes, this kidney can be processed for glomeruli. ONCE THE KIDNEY HAS BEEN REMOVED, DO NOT REMOVE THE HEMOSTAT FOR THE REMAINDER OF THE PERFUSION.

14. Continue cutting the skin until the xiphoid process is reached. At this point, cut through the outer part of the ribcage to expose the heart. Be careful not to puncture the heart or any other organs while cutting. Once enough of the ribcage has been cut, it can be held down to keep the chest exposed with a hemostat.

15. With the heart exposed, use the 18g needle to puncture the left ventricle. The needle should piece the lower right portion of the heart at a slight angle so as to get the needle into the aorta.

16. When the needle has been placed, turn the pump on and begin perfusing with PFA. The pump speed should be around 1.5-2 on the pump we use, but this can be increased or decreased according to preference. The needle should be held in place for the remainder of the perfusion.

17. Using a pair of scissors, cut the right atrium. This should be the bulging structure on the top left of the heart.

18. Perfuse 150-300 mL of PFA until the vasculature, liver, and kidney become pale. In a good perfusion, the liver should start turning pale very quickly.

19. Turn off the pump. Remove the perfused kidney and place into the 50mL tube with ice cold PFA. NOTE: If the perfusion went poorly, and there was very little or no change in liver/vasculature, then prior to placing the kidney into the PFA cut it up into cortical sections with the disposable razors.
20. Remove the hemostats from the rat and place the body into the red biohazard bag.

If sacrificing via Decapitation:

Materials

- Guillotine
- Red biohazard bag
- Isoflurane.
- Ice

Methods

1. Weigh rat. Calculate dose of Ketamine/Xylazine mixture to be used. See Ketamine / Xylazine preparation for how to make this cocktail. We currently use 40mg ketamine per kg rat.
2. Observe the rat for sedation. After a few minutes, the rat should be unresponsive to stimulus. Confirm that the rat is sufficiently sedated by performing pinch paw test. If not sedated, leave in chamber a few more minutes or add more isoflurane.

3. Take rat to guillotine and decapitate. Immediately transfer the body to the cold room and put on ice.

4. Leave the rat on ice in the cold room for 5 minutes.

5. Remove kidneys from the rat and begin glomerular isolation.

Glomerular Isolation:

Materials

- 60mm cell culture dish
- Disposable razors
- Tweezers
- 3 Meshes and mesh holders (#75,#150,#300). (Note: do not trust mesh labels. When in doubt, verify by checking mesh size under microscope.)
- Amber collection jug
- 3-4L Sterile PBS per rat (Note: should be put in cold room overnight. If not, place in ice water bath for 30-60 mins prior to experiment.)
- Tissue grinder
- 2x50mL conical tube
- 2% BSA
- 3 Protein LoBind Eppendorf tubes per rat
- 1000 uL pipette
- 25mm cell culture dish
- PBS with 2x Halt protease inhibitor cocktail
- Liquid nitrogen
- Styrofoam container

All equipment and PBS except for plasticware should be autoclaved prior to use.

Methods

1. Set up the meshes for serial sieving. Place the 300 mesh (in the holder) on the bottom, with the 150 mesh in the middle, and 75 mesh on top. Pour a small amount of PBS to chill the metal meshes. Place all meshes on top of the collecting vessel, which should be seated in the middle of a tray containing ice.

2. Place kidney(s) on a cell culture dish. Using the disposable razors and tweezers, cut off any excess fat and tissue still attached to the kidney. Additionally, the renal capsule is a membrane surrounding the kidney. Puncture it with sharp tweezers remove it. Discard the membrane and non-kidney tissue that has been removed.

3. Using the disposable razor, mince the kidney up until it is a fine paste. This process should take about 5-10 minutes and the kidney tissue should have a paste like consistency with no chunks.

4. Transfer the paste to the 75 mesh. Use PBS to wash any remaining tissue on the dish into the mesh.

5. At regular intervals, pour PBS into the 75 mesh and gently push the tissue through with the tissue grinder. As you are pouring, check the second and third meshes to see if they are filling up and functioning properly. You will regularly have to lift the third mesh from the container as otherwise it will form a vacuum and seriously diminish the flow rate.

6. Keep pouring and pushing tissue on the 75 mesh until it is a white or off-white color. This should take 20-40 mins and 2-4 L of PBS depending on the rat and health of the kidney.

7. Remove the top (75) mesh and set it aside.

8. Pour 5-10 more times into the second (150) mesh directly. Do not use the tissue grinder, as this mesh is not as sturdy as the 75 mesh.

9. Remove the second (150) mesh.

10. If you look on the 300 mesh, you should see small white granular pieces resembling sand. Those are the glomeruli. Collect the glomeruli using a 1mL pipette to transfer from the third mesh into a labelled 50 mL conical tube. Optional: Pipet 2% BSA up and down in the pipette tip to prevent glomeruli from sticking to it.

11. Add approximately 20-30 uL of 2% BSA into the 50 mL conical tube with the glomeruli. This helps prevent glomeruli from sticking to the plastic. NOTE: the 50mL tube can also be precoated with BSA prior to the experiment.

12. Pour PBS into the mesh holder and then place it on its side. Collect as many glomeruli as you can. Rinsing PBS over the mesh at an angle can help push the glomeruli to the sides where they can be collected.

13. Create a counterbalance for the tube containing the glomeruli.

14. Spin down the gloms @ 500g for 5 minutes @ 4 degrees. Aspirate excess PBS.

15. Add ~5 mL of PBS with 2x Halt.

16. Agitate the suspension, and transfer 20-30 uL onto a 25mm dish and view under a BF microscope at 4x and/or 10x magnification to check for quality. There should be glomeruli visible and the ratio of glomeruli to tubule and other tissue should be high. Save and label these images.

17. Agitate the tube containing gloms. Transfer equal amounts to the 3 Eppendorf tubes by . Prior to transferring, coat the pipette tip with 2% BSA by pipetting it up and down a few times. Be sure to transfer as many of the glomeruli as possible. If there is still a lot stuck to the sides, add a small amount of PBS and agitate. In cases of extreme sticking, more BSA might need to be added to the tube.

18. Spin down the Eppendorf tubes for 5 minutes @ 500xg and 4 degrees Celsius and aspirate.

19. Add 500 uL of Cryostor to one of the tubes. The other two should just contain the pellet.

20. Flash freeze all 3 tubes with liquid nitrogen and store in -80ºC freezer.