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Mouse transcardial perfusion

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We use this protocol and it's working.

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Abstract

Protocol for performing pump-free transcardial perfusion in mice.

Materials

MATERIALS

- 20 mL syringe
- Hemostats **Roboz Catalog #**RS7117(curved) RS7116(straight)
- Scissors surgical. **Fine Science Tools Catalog #**14028-10
- Small curved hemostats **Fine Science Tools Catalog #**FST 13003-10

Troubleshooting

Preparation

1 Make sure there are enough sterile surgical instruments and other materials required to carry out the procedure (See list of supplies listed below).

1.1 **Supplies**
2, 20ml syringes
1, 23 gauge butterfly needle with 12 inch tubing
2 pairs of hemostat clamps; 1 small and 1 large
1, blunt/sharp scissor
1, fine microdissection scissor or Irridectomy scissors
1 blunt ended forceps
1-2 metal spatula/scoop
1 small rongeurs
5-6 inch Metal or plastic tray
Container filled with cold 1x PBS on ice (Amount varies depending on number of mice being perfused. 15-20 ml/mouse
Container filled with cold NBF (Normal buffered formalin) on ice (Amount varies depending on number of mice being perfused. 20 ml/mouse. Any portion using NBF should be performed in fume hood.

2 Prepare work area for transcardial perfusion. Clean the workspace with 70% ethanol or any other available cleaner.

3 Set out tray or other platform on which to perform perfusion; place nose cone attached to anesthesia apparatus in convenient location.
Set out all scissors, forceps, and hemostat clamps in easy to reach locations around tray or platform. Set spatula/scoop, rongeurs and glass vial filled with NBF aside. They will be used last.

4 5.Prepare a 20 ml syringe filled with cold PBS making sure they are free of bubbles. Do the same to prepare another syringe with 10% Neutral buffered formalin (NBF). Place them on ice to keep it cold till it's needed in the procedure. If multiple mice need to be perfused in a single session, prepare the amount of PBS and NBF accordingly.

Anesthesia

5 Determine and confirm the mouse to be perfused. Anesthetize mouse with isoflurane in induction chamber at ~5% Isoflurane and 2% Oxygen. Mouse should be well into the surgical plane of anesthesia but breathing regularly.

6 Place mouse ventral side up on the tray, make sure the area is well-lit. Secure mouse in place- this can be done by using thumb pins or tape – (generally not needed).

7 Perform toe and nail pinch to ensure that the mouse is unresponsive. Better to wake the mouse now than further in the procedure.

8 Maintain isoflurane and oxygen at ~5% and 2%, respectively.

Surgical opening

9 Be sure all the surgical instruments are within reach and easy to pick-up. Once you start opening the mouse you have to move quickly and at a steady pace.

10 Use blunt/sharp scissors to cut laterally into the area just below the chest cavity. Next, make a vertical incision from this cut going in the diaphragm (looks like an upside-down T). Keep the scissor tips up to ensure not to cut any organs.

11 Incise along the rib cage both the left and right side. Clamp the end of the rib cage with the hemostat and place it over the head.

12 At this point you should see the exposed chest cavity, and the heart covered by a wall of thin connective tissue. Cut through this **with extreme caution**. The heart generally rests on the other side of the wall, you do not want to accidentally cut through the heart.

13 Once the heart is exposed, ready the winged needle connected to the syringe with 20 ml of cold PBS.

Perfusion

14 Identify the apex of the heart, left ventricle, and the right atrium of the heart.

15 Insert needle into the left ventricle – do not push needle far into ventricle. Clamp the heart and the needle with a hemostat to secure its position.

16 As soon as you insert the needle, cut the right atrium with iridectomy scissors. Blood will then start flowing from this area. At this point, push the syringe plunger at a moderate and steady pace. Once done injecting 20 ml PBS, check to see if the liver is cleared of blood.

17 Quickly switch from the PBS syringe to the syringe with cold NBF and use the same moderate and steady pace to fix the mouse with NBF. Observe tremors at the extremities as an evidence of fixation taking place.

18 Once 20 ml of NBF has been used up, check to see if the mouse body is stiff. If the mouse does not feel stiff and/or did not convulse during perfusion, perfuse with another 10-20 ml of NBF.

Brain dissection

19 Use clean spatula to free the brain of ocular and olfactory connections. Tease the brain away from the skull and remove it gently.

20 Remove the skin over the skull. Use ronguers to remove skull part by part; watch for dura mater as it can tear the brain.

21 Use clean spatula to free the brain of ocular and olfactory connections. Tease the brain away from the skull and remove it gently.

Post-dissection

22 Place the brain in a labeled vial of NBF. Let sit for a minimum of 48 hours and then move it in 70% ethanol solution before carrying out histological procedures such as paraffin embedding and sectioning.