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Mouse Surgery – Transplantation of human thymus into the kidney capsule of NSG mice V.2

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Human Islet Research Ne…

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We use this protocol and it's working

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Abstract

This protocol details our minimally invasive approach for implanting human thymus tissue under the kidney capsule of NSG mice. In contrast to our open abdominal approach, this approach is from the dorsal aspect of the mouse and requires only two interrupted sutures and 1-2 staples to close the incision. The technique can be applied to other strains of mice, though we have found the NSG kidney capsule to be more delicate, and thus more challenging to manipulate during surgery.

Note

The changes of this protocol was made by Austin Chen on Markus Holzl original protocol.

Note

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Materials

Required material (everything that comes in contact with the mouse has to be sterilized)

¤ Scissors (sharp)
¤ 2 Forceps (blunt)
¤ 2 Forceps (pointed)
¤ Sewing Forceps
¤ Forceps (bent)
¤ PBS
¤ Wound Stapler
¤ Surgical thread
¤ Alcohol
¤ Wound staples
¤ Thymic tissue
¤ Lidocaine (local painkiller)
¤ Syringe and needle (various)
¤ Anesthesia (ketamine or isoflurane)
¤ Gentamicin
¤ Buprenorphine
¤ Sterile Napkins
¤ Iodine Sweeper
¤ Alcohol preps
¤ Gauze (sterile)
¤ cotton tipped applicators (sterile)
¤ 3 cm **petri dish**
¤ Electrical shaver
¤ Syringe
¤ Request T-cell depleting AB
¤ Restrainer
¤ Request medicated water

- ·Sterilization of tools in 70% EtOH for 30 minutes prior to surgery
- ·Let tools air dry in hood after 30 minute soak. Drying takes ~15-20 minutes.
- ·For surgical thread, we use absorbable 4-0 Vicryl (**McKesson Pharmaceutical; Cat: J214H**)

Concentrations of the agents:

- ·Isoflurane: 2-5%

- ·Ketamine/Xylazine solution: 0.8 mL Ketamine (stock 100mg/ml)+ 0.6 mL Xylazine (stock 20mg/ml) + 8.6 mL PBS = 10 mL total

- ·Lidocaine: 2% (200 µL in 9800 µL PBS)

- ·LoCD2 antibody: Prepare a working solution that will allow you to inject (intraperitoneally) 400 ng/mouse in a volume of 1000 µL per mouse

- ·Buprenorphine/Gentamicin solution:
  o Working solution concentration: 12.5 ug/mL buprenorphine, 2.5 mg/mL gentamicin in 1x PBS

  o Buprenorphine: (stock: 0.3mg/ml; Buprenex CIII 0.3mg/mL; Henry Schein; cat: 055175)
    ◊ Add 1 mL buprenorphine to 11 mL 1x PBS (this is now 25 ug/mL buprenorphine)

  o Gentamicin: (stock: 50mg/ml; **ThermoFisher; cat: 15750078**)
    ◊ Add 1 mL gentamicin to 9 mL 1x PBS (this is now 5 mg/mL gentamicin)

  o Aliquot the 25 ug/mL buprenorphine with 5 mg/mL gentamicin in equal parts into 2 mL cryovials to maintain sterility
    ◊ 950 uL of 25 ug/mL buprenorphine + 950 uL of 5 mg/mL gentamicin
    ◊ Resulting concentration is 12.5 ug/mL buprenorphine and 2.5 mg/mL gentamicin

- ·Dosage: Approximately 0.1 ug buprenorphine per gram of mouse and 0.5mg gentamicin/mouse is necessary
  o Inject subcutaneously each mouse 200 uL of this 12.5 ug/mL buprenorphine and 2.5 mg/mL gentamicin solution
    ◊ With this working solution and injection of 200 uL, it gives about 2.5 ug buprenorphine per mouse and 2.5 mg of gentamicin per mouse. This is exactly enough buprenorphine for a 25g mouse, but it seems to be tolerated well for any weight mouse.

Day 0 or 1, Irradiation

1 1. Put the mice into an irradiation cage

2. Irradiate the mouse with indicated grey
120 – 150 for NSG
250-270 for NS

3. Put the mice back into their original cage

Day 1, Thymus surgery (all of these steps take place within the hood)

2 **Make sure to read and understand steps 14-19 prior to starting the protocol as these steps take place in quick succession and require strategic placement of forceps, cotton tipped applicators, other tools, and the tissue within the hood.**

1. Thaw the thymic tissue according to protocols. In brief, put thymus into warm BM Medium for 15 minutes. Transfer into new BM Medium for 15 min. Make 1 mm³ pieces.
- ***Additionally it can be discussed to vigorously pipette the tissue, thereby, excess of thymocytes can be removed.***

2. Place the thymic tissue into medium and a 3 cm petri-dish and cut into appropriate pieces (1 mm³ pieces are usually used)

3. Anesthetize mice using ketamine/xylazine ip injection (or isoflurane)
- (See **anesthesia protocol**)

4. ***For surgery via the back***: Shave the left dorsal aspect of the mouse with the electric shaver.

5. Wipe the shaved area with iodine and then wipe excess iodine with an alcohol wipe

6. Find the approximate location of the kidney underneath the skin palpating the dorsal area and looking for the kidney. Both spleen and kidney appear dark red and it can be hard to differentiate the two.
- ·The kidney appears rounder and more plump while the spleen is thinner and longer.
- ·It may be hard to locate the kidney

7. With a pointed scissor, make an approximately 1 cm cut into the left side of the back, parallel to the spine. To make a cut parallel to the spine, you must pinch the skin perpendicular to the spine. If you then cut this perpendicular fold, the result will be a parallel incision.

- ·Do this by pinching the skin together with the forceps so that there is about 0.5 cm on each side of the pinch. Cut the fold to make a 1 cm incision.

8. Gently put the scissors below the skin, being careful not to puncture the peritoneum. Open the scissors to separate the fascia from the skin.
- ·Do this a few times to give maneuverability between the skin and the fascia

9. Again, try to locate the kidney underneath the peritoneum
- ·It may not be possible to see the kidney, and only the spleen may be visualized
- ·If this is the case, making the next cut slightly away from the spleen, closer to the spine, may be helpful as one difficulty later on is having the spleen continually protrude out of the hole, making it difficult to isolate the kidney.

10. With a pointed scissor, cut an approximately 0.5 cm cut into the peritoneum, parallel to the spine. This cut is essential. If it is too small, the kidney cannot pop out. If it is too big, the kidney slips back in. This requires some practice and each mouse kidney is a different size; sometimes adjustments to the cut must be made.

11. Squeeze the kidney out by pushing the belly with your thumbs with counter pressure from your index fingers near the dorsum of the mouse. If perfect, the kidney will pop out. Oftentimes, intestines or spleen will protrude out of the hole with the kidney. If this is the case, use a Q-tip soaked in 1x PBS to gently push the other viscera back into the hole while keeping the kidney out. This step may require a few tries and a few Q-tips.
- ·If done correctly, the kidney should remain above the peritoneum and will not slip back in.

12. If the kidney is slips back, you can use a wet Q-tip pressed against the mouse to prevent the kidney from slipping back into the hole while you operate.

13. When the kidney is out, it is **essential** to wet the kidney every 20-30 seconds with 1x PBS using a wet Q-tip.

14. Create a hole in the kidney capsule by using 2 forceps to gently lift the capsule and then poke a hole.
- ·This can be done with 2 sharp forceps or 1 sharp forcep and 1 blunt forcep.
  i. The sharp forcep can be used to create the hole.
  ii. Alternatively, a 28 gauge needle can also be used to poke the hole.

15. Continue holding the capsule with the forcep that was not used to poke the hole in order to keep track of the location of the hole.

16. While holding the capsule, insert the space making tool (a blunted pipette tip) under the kidney and make space. If done right, bleeding is minimal and the capsule stays intact.
- ·If you do not have a space making tool, you can use a blunt straight forcep to make space instead keeping the forcep closed and then probing under the capsule and sliding **very gently** back and forth and forward and back.

17. With a blunt, straight forceps, insert a 1mm³ piece of thymic tissue under the kidney capsule
- ·To prevent it from slipping out, try to insert the tissue around 0.5 cm or more past the entry of the hole. The balance is pushing the thymic piece in while not tearing the kidney capsule.
- ·Once you release the thymic piece and retract the forceps, you may then have to re-enter with the blunt forceps closed to nudge the piece back in as the thymic pieces are sticky and may get stuck on the forceps as you try to release and retract.

18. Check that the thymic piece is indeed under the kidney capsule.

19. Gently push the kidney into the retro peritoneum by using forceps to hold and slightly widen the opening to allow the kidney to fall in.

20. Sew the peritoneum closed with 2 interrupted sutures using the absorbable 4-0 vicryl.

21. Close the skin with the stapler.

22. Inject mice subcutaneously with 200 µL of Buprenorphine (12.5 ug/mL) + Gentamicin (2.5 mg/mL) solution

23. Add topical eye ointment to the mouse's eyes

24. Carefully place the mouse into the cage and put it on a cage heater

25. Mice should awaken in ~30 minutes

Day 2, Medical Treatment

3 ***After Surgery, antibiotics, analgesics, loCD2, and CD34+ cells***

1. Inject another dose **subcutaneously** of 200 µL of Buprenorphine and Gentamicin 1 day after surgery.

2. Inject **intraperitoneally** 400 ng of loCD2 antibody into each mouse.

a. We prepare working solutions so that we can inject 1000 µL of solution for each mouse.

For example, prepare 4000 ng/mL solution in 10 mL for 9 mice.

i. We prepare 1 mL extra to ensure that each mouse gets enough.

b. Only inject loCD2 antibody if you are implanting tissue that may contain remnant thymocytes, such as fetal thymus pieces.

3. Each mouse must receive CD34+ cells since they were irradiated. Inject **intravenously** 100,000-200,000 CD34+ cells.

a. More CD34+ cells leads to quicker reconstitution.