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Title: Blocking Lymph Flow by Suturing Afferent Lymphatic Vessels in Mice

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   No

   If **No**, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Olympus S261 (522-STS OH141791) with light source: Olympus Highlight 3100

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **no**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Shan Liao:** This technique interrupts lymph flow with minimal damage to lymphatic endothelial cells and with precise control of blockade timing. It can be used to study how lymph flow impacts homeostasis and immune responses in the lymph node.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Vid NOTE: Author did not want to remove mask, Take 1 NG, Take 2 good MS and CU

Introduction of Demonstrators on Camera

1.2. **Shan Liao:** Helping to demonstrating the procedure will be Jingna Xue, a graduate student from my laboratory.

1.2.1. INTERVIEW: Author saying the above.

1.2.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera. Vid NOTE: take 1 NG. take 2 good

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Calgary.

Protocol

2. Preparation of Materials

2.1. To prepare an injection apparatus, cut approximately 30 centimeters of polyethylene tubing **[1]**. Connect the tip of needle A to one end of the tubing **[2-TXT]**. Carefully dislodge another needle and connect the broken side to the other end of polyethylene tubing **[3]**. Then, attach needle A to a 1-milliliter tuberculin syringe **[4]**.

2.1.1. Talent cuts a piece of tubing.
2.1.2. Talent connects needle A to the tubing. **TEXT: Needle A: 30 G x 1⁄2**
2.1.3. Talent dislodges another needle and connects the broken side to the tubing.
2.1.4. Talent attaches needle A to a syringe. Vid NOTE: Use 2nd reset near the end of clip

2.2. Immediately before use, prepare a 10 to 1 ketamine-xylazine mixture in saline **[1]**.

2.2.1. Talent prepares mixture of ketamine and xylazine in saline. NOTE: This reagent is pre-mixed, only the tube with names were shot

3. Preparation of the Animal for Surgery

3.1. After anesthetizing the mouse by injecting 250 microliters of the ketamine-xylazine mixture intraperitoneally **[1]**, ensure full anesthetization with a toe pinch **[1a]**.

3.1.1. Talent checks mouse for anesthetization.
3.1.1a Added shot: CU of pinch

3.2. Shave the fur around the legs with hair clippers **[1]**. Then, apply depilatory cream around the leg **[2]**.

3.2.1. Talent shaves the fur around the legs.
3.2.2. Talent applies depilatory cream.

3.3. After 5 minutes, wipe off the residual fur and the depilatory cream using a moist tissue, then clean the leg with sterile water **[1]**. Spray 70 percent ethanol around the leg to sterilize the operating area **[2]**.

3.3.1. Talent wipes off fur and depilatory cream, ~~and cleans the leg with sterile water~~.
3.3.2. Talent sprays ethanol around the leg.

4. Surgical Suturing of Afferent Lymphatic Vessels

4.1. Place the mouse in a prone position and use surgical tape to expose the operation area on the right leg **[1]**. NOTE: Switch order of 4.1 and 4.2

4.1.1. With mouse in a prone position, talent uses surgical tape to expose the operation area.

4.2. Intradermally inject 5 microliters of 1 percent Evans blue dye, or 9 centimeters of the fluid from the injection apparatus tubing, into the right footpad of the mouse **[1]**. Gently massage the footpad to help the fluid enter the lymphatic vessels **[2]**. NOTE: This step should be moved above step 4.1

4.2.1. Talent injects Evans blue dye into mouse.
4.2.2. Talent massages the footpad of the mouse.

4.3. Under a dissecting microscope, locate an incision site 5 millimeters from the bottom edge of the popliteal fossa **[1]**. Using a pair of scissors, make a small incision, approximately 5 millimeters in length **[2]**. *Videographer: This step is important!*

4.3.1. SCOPE: Talent points to incision site. Vid NOTE: Alternate SCOPE angle for 4.3.1 – 4.7.2 available
4.3.2. SCOPE: Tip of scissors making incision.

4.4. Then, use fine operation forceps to stretch the incision and expose the collecting lymphatic vessels **[1]**. Identify both of the afferent lymphatic vessels leading to the popliteal lymph nodes **[2]**.

4.4.1. SCOPE: Talent uses forceps to expose the collecting lymphatic vessels.
4.4.2. SCOPE: Talent points out the afferent lymphatic vessels.

4.5. Using a needle holder, cautiously insert the suture needle between the afferent lymphatic vessel and the Saphenous artery. Gently pull the needle out around the afferent lymphatic vessel **[1]**. Carefully pull the suture string, leaving about 2 centimeters of the suture string behind **[2]**. *Videographer: This step is important!*

4.5.1. SCOPE: Talent inserts suture needle and pulls the needle out.
4.5.2. SCOPE: Talent pulls the suture string.

4.6. Use the needle holder to help tie a surgeon's knot **[1]**. Gently massage the footpad to ensure no Evans Blue dye passes the suture site **[2]**. Then, cut the excess string with scissors **[3]**. *Videographer: This step is important!*

4.6.1. SCOPE: Talent ties a surgeon's knot.
4.6.2. Talent massages the footpad.
4.6.3. SCOPE: Talent cuts excess string.

4.7. Suture the other afferent lymphatic vessel **[1]**. Then, close the skin incision with the same suture that was used for the lymphatic vessels **[2]**. *Videographer: This step is important!*

4.7.1. SCOPE: Talent begins suturing the other afferent lymphatic vessel.
4.7.2. SCOPE: Talent sutures closed the skin incision.

NOTE: A cut happened here due because mouse woke up from the anesthesia. Another mouse was used for shooting the sham control 4.8

4.8. For the sham control, intradermally inject 5 microliters of 1 percent Evans blue dye in the left footpad and massage the footpad **[1]**. Open the skin with an excision, and then close the wound without suturing the vessel **[2]**.

4.8.1. Talent injects dye in the left footpad, and massages the footpad. Vid NOTE: Take 1 NG, take 2 good
4.8.2. SCOPE: Talent cuts the skin, and then sutures the wound closed. Vid NOTE: Alternate scope angle available

4.9. When monitoring the mouse post-surgery, the right leg should show edema, with Evans Blue dye spread to the thigh, while the control leg will show Evans blue dye restricted to the footpad **[1]**.

4.9.1. Mouse legs, with right leg swollen.

5. Tracking the Lymph Flow

5.1. Immediately after the surgery, intradermally inject 10 microliters of 2 percent FITC in both the right footpad and the left **[1-TXT]**.

5.1.1. Talent injects FITC in both feet of mouse. **TEXT: FITC: fluorescein isothiocyanate**

5.1.1a Added shot: CU of bottle

5.2. Two, six, and twelve hours after FITC injection, collect the popliteal lymph nodes from the fossa of euthanized mice **[1-TXT]**. Carefully remove the perinodal adipose tissue around the pLNs **[2]**.

5.2.1. Talent collects popliteal lymph node from mouse. **TEXT: pLN: popliteal lymph node**
5.2.2. ~~SCOPE: Talent removes tissue from around the pLNs.~~ NOTE: This step is same as 5.2.1

5.3. Embed the pLNs in optimal cutting temperature compound, with the medullary sinus area facing to the side of the cryomold **[1]**. Use a cryotome to prepare 20-micron frozen sections **[2]**.

5.3.1. SCOPE: Talent places pLN in cryomold with optimal cutting compound.

~~5.3.2.~~ Talent uses cryotome to make slices.~~-~~NOTE: machine broken; replaced by a 'walking-out' shot

5.4. To determine the FITC distribution in the pLNs, image the cryosections under a confocal microscope **[1]**.

5.4.1. Talent places a cryosection under a microscope.

Results

6. Results: Selective Prevention of Lymph Flow

6.1. Confocal images captured 2, 6, and 12 hours after FITC injection show substantially reduced FITC accumulation in the popliteal lymph nodes after suturing the afferent lymphatic vessels. The residual FITC in the pLNs was preferentially accumulated in the lymph node sinuses **[1]**.

6.1.1. LAB MEDIA: Figure 2.

6.2. Confocal images of the perinodal adipose tissue show when lymphatic vessels are blocked by suturing, FITC enters the tissue and the lymph node sinuses, but is not effectively distributed throughout the lymph nodes **[2]**.

6.2.1. LAB MEDIA: Figure 3.

Conclusion

7. Conclusion Interview Statements

7.1. **Shan Liao:** When attempting this technique, it is important to remember that inserting the suture needle between the blood vessel and the lymphatic vessels is critical. Failure of this step might break the vessels. Vid NOTE: 7.1.1 – 7.3.1 Good MS and CU

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.5.1 and 4.5.2.*

7.2. **Shan Liao:** After performing this method, it is possible to immunize mice with infection or other immune stimulation to study how lymph flow regulates immune protection.

7.2.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.

7.3. **Shan Liao:** The function of lymph flow is not well understood. This technique can be used to study cell migrations in the lymph node in the absence of lymph flow.

7.3.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.