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Title: A Murine Tail Lymphedema Model

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **YES**

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Leica , Model- MSV266

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

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

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

Current Protocol Length

Number of Steps: 15
Number of Shots: 28

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Al Hassnein:** Lymphedema is a chronic condition with no cure. It affects one-third of patients who have axillary lymph node surgery and radiation in the treatment of breast cancer. Having a valid animal model facilitates understanding of the mechanism and development of novel treatment strategies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Mithun Sinha:** The mouse tail model of lymphedema is reliable and reproducible, with a rapid onset. It exhibits histological changes consistent with human lymphedema.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~**OPTIONAL:**~~

~~1.3. **Al Hassanein:** We use a novel targeted gene-based therapeutic approach that employs tissue nanotransfection technology, or TNT. TNT facilitates focal, non-viral gene delivery using the TNT 2.0 chip with nanochannel poration through a rapid, focused electric field.~~

~~1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.~~

NOTE: Optional statement was not captured

Introduction of Demonstrator on Camera

1.4. **Al Hassanein:** Demonstrating the procedure will be Dr. Ganesh Mohan, a post-doctorate from my laboratory.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. All animal experiments were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee.

Protocol

2. Surgical Disruption of Mouse Tail Lymphatics

2.1. To begin, position the eight-week-old sedated mouse dorsally **[1-TXT]** and prep the tail with 70% isopropyl alcohol **[2]**.

2.1.1. Talent placing the mice dorsally. **TEXT: Use 8-week-old C57BL/6 mice**

2.1.2. Talent applying alcohol to the tail.

2.2. Use a caliper to measure the tail diameter at 5 millimeter increments starting 20 millimeters from the base of the tail **[1]**.

2.2.1. Talent measuring the tail diameter using caliper.

2.3. Mark a 3-millimeter circumferential excision on the tail 20 millimeters from the base **[1]**.

2.3.1. Talent marking the tail for cricumferential excision.

2.4. Under surgical microscopic magnification, perform a meticulous 3-millimeter full-thickness skin excision, leaving all the underlying vasculature intact. Incise the superior circumferential mark first through the dermis followed by a circumferential full thickness incision 3 millimeters distal to the first incision **[1]**. *Videographer: This step is difficult and important!*

2.4.1. SCOPE: Talent making the 2 circumferential incisions.

2.5. Make a perpendicular full thickness vertical incision to connect the two incisions, then use a toothed fine pickup to grasp a leading edge **[1]** and dissect deep within the avascular plane to the dermis and superficial to the vein adventitia with microscissors **[2]**.

2.5.1. SCOPE: Talent making the perpendicular incision connecting two incisions.

2.5.2. SCOPE: Talent dissecting within the avascular plane to the dermis.

2.6. Inject 0.1 milliliter of 1% isosulfan blue subcutaneously proximal to the tip of the tail **[1]**. Identify the two lymphatic channels, appearing blue due to the injection, adjacent to the lateral tail veins **[2]**. *Videographer: This step is important!*

2.6.1. SCOPE: Talent injecting isosulfane blue to the tail.

2.6.2. SCOPE: Lymphatic channels.

2.7. Transect the lymphatics carefully, dissecting a plane between the lateral vein and the lymphatic with straight microsurgical scissors **[1]**. Pass the tip of one scissor blade between the lymphatic vessel and the lateral vein and close the blades to transect the lymphatic vessel **[2]**.

2.7.1. SCOPE: Talent transecting the lymphatics.

2.7.2. SCOPE: Talent transecting the lymphatic vessel.

2.8. When finished, dress the tail wound with an adherent clear dressing **[1]**.

2.8.1. Talent dressing the tail wound.

3. Functional lymphatic evaluation with near infrared laser angiography ~~and focal delivery of nucleic acid cargo to mouse tail using TNT~~ NOTE: Avoid using TNT term or procedure anywhere

3.1. Administer indocyanine green 0.1 milliliter subcutaneously into the distal mouse tail near the tip **[1-TXT]**.

3.1.1. Talent administering the dye in distal tail. **TEXT: 25 mg/10 mL**

3.2. Dim the room lights **[1]**, then place near-infrared laser angiography in buffering setting and perform live imaging **[2]**.

3.2.1. Talent dimming the room lights.

3.2.2. Talent setting the angiography machine.

NOTE: 3.3-3.7 was not captured to exclude TNT procedure

~~3.3. For TNT, exfoliate the anaesthetized mouse tail using topical skin exfoliation cream **[1-TXT].**~~

~~3.3.1. Talent applying exfoliation cream to the tail. **TEXT: TNT-Tissue nanotransfection technology**~~

~~3.4. Immerse the mouse tail in 10 milligrams per milliliter collagenase solution at 37 degrees Celsius for 5 minutes **[1].** Load DNA into the TNT chip reservoir **[2].**~~

~~3.4.1. Talent immersing the tail in collagenase solution.~~

~~3.4.2. Talent loading the DNA in TNT chip reservoir.~~

3.5. Place the TNT silicone chip device over the desired focal site of delivery on the tail with the nanoneedles in contact with the tail **[1]**.

3.5.1. Talent placing the chip device over focal site using nanoneedles.

3.6. Place a positive electrical probe in the reservoir **[1]**. Attach the negative probe to a 30-gauge needle **[2]** and insert the needle subcutaneously into the tail to the site of delivery **[3]**. *Videographer: This step is important!*

3.6.1. Talent placing a positive electrical probe in reservoir.

3.6.2. Talent attaching the negative probe to 30 G needle.

3.6.3. Talent inserting the needle in the site of delivery.

3.7. Apply square wave pulse electric stimulation of 10 by 10 millisecond pulses at 250 volts and 10 milliamperes **[1]**. *Videographer: This step is important!*

3.7.1. Talent applying electric pulse simulation.

Results

4. Assessment of progressive swelling, lymphatic function, and focal delivery of genetic cargo in the mouse tail lymphedema model

4.1. After administration of isosulfan blue into the tail tip, the lymphatics exhibited blue color **[1]**. The lymphatics were disrupted while adjacent lateral veins were preserved **[2]**.

4.1.1. LAB MEDIA: Figure 1C. *Video editor focus on the yellow arrow.*

4.1.2. LAB MEDIA: Figure 1C. *Video editor focus on the white arrow.*

4.2. The progressive swelling and sustained persistant lymphedema in the mouse tail after lymphedema induction is shown here **[1]**.

4.2.1. LAB MEDIA: Figure 2A.

4.3. The mouse tail volume, as calculated by the truncated cone equation, peaked at week 4 and plateaued to week 6 followed by gradual improvement that was sustained until week 15 **[1]**.

4.3.1. LAB MEDIA: Figure 2B and 2C. *Video editor focus on the bar and line graph corresponding to 28 days.*

4.4. High resolution laser speckle contrast imaging was done to confirm mouse tail perfusion in the lymphedema tail model showing injured lateral veins **[1]** and intact lateral veins for assessment of tail vasculature patency **[2]**.

4.4.1. LAB MEDIA: Figure 3A. *Video editor focus on the black arrow showing injured veins.*

4.4.2. LAB MEDIA: Figure 3B. *Video editor focus on the black arrow showing intact veins.*

4.5. Near infrared laser lymphangiography demonstrated preoperative intact lymphatics **[1]** and no ICG transit beyond surgical site postoperatively, thereby confirming that swelling was caused by lymphatic dysfunction **[2]**.

4.5.1. LAB MEDIA: Figure 4. *Video editor focus on the day 0 images.*

4.5.2. LAB MEDIA: Figure 4. *Video editor focus on the ICG images of day 19 and 35.*

4.6. The efficiency of genetic cargo delivery using tissue nanotransfection technology was demonstrated by delivering fluorescein amidite labeled DNA to the murine tail **[1]**.

4.6.1. LAB MEDIA: Figure 5C.

Conclusion

5. Conclusion Interview Statements

5.1. **Al Hassanein:** Using tissue nanotransfection technology to deliver genes to the lymphedema mouse tail is an exciting prospect for novel targeted therapies with a potential clinical translation to treat lymphedema. NOTE: Conclusion statement was altered to exclude "tissue nanotransfection technology"

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.