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Title: Delayed Intramyocardial Delivery of Stem Cells After Ischemia Reperfusion Injury in a Murine Model

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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?

   Yes

   We have our own microscope camera

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**

Current Protocol Length

Number of Steps: 19
Number of Shots: 44

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Michaela Olthoff:** Ischemic damage induces a hostile pro-inflammatory environment affecting the survival of transplanted cells. Our protocol presents an alternative method for providing biologics by circumventing the initial immune response to injury.

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

1.2. **Michaela Olthoff:** The main advantage of this protocol is that it makes it possible to administer therapeutics one week following injury, mimicking treatment in the clinical environment.

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

OPTIONAL:

1.3. **Michaela Olthoff:** Our primary focus is the ischemia reperfusion injury model and transplantation of stem cells. However, this approach can be utilized in models of inflammatory disease that involve introduction of biological therapies.

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

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the Mayo Clinic College of Medicine.

Protocol

2. Preparation and Intubation

2.1. Begin by autoclaving all surgical instruments. If multiple surgeries are to be performed in one session, clean the instruments after each animal and re-sterilize them using a hot bead sterilizer **[1]**.

2.1.1. WIDE: Establishing shot of talent walking to the autoclave and taking out the surgical instruments.

2.2. Subcutaneously administer analgesic **[1-TXT]**, weigh the animal **[2]**, and input the weight into the ventilator **[3]**. Shave the left side of the chest from the sternum to the level of the shoulder and apply depilatory cream to remove excess fur **[4]**.

2.2.1. Talent administering analgesic. **TEXT: Buprenorphine SR 1 mg/kg**

2.2.2. Talent weighing the mouse.

2.2.3. Talent inputting the weight on the ventilator.

2.2.4. Talent shaving and removing fur. Videographer NOTE: broken up into 2 different shots, slated as 2.2.4 and 2.2.4A

2.3. To prevent lung collapse, maintain the positive end-expiratory pressure at 2 centimeters of water for the ischemia reperfusion procedure and change it to 3 centimeters of water for the delayed injection of cells procedure **[1]**.

2.3.1. Talent setting the PEEP.

2.4. After anesthetizing the mouse, intubate it with a 20-gauge endotracheal tube **[1]** and transfer it to a controlled heating pad to maintain a body temperature of 35 to 37 degrees Celsius **[2]**. *Videographer: This step is important!*

2.4.1. Talent intubating the animal. Videographer NOTE: For timeliness purposes, these shots were combined into one clip, but slated with 2.4.1 and 2.4.2. After the reframe is where 2.4.2 is. For 2.5.1, the mouse is never positioned on the ventilator, but remains on the heating pad.

2.4.2. Talent putting the mouse on a heating pad.

2.5. Place the mouse in lateral recumbency with cranial end on the left and caudal end on the right **[1]**. Scrub the surgical area, alternating between povidone-iodine and alcohol swabs three times **[2]**, and apply ophthalmic ointment to both eyes **[3]**.

2.5.1. Talent positioning the mouse on the ventilator.

2.5.2. Talent cleaning the surgical area.

2.5.3. Talent applying ointment to eyes.

Videographer NOTE: There were a lot of shots called out as SCOPE. The talent has a Scope camera that can record, so will send all of the SCOPE shots. I did shoot some miscellaneous B-Roll of operating the microscope and making "fake" incisions

3. Ischemia Reperfusion Injury

3.1. Use a number 10 blade scalpel to make a vertical incision 2.5 millimeters to the right of the leftmost nipple in the field of view **[1]**, then use scissors to cut through the superficial muscle layers until the intercostal muscles and ribs are visible **[2]**. *Videographer: This step is important!*

3.1.1. SCOPE: Ischemia Reperfusion Injury_1.mp4. 0:00 – 1:15. Talent making the incision. NOTE: Authors have uploaded the microscope footage to their project page. The videos are long, but there are parts where nothing is going on (talent swapping tools or adjusting focus) that can be cut. Please feel free to get in touch with scriptwriter if help is needed in figuring out what to cut.

3.1.2. SCOPE: Ischemia Reperfusion Injury_1.mp4. 1:15 – 2:40. Talent cutting through muscle.

3.2. While lifting the ribs and surrounding tissue, cut through the intercostal space between the 4th and 5th ribs, then insert the eyelid retractor into the open space **[1]**. Retract the pericardium using curved forceps, moving the lung upwards and out of view **[2]**.

3.2.1. SCOPE: Ischemia Reperfusion Injury_1.mp4. 2:40 – 4:27. Talent cutting through the intercostal space and inserting the eyelid retractor.

3.2.2. SCOPE: Ischemia Reperfusion Injury_1.mp4. 4:27 – 5:17. Talent retracting the pericardium.

3.3. Pass a 9-0 nylon suture through the myocardium beneath the LAD artery 2.5 millimeters distal to the left auricle, maintaining smooth and consistent movement to prevent tearing of the tissue or puncturing a major blood vessel. Tie a loose square knot **[1]**, then place 1 centimeter of polyethylene tubing within the loose knot **[2]**. *Videographer: This step is difficult and important!*

3.3.1. SCOPE: Ischemia Reperfusion Injury_1.mp4. 5:17 – 8:14. Talent passing the suture and tying the knot.

3.3.2. SCOPE: Ischemia Reperfusion Injury_1.mp4. 8:14 – 8:38. Talent placing tubing within the knot.

3.4. Secure the suture around the tubing and confirm ischemia by pallor and ventricular arrhythmia **[1]**, then release after 35 minutes **[2]**. After removing the tubing, wait for 5 minutes to confirm reperfusion of the myocardium **[3]**. *Videographer: This step is important!*

3.4.1. SCOPE: Ischemia Reperfusion Injury_1.mp4. 8:38 – end. Talent securing the suture.

3.4.2. SCOPE: Ischemia Reperfusion Injury_2.mp4. 0:02 – 0:08. Talent releasing the suture.

3.4.3. SCOPE: Ischemia Reperfusion Injury_2.mp4. 0:08 – 2:13. Reperfusion of myocardium.

3.5. Place a 24-gauge IV *('four')* catheter tube into the thoracic cavity one intercostal space to the right of the opening **[1]**. Use 6-0 absorbable sutures to close the intercostal incision in a simple interrupted pattern **[2]** and the muscle layer in a continuous suture pattern **[3]**.

3.5.1. SCOPE: Ischemia Reperfusion Injury_2.mp4. 2:13 – 3:30. Talent placing the tube.

3.5.2. SCOPE: Ischemia Reperfusion Injury_2.mp4. 3:30 – 8:16. Talent closing the intercostal incision.

3.5.3. SCOPE: Ischemia Reperfusion Injury_2.mp4. 8:16 – 13:00. Talent closing the muscle layer.

3.6. After closing the superficial muscle layer, remove the chest tube while withdrawing the air from thoracic cavity with a 1-milliliter tuberculin syringe **[1]**. Close the skin incision with a 6-0 absorbable suture in a continuous horizontal mattress pattern **[2]**.

3.6.1. SCOPE: Ischemia Reperfusion Injury_2.mp4. 13:00 – 13:38. Talent removing the chest tube while withdrawing air.

3.6.2. SCOPE: Ischemia Reperfusion Injury_2.mp4. 13:39 – end. Talent closing the skin incision. *Videographer: Obtain multiple usable takes of this shot because it will be reused in 4.7.1.*

3.7. When finished, administer 1.5 milliliters of warm saline subcutaneously **[1]** and apply triple-antibiotic ointment to the incision site to prevent infection **[2]**. Transfer the mouse to a bedding-free cage or a cage with covered bedding on a warm pad with a temperature of 35 to 37 degrees Celsius until it is fully recovered **[3]**.

3.7.1. Talent administering warm saline. Videographer NOTE: There are 2 clips, but the second was not slated, it immediately follows the slated 3.7.1. *Videographer: Obtain multiple usable takes of this shot because it will be reused in 4.7.2.*

3.7.2. Talent applying antibiotic. *Videographer: Obtain multiple usable takes of this shot because it will be reused in 4.7.3.*

3.7.3. Talent transferring the mouse to the cage. *Videographer: Obtain multiple usable takes of this shot because it will be reused in 4.7.4.*

4. Mouse Mesenchymal Stem Cell Delivery

4.1. After preparing and intubating the mouse as previously described, remove the suture from the skin layer with scissors and forceps **[1]**, then use a number 10 scalpel to make an incision in the same location as the previous surgery **[2]**.

4.1.1. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 0:02 – 0:33. Talent removing suture from the skin.

4.1.2. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 0:33 – 1:25. Talent making the incision.

4.2. Continue to cut through scar tissue until the muscle layer suture is visible **[1]**, then use the scissors and forceps to remove the suture and cut the muscle layer open **[2]**. Remove the sutures holding the ribs together and continue cutting through the intercostal muscle from the previous incision **[3]**. *Videographer: This step is important!*

4.2.1. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 1:25 – 2:26. Talent cutting through scar tissue.

4.2.2. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 2:26 – 4:40. Talent removing the suture from the muscle layer and cutting it open.

4.2.3. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 4:40 – 7:14. Talent removing the sutures from the ribs and cutting through the intercostal muscle.

4.3. Place the eyelid retractor into the intercostal space and locate the area of the previous ligation **[1]**.

4.3.1. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 7:14 – 7:35. Talent placing the eyelid retractor.

4.4. Load 300,000 mesenchymal stem cells suspended in 20 microliters of PBS into a 30-gauge insulin syringe **[1]** and bend the needle slightly to achieve the proper angle for injection **[2]**.

4.4.1. Talent loading the syringe.

4.4.2. Talent bending the needle.

4.5. Moving in the direction from the apex towards the base of the heart insert the syringe into the peri-infarct region until the needle opening is completely inside the myocardium **[1]**. Then, slowly inject the cells into the myocardium, wait 3 seconds, and remove the needle **[2]**. *Videographer: This step is important!*

4.5.1. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 7:35 – 7:54. Talent inserting the syringe.

4.5.2. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 7:54 – 8:15. Talent injecting the cells, waiting, and removing the syringe.

4.6. Observe the heart closely for 3 minutes to make sure that there is no abnormal reaction to the cells, such as ventricular fibrillation **[1]**. After 3 minutes, place a 24-gauge IV catheter tube into the thoracic cavity one intercostal space to the right of the opening **[2]**.

4.6.1. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 8:15 – 8:29. Heart with no abnormal reaction.

4.6.2. SCOPE: Mouse Mesenchymal Stem Cell Delivery_1. 8:29 – 9:06. Talent placing the catheter tube.

4.7. Close the intercostal, muscle, and skin layers and remove the chest tube as previously described **[1]**. Administer 1.5 milliliters of warm saline subcutaneously **[2]** and apply triple-antibiotic ointment to the incision site to prevent infection **[3]**. Transfer the mouse to a bedding-free cage on a warm pad until it is fully recovered **[4]**.

4.7.1. SCOPE: Mouse Mesenchymal Stem Cell Delivery_2. 3:34 – 3:41.

4.7.2. *Use 3.7.1.*

4.7.3. *Use 3.7.2.*

4.7.4. *Use 3.7.3.*

Results

5. Results: Heart Function after Ischemic Injury

5.1. Post-operative echocardiogram and electrocardiogram were performed on the day preceding stem cell implantation, confirming infarction and decreased ventricular contractile function **[1]**.

5.1.1. LAB MEDIA: Figure 1.

5.2. Further examination of the data showed the ejection fraction and fractional shortening were decreased in mice that received ischemic injury **[1]**, while the end-diastolic and systolic volumes increased **[2]**.

5.2.1. LAB MEDIA: Table 1. *Video Editor: Emphasize the EF% and FS% columns.*

5.2.2. LAB MEDIA: Table 1. *Video Editor: Emphasize the EDV and ESV columns.*

5.3. Masson Trichrome staining of myocardial tissue 7 days post-injury showed increased collagen deposition and thinning of the left ventricular wall in hearts of mice that received ischemic injury **[1-TXT]**.

5.3.1. LAB MEDIA: Figure 2. *Video Editor: Label A "***Normal***" and B "***Injured***".*

5.4. In vivo bioluminescent imaging, or BLI, was performed on the day after mesenchymal stem cell implantation **[1]**. Successful delivery of MSCs is exemplified by the BLI signal **[2]**, compared to mice that had induced ischemia reperfusion injury but did not receive MSCs **[3]**.

5.4.1. LAB MEDIA: Figure 3.

5.4.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize B.*

5.4.3. LAB MEDIA: Figure 3. *Video Editor: Emphasize A.*

Conclusion

6. Conclusion Interview Statements

6.1. **Michaela Olthoff:** When attempting this protocol, keep in mind that small, intentional movements when cutting through the intercostals, ligating the vessel, and injecting cells are vital for preventing undue damage to the heart or lung in the field of view.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Anything from 3.2 – 3.3.*

6.2. **Michaela Olthoff:** These procedures offer the ability to monitor cardiac function after transplantation through ultrasound, cell viability via bioluminescence imaging, or further molecular or biochemical techniques depending on the therapy used.

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