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Scriptwriter Name: Gaurav Vaidya
Supervisor Name: Anastasia Gomez
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Title: A Modified Surgical Model of Hind Limb Ischemia in ApoE$^{-/-}$ Mice Using a Miniature Incision

Authors and Affiliations:

Kaixuan Yan[1,2] , Jiaxing Zheng[1,2] , Frank G. Zöllner[3,4] , Kay Schwenke[1] , Prama Pallavi[1,2] , Michael Keese[1,2]

[1]Department of Surgery, Medical Faculty Manheim, Mannheim University Medical Centre, University of Heidelberg, Mannheim, Germany
[2]European Center of Angioscience ECAS, Medical Faculty Mannheim of the University of Heidelberg, Mannheim, Germany
[3]Computer-Assisted Clinical Medicine, Mannheim Institute for Intelligent Systems in Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[4]Cooperative Core Facility Animal Scanner ZI, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Corresponding Authors:
Michael Keese (Michael.keese@umm.de)

Email Addresses for All Authors:

Kaixuan.Yan@medma.uni-heidelberg.de
Jiaxing.Zheng@medma.uni-heidelberg.de
Frank.Zoellner@medma.uni-heidelberg.de
kay.schwenke@umm.de
Prama.pallavi@medma.uni-heidelberg.de
Michael.keese@umm.de

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, we need dissecting or stereomicroscope for performing DLFA.

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

   No

   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.

   We have a Leica surgical microscope (M651, Leica, Germany).

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage?

   No, we do not require filming of software usage.

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: 11
Number of Shots: 23

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Michael Keese:** This method displays a modified, simplified, and surgically efficient approach to establish the hind limb ischemia model in ApoE$^{-/-}$ mice. This method can be easily implemented in any basic animal laboratory.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 2.9.2.*

1.2. **Michael Keese:** The main advantage of this technique is the low invasiveness. It helps model acute ischemia in mice by inducing hind limb ischemia with an incision of less than 5 millimeters.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 2.3.3.*

OPTIONAL:

1.3. **Michael Keese:** The technique does require some microsurgical expertise and has a learning curve; therefore, it is advised to first practice on dead animals before starting on animals included in the experimental groups.

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

Introduction of Demonstrator on Camera

1.4. **Michael Keese:** Demonstrating the procedure will be Dr. Kaixuan Yan, a medical student from my laboratory.

1.4.1. INTERVIEW: Author saying the above.

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

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the University Committee on animal care at Heidelberg University and were performed according to the NIH guideline for the care and use of laboratory animals.

Protocol

2. Induction of HLI in ApoE$^{-/-}$ Mice

2.1. Begin by preparing the required equipment and tools for the surgery **[1]**. After administering the anesthesia to the mouse, apply vet ointment on the eyes to prevent dryness **[2-TXT]**.

2.1.1. WIDE: Establishing shot of the talent preparing the equipment and tools for surgery.

2.1.2. Talent applying the vet ointment on the eyes. **TEXT- Anesthesia: 5 mg/kg midazolam, 0.05 mg/ml/kg medetomidine, and 0.5 mg/kg fentanyl**

2.2. Then, place the mouse on a heating pad to keep the core body temperature at approximately 37 degrees Celsius **[1]**. Using a cotton swab and hair removal cream, carefully remove hair from the hind limb skin on the right side **[2]**

2.2.1. Talent placing the mouse on the heating pad.

2.2.2. Talent using the cotton swab to remove hair from the hind limb skin.

2.3. Lay the mouse in the supine position on the heating pad under a dissecting microscope **[1]**, use alcohol to disinfect the skin and wipe with the cotton away from the planned site of incision **[2]**. Using the pointed forceps and surgical scissors, make an incision approximately 3 to 4 millimeters in the middle of the inguinal region **[3]**.

2.3.1. Talent placing the mouse under the dissecting microscope.

2.3.2. Talent disinfecting the skin and wiping the skin with the cotton away from the planned site of incision

2.3.3. SCOPE: Talent making an incision in the inguinal region.

2.4. Carefully remove the subcutaneous fat tissue with the help of fine pointed forceps to expose the proximal femoral neurovascular bundle **[1]**. Using a cotton swab moistened with saline, carefully move the femoral artery away from the femoral nerve and femoral vein **[2.5.1]**. Then, using the tip of a 7.0 suture carefully pierce the membrane of the femoral sheath **[2]**. NOTE: 2.5.1 follows 2.4.1, hence VO sequence changed accordingly

2.4.1. SCOPE: Talent removing the subcutaneous fat tissue to expose the proximal femoral neurovascular bundle.

2.5.1 SCOPE: Talent moving the femoral artery away from the femoral nerve and femoral vein. NOTE: This shot earlier was 2.5.1, but it will come after 2.4.1

2.4.2. SCOPE: Talent piercing the membrane of the femoral sheath the tip of a 7.0 suture.

2.6 Then, pass two 7-0 absorbable sutures through the proximal femoral artery **[1]**, and make double knots using the spring scissors. Afterwards transect the femoral artery between the two ties **[2]**.

2.6.1 SCOPE: Talent suturing the proximal FA.

2.6.2 SCOPE: Talent making double knots to transect the FA. *Videographer: This step is important!*

2.7 Pass a 6-0 absorbable suture through the lower edge of the incision **[1]** and gently drag the incision to the region of the right side of the knee of the hind limb to expose the distal femoral artery **[2]**.

2.7.1 SCOPE: Talent passing a suture through the lower edge of the incision.
2.7.2 SCOPE: Talent dragging the incision to the region of the right side of the knee of the hind limb.

2.8 Carefully move the subcutaneous tissue aside to expose the neurovascular bundle **[1]**. Dissect the femoral artery from the femoral vein and femoral nerve and use cotton to protect the nerve **[2]** ~~**[3]**~~. Then, using the tip of 7.0 suture, pierce the membrane of the femoral sheath ~~**[2]**~~ **[3].** NOTE: While editing, please take a note of the shot and VO sequence, it was altered by the authors

2.8.1 SCOPE: Talent moving the subcutaneous tissue aside.
2.8.2 SCOPE: Talent dissecting the femoral artery from the femoral vein and femoral nerve and use cotton to protect the nerve
2.8.3 SCOPE: Talent piercing the membrane of the femoral sheath.

2.9 Pass two 7-0 absorbable sutures through the distal femoral artery **[1]** and make double knots using the spring scissors to transect the femoral artery between the two ties **[2]**.

2.9.1 Talent passing a suture through the distal femoral artery
2.9.2 Talent making double knots to transect the femoral artery. *Videographer: This step is important!*

2.10 Afterward, stitch the incision using the 6-0 absorbable sutures **[1]**. Place the mouse on a heating pad in a clean cage **[2]** and continue to monitor its breathing and heartbeat until it fully recovers **[3]**.

2.10.1 SCOPE: Talent stitching the incision. *Videographer: This step is important!*
2.10.2 Talent placing the mouse on a heating pad inside the cage.
2.10.3 Talent monitoring mouse's heartbeat and breathing.

2.11 Determine the success of double ligation of the femoral artery, or DLFA, on the next day via MRI **[1]**.

2.11.1 SCREEN: ApoE with Arrow.mp4 00:00-0:16

Results

3. Results: DLFA Surgery Using Miniature Incision to Establish HLI Model

3.1. Before the DLFA procedure, weights of mice ranged from 29.6 to 38.0 grams. Seven days after the DLFA surgery, the weights ranged from 26.5 to 34.1 grams, significantly lower than the pre-DLFA weights **[1]**.

3.1.1. LAB MEDIA: Figure 3A *Video Editor: Emphasize the Post-DLFA boxplot.*

3.2. The proximal and distal regions of the right FA showed no perfusion **[1]**. The Tarlov Scale results were significantly decreased one day after the surgery. Although they slowly increased over the following days, they were still lower than baseline until day 7 **[2]**.

3.2.1. LAB MEDIA: Figure 3C *Video Editor: Emphasize the absence of perfusion on the right FA.*

3.2.2. LAB MEDIA: Figure 3B *Video Editor: Emphasize the increase in Tarlov scale values from left to right, starting at day 1.*

3.3. The paws of the ischemic hind limbs in 7 mice were unable to stretch naturally compared to the contralateral side **[1].** In addition, 4 of the mice exhibited slight discoloration of the paws compared to the contralateral side **[2]**.

3.3.1. LAB MEDIA: Figure 3D *Video Editor: Emphasize the two images on the upper left only.*

3.3.2. LAB MEDIA: Figure 3D *Video Editor: Emphasize the two images on the upper right only.*

3.4. HE staining of the right Gm muscle revealed myofibers that exhibited irregular ischemic necrosis where proliferating satellite cells had replaced the necrotic myofibers and were distributed in a mass or with irregular dispersion **[1]**.

3.4.1. LAB MEDIA: Figure 4A

3.5. Myofibers exhibiting inflammatory infiltration by multinucleated macrophages had lost their normal morphological characteristics, with very few regenerated myofibers being present **[1].**

3.5.1. LAB MEDIA: Figure 4B

3.6. Transverse sections of these regenerated myofibers were round, the cytoplasm was stained red, and one small nucleus or multiple nuclei were located at the center **[1]**. However, this kind of inflammatory pattern was not observed in the left Gm **[2].**

3.6.1. LAB MEDIA: Figure 4C
3.6.2. LAB MEDIA: Figure 4D

3.7. CD31 antibody staining performed to observe microvascular density indicated that ischemic hind limbs exhibited significantly more microvascular density than the non-ischemic side **[1]**.

3.7.1. LAB MEDIA: Figure 5C *Video Editor: Emphasize boxplot on the right.*

Conclusion

4. Conclusion Interview Statements

4.1. **Dr. Prama Pallavi:** The most important thing is the dissection of the femoral artery. The operator needs to have sufficient microsurgical experience and familiarity with the anatomy of the mouse hind limb.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1. , 2.8.3.*

4.2. **Dr. Prama Pallavi:** In principle, this method would also help study ischemia and reperfusion in the leg. Herby, the artery could only be transiently occluded by a soft vessel clamp before allowing reperfusion.

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