FINAL SCRIPT APPROVED FOR FILMING

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Title: Operating Transverse Aortic Constriction with Absorbable Suture to Obtain Transient Myocardial Hypertrophy

Authors and Affiliations:

Yi Lao[1], Cankun Zheng[1], Hailin Zhu[1,2], Hairuo Lin[1], Xiaoxia Huang [1], Yulin Liao[1]

[1]Department of Cardiology, State Key Laboratory of Organ Failure Research, Nanfang Hospital, Southern Medical University, Guangzhou, China
[2]Department of Geriatrics, Pingxiang People's Hospital of Southern Medical University, Pingxiang, Jiangxi, China

Corresponding Authors:

Yulin Liao (liao18@msn.com)

Email Addresses for All Authors:

yilaoyilao@126.com
ckzheng92@126.com
498612570@qq.com
574087324@qq.com
1053947171@qq.com
liao18@msn.com

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? **No**

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

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps.

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: 20
Number of Shots: 37

Introduction

1. Introductory Interview Statements

1.1. **Yi Lao**: This protocol presents an improved method to obtain transient myocardial hypertrophy using absorbable sutures, which simulates left ventricular hypertrophy decrease after removing pressure overload.

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

1.2. **Cankun Zheng**: Using this protocol, researchers can easily master the model with a lower operation mortality.

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

Introduction of Demonstrator on Camera

1.3. **Cankun Zheng:** Demonstrating the procedure will be Xiaoxia Huang, a technician from my laboratory.

1.3.1. INTERVIEW: Author saying the above.

1.3.2. The named demonstrator looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.4. All procedures were carried out following the guidelines of the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Protocol

2. Preoperative preparation of surgical instruments

2.1. Begin by pinching off the tip of a 25-gauge needle **[1]** with a needle holder and blunting it **[2]**. *Videographer: This step is important!*

2.1.1. Talent pinching off the needle.

2.1.2. Talent blunting the needle.

2.2. Then, pass a 5 by 0 absorbable suture through this needle **[1]** and curve the blunt needle to a 90-degree angle using the holder **[2]**.

2.2.1. Talent passing the suture through the needle.

2.2.2. Talent bending the curved needle.

2.3. Pinch off the tip of another 25-gauge needle **[added, 1]**, then curve it to a 120-degree angle **[2]** and smoothen the tip with a holder to be used as a spacer in the ligation step **[3]**.

2.3.1. Added shot: Talent pinching off another needle.

2.3.2. Talent bending the needle.

2.3.3. Talent smoothening the tip of the needle using the holder.

2.4. Prepare sterilized surgical instruments **[1]** including 1 ophthalmic scissors, 1 micro scissors, 2 microsurgical elbow tweezers, 1 needle holder, and 1 micro needle holder **[2]**.

2.4.1. Talent sterilizing the instruments

2.4.2. Sterilized instruments on the lab bench.

3. Surgery

3.1. To begin the surgery, confirm complete anesthesia of the mouse with the pedal withdrawal reflex **[1]**.

3.1.1. Talent confirming the anesthesia the mice.

3.2. Keep the mouse in supine position by fixing the incisors with a suture and fixing the limbs with adhesive tape **[1]**.

3.2.1. Talent placing the mice and fixing their limbs on the bench.

3.3. Remove the hair from the neck **[1]** and disinfect the area with iodine and 75% alcohol **[2]**.

3.3.1. Talent shaving the hair from the neck area.
3.3.2. Talent disinfecting the area of incision.

3.4. Start the surgery by making an incision over 10 millimeters at the midline position between the supra-sternal notch and chest **[1]**. Then, separate the skin and the superficial fascia **[2]**.

3.4.1. Talent making an incision on the mice.

3.4.2. Talent separating the superficial skin to expose the ribs.

3.5. Make an incision in the first intercostal space, as close as possible to the sternum **[1]**, and bluntly penetrate to open this space with elbow tweezers **[2]**. *Videographer: This step is important!*

3.5.1. Talent making incision at the ribs section.

3.5.2. Talent using elbow tweezers to open the incised space.

3.6. Gently separate the parenchyma and the thymus until the transverse aortic arch is visible **[1]**. Pass a 5 by 0 absorbable suture under the aortic arch between the brachiocephalic artery and the left common carotid artery with a latch needle **[2]**. *Videographer: This step is difficult and important!*

3.6.1. Talent separating the muscle layers to expose the aortic arch.

3.6.2. Talent passing the suture with the latch needle at the target area.

3.7. Place the previously prepared spacer on the transverse aorta and make a double knot on the spacer with the suture **[1].** *Videographer: This step is difficult and important!*

3.7.1. Talent placing the spacer and making the double knot.

3.8. Gently but quickly remove the spacer **[1]** and cut the ends of the suture **[2]**.

3.8.1. Talent removing the spacer from the transverse aorta.

3.8.2. Talent cutting the end of the sutures.

3.9. Close the first intercostal space and skin using 5 by 0 nylon sutures **[1]**. Disinfect the skin again with 75% alcohol **[2]**.

3.9.1. Talent closing the wound using sutures.

3.9.2. Talent disinfecting the skin.

4. Echocardiographic assessment of successful ligation and measurements

4.1. Seven days after surgery, place the mouse in supine position, maintaining it at 37 degrees Celsius **[1]**, and tape its limbs to the electrode **[2]**.

4.1.1. Talent placing the mouse in supine position.

4.1.2. Talent placing the electrodes.

4.2. Remove chest hair **[1]** and apply ultrasonic coupling agent to the mouse's chest **[2]**, then assess TAC *(pronounce 'T-A-C')* with a 30 mega Hertz probe **[3-TXT]**.

4.2.1. Talent shaving the chest hair.

4.2.2. Talent applying coupling agent to the chest.

4.2.3. Talent assessing the transverse aortic constriction. **TEXT: TAC- transverse aortic constriction**

4.3. Tilt the platform to the far left **[1]**. Keep the probe in vertical position and lower it on the chest along the right parasternal line **[2]**. Adjust the X- and Y-axis under B-mode until the aortic arch and constriction is clearly visible **[3]**.

4.3.1. Talent tilting the platform to the left.

4.3.2. Talent lowering the probe on the chest vertically.

4.3.3. Talent adjusting the probe to view the aortic arch.

4.4. Using Doppler mode, measure the peak velocity, dimensions, and contractility of the left ventricle. Select the mice with a velocity of more than 3000 millimeters per second as the TAC group **[1]**.

4.4.1. Talent measuring parameters in color Doppler mode.

4.5. Using a 30 mega Hertz probe, assess the left ventricular dimensions and contractility **[1]**.

4.5.1. Talent assessing the left ventricular dimensions and contractility

4.6. Reset the platform to horizontal position **[1]**, keeping the probe at 30 degrees counterclockwise to the left parasternal line **[2]**.

4.6.1. Talent resetting the platform from vertical to horizontal position.

4.6.2. Talent moving the probe to the left parasternal line.

4.7. Obtain a short axis view of the heart by manipulating the X and Y axis in B-mode **[1]**, then Press M-mode to show the indicator line and acquire images with Cine Store and Frame Store for later measurement of the LV chamber dimension, fractional shortening, and LV wall thickness **[2]**. *Videographer: Film the talent at the computer here and focus on the screen.*

4.7.1. Talent obtaining short axis view using adjustments in B-mode.

4.7.2. Talent acquiring images of the left ventricle.

Results

5. Assessment of left ventricular physiological and functional parameters post-TAC

5.1. Pulse wave Doppler imaging performed after 14 days of TAC showed that the blood flow velocity at the constriction was greater than 3000 millimeters per second even though an absorbable suture had been used to constrict the aortic arch **[1]**.

5.1.1. LAB MEDIA: Figure 1a. *Video Editor: Emphasize on the 14d graph.*

5.2. The pressure gradient of blood flow after 14 days of TAC was maintained above 40 millimeters of mercury **[1]**. Interestingly, there was no constriction in the fourth week after the surgery, indicating that the absorbable suture had been completely absorbed **[2]**.

5.2.1. LAB MEDIA: Figure 1b

5.2.2. LAB MEDIA: Figure 1a, just the TAC 28d images for both silk and absorbable sutures, next to Figure 1b. *Video Editor: Emphasize the absorbable suture image in a and the 28d data points in b.*

5.3. The left ventricular parameters in the silk suture group and the absorbable suture group on Days 0, 14, and 28 after TAC on M-mode imaging are shown here **[1].**

5.3.1. LAB MEDIA: Figure 2a

5.4. On Day 14 after TAC, the left ventricular posterior wall thickness at end-diastole increased **[1]** while the left ventricular internal diameter at end-diastole slightly decreased **[2]**.

5.4.1. LAB MEDIA: Figure 2b. *Video Editor: Emphasize on the 14d bar-graph.*

5.4.2. LAB MEDIA: Figure 2c. *Video Editor: Emphasize on the 14d bar-graph.*

5.5. The ejection fraction of the left ventricle was unaffected by the use of absorbable sutures **[1]**.

5.5.1. LAB MEDIA: Figure 2d

5.6. The heart weight to body weight ratio comparison of the silk suture group, absorbable suture group, and the sham group is shown here **[1]**.

5.6.1. LAB MEDIA: Figure 3a

5.7. Histological slices of the heart showed that cardiomyocytes significantly enlarged from day 14 to day 28 in the silk suture group **[1]** but mostly regressed on day 28 in the absorbable suture group **[2]**.

5.7.1. LAB MEDIA: Figure 3b *Video Editor: Emphasize the Silk Suture images.*

5.7.2. LAB MEDIA: Figure 3b. *Video Editor: Emphasize the Absorbable Suture images.*

Conclusion

6. Conclusion Interview Statements

6.1. **Yi Lao:** When attempting this protocol, it is most important to not damage the parietal pleura to avoid pneumothorax. Make sure to blunt the tip of the needle as much as possible and perform the procedure gently.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Shots from 3.4 – 3.5.*

6.2. **Cankun Zheng:** There are many potential applications for this method such as establishing the model of myocardial hypertrophic preconditioning, exploring the mechanism of hypertrophy regression, and investigating the time of left ventricular reversible remodeling.

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

