FINAL SCRIPT: APPROVED FOR FILMING

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Title: A Simplified Operation for the Endovascular Perforation Murine Model of Subarachnoid Hemorrhage

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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 **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.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

OLYMPUS SZ61

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

SCOPE shots: 2.3.1, 2.3.2, 2.4.1, 2.5.2, 2.6.1, 2.7.1, 2.7.2, 2.9.1, 2.9.2, 2.10.1, 2.10.2, 2.11.1, 2.12.1, 2.13.2.

Videographer: Please film the above-mentioned shots using the scope kit

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: 20
Number of Shots: 32

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

REQUIRED:

1.1. **Wenhao Ding:** Our research established a simplified operation for endovascular perforation-induced subarachnoid hemorrhage model in mice, which may facilitate its application in transgenic mice and greater sample size in molecular mechanism studies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.2*

What advantage does your protocol offer compared to other techniques?

1.2. **Wenhao Ding:** Our protocol for establishing the SAH mouse model is easier for new manipulators to operate and increases their efficiency.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6.1*

What research questions will your laboratory focus on in the future?

1.3. **Wenhao Ding:** In the future, we will use this model to explore the molecular mechanisms underlying and the pharmacological effects of subarachnoid hemorrhage.

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

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Animal Ethics Committee at the Longhua Hospital

Protocol

2. Subarachnoid Hemorrhage (SAH) Induction in Mouse

Demonstrator: Wenhao Ding

2.1. To begin, place the mouse on a heating pad preheated to 37 degrees Celsius and maintain this temperature until the surgery is completed **[1]**.

2.1.1. WIDE: Talent placing the mouse on the heated pad and monitoring the temperature.

2.2. After positioning the mouse in a supine position, secure all the limbs using tape **[1-TXT]**.

2.2.1. Talent taping each limb of the supine anesthetized mouse.

TXT: Anesthesia:

Induction: 2 - 2.5% Isoflurane

Maintenance: 1 - 1.5% Isoflurane (0.4 - 0.6 L/min flow rate)

2.3. Now, using scissors, incise the skin along the midline of the anterior neck **[1]**. Dissect the connective tissue and expose the left common carotid artery and its bifurcations **[2]**.

2.3.1. SCOPE: performing a precise incision along the anterior neck midline with scissors.

2.3.2. SCOPE: using forceps to spread tissue and reveal the common carotid artery and its branches.

2.4. Loop the common carotid artery with a 6-0 *(6-oh)* nylon suture **[1]** and leave both ends free without tying them **[2]**.

2.4.1. SCOPE: looping the suture around the artery.

2.4.2. Shot of the end of the suture left free.

2.5. Then, attach both ends of the suture to tape **[1]** and gently pull them downward and to the right at a 45-degree angle from the horizontal **[2]**. Now, secure the tape to the operating table to temporarily block the artery **[3]**.

2.5.1. Talent attaching both ends of the suture to tape.

2.5.2. SCOPE: pulling them downward and to the right at a 45-degree angle from the horizontal.

2.5.3. Talent securing the tape to the operating table.

2.6. Next, ligate the external carotid artery using a nylon suture **[1]**.

2.6.1. SCOPE: Tying off the external carotid artery with a nylon suture.

2.7. Using an electrocautery pen, fuse the external carotid artery distal to the ligation site **[1]**. Pull the artery downward to expose the internal carotid artery and align both vessels into a straight line **[2]**.

2.7.1. SCOPE: sealing the distal artery with the electrocautery pen.

2.7.2. SCOPE: gently pulling and aligning the external and internal carotid arteries in a straight line.

2.8. With a sharpened tip of the filament, locate the black mark positioned 8 millimeters from the tip **[1]**.

2.8.1. Talent trimming the filament and checking the placement of the 8 millimeter mark.

NOTE: The timestamps for the SCOPE shots were provided by the videographer. The postshoot integrator hasn't reviewed the footage.

2.9. Use scissors to make a small incision for inserting the filament into the external carotid artery **[1]** and insert the filament using forceps **[2]**.

2.9.1. SCOPE: making the incision with scissors near the artery. Videographer's NOTE: We did a long take with the scope footage, so here's the timecode for Clip "IMG_4371": STEP 2.9.1 IMG_4371 18:38

2.9.2. SCOPE: inserting the filament precisely using forceps. Videographer's NOTE: We did a long take with the scope footage, so here's the timecode for Clip "IMG_4371": STEP 2.9.2/2.10.1/2.10.2 IMG_4371 27:25.

2.10. Slowly advance the filament with forceps until the black mark passes completely through the carotid bifurcation **[1]**. Then, advance it an additional 2 millimeters to perforate the vessel **[2]**.

2.10.1. SCOPE: guiding the filament through the artery to the marked position. Videographer's NOTE: We did a long take with the scope footage, so here's the timecode for Clip "IMG_4371": STEP 2.9.2/2.10.1/2.10.2 IMG_4371 27:25.

2.10.2. SCOPE: slightly advancing filament further and inducing perforation of the vessel.

2.11. Immediately retract the filament following perforation **[1]**.

2.11.1. SCOPE: quickly pulling the filament back after completing the perforation. Videographer's NOTE: We did a long take with the scope footage, so here's the timecode for Clip "IMG_4371": STEP 2.11.1 IMG_4371 30:09

2.12. Then, fuse the external carotid artery using an electrocautery pen **[1]**.

2.12.1. SCOPE: applying the electrocautery pen to seal the external carotid artery. Videographer's NOTE: We did a long take with the scope footage, so here's the timecode for Clip "IMG_4371": STEP 2.12.1 IMG_4371 29:14

2.13. Remove the tape and withdraw the nylon suture from the common carotid artery to restore blood flow **[1]** and observe the clear pulsation of the artery **[2]**.

2.13.1. Talent removing the securing tape and gently pulling out the nylon suture.

2.13.2. SCOPE: Shot of the artery's pulsation. Videographer's NOTE: We did a long take with the scope footage, so here's the timecode for Clip "IMG_4371": STEP 2.13.2 IMG_4371 32:31

2.14. Now, close the neck incision using 5-0 *(5-oh)* absorbable sutures **[1-TXT]**.

2.14.1. Talent stitching the neck incision with 5-0 absorbable sutures. **TXT: Sham group: Partially advance the filament; Do not puncture**

3. Post-Operative Procedures and Tests

3.1. 24 hours post-surgery, assess neurological performance using a modified scoring system in a blinded manner **[1]**. Evaluate spontaneous activity, movement of all limbs, forelimb strength, ability to climb a wire cage, tactile response on both sides of the trunk, and reaction to vibrissae stimulation **[2]**.

3.1.1. Talent examining the animal after surgery.

3.1.2. LAB MEDIA: Table 1.

3.2. After anesthetizing the mouse, place the mouse in a supine position **[1-TXT]** and tape its limbs to the surgical table **[2]**. Using scissors, make a midline abdominal incision, cut through the abdominal wall, and carefully expose the thoracic cavity **[4]**. Then, cut the sternum to reveal the heart **[4]**.

3.2.1. Talent positioning the anesthetized mouse supine on the operating table. **TXT:**

Anesthesia: Isoflurane

3.2.2. Talent taping the mouse limbs. Videographer's NOTE: Step 3.2.2 and step 3.2.3 were combined in one clip.

3.2.3. Talent performing abdominal and thoracic incisions.

3.2.4. Talent cutting the sternum, exposing the heart.

3.3. Now, carefully insert the infusion needle into the left ventricle, ensuring it remains stable and does not puncture the heart **[1]**.

3.3.1. Talent guiding the infusion needle into the ventricle with precision, checking for stability. Videographer's NOTE: Steps 3.3.1 and 3.4.1 were combined in one clip.

3.4. Begin perfusion using precooled 1x PBS at 4 degrees Celsius at a flow rate of approximately 10 milliliters per minute **[1-TXT]**.

3.4.1. Talent operating the perfusion. **TXT: Continue until the exiting liquid turns clear** Videographer's NOTE: Steps 3.3.1 and 3.4.1 were combined in one clip.

3.5. Once perfusion is complete, remove the infusion needle and stop the fluid flow. Proceed with the collection of brain tissue **[1]**.

3.5.1. Talent extracting the needle and picking up tools for brain extraction procedure.

Results

4. Results

4.1. The average neurological score 24 hours after surgery was significantly lower in the subarachnoid hemorrhage group compared to the sham group **[1]**.

4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the SAH data points*.

4.2. Blood clots visibly accumulated around the circle of Willis in the subarachnoid hemorrhage group **[1]**, while no such accumulation was observed in the sham group **[2]**.

4.2.1. LAB MEDIA: Figure 3B. *Video editor: Zoom in on the right panel (SAH) showing red clots*
LAB MEDIA: Figure 3B. *Video editor: Zoom in on the left panel (sham).*

Pronunciation Guides:

1. Isoflurane
Pronunciation link:
https://www.merriam-webster.com/medical/isoflurane
IPA: /ˌaɪsəˈflʊreɪn/
Phonetic Spelling: eye-suh-floo-rayn
2. Carotid
Pronunciation link:
https://www.merriam-webster.com/dictionary/carotid
IPA: /kəˈrɑːtɪd/
Phonetic Spelling: kuh-raa-tid
3. Bifurcation
Pronunciation link:
https://www.merriam-webster.com/dictionary/bifurcation
IPA: /ˌbaɪfɚˈkeɪʃən/
Phonetic Spelling: bye-fer-kay-shun
4. Electrocautery
Pronunciation link:
https://www.merriam-webster.com/medical/electrocautery
IPA: /ɪˌlɛktroʊˈkɔːtəri/
Phonetic Spelling: ih-lek-troh-kaw-tuh-ree
5. Filament
Pronunciation link:
https://www.merriam-webster.com/dictionary/filament

IPA: /ˈfɪləmənt/
Phonetic Spelling: fih-luh-muhnt
6. Pulsation
Pronunciation link:
https://www.merriam-webster.com/dictionary/pulsation
IPA: /ˌpʌlˈseɪʃən/
Phonetic Spelling: puhl-say-shun
7. Absorbable
Pronunciation link:
https://www.howtopronounce.com/absorbable
IPA: /əbˈzɔːrbəbl̩/
Phonetic Spelling: ub-zor-buh-buhl
8. Subarachnoid
Pronunciation link:
https://www.howtopronounce.com/subarachnoid
IPA: /ˌsʌbəˈrækˌnɔɪd/
Phonetic Spelling: suh-buh-rak-noid
9. Vibrissae
Pronunciation link:
https://www.merriam-webster.com/dictionary/vibrissa
IPA: /vaɪˈbrɪsi/
Phonetic Spelling: vai-brih-see
10. Perfusion
Pronunciation link:
https://www.merriam-webster.com/dictionary/perfusion
IPA: /pərˈfjuːʒən/
Phonetic Spelling: per-fyoo-zhun
11. Sternum
Pronunciation link:
https://www.merriam-webster.com/dictionary/sternum
IPA: /ˈstɝːnəm/
Phonetic Spelling: stur-nuhm
12. Thoracic
Pronunciation link:
https://www.merriam-webster.com/dictionary/thoracic
IPA: /θəˈræsɪk/
Phonetic Spelling: thuh-ra-sik