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Title: A Murine Model of Carotid Aneurysm Formation

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Interview statements: Which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees self-record interview statements. JoVE can provide support for this option.

3. Proposed interview filming date: Please indicate the proposed date that your group will **self-film** interviews: **MM/DD/YYYY**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 08
Number of Shots: 23

Introduction

INTRODUCTION:

1.1. **Keishi Kohyama:** Our research develops a reproducible murine carotid aneurysm model to investigate mechanisms and evaluate new therapeutic strategies for aneurysm treatment.

> 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:2.3.1*

CONCLUSION:

1.2. **Keishi Kohyama:** Our results show that enzymatic vessel wall degradation combined with altered hemodynamics reproduces key pathological features seen in human aneurysms.

> 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

1.3. **Keishi Kohyama:** Compared with intracranial models, our approach is faster, more accessible, and allows testing of endovascular or pharmacologic interventions in mice.

> 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

1.4. **Keishi Kohyama:** This standardized murine model promotes cross-study comparability and helps accelerate therapeutic testing for aneurysm prevention and treatment.

> 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.1*

Ethics Title Card

This research has been approved by the University of Pittsburgh Institutional Animal Care and Use Committee

Protocol

2. Common Carotid Artery Ligation

Demonstrator: Keishi Kohyama

2.1. To begin, prepare the surgical site on the animal with betadine and 70 percent ethanol solutions **[1-TXT]** and place the surgical drape **[2]**.

    2.1.1. LAB MEDIA: 3rd-2.mp4 07:50 – 08:00 **TXT: Anesthesia: Isoflurane; Induction: 3 - 4% ; Maintenance: 1 – 3%**

    2.1.2. LAB MEDIA: 3rd-2.mp4 12:18-12:39

2.2. Using surgical scissors, make a 1-centimeter vertical incision along the midline of the neck, starting from just below the jawline **[1]**. Cut through the dermis and underlying fascia **[2]**.

    2.2.1. LAB MEDIA: 3rd-2.mp4 14:15 – 14:20

    2.2.2. LAB MEDIA: 3rd-2.mp4 17:16-17:24

2.3. Use blunt forceps to mobilize the submandibular gland laterally, taking care not to damage surrounding tissues **[1]**. Gently lift the gland and separate it from the sternum by blunt dissection **[2]**. Secure the gland with a small sterile retractor or clip to maintain a clear surgical field **[3]**.

    2.3.1. LAB MEDIA: 3rd-2.mp4 24:52-24:58

    2.3.2. LAB MEDIA: 3rd-2.mp4 25:28-25:48

    2.3.3. LAB MEDIA: 3rd-2.mp4 30:35-30:47

2.4. Identify the ipsilateral sternocleidomastoid muscle **[1]**. Use blunt forceps to lift the muscle gently and thread a 5-0 *(5-oh)* braided silk suture underneath **[2]**. Reflect the muscle laterally by gently pulling the suture to expose the carotid sheath **[3]**.

    2.4.1. LAB MEDIA: 3rd-2.mp4 38:20-38:35

    2.4.2. LAB MEDIA: 3rd-2.mp4 38:40-38:54

    2.4.3. LAB MEDIA: 3rd-2.mp4 39:15-39:25

2.5. Open the carotid sheath using sharp forceps **[1]**. Carefully separate the carotid artery from the adjacent jugular vein and vagus nerve **[2]**. To improve visibility, insert a sterile

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silicone cuff or retractor beneath the carotid artery. Avoid causing trauma to the artery or surrounding structures **[3]**.

    2.5.1.   LAB MEDIA: 3rd-2.mp4 43:12-43-22

    2.5.2.   LAB MEDIA: 3rd-2.mp4 49:08-49:28

    2.5.3.   LAB MEDIA: 3rd-2.mp4 01:07:56-01:08:12

2.6.   Using sharp forceps, scoop the carotid artery and thread a 9-0 suture underneath **[1]**. Tie a secure double knot to ligate the artery, ensuring proper tension to occlude blood flow without damaging the vessel **[2]**. Trim the suture ends to minimize tissue irritation **[3]**.

    2.6.1.   LAB MEDIA: 3rd-2.mp4 01:03:48-01:04:00

    2.6.2.   LAB MEDIA: 3rd-2.mp4 01:05:13-01:05:23

    2.6.3.   LAB MEDIA: 3rd-2.mp4 01:06:10-01:06:21

2.7.   Using a syringe, apply a couple of drops of sterile porcine elastase solution at 10 units diluted in 1 milliliter of saline solution, over 10 or 20 minutes **[1]**. Once the elastase treatment time is completed, use a sterile cotton swab to remove excess elastase **[2]**. If desired, use sterile normal saline to wash out the remaining elastase solution **[3]**. Finally, remove any retaining sutures or clips to prepare for closure **[4]**.

    2.7.1.   LAB MEDIA: 3rd-2.mp4 01:11:35-01:11:45

    2.7.2.   LAB MEDIA: 3rd-2.mp4 01:20:10-01:20:22

    2.7.3.   LAB MEDIA: 3rd-2.mp4 01:24:37-01:24:57

    2.7.4.   LAB MEDIA: 3rd-2.mp4 01:26:07-01:26:19

2.8.   Approximate the skin edges using sterile nylon suture 5-0 or surgical staples **[1]**. Finally, apply gentle pressure with sterile gauze to ensure hemostasis **[2]**.

    2.8.1.   LAB MEDIA: 3rd-2.mp4 01:27:05-01:27:19

    2.8.2.   LAB MEDIA: 3rd-2.mp4 01:32:48-01:32:55

Results

3. **Results**

3.1. A total of 15 female wild-type mice underwent the aneurysm induction procedure following the standardized protocol. Two weeks after surgery, aneurysm formation was successfully achieved in approximately 87% of the mice, as confirmed by gross morphological examination **[1]**.

    3.1.1. LAB MEDIA: Figure 2.

3.2. Aneurysm formation was also visualized under microscopy by disruption of the internal elastic lamina **[1]**.

    3.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the area "disrupted internal elastic lamina"*

- Submandibular (as in "submandibular gland")
Pronunciation link: https://www.howtopronounce.com/submandibular-glands How To Pronounce+1
IPA: /ˌsʌbmænˈdɪbjʊlər/
Phonetic spelling: suh-bman-dih-byoo-luh(r)

- Sternocleidomastoid (as in "sternocleidomastoid muscle")
Pronunciation link: https://www.howtopronounce.com/sternocleidomastoid How To Pronounce
IPA: /ˌstɜːrnoʊˌklaɪd.oʊˈmæstəɪd/
Phonetic spelling: ster-noh-klyd-oh-mas-toyd

- Elastase (as in "porcine elastase solution")
Pronunciation link: https://www.howtopronounce.com/elastase How To Pronounce+1
IPA: /ɪˈlæsteɪs/
Phonetic spelling: ih-las-tays

- Porcine (as in "porcine elastase")
Pronunciation link: https://dictionary.cambridge.org/us/pronunciation/english/porcine Cambridge Dictionary+1
IPA: /ˈpɔːr.saɪn/
Phonetic spelling: por-sine

- Dermis
Pronunciation link: https://dictionary.cambridge.org/us/pronunciation/english/dermis Cambridge Dictionary+2Oxford Learner's Dictionaries+2
IPA: /ˈdɝː.mɪs/
Phonetic Spelling: DUR-mis

- Fascia
Pronunciation link: https://dictionary.cambridge.org/us/pronunciation/english/fascia Cambridge Dictionary+1
IPA: /ˈfæʃ.ə/
Phonetic Spelling: FASH-uh

- Submandibular
Pronunciation link: https://www.howtopronounce.com/submandibular-glands (No Merriam-Webster or Oxford page found) → No confirmed link found
IPA: /ˌsʌb.mænˈdɪb.jə.lər/
Phonetic Spelling: sub-MAN-di-byoo-luh(r)

- Sternocleidomastoid
Pronunciation link:
https://dictionary.cambridge.org/us/pronunciation/english/sternocleidomastoid Cambridge Dictionary+1
IPA: /ˌstɝː.noʊˌklaɪ.doʊˈmæs.tɔɪd/
Phonetic Spelling: stur-noh-KLY-doh-MAS-toyd

- Carotid
Pronunciation link: https://dictionary.cambridge.org/us/pronunciation/english/carotid Cambridge Dictionary
IPA: /kəˈrɑː.tɪd/
Phonetic Spelling: kuh-RAH-tid

- Carotid sheath
Pronunciation link: https://www.howtopronounce.com/carotid-sheath How To Pronounce
IPA: /kəˈrɑː.tɪd ʃiːθ/
Phonetic Spelling: kuh-RAH-tid SHEETH

- Elastase
Pronunciation link: https://www.howtopronounce.com/elastase How To Pronounce
IPA: /ɪˈlæ.steɪs/
Phonetic Spelling: ih-LAS-tays