

Submission ID #: 72015

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21964623>

Title: Reliable Creation of a Murine Abdominal Aortic Aneurysm Model Using Elastin Sensitization and Perivascular Elastase

Authors and Affiliations:

Rohan Basu, Ethan Chernivec, Mackenzie Madison, Carol Sampson, Jennifer Stashevsky, Chang-Hyun Gil, Ping Li, Steven Miller, Michael Murphy

Division of Vascular Surgery, Department of Surgery, Indiana University School of Medicine

Corresponding Authors:

Michael Murphy mipmurph@iu.edu

Email Addresses for All Authors:

Rohan Basu	basur@iu.edu
Ethan Chernivec	etcherni@iu.edu
Mackenzie Madison	madisonm@iu.edu
Carol Sampson	sampsonc@iu.edu
Jennifer Stashevsky	jstashev@iu.edu
Chang-Hyun Gil	chgil@iu.edu
Ping Li	pili@iu.edu
Steven Miller	sjmiller@iu.edu
Michael Murphy	mipmurph@iu.edu

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, all done**

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

Yes

SCOPE shots: 4.1 to 4.15

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? **Yes**

If **Yes**, how far apart are the locations? Approximately 500ft

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 25

Number of Shots: 50 (22 Scope)

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED:~~ Summarize your research focus and the main questions it aims to answer.

- 1.1. **Rohan Basu:** We developed a model of aortic aneurysm that includes an immune-sensitization component to study novel preventative therapies.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Rohan Basu:** These models are complicated due to high mortality rates or inconsistent aortic dilation. We use elastin sensitization to limit these complications.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Rohan Basu:** This protocol allows the study of aortic dilation, inflammatory changes, immune responses, and the effects of preventative therapies in aortic aneurysm.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Rohan Basu:** Balancing adequate exposure of the abdominal aorta with survival of the animal is the key judgment step in the protocol.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Rohan Basu**: Future studies can evaluate various elastin degradation product preparations, cell therapies, and biologic therapies to limit aortic aneurysm progression.
- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

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

Ethics Title Card

This research has been approved by the Indiana University Institutional Animal Care and Use Committee. All studies followed the National Research Council's Guide for the Care and Use of Laboratory Animals and were compliant with animal welfare ARRIVE guidelines

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Initial Elastin Sensitization

Demonstrator: Carol Sampson

- 2.1. To begin, wear personal protective equipment, including gloves, eyewear, and a facemask **[1]**. Obtain a formulation of porcine pancreatic elastase in a suspension at 5.1 milligrams protein per milliliter **[2-TXT]**.
 - 2.1.1. WIDE: Talent donning personal protective equipment, including gloves, eye protection, and facemasks. ***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent placing the vial with enzyme on the work bench. **TXT: Elastase: ≥ 4 units/mg protein**
- 2.2. Add 168 microliters of elastase to 5 milligrams of murine elastin **[1]**. Then, add 200 microliters of 1x PBS to the mixture **[2]**. Incubate the elastase and elastin mixture at 37 degrees Celsius for 20 minutes with intermittent gentle agitation **[3]**.
 - 2.2.1. Talent adding 168 microliters of elastase to the murine elastin.
 - 2.2.2. Talent adding 200 microliters of PBS to the mixture and pipetting up and down.
 - 2.2.3. Talent placing the elastase and elastin mixture into the incubator.
- 2.3. Next, add 632 microliters of cold PBS to the elastase and elastin mixture **[1]**, followed by 1 milliliter of complete Freund's adjuvant **[2]**.
 - 2.3.1. Talent adding cold PBS to the elastase and elastin mixture.
 - 2.3.2. Talent adding complete Freund's adjuvant to the mixture.
- 2.4. Place the anesthetized 13-week-old male C57BL-6J mouse in a prone position on a pre-heated stage **[1-TXT]**. Position the mouse's nose in the anesthesia nose cone **[2]**. Inject 50 microliters of the prepared elastin degradation product or EDP mixture subcutaneously into each of the mouse flanks **[3-TXT]**.

- 2.4.1. Talent placing the mouse in a prone position on the heated stage. **TXT: Anesthesia: 1.8 - 2% Isoflurane**
- 2.4.2. Talent positioning the mouse's nose in the anesthesia nose cone.
- 2.4.3. Talent injecting elastin degradation products subcutaneously into one flank of the mouse. **TXT: 250 µg EDP mixture/mouse** **Authors: Please check if this correct**
- 2.5. Place the mouse in a recovery basket lined with towels on a stage heated to 37 degrees Celsius [1]. Monitor the mouse until it emerges from anesthesia [2].
 - 2.5.1. Talent placing the mouse in a towel-lined recovery basket on the heated stage.
 - 2.5.2. Talent monitoring the mouse during recovery from anesthesia.

3. Preparation for Aneurysm Induction Surgery

Demonstrator: Rohan Basu

- 3.1. Seven days after initial elastin sensitization, set up the surgical station for aneurysm induction surgery [1]. Prepare a working elastase solution by mixing 27 microliters of 5.1 milligrams per milliliter porcine pancreatic elastase with 73 microliters of PBS [1-TXT].
 - 3.1.1. Talent showing the fully prepared surgical station. **Authors: As we are already at the upper-shot limit, let us only show the fully prepared station**
 - 3.1.2. Talent adding porcine pancreatic elastase into PBS and mixing. **TXT: Concentration: 1.38 mg/mL**
- 3.2. Position the anesthetized mouse supine on a heated stage at 37 degrees Celsius [1-TXT]. Position the mouse's nose in the anesthesia nose cone [2] and inject 75 microliters of 1.3 milligrams per milliliter extended-release buprenorphine subcutaneously prior to surgery [3].
 - 3.2.1. Talent placing the mouse in a supine position on the heated stage. **Anesthesia: 1.8 - 2% Isoflurane**
 - 3.2.2. Talent positioning the mouse's nose in the anesthesia nose cone.
 - 3.2.3. Talent injecting buprenorphine subcutaneously.

3.3. Tape the hindlimbs to the stage with labeling tape [1]. Apply a lubricant to the eyes [2] and confirm the depth of anesthesia by pinching the animal's toe [3]. Remove the abdominal hair [4] and prepare the surgical site [5].

3.3.1. Talent taping the mouse's hindlimbs to the stage with labeling tape.

3.3.2. Talent applying eye lubricant.

3.3.3. Talent pinching the animal's toe to check the absence of reflex.

3.3.4. Talent removing the abdominal hair.

3.3.5. TEXT ON PLAIN BACKGROUND:

Preparing the Surgical Site

- Depilate the abdomen and wait 30 s
- Remove hair with gauze
- Disinfect the surgical site with povidone-iodine
- Wipe with alcohol and reapply povidone-iodine

4. Aneurysm Induction by Perivascular Elastase Application

4.1. Make an approximately 3-centimeter midline abdominal incision using a surgical blade. Start 0.5 to 1 centimeter cephalad to the penis and extend to 0.5 centimeter caudad to the xiphoid [1].

4.1.1. SCOPE: 4-1-1.

4.2. Using toothed forceps, grasp the skin and gently pull up [1].

4.2.1. SCOPE: 4-2-1--2.

4.3. Identify and incise the linea alba with scissors while continuing to pull the skin up [1-TXT].

4.3.1. SCOPE: .4-3-1 00:00-00:06 and 00:18-00:21 TXT: **Avoid injury to intra-abdominal structures**

4.4. Place magnetic self-retaining retractors to retract the abdominal wall and the caudal structures, such as the bladder and seminal vesicles [1].

4.4.1. SCOPE: 4-4-1 .

4.5. Using moistened cotton-tipped applicators, eviscerate the bowel away [1]. Cover the bowel with a moist 5 by 5 centimeter gauze [2].

4.5.1. SCOPE: 4-5-1--2. 00:10-00:25

4.5.2. SCOPE: 4-5-1--2. 00:33-00:38

4.6. Identify the inferior vena cava and abdominal aorta in the posterior midline [1]. Using cotton-tipped applicators, enter the retroperitoneum by gently opening the tissue plane covering the posterior vascular structures [2].

4.6.1. SCOPE: 4-6-1.

4.6.2. SCOPE: 4-6-2. 00:15-00:28

4.7. Then, place the lateral retractors deeper to improve traction and aortic visualization [1]. Use cotton-tipped applicators to separate the inferior vena cava from the infrarenal abdominal aorta. Avoid releasing the lateral aortic attachments at this stage [2].

4.7.1. SCOPE: 4-7-1. 00:12-00:22

4.7.2. SCOPE: 4-7-2-2. 00:22-00:28

4.8. Develop the plane between the inferior vena cava and the aorta until the triangular space becomes visible [1]. Using blunt dissection or sharp forceps, release the lateral aortic attachments by pulling the aorta apart from the connective tissue and lumbar musculature on the mouse's left [2].

4.8.1. SCOPE: 4-8-1. 00:15-00:22

4.8.2. SCOPE: 4-8-2.01:32-01:50

4.9. Next, place the angled sharp forceps into the triangular space [1]. Gently open and close the forceps in a repeated fashion to open the posterior aortic attachments [2].

4.9.1. SCOPE: 4-9-1--2. 00:15-00:25

4.9.2. SCOPE: 4-9-1--2. 00:26-00:35

4.10. Use the same opening-and-closing motion to develop the posterior plane along the aorta from cephalad to caudal [1]. Then, obtain a video micrometry image of the entire aorta and save it for analysis [1].

4.10.1. SCOPE: 4-10-1. 00:20-00:31

4.10.2. LAB MEDIA: 4-10-2.

4.11. Using a pipette, drop 10 microliters of the elastase solution along the length of the exposed aorta [1-TXT]. Place moistened 5 by 5 centimeter gauze pads over the open abdomen and wait for 10 minutes [2].

4.11.1. SCOPE: 4-11-1 00:06-00:13. TXT: ~13.8 µg elastase protein/mouse

4.11.2. SCOPE: 4-11-2.

4.12. Then, remove the gauze and irrigate the abdomen with sterile PBS three times to remove the residual elastase solution [1].

4.12.1. SCOPE: 4-12-1-2. 00:00-00:02 AND 00:17-00:23

4.13. Return the abdominal organs to their original positions and correct any bowel twisting or kinking [1].

4.13.1. SCOPE: 4-13-1--2. 00:32-00:35 AND 01:01-01:05

4.14. Using toothed forceps, peel the rectus muscle and fascia away from the skin to create a 0.25-centimeter skin flap [1].

4.14.1. SCOPE: 4-14-1. 00:011-00:16 and 00:28-00:31

4.15. Close the abdominal fascia with a 4-0 (4-Oh) braided polyglycolic acid suture in a simple running pattern. Incorporate the corners of the incision and space the bites less than 2 millimeters apart [1]. Staple the overlying skin together using 3 to 4 staples [2].

4.15.1. SCOPE: 4-15-1--2. 00:07-00:12 and 07:35-07:45

4.15.2. SCOPE: 4-15-3. 00:11-00:25

4.16. Remove the mouse from the surgery stage and place it in a towel-lined warming recovery basket on a stage heated to 37 degrees Celsius [1]. Monitor the mouse until it emerges from anesthesia [2].

4.16.1. Talent placing the mouse in a towel-lined warming recovery basket on the heated stage.

4.16.2. Talent monitoring the mouse during recovery from anesthesia.

4.17. Seven days after the surgery, perform an ultrasound to monitor the aneurysm growth [1]. Then, prepare a fresh EDP mixture using incomplete Freund's adjuvant [2] and repeat the elastin sensitization procedure as demonstrated earlier [3]. On postoperative day 21, collect blood and tissue samples for morphological and molecular analyses [4].

4.17.1. SCREEN: 4.17.1. **Authors: This is just a representative shot**

4.17.2. Reuse 2.3.1

4.17.3. Reuse 2.4.3

4.17.4. Talent harvesting the tissues or talent showing any existing harvested tissue or showing a tube with blood. **Authors: This is just a representative shot**

Results

5. Results

- 5.1. The mean aortic diameter ratio versus baseline at day 21 was significantly higher in the aneurysm group [1] than in sham surgery controls by ultrasound and video micrometry [2].
 - 5.1.1. LAB MEDIA: Figure 3AB. *Video editor: Highlight the dark gray bars labelled 'Aneurysm'.*
 - 5.1.2. LAB MEDIA: Figure 3AB. *Video editor: Highlight the light gray bars labelled 'Sham Surgery'.*
- 5.2. Representative histological images of aortic sections after tissue harvest on day 21 showed cellular infiltration, with a thickened intima [1].
 - 5.2.1. LAB MEDIA: Figure 4Av2. *Video editor: Highlight the thick pink ring surrounding the white center. Authors: Can you please label the areas of interest in the original figures with arrows for clarity?*
- 5.3. Substantial collagen deposition was seen surrounding an aneurysmal aorta [1].
 - 5.3.1. LAB MEDIA: Figure 4B.
- 5.4. Thinned, fragmented, or absent lamellar elastin was seen in the aneurysm model [1], compared to thick and well-defined lamellar elastin layers in a non-aneurysmal section [2].
 - 5.4.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the bottom inset with thin black curved lines.*
 - 5.4.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the top inset with thick black curved lines.*
- 5.5. Compared to the normal aorta [1], the aneurysmal aorta was grossly larger in the entire infrarenal segment [2], with an area of greater dilation near the aortic bifurcation [3].
 - 5.5.1. LAB MEDIA: Figure 5. *Video editor: Highlight A.*
 - 5.5.2. LAB MEDIA: Figure 5. *Video editor: Highlight the wider specimen in panel B.*
 - 5.5.3. LAB MEDIA: Figure 5. *Video editor: Highlight the wider right end of the specimen in panel B.*

1. Porcine
Pronunciation link: <https://www.merriam-webster.com/dictionary/porcine>
IPA: /'pɔːr,sam/
Phonetic Spelling: por·syne
2. Pancreatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/pancreatic>
IPA: /,pæŋkri'ætrɪk/
Phonetic Spelling: pang·kree·at·ik
3. Elastase
Pronunciation link: <https://www.merriam-webster.com/dictionary/elastase>
IPA: /ɪ'læs,tɛɪs/
Phonetic Spelling: ih·lass·tays
4. Suspension
Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>
IPA: /sə'spen(t)-shən/
Phonetic Spelling: suh·spen·shuhn
5. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkroʊ,li:tər/
Phonetic Spelling: my·kroh·lee·ter
6. Murine
Pronunciation link: <https://www.merriam-webster.com/dictionary/murine>
IPA: /'mjʊr,am/
Phonetic Spelling: myoor·ine
7. Elastin
Pronunciation link: <https://www.merriam-webster.com/dictionary/elastin>
IPA: /ɪ'læstɪn/
Phonetic Spelling: ih·lass·tin
8. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /'ɪŋkjə,bert/
Phonetic Spelling: ing·kyuh·bayt
9. Agitation
Pronunciation link: <https://www.merriam-webster.com/dictionary/agitation>
IPA: /,ædʒə'teɪʃən/
Phonetic Spelling: aj·uh·tay·shuhn
10. Freund's
Pronunciation link: <https://www.merriam-webster.com/dictionary/Freund>
IPA: /frɔɪnd/
Phonetic Spelling: froynd
11. Adjuvant
Pronunciation link: <https://www.merriam-webster.com/dictionary/adjuvant>
IPA: /'ædʒəvənt/
Phonetic Spelling: aj·uh·vuhnt

12. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /əˈnesθəˌtaɪz/
Phonetic Spelling: uh·ness·thuh·tyze
13. Subcutaneously
Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneously>
IPA: /ˌsʌbkjuːˈteɪniəsli/
Phonetic Spelling: sub·kyoo·tay·nee·uhs·lee
14. Degradation
Pronunciation link: <https://www.merriam-webster.com/dictionary/degradation>
IPA: /ˌdeɪɡrəˈdeɪʃən/
Phonetic Spelling: deg·ruh·day·shuhn
15. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
IPA: /ˌaɪsoʊˈfloʊrən/
Phonetic Spelling: eye·soh·floor·ayn
16. Sensitization
Pronunciation link: <https://www.merriam-webster.com/dictionary/sensitization>
IPA: /ˌsen(t)səˈteɪʒən/
Phonetic Spelling: sen·suh·tuh·zay·shuhn
17. Buprenorphine
Pronunciation link: <https://www.merriam-webster.com/dictionary/buprenorphine>
IPA: /ˌbjuːprəˈnɔːfɪn/
Phonetic Spelling: byoo·pruh·nor·feen
18. Depilate
Pronunciation link: <https://www.merriam-webster.com/dictionary/depilate>
IPA: /ˈdepəˌleɪt/
Phonetic Spelling: dep·uh·layt
19. Povidone-iodine
Pronunciation link: <https://www.merriam-webster.com/dictionary/povidone-iodine>
IPA: /ˈpəvəˌdoʊn ˈaɪəˌdaɪn/
Phonetic Spelling: pov·uh·dohn eye·uh·dyne
20. Cephalad
Pronunciation link: <https://www.merriam-webster.com/dictionary/cephalad>
IPA: /ˈsefəˌlæd/
Phonetic Spelling: sef·uh·lad
21. Caudad
Pronunciation link: <https://www.merriam-webster.com/dictionary/caudad>
IPA: /ˈkɔdˌæd/
Phonetic Spelling: kawd·ad
22. Xiphoid
Pronunciation link: <https://www.merriam-webster.com/dictionary/xiphoid>
IPA: /ˈzaɪˌfɔɪd/
Phonetic Spelling: zy·foyd

23. Linea alba
Pronunciation link: <https://www.merriam-webster.com/dictionary/linea%20alba>
IPA: /'lɪniə 'ælbə/
Phonetic Spelling: lin·ee·uh al·buh
24. Retractors
Pronunciation link: <https://www.merriam-webster.com/dictionary/retractor>
IPA: /rɪ'træktər/
Phonetic Spelling: ree·trak·ter
25. Seminal
Pronunciation link: <https://www.merriam-webster.com/dictionary/seminal>
IPA: /'semənəl/
Phonetic Spelling: sem·uh·nul
26. Vesicles
Pronunciation link: <https://www.merriam-webster.com/dictionary/vesicle>
IPA: /'vesɪkəl/
Phonetic Spelling: vess·ih·kul
27. Eviscerate
Pronunciation link: <https://www.merriam-webster.com/dictionary/eviscerate>
IPA: /i'vɪsə'reɪt/
Phonetic Spelling: ee·viss·uh·rayt
28. Inferior vena cava
Pronunciation link: <https://www.merriam-webster.com/dictionary/vena%20cava>
IPA: /ɪn'fɪriər 'vi:nə 'keɪvə/
Phonetic Spelling: in·feer·ee·er vee·nuh kay·vuh
29. Aorta
Pronunciation link: <https://www.merriam-webster.com/dictionary/aorta>
IPA: /eɪ'ɔrtə/
Phonetic Spelling: ay·or·tuh
30. Retroperitoneum
Pronunciation link: <https://www.merriam-webster.com/dictionary/retroperitoneum>
IPA: /,retɹə'perətə'ni:əm/
Phonetic Spelling: reh·troh·pair·uh·tuh·nee·uhm
31. Infrarenal
Pronunciation link: <https://www.merriam-webster.com/dictionary/infrarenal>
IPA: /,ɪnfɹə'ri:nəl/
Phonetic Spelling: in·fruh·ree·nul
32. Micrometry
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometry>
IPA: /maɪ'kɑ:mətri/
Phonetic Spelling: my·kom·uh·tree
33. Polyglycolic
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyglycolic>
IPA: /,pəlɪɡlaɪ'kəlɪk/
Phonetic Spelling: pol·ee·glye·kol·ik

34. Suture
Pronunciation link: <https://www.merriam-webster.com/dictionary/suture>
IPA: /'su:tʃər/
Phonetic Spelling: soo·cher
35. Ultrasound
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasound>
IPA: /'ʌltrəˌsaʊnd/
Phonetic Spelling: ul·truh·sound
36. Morphological
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphological>
IPA: /ˌmɔrfə'lɒdʒɪkəl/
Phonetic Spelling: mor·fuh·loj·ih·kul
37. Histological
Pronunciation link: <https://www.merriam-webster.com/dictionary/histological>
IPA: /ˌhɪstə'lɒdʒɪkəl/
Phonetic Spelling: hiss·tuh·loj·ih·kul
38. Infiltration
Pronunciation link: <https://www.merriam-webster.com/dictionary/infiltration>
IPA: /ˌɪnfɪl'treɪʃən/
Phonetic Spelling: in·fil·tray·shuhn
39. Intima
Pronunciation link: <https://www.merriam-webster.com/dictionary/intima>
IPA: /'ɪntəmə/
Phonetic Spelling: in·tuh·muh
40. Collagen
Pronunciation link: <https://www.merriam-webster.com/dictionary/collagen>
IPA: /'kɒlədʒən/
Phonetic Spelling: kol·uh·jun
41. Aneurysmal
Pronunciation link: <https://www.merriam-webster.com/dictionary/aneurysmal>
IPA: /ˌænjə'rɪzməl/
Phonetic Spelling: an·yuh·riz·mul
42. Lamellar
Pronunciation link: <https://www.merriam-webster.com/dictionary/lamellar>
IPA: /lə'melər/
Phonetic Spelling: luh·mel·er
43. Bifurcation
Pronunciation link: <https://www.merriam-webster.com/dictionary/bifurcation>
IPA: /ˌbaɪfər'keɪʃən/
Phonetic Spelling: by·fer·kay·shuhn