Submission ID #: 69522
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/LAB MEDIA-uploader?src=21204258

Title: The Stroke Preclinical Assessment Network Multi-Laboratory Model of Thromboembolic Stroke with Thrombolysis: TE-MCAo

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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? **No**

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**

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.

Current Protocol Length

Number of Steps: 18
Number of Shots: 28

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Mozammel Bhuiyan:** The scope of our research is to develop a thromboembolic stroke model with controlled thrombolysis for use in rigorous multi-laboratory preclinical therapeutic testing.

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

~~What research gap are you addressing with your protocol?~~

1.2. **Carly McCurry:** Our protocol addresses the absence of a simple, reproducible thromboembolic stroke model that mimics clinical thrombolysis and supports multicenter preclinical trials.

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

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

1.3. **Mozammel Bhuiyan:** Our protocol mimics human thrombus and thrombolysis, reduces animal use, standardizes surgery, and achieves reliable multicenter reproducibility.

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

~~What significant findings have you established in your field?~~

1.4. **Carly McCurry:** As a significant outcome of our research, we created a scalable, reproducible TE-MCAo model usable across multiple labs with consistent outcomes and structured quality control.

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

~~How will your findings advance research in your field?~~

1.5. **Mozammel Bhuiyan:** This model can be consistently used in both single and multi-laboratory stroke studies improving therapeutic screening and accelerating translation of candidate cerebroprotective treatments.

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. Enter author name, Enter author title: (authors will present their testimonial statements live)

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. Enter author name, Enter author title: (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at University of Southern California, University of Iowa, Augusta University, Duke University, University of California, San Diego, Yale University and Massachusetts General Hospital

Protocol

NOTE: Protocol was scripted from author provided footage

2. Collection of Donor Blood from Femoral Artery Catheterization and Thrombus Preparation

Demonstrator: Mozammel Bhuiyan and **Carly McCurry**

2.1. To begin, render the PE-50 (*P-E-Fifty)* tubing blunt by cutting the end at a beveled angle **[1]**. Trim off the very tip of the bevel point using micro scissors **[2]**.

2.1.1. LAB MEDIA: Trim_PE10.mov 00:04 – 00:18

2.1.2. LAB MEDIA: Trim_PE10.mov 00:18 – 00:33

2.2. Make a vertical incision on the inner thigh approximately 1.5 to 2 centimeters long along the natural fold of the inguinal intersection with the leg **[1-TXT]**.

2.2.1. LAB MEDIA: FemoralArteryDissection.mov 09:12 – 09:27
TXT: Incision should be orthogonal to expected anatomic course of femoral artery

2.3. Using blunt dissection, carefully dissect down to the femoral vessels **[1]**. Then separate the femoral artery from the vein and nerve that run in the same bundle **[2]**.

2.3.1. LAB MEDIA: FemoralArteryDissection.mov 09:46 – 10:00

2.3.2. LAB MEDIA: FemoralArteryDissection.mov 10:33 – 10:49

2.4. Distally occlude the femoral artery with a 5-0 *(Five-Oh)* silk ligature and use the tails of the suture to provide slight tension distally with hemostatic clamps **[1-TXT]**. Loosely place a loop of 5-0 silk around the femoral artery and use the tails to provide gentle tension on the proximal end of the artery **[2]**.

2.4.1. LAB MEDIA: FemoralArteryDissection.mov 11:18 – 11:40
TXT: Skeletonize femoral artery as proximal as possible

2.4.2. LAB MEDIA: FemoralArteryDissection.mov 12:30 – 12:51

2.5. About two-thirds of the distance from the proximal suture loop, place a piece of silk suture under the artery and make a small arteriotomy distal to the loose suture using micro scissors **[1]**.

2.5.1. LAB MEDIA: FemoralArteryDissection.mov 12:52 – 13:33

2.6. Using sharp forceps, lift the top opening of the arteriotomy and introduce the blunt bevelled PE-50 catheter into the opening **[1]**. Advance the catheter as far proximal as possible **[2-TXT]**.

2.6.1. LAB MEDIA: BloodDraw_Femoral_Artery 01:05 – 01:20

2.6.2. LAB MEDIA: BloodDraw_Femoral_Artery 01:21 – 01:34
TXT: When catheter has advanced enough, release tension from suture tails

2.7. Advance the catheter enough so that the loose loop, when tightened, encompasses the catheter **[1]**. Tighten the suture gently, ensuring it does not occlude the catheter or sit on the bevel **[2]**.

2.7.1. LAB MEDIA: BloodDraw_Femoral_Artery 01:42 – 01:46

2.7.2. LAB MEDIA: BloodDraw_Femoral_Artery 01:47 – 01:57

2.8. Release the rubber-shod clamp. The catheter should immediately fill with blood **[1-TXT]**.

2.8.1. LAB MEDIA: BloodDraw_Femoral_Artery 02:06 – 02:16
TXT: Fill three 1.5mL centrifuge tubes with blood; Store at 4°C

2.9. Once the tubes are filled, remove the PE-50 catheter from the femoral artery **[1]**. Ligate the proximal end and close the incision with interrupted 5-0 prolene suture **[2]**.

2.9.1. LAB MEDIA: BloodDraw_Femoral_Artery 04:20-04:30

2.9.2. LAB MEDIA: BloodDraw_Femoral_Artery 05:00 – 05:15

2.10. Now draw blood from either one of the 1.5 milliliter microcentrifuge tubes, if using freshly drawn blood, or from one of the pediatric EDTA microtubes, if using stored donor blood **[1]**.

2.10.1. LAB MEDIA: Image3.updated.jpg

2.11. To make clots from stored donor blood, aspirate blood from the microcentrifuge tube into a 100-centimeter PE-50 *(P-E-Fifty)* tubing using gentle suction **[1-TXT]**. Incubate the coiled tubing in pre-warmed PBS at 37 degrees Celsius for 2 hours in a table-top oven **[2]**.

2.11.1. LAB MEDIA: Image4. updated.jpg
TXT: Reverse anticoagulation with $CaCl_2$; PE: polyethylene

2.11.2. LAB MEDIA: Image5. updated.jpg 00:00 – 00:32.

2.12. Using a razor blade, cut sections of thrombus approximately 6 centimeters in length **[1]**. Draw each thrombus gently into a PE-50 catheter and fully expel it 5 times to wash it **[2]**.

2.12.1. LAB MEDIA: Video1_CuttingClot.mov. 00:00-00:07

2.12.2. LAB MEDIA: Video2_WashingClot_PE50.mov 00:01-00:15

2.13. Wash the thrombus again by drawing it gently into a PE-10 *(P-E-Ten)* catheter and fully

expelling it 15 times **[1]**.

2.13.1. LAB MEDIA: Video4_WashingClot_PE10.mov. 00:00-00:20

2.14. Then pipette 400 microliters of Evan's Blue Dilution Number 1 into a separate petri dish filled with 20 milliliters of PBS to create Evan's Blue Dilution Number 2 **[1]**.

2.14.1. LAB MEDIA: Image6.updated.jpg

2.15. Place the clot in the more concentrated Evan's Blue petri dish solution for 1 second **[1]**, then transfer it to the diluted Evan's Blue petri dish **[2]**.

2.15.1. LAB MEDIA: Video6_TransferringClot_mb2.mov 00:00 – 00:14

2.15.2. LAB MEDIA: Video6_TransferringClot_mb2.mov 00:15 – 00:20

2.16. Load one washed thrombus into prefabricated microcatheters without air emboli using the wet-to-wet method. **[1]**.

2.16.1. LAB MEDIA: Video11_AspiratingClot.mov 00:00 – 00:11

2.17. Once the thrombus is fully loaded, extrude enough thrombus so that exactly 5 centimeters remains in the microcatheter **[1]**. Trim the excess with a razor blade **[2]**.

2.17.1. LAB MEDIA: Video12_CuttingExcessClot_updated.mp4 00:00-00:08.

2.17.2. LAB MEDIA: Video12_CuttingExcessClot_updated.mp4. 00:13-00:23

2.18. Mark the microcatheter 16 millimeters from the tip to demarcate the extent of catheter that should be advanced intraluminally **[1]**.

2.18.1. LAB MEDIA: Video13_WhiteOut.mov 00:00-00:05

Results

3. Results

3.1. Among the 135 enrolled subjects, magnetic resonance imaging was obtained in 102 animals, indicating a 75% scan completion rate across all 6 laboratories **[1]**. Animal loss before magnetic resonance imaging on Day 3 was consistent across all 6 laboratories **[2]**.

3.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the row "MRI Done"*

3.1.2. LAB MEDIA: Table 1. *Video editor: Highlight both rows "Sample Size (N)" and "MRI Done"*

3.2. The mean lesion volume across all sites was 13 percent of the ipsilateral hemisphere, with some variation across the 6 laboratories **[1]**.

3.2.1. LAB MEDIA: Table 2. *Video editor: Highlight the "Lesion Volume (%) Mean ± SD" column*

Pronunciation Guide:

 Thromboembolic
Pronunciation link: https://www.howtopronounce.com/thromboembolic
IPA: /θɹɑː.mbo..ʊm.bˈɑːlɪk/
Phonetic Spelling: thrah·mbo·um·BAH·lik
 Thrombolysis
Pronunciation link: https://www.howtopronounce.com/thrombolysis
IPA: /θɹə.mbˈɑ.ː.ləsɪs/
Phonetic Spelling: thruh·MBAH·luh·sis
 Catheterization
Pronunciation link: https://www.howtopronounce.com/catheterization
IPA: /kæ.θ.ɪ.ɾə-..ɹ..aɪˈzeɪʃən/
Phonetic Spelling: ka·thi·rur·RYE·zay·shuhn
 Arteriotomy
Pronunciation link: https://www.howtopronounce.com/arteriotomy
IPA: /ɑː.ɹt.ɪ.ɹ.iˈ.ɑːɾəmi/
Phonetic Spelling: ah·rti·REE·ah·ruh·mee
 Femoral
Pronunciation link: https://www.howtopronounce.com/femoral
IPA: /fˈɛmə-.ɹəl/
Phonetic Spelling: FEM·ur·ruhl
 Inguinal
Pronunciation link: https://www.howtopronounce.com/inguinal
IPA: /ɪ.ŋgˈ.ɪnəl/
Phonetic Spelling: in·GWIN·uhl
 Hemostatic
Pronunciation link: https://www.howtopronounce.com/hemostatic
IPA: /hiː.mo..ʊ.stˈærɪk/
Phonetic Spelling: hee·mo·uh·STAH·rik
 Intraluminal
Pronunciation link: https://www.howtopronounce.com/intraluminal
IPA: /ɪ.ntɹ.əl.ˈu.ːmɪnəl/
Phonetic Spelling: in·truh·lOO·mi·nuhl
 Ipsilateral
Pronunciation link: https://www.howtopronounce.com/ipsilateral
IPA: /ˈɪ.ps.ɪ.l.ærə-ɹəl/
Phonetic Spelling: IP·sih·luh·RARE·uhl
 Anticoagulation
Pronunciation link: https://www.howtopronounce.com/anticoagulation
IPA: /æ.nt.ɪk...o.ʊæ..gjʊlˈeɪʃən/
Phonetic Spelling: an·tik·oh·uh·GYOO·lay·shuhn

 Thrombus
Pronunciation link: https://www.howtopronounce.com/thrombus
IPA: /θɹˈɑː.mbəs/
Phonetic Spelling: THRAHMB·uhs
 Microcentrifuge
Pronunciation link: https://www.howtopronounce.com/microcentrifuge
IPA: /maɪ..kɹ..oʊsɛˈ.ntɹɪfjuːdʒ/
Phonetic Spelling: mye·kroh·sen·TRIF·yooj
 EDTA
Pronunciation link: https://www.howtopronounce.com/edta
IPA: /ˈɛdtə/
Phonetic Spelling: ED·tuh
 Calcium chloride
Pronunciation link: https://www.howtopronounce.com/calcium-chloride
IPA: /kˈæ.ls..iəm .klo.ːɹaɪd/
Phonetic Spelling: KAL·see·uhm·KLOR·ide
 Magnetic resonance imaging
Pronunciation link: https://www.howtopronounce.com/magnetic-resonance-imaging
IPA: /mæ.gnˈɛr.ɪk ɹɛ.z.ənə.n.s ɪmɪdʒɪŋ/
Phonetic Spelling: mag·NEH·rik·REZ·uh·nuhns·IM·ih·jing
 Polyethylene
Pronunciation link: https://www.howtopronounce.com/polyethylene
IPA: /pɑː.l.ɪɛˈ.θɪliːn/
Phonetic Spelling: pah·lye·ETH·uh·leen
 Evans
Pronunciation link: https://www.howtopronounce.com/evans-
IPA: /ˈɛvənz/
Phonetic Spelling: EV·uhnz