FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 69563
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=21216763

Title: Establishment of a Modified Ferric Chloride-Induced Superior Sagittal Sinus Thrombosis

Authors and Affiliations:

Shuling Wan[1], Ran Meng[1], Xunming Ji[2], Min Li[1]

[1]Department of Neurology, Xuanwu Hospital Capital Medical University
[2]Department of Neurosurgery, Xuanwu Hospital Capital Medical University

Corresponding Authors:
Min Li 812879698@qq.com

Email Addresses for All Authors:
Shuling Wan 15901589718@163.com
Ran Meng ranmeng2011@126.com
Xunming Ji jixm@ccmu.edu.cn
Min Li 812879698@qq.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? **Yes**

Videographer: Please record the computer screen for the shots labeled as SCREEN

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? **About a 5-minute walk.**

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: 24
Number of Shots: 53 (17 SC)

Introduction

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 statements with sufficient context. We have selected 4 best questions and **reordered them according to your suggestion**.

INTRODUCTION:

~~What are the most recent developments in your field of research~~?

1.1. **Shuling Wan:** In our field, several modeling methods for superior sagittal sinus thrombosis are used. However, the ferric chloride induction method is widely used due to its simplicity and low cost.

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

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

1.2. **Shuling Wan:** We aim to refine the ferric chloride-induced superior sagittal sinus thrombosis model by reducing cortical injury and visualizing sinus thrombus.

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. **Shuling Wan:** Our refined model is less invasive and more compatible with ultrasound imaging, enabling more reliable, direct therapeutic development and evaluation.

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. **Shuling Wan:** We show that intraoperative laser speckle contrast imaging confirms model success, and postoperative high-resolution ultrasound dynamically monitors thrombus formation/lysis and lumen recanalization.

1.4.1. 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 Animal Experiments and Experimental Animal Welfare Committee at the Capital Medical University

Protocol

2. Establishment of a Modified $FeCl_3$-Induced Rat Superior Sagittal Sinus Thrombosis (SSST) Model

Demonstrator: Shuling Wan

2.1. To begin, apply ophthalmic ointment to both eyes of the rat after inducing anesthesia **[1-TXT]** and then, shave the scalp hair carefully **[2]**.

2.1.1. WIDE: Talent applying ophthalmic ointment to the rat's eyes. **TXT: Anesthesia: 2% Pentobarbital (50 mg/kg, IP)**

2.1.2. Talent shaving the scalp hair.
Authors: We do not show the animal being anesthetized in our videos. Hence we just mention 1 line text on screen stating the drug that was used. We start directly from the next step. So please anesthetize the animal before this step and anesthesia induction will **NOT** be filmed

2.2. Then, secure the rat in a prone position on a rodent operating platform using medical tapes **[1]**. Maintain the body temperature at around 37 degrees Celsius with a heating pad **[2-TXT]**.

2.2.1. Talent positioning the rat prone and securing it with medical tapes on the operating platform.

2.2.2. Talent placing the animal over a heating pad. **TXT: Continuously monitor the core temperature**

2.3. Disinfect the shaved scalp thoroughly using 5 percent povidone-iodine **[1]**.

2.3.1. Talent applying povidone-iodine to the scalp with sterile technique.

2.4. Now, make a 15-millimeter paramedian skin incision on the scalp **[1]**. Using blunt dissection, separate the underlying fascia and periosteum to expose the skull fully **[2]**.

2.4.1. Talent creating a paramedian scalp incision with a surgical instrument.

2.4.2. Talent bluntly dissecting fascia and periosteum to expose the skull.

2.5. Thin the skull using a cranial drill until the superior sagittal sinus is clearly exposed **[1]**, starting from the lambda and extending 10 millimeters anteriorly along the sagittal

suture **[2]**. Employ an intermittent drilling technique to avoid damage to the underlying dura mater and superior sagittal sinus **[3]**, and repeatedly irrigate the drill bit with normal saline to prevent thermal injury to the cortex **[4-TXT]**.

2.5.1. Talent drilling the skull to thin the bone over the superior sagittal sinus.

2.5.2. Close-up showing fully drilled area.

2.5.3. Talent pointing to the preserved dura mater and superior sagittal sinus.

2.5.4. Talent irrigating the drill bit and skull surface with normal saline. **TXT: Perform laser speckle contrast imaging; Record the baseline superior sagittal sinus condition**

Authors: I have added a text on the shot 2.5.4 and also given some context in the next line at 2.6 to record the baseline

2.6. After imaging the baseline conditions, apply a 10-millimeter segment of 2-0 *(2-oh)* silk suture soaked with 40 percent ferric chloride solution onto the exposed superior sagittal sinus surface for 5 minutes **[1]**. Replace the suture with a newly soaked segment and apply for another 5 minutes **[2]**.

2.6.1. Talent placing the ferric chloride–soaked silk suture onto the exposed superior sagittal sinus.

2.6.2. Talent removing the suture and placing a newly soaked segment onto the same site.

2.7. Then, rinse the surgical field with 0.5 milliliters of normal saline three times to remove residual ferric chloride **[1]**.

2.7.1. Talent irrigating the surgical field with measured volumes of normal saline.

2.8. Close the skin using interrupted sutures with 4-0 *(4-oh)* silk suture **[1]** and disinfect the area with povidone-iodine **[2]**.

2.8.1. Talent suturing the skin incision with interrupted stitches.

2.8.2. Talent applying povidone-iodine over the closed incision.

2.9. Observe the rat until it is fully awake **[1]**, then return it to its cage **[2]**.

2.9.1. Shot of the rat during recovery from anesthesia.

2.9.2. Talent placing the recovered rat back into its cage.

3. Assessment of Successful Model Establishment

3.1. Turn on the laser speckle contrast imaging system and the corresponding software RFLSI version 5 **[1]**. Press the **Online mode** button to activate live imaging **[2]**.

3.1.1. Talent powering on the laser speckle contrast imaging system and launching the RFLSI software on the computer.

3.1.2. SCREEN: Show the RFLSI version 5.0 software interface as the talent clicks the **Online mode** button to enable live imaging. ***Videographer: Please record the computer screen for the shots labeled as SCREEN***

3.2. Adjust the height and position of the instrument **[1]** until the indicator laser is centered within the field of view **[2]**.

3.2.1. Talent adjusting the vertical height and lateral position of the imaging instrument.

3.2.2. Close-up showing the indicator laser becoming centered in the field of view.

3.3. Place the rat on a foam platform **[1]** and adjust its position until the superior sagittal sinus aligns with the indicator laser **[2]**.

3.3.1. Talent placing the rat onto the foam platform beneath the imaging system.

3.3.2. Talent carefully repositioning the rat so the superior sagittal sinus aligns with the indicator laser.

3.4. Adjust the magnification and focus until the image is displayed clearly **[1]**. Set the lower and upper limits of the pseudocolor threshold to 10 and 200 to optimize the visual appearance of the pseudocolor image **[2]**.

3.4.1. Talent adjusting the magnification and focus controls on the imaging system.

3.4.2. SCREEN: set the pseudocolor threshold lower limit and upper limits appropriately.

3.5. Now, press the **Set ROI** button **[1]**, select the circular tool **[2]**, and delineate the superior sagittal sinus region **[3]**.

3.5.1. SCREEN: Show the cursor clicking the **Set ROI** button in the software interface.

3.5.2. SCREEN: Show the selection of the circular region of interest tool.

3.5.3. SCREEN: Show the circular region of interest being drawn around the superior sagittal sinus.

3.6. Then, press the **Record** button to obtain the venous blood flow **[1]** and capture both the original and pseudocolor images **[2]**.

3.6.1. SCREEN: Show the talent clicking the **Record** button in the software interface to begin data acquisition.

3.6.2. SCREEN: Display the recorded original image alongside the pseudocolor image representing venous blood flow.

4. Assessment of Thrombus Area/Volume and Hemodynamic Changes

4.1. Prepare isoflurane, ultrasound gel, disposable razor blades, medical tapes and check all the instruments required **[1]**.

4.1.1. Talent arranging drugs, consumables, instruments, and imaging equipment on the preparation bench.

4.2. Install the three-dimensional acquisition motor onto the imaging system **[1]**. Then, install the UHF57x transducer **[2]** and initialize the motor **[3]**.

4.2.1. Talent mounting the three-dimensional acquisition motor on the imaging system.

4.2.2. Talent attaching the UHF57x transducer to the motor.

4.2.3. SCREEN: Show the system interface as the motor initialization process is started and completed.

4.3. After anesthetizing the rat, place it in a prone position on the thermostatic imaging plate tempered at 37 degrees Celsius **[1-TXT]** and gently secure the head and limbs using medical tapes **[2]**.

4.3.1. Talent positioning the rat prone on the heated imaging plate. **TXT: Anesthesia:**
Induction: 3% Isoflurane with 1 L/min O_2
Maintenance: 1.5 - 2% Isoflurane via nose cone

4.3.2. Talent securing the rat's head and limbs with medical tapes.

4.4. Apply ultrasound gel evenly over the exposed scalp **[1]**.

4.4.1. Talent applying ultrasound gel to the exposed scalp area.

4.5. Adjust the position and orientation of the rat relative to the transducer **[1]** until the superior sagittal sinus and intraluminal thrombus are clearly visualized **[2]**.

4.5.1. Talent adjusting the rat and transducer alignment.

4.5.2. SCREEN: Show the ultrasound image as the superior sagittal sinus and thrombus come into clear view.

4.6. Acquire sequential tomographic images of the superior sagittal sinus in the sagittal plane using three-dimensional imaging in B-mode **[1-TXT],** followed by tomographic images in the coronal plane using the same settings **[2]**.

4.6.1. SCREEN: Show B-mode three-dimensional imaging as sagittal tomographic images are acquired. **TXT: Step size: 0.04 mm**

4.6.2. SCREEN: Show B-mode three-dimensional imaging as coronal tomographic images are acquired.

4.7. Now, use **Color Doppler** mode to observe blood flow in the superior sagittal sinus **[1]** and apply **Pulsed-Wave Doppler** mode to measure blood flow velocity **[2]**.

4.7.1. SCREEN: Show the Color Doppler mode displaying blood flow within the superior sagittal sinus.

4.7.2. SCREEN: Show the Pulsed-Wave Doppler mode measuring blood flow velocity.

4.8. Discontinue anesthesia and observe the rat until it is fully awake **[1]**. Then, return the rat to its cage **[2]**.

4.8.1. Talent monitoring the rat during recovery from anesthesia.

4.8.2. Talent placing the fully awake rat back into its cage.

4.9. Finally, export the acquired images **[1]** and analyze them using Vevo LAB software **[2]**. Record the maximum sagittal and coronal cross-sectional areas and the volume of the thrombus in the superior sagittal sinus **[3]**, along with the maximum blood flow velocity of the sinus **[4]**.

4.9.1. SCREEN: Show the image export process from the imaging system.

4.9.2. SCREEN: Show Vevo LAB software opening and loading the acquired datasets.

4.9.3. SCREEN: Show measurement of sagittal and coronal cross-sectional areas and thrombus volume.

4.9.4. SCREEN: cursor hovering over the maximum blood flow velocity.

Results

5. Results

5.1. After local application of ferric chloride, laser speckle contrast imaging detected a significant reduction in local blood flow in the superior sagittal sinus compared with baseline **[1]**.

5.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the bottom panel images "Post-FeCl3" and emphasize the area indicated by the black arrows*.

5.2. On postoperative day 7, small-animal ultrasound confirmed thrombus formation in the superior sagittal sinus **[1]** with partial luminal recanalization **[2]**.

5.2.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the region marked by the red arrow* .

5.2.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the region marked by the light blue arrow for the remaining open lumen*.

5.3. Pulsed-wave Doppler ultrasound demonstrated measurable blood flow velocity within the residual lumen of the superior sagittal sinus **[1]**.

5.3.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the colored flow region indicated by the yellow arrow and the velocity waveform at the bottom of the image*.

5.4. Three-dimensional ultrasound imaging enabled volumetric measurement of the superior sagittal sinus thrombus using serial image slices with a thickness of 0.04 millimeters **[1]**.

5.4.1. LAB MEDIA: Figure 4.

- **Povidone-iodine**

Pronunciation guide: *pah-vih-dohn EYE-uh-dyne*
IPA: /ˈpɑː.vɪˌdoʊn ˈaɪ.əˌdaɪn/
https://www.merriam-webster.com/medical/povidone-iodine

- **Paramedian**

Pronunciation guide: *pair-uh-MEE-dee-uhn*
IPA: /ˌpɛr.əˈmiː.di.ən/
https://www.merriam-webster.com/medical/paramedian

- **Periosteum**

Pronunciation guide: *pair-ee-OSS-tee-um*
IPA: /ˌpɛr.iˈɑːs.ti.əm/
https://www.merriam-webster.com/medical/periosteum

- **Superior sagittal sinus**

Pronunciation guide: *soo-PEER-ee-or SAJ-ih-tuhl SIGH-nuhs*
IPA:

- Superior: /suːˈpɪr.i.ɚ/
- Sagittal: /ˈsædʒ.ɪ.ɾəl/
- Sinus: /ˈsaɪ.nəs/
  https://www.merriam-webster.com/medical/sagittal
  https://www.merriam-webster.com/medical/sinus

- **Ferric chloride**

Pronunciation guide: *FAIR-ik KLOR-ide*
IPA: /ˈfɛr.ɪk ˈklɔːr.aɪd/
https://www.merriam-webster.com/medical/ferric
https://www.merriam-webster.com/medical/chloride

- **Isoflurane**

Pronunciation guide: *EYE-so-FLOOR-ane*
IPA: /ˌaɪ.soʊˈflʊr.eɪn/
https://www.merriam-webster.com/medical/isoflurane

- **Pseudocolor**

Pronunciation guide: *SOO-doh-kuh-ler*
IPA: /ˈsuː.doʊˌkʌl.ɚ/
https://www.merriam-webster.com/dictionary/pseudocolor

- **Tomographic**

Pronunciation guide: *toh-muh-GRAF-ik*
IPA: /ˌtoʊ.məˈgræf.ɪk/
https://www.merriam-webster.com/medical/tomographic

- **Coronal**

Pronunciation guide: *kuh-ROH-nuhl*
IPA: /kəˈroʊ.nəl/
https://www.merriam-webster.com/medical/coronal

- **Sagittal**

Pronunciation guide: *SAJ-ih-tuhl*
IPA: /ˈsædʒ.ɪ.ɾəl/
https://www.merriam-webster.com/medical/sagittal

- **Ischemia**

Pronunciation guide: *iss-KEE-mee-uh*
IPA: /ɪsˈkiː.mi.ə/
https://www.merriam-webster.com/medical/ischemia

- **Recanalization**

Pronunciation guide: *ree-kan-uh-luh-ZAY-shun*
IPA: /ˌriːˌkæn.əˌləˈzeɪ.ʃən/
https://www.merriam-webster.com/medical/recanalization