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Establishment of a Rat Model of Superior Sagittal-Sinus Occlusion via a Thread-Embolism Method

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TITLE:

Establishment of a Rat Model of Superior Sagittal-Sinus Occlusion via a Thread-Embolism Method

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KEYWORDS:

cerebral venous sinus thrombosis, animal model, Sprague-Dawley rat, thread plug, superior sagittal sinus, laser-speckle blood-flow imaging, blood flow, small animal, magnetic resonance imaging

SUMMARY:
Here, we establish a novel Sprague-Dawley (SD) rat model of superior sagittal sinus (SSS) thrombosis via a thread-embolization method, and the stability and reliability of the model were verified.

ABSTRACT
The mechanisms contributing to the natural onset of cerebral venous sinus thrombosis (CVST) are mostly unknown, and a variety of uncontrollable factors are involved in the course of the disease, resulting in great limitations in clinical research. Therefore, the establishment of stable CVST animal models that can standardize a variety of uncontrollable confounding factors have helped to circumvent shortcomings in clinical research. In recent decades, a variety of CVST animal models have been constructed, but the results based on these models have been inconsistent and incomplete. Hence, in order to further explore the pathophysiological mechanisms of CVST, it is necessary to establish a novel and highly compatible animal model, which has important practical value and scientific significance for the diagnosis and treatment of CVST. In the present study, a novel Sprague-Dawley (SD) rat model of superior sagittal sinus (SSS) thrombosis was established via a thread-embolization method, and the stability and reliability of the model were verified. Additionally, we evaluated changes in cerebral venous blood flow in rats after the formation of CVST. Collectively, the SD-rat SSS-thrombosis model represents a novel CVST animal model that is easily established, minimizes trauma, yields good stability, and allows for accurately controlling ischemic timing and location.

INTRODUCTION

Cerebral venous sinus thrombosis (CVST) is a rare disease of the cerebral venous system that accounts for only 0.5–1.0% of all causes of stroke but has a relatively high occurrence rate in children and young adults[1]. During autopsy, CVST was found to be the cause of 10% of cerebrovascular disease deaths[2]. Thrombosis can occur in any part of the intracranial venous system. The superior sagittal sinus (SSS) is one of the most commonly affected areas in CVST and can involve multiple blood vessels. Owing to stenosis or occlusion of the venous sinuses, intracranial venous return is blocked, which is often accompanied by increased intracranial pressure[3]. The clinical manifestations of CVST are complex and vary over time; although there is a lack of specificity of symptoms, the most common symptoms include headache (77.2%), seizures (42.7%), and neurological deficits (39.9%). In severe cases, coma and even death may occur[4,5]. In recent years, due to the overall improvement of medical and health standards and public health awareness, the proportion of related risk factors has changed, the proportion of trauma and infection has decreased, and the proportion of CVST caused by pregnancy, puerperium, oral contraceptives, and other reasons has gradually increased[5].

At present, the pathogenesis of CVST is still not well understood. To explore CVST in depth, further pathophysiological research is needed. However, most of these research methods are invasive and therefore difficult to implement clinically. Owing to many limitations of clinical

research, animal models have irreplaceable advantages in terms of basic and translational research.

The cause of CVST is complex, as its initial onset is often unrecognized and the location of thrombus formation is highly variable. Fortunately, animal models can achieve better control of these factors. In the past few decades, a variety of CVST animal models have been established, and each model has its own disadvantages. According to different production methods, they can be roughly divided into the following categories: the simple SSS-ligation model[6,7]; the SSS internal-injection-accelerator model[8]; the ferric-chloride-induced SSS thrombosis model[9]; the photochemical-induced SSS thrombosis model[10]; and the self-made embolism-occlusion SSS model[11]. However, most of these models are unable to circumvent invasive damage to the animal's cerebral cortex and are not able to accurately control the ischemic time and location. In some models, the thrombus will recanalize spontaneously; in other models, the SSS becomes permanently occluded. In addition, complicated operations and/or serious injuries may affect subsequent pathophysiological findings in these models.

In the present study, a thread plug was inserted into the SSS of Sprague-Dawley (SD) rats to successfully establish a CVST model that minimized damage, enabled precise controllability, and yielded good stability. Additionally, small-animal magnetic resonance imaging (MRI) and laser-speckle blood-flow imaging were combined to verify the model’s effectiveness. We evaluated changes in cerebral blood flow before and after establishment of our model, as well as evaluated the stability of our model, laying a foundation for further studies exploring the occurrence, development, and related pathophysiological mechanisms of CVST.

PROTOCOL

Procedures involving animal subjects have been approved by the Medical Norms and Ethics Committee of Wenzhou Medical University and are in accordance with the China legislation on the use and care of laboratory animals.

1. Preparation of the thread plug, SD rats, and experimental equipment

1.1. Use a nylon thread with a diameter of 0.28 mm as the main body of the thread plug.

NOTE: The softness and hardness of the nylon thread should be moderate.

1.2. Cover one end of the nylon thread with silicone material. The length of the silicone part of the thread plug is approximately 1.2 cm, and the diameter is approximately 1.2 mm. The head end is tapered, and the silicone part is cylindrical. Reserve another 5–7 cm. The nylon thread is easy to clamp and can be cut according to specific needs after the operation.

1.3. Use 75% ethanol to soak the thread plug for 3 min before the operation and rinse any residual ethanol with normal saline before plugging.

1.4. Select 12 male SD rats weighing between 280 and 320 g, and randomly divide them into a sham group and experimental group (n = 6 per group). After a week of environmental adaptation, fast the rats for 12 h and water-deprive them for 4 h before the operation.

1.5. Prepare the following experimental equipment needed for the experiment: a small animal anesthesia machine, a brain stereotaxic instrument, a dissecting microscope, a high-speed skull drill, scissors, tweezers, vascular forceps, a needle holder, a needle thread, a 2 mL syringe, a laser-speckle blood-flow imaging system, and a small-animal MRI scanner.

2. Construction of SD-Rat SSS-Embolization Model via Thread Embolization

2.1. Place the SD rat into an anesthesia-induction box and use a small-animal anesthetic machine to deliver 4% isoflurane to induce anesthesia. Thereafter, use forceps to confirm that the SD rat's hind limbs and toes do not respond to moderate pinching.

2.2. Quickly fix the SD rat with shaved top hair in the prone position on a brain stereotaxic device. Maintain anesthesia with 1.5–2.0% isoflurane (at a speed of 0.5 L/min), and stabilize the respiratory rate at 40–60 breaths/min. Stabilize the rat’s body temperature via a heating pad at 37±0.2 °C.

2.2.1. Apply sterile ophthalmic eye lubricant after the rat is placed on the stereotaxic frame to protect the corneas from drying during anesthesia.

2.3. Sterilize the surface on the top of the rat’s head with 5% povidone iodine alternating three times with 75% ethanol. Make a skin incision (2.0-cm long) in the middle of the head, and then carefully peel off the top fascia and periosteum to fully expose the skull.

2.3.1. Confirm the positions of the anterior fontanelle, posterior fontanelle, coronal suture, sagittal suture, and herringbone suture.

2.4. Use the area between the coronal suture and the herringbone suture as the blood-flow observational area. To prevent the skull from affecting observation during laser-speckle blood-flow imaging, thin the skull in the observation area until the blood vessels are clearly visible. The size of the thinned skull should be approximately 1.0 cm × 1.0 cm. This step and the following are all performed under a dissecting microscope.

2.4.1. During skull grinding, use normal-temperature saline to rinse the drill repeatedly to avoid high-temperature burns to the cerebral cortex.

2.5. Use a high-speed drill to grind the skull within a 6.0 mm x 4.0 mm bone window centered at bregma to expose the SSS of the bregma area.

2.5.1. Use normal saline to cool the skull during grinding. When the skull becomes thin, use tweezers to carefully remove the remaining bone pieces to avoid tearing the SSS.

2.6. Choose a suitable thread plug, use the SSS bregma point as the plug point, carefully puncture it with a 2 mL syringe needle, and quickly insert the thread plug head into the plug point.

2.6.1. At this time, the angle between the end of the thread-plug head and the SSS should be approximately 30–45°; then adjust the angle between the end of the thread plug and the SSS to 0–10 degrees, and slowly insert the SSS into the center until the head reaches the posterior edge of the sinus confluence. Thereafter, cut off the excess part of the tail.

NOTE: Rapid bleeding may occur when the SSS is punctured. If the end of the thread plug cannot be quickly inserted into the plug point at one time, use a small gauze or cotton ball to gently press the plug point while slowly sliding down to expose the plug point carefully, and then quickly insert the end of the wire bolt into the SSS. After the thread plug is inserted, if there is bleeding at the plug point, hemostatic materials such as a gelatin sponge can be used to stop the bleeding.

3. Detection of Blood Flow on the Brain Surface of SD Rats

3.1. Use a laser light source to uniformly illuminate the blood-flow observational area. The reflected light is collected by a camera and is transmitted to a computer for analysis. Use the following parameter settings for the laser-speckle blood-flow imaging system: wavelength: λ = 785 nm; and image exposure time: T = 10 ms.

3.2. Center the SD-rat blood-flow observational area into the field of view of the laser-speckle blood-flow imaging system and conduct continuous monitoring of blood flow on the brain surface for 2 min. Collect and process the blood flow data before and after embolization for each SD rat, and obtain the laser-speckle blood-flow map of the observed area.

3.3. Repeatedly rinse the operated area with normal saline to wash away bone debris and residue. Suture the skin (0# thread) and disinfect with iodophor.

3.4. Maintain body temperature until the rat awakens after surgery and then house in a single cage with food and water provided *ad libitum*. The sham group cannot be plugged.

3.5. After data collection is completed, perform post-processing.

3.5.1. Complete selection of the region of interest (ROI) via the tools provided by the laser-speckle blood-flow imaging system software. The values obtained are the average blood-flow value in the ROI, and the local cerebral-blood-flow values before and after embolization. Use the blood-flow value before embolization as the baseline value.

3.5.2. Select four ROIs and measure the relative change in cerebral blood flow in each ROI,

expressed as the percent change from the baseline value.

4. Detection of thread position on small animals MRI

4.1. Use the following T2-weighted imaging (T_2WI) parameters for MRI imaging system: time of echo (TE) = 33 ms, time of repeat (TR) = 3000 ms, number of excitation (NEX) = 4, Slices = 28, slice thickness = 0.8 mm, matrix size = 256*256 mm^2, flip angle = 80°, field of view (FOV) = 30*30 mm^2, scan time = 6 min 24 s; magnetic resonance angiography (MRA) parameters were set as follows: TE = 4.4 ms, TR = 12 ms, NEX = 4, slices = 80, slice thickness = 0.4 mm, matrix size = 256*256 mm^2, flip angle = 80°, FOV = 30*30 mm^2, scan time = 16 min 23 sec 40 ms.

4.2. Fix the animal on the MRI scanning table, calibrate the brain position by positioning scanning, and perform T_2WI and MRA sequence scanning after confirming the position.

4.3. Use continuous anesthesia via the animal anesthesia machine during the detection. Then, euthanize the SD rats by intraperitoneal injection of excessive pentobarbital.

4.4. Image acquisition and post-processing: after collecting the image data, in order to observe the state of the thread plug in the SSS more clearly, use the pseudo color enhancement method to display the T_2WI image of the rat brain.

Representative Results

To establish the SD-rat SSS-thrombosis model via the suture method, the suture should be prepared in advance (**Figure 1A**), and the equipment required for the experiment (**Figure 1B**) should be prepared. Due to the delicate nature of the operation, the preparation of the model needs to be completed under a dissecting microscope. The main steps are shown in **Figure 2**. To facilitate the description of the specific details of the blood-flow observation of the model, **Figure 3B** marks the blood-flow observational area, the bone window, and the plug point, and also shows the state of the thread plug inserted into the SSS (**Figure 3C**). After the preparation of the model is completed, laser-speckle blood-flow imaging is used to detect the blood flow on the brain surface of SD rats (**Figure 4A, B). Figure 4C** shows that the blood flow in the SSS and bridge veins (BVs) were decreased significantly compared with those in the middle cerebral artery (MCA) and capillaries (CAPs). Next, small-animal MRI is used to detect the state of the thread plug in the SSS from the horizontal position (**Figure 5A**), sagittal position (**Figure 5B**), and coronal position (**Figure 5C**). The images show that the thread plug is in place, which confirms embolization of the SSS.

Figure 1. Picture of thread embolism and experimental conditions. (**A**) Image of the self-made thread embolism (a: thread-embolism head, b: thread- embolism body). Scale bar = 5 mm. (**B**) Main equipment required for this experiment.

Figure 2. The main steps of the SD-rat SSS-thrombosis model. (**A**) Clamp the toes of the hind limbs of the SD rat with forceps to verify successful anesthesia. (**B**) Fix the SD rat on a

stereotaxic device and sterilize the surface on the top of the head. (**C**) Cut the skin. (**D**) Peel off the top fascia and periosteum, and fully expose the skull. (**E**) Drill and polish the observation area and the skull of the bone window until the blood vessels are clearly visible. (**F**) Carefully remove the bone fragments at the bone window with forceps to avoid tearing the SSS. (**G**) Select a suitable thread plug. (**H**) Use a syringe needle to pierce the plug point and insert the thread plug quickly. (**I**) Adjust the angle between the thread plug and the SSS. (**J**) Insert the suture slowly. (**K**) until the tip reaches the posterior edge of the sinus confluence. (**L**) Suture the scalp.

Figure 3. Observation and operation of SD rats. (**A**) Anatomical landmarks of the top skull of SD rats were displayed to facilitate the location of SSS (a: bregma, b: posterior bregma). (**B**) The red rounded-rectangular area is the blood flow observation area, and the blue rounded-rectangular area is the bone window. The red circular area is the plug point, and the arrow points to the superior sagittal sinus. (**C**) State of the plug inserted into the SSS from the plug point (blue arrow). The white arrow points to the body of the plug, while the red arrow points to the thread head. Scale bar = 2 mm.

Figure 4. Comparison of blood flow before and after thread-embolism insertion. Laser-speckle blood-flow imaging of the cerebral blood flow of SD rats (**A**) before and (**B**) after embolization. The blue-to-red column on the right represents the range of blood flow values from small to large. In (**A**), the selected ROI is marked (a: SSS, b: BV, c: MCA, d: CAP). (**C**) A bar graph of relative cerebral blood flow (CBF) is shown. One-way analysis of variance revealed significantly decreased blood flow in the SSS and BV (# $P < 0.001$ vs. MCA and CAP; * $P < 0.001$ vs. MCA and CAP). Scale bar = 1 mm.

Figure 5. MRI verification results. Small-animal MRI shows the state of the thread plug (shown by the black arrow) in the SSS from horizontal (**A**), sagittal (**B**), and coronal (**C**) positions. Scale bar = 2 mm.

Table 1: Disadvantages of CVST animal models.

DISCUSSION

In this study, a new type of CVST model was successfully established by inserting a self-made thread plug into the SSS of SD rats. Additionally, laser-speckle blood-flow imaging and small-animal MRI were combined to monitor changes in blood flow on the brain surface of SD rats before and after the embolization in order to standardize ischemic timing and location.

In 1989, Longa et al. made a reversible MCA occlusion model by retrogradely inserting a self-made nylon suture into the external carotid artery of rats[12]. To improve the stability of this model, several improved methods have since been introduced[13]. This MCA occlusion model enables precise ischemic timing and location, is easy to recanalize, and has been widely used in basic research investigating cerebral arterial stroke[14,15]. In the present study, building upon the design of the MCAO model, a novel CVST model was established by

inserting a self-made thread plug into the center of the SSS starting position.

To elucidate the pathogenic mechanisms of CVST, a variety of animal models have been established and implemented (**Table 1**). The simple SSS-ligation model cannot avoid damage to the animal's cerebral cortex[6,7]. The ferric-chloride-induced SSS-thrombosis model also inevitably causes damage to the animal's cerebral cortex due to the toxicity of ferric chloride[9]. As an alternative model, a thread plug can be inserted into the SSS in order to completely avoid contact with the cerebral cortex, which can help to mitigate any cortical damage. The SSS internal-injection-accelerator model uses a photochemical method to induce thrombosis with the possibility of recanalization[8]; this model uses a self-made nylon-thread plug to induce reliable embolization at a fixed location. The embolism used in the self-made embolism-occlusion model is difficult to establish, which increases damage to vascular endothelial cells and cannot be recanalized[11]. The self-made thread plug used in this model is made of nylon thread and silica gel. These materials have a flexible texture and a relatively smooth surface, which minimize the damage caused by the material itself to the cerebral cortex and SSS endothelial cells during the modeling process. There are individual differences in the diameter of the SSS in SD rats. To rule out the influence of this parameter, the specification of the thread plug is determined based on the data obtained from multiple measurements of the SSS and one's operating experience. In addition, such an injury easily recovers after the plug is pulled out, which provides the possibility for subsequent SSS recanalization.

This present study successfully established a novel CVST model that minimized damage, enabled precise controllability, and yielded good stability. Additionally, small-animal MRI and laser-speckle blood-flow imaging were combined to verify the stability of the model and evaluate cerebral blood before and after embolization. Taken together, the present findings and protocol provide a foundation for further studies exploring the occurrence, development, and related pathophysiological mechanisms of CVST.

DISCLOSURES:

The authors declare that they have no competing financial interests.

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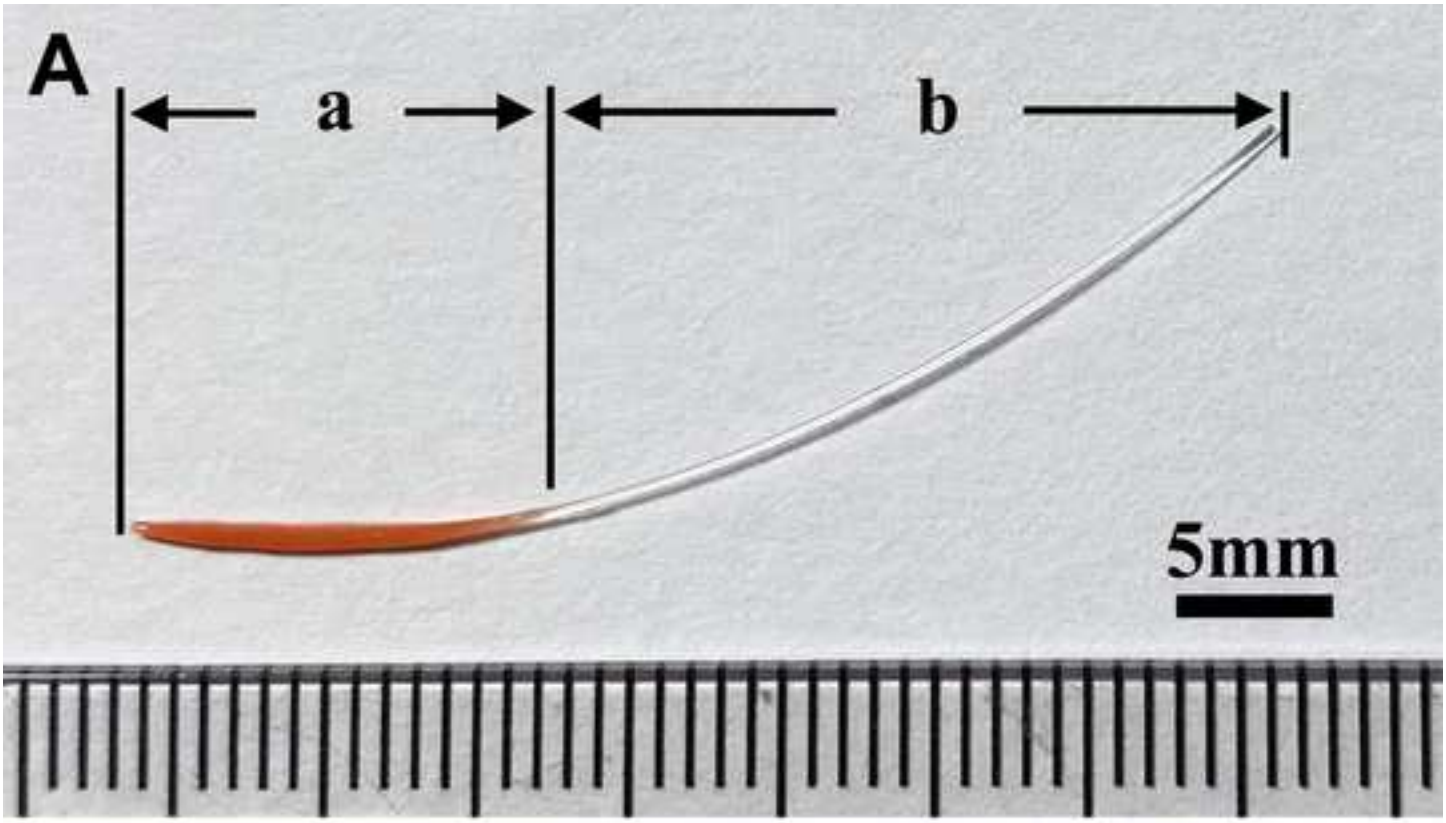
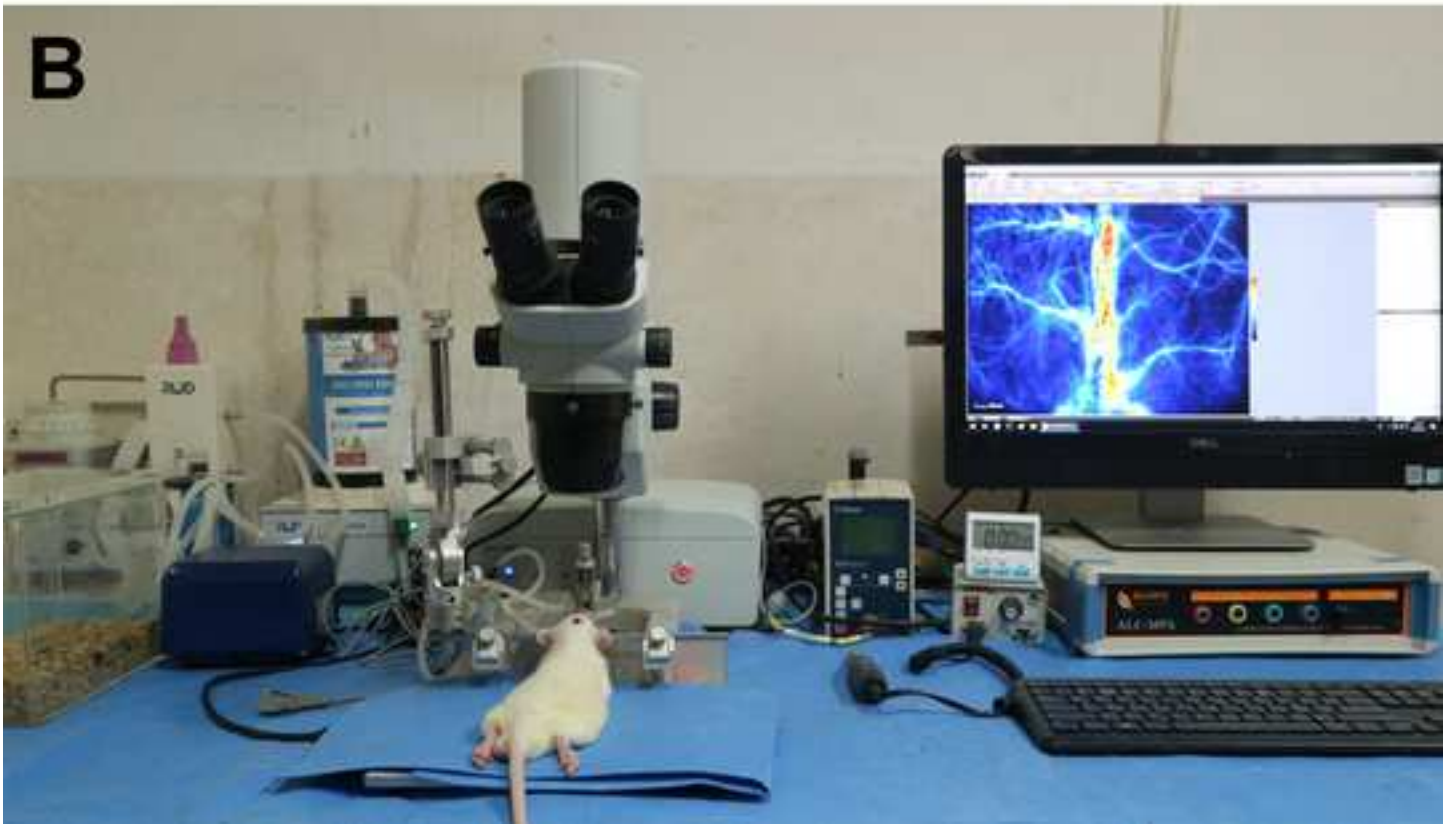
REFERENCES

1. Bousser, M. G., Ferro, J. M. Cerebral venous thrombosis: an update. *Lancet Neurology*. **6** (2), 162-170 (2007).
2. Guenther G, Arauz A. Cerebral venous thrombosis: A diagnostic and treatment update. *Neurologia*. **26** (8), 488-498 (2011).
3. Stam, J. Thrombosis of the cerebral veins and sinuses. *New England Journal of Medicine*. **352** (17), 1791-1798 (2005).
4. Einhäupl, K. et al. EFNS guideline on the treatment of cerebral venous and sinus

thrombosis in adult patients. *European Journal of Neurology*. **17** (10), 1229-1235 (2010).
5. Coutinho, J. M., Zuurbier, S. M., Stam, J. Declining mortality in cerebral venous thrombosis: a systematic review. *Stroke*. **45** (5), 1338-1341 (2014).
6. Gotoh, M., Ohmoto, T., Kuyama, H. Experimental study of venous circulatory disturbance by dural sinus occlusion. *Acta Neurochir (Wien)*. **124** (2-4), 120-126 (1993).
7. Miyamoto, K., Heimann, A., Kempski, O. Microcirculatory alterations in a mongolian gerbil sinus-vein thrombosis model. *Journal of Clinical Neuroscience*. **8** (4), (2001).
8. Ungersböck, K., Heimann, A., Kempski, a. O. Cerebral Blood Flow Alterations in a Rat Model of Cerebral Sinus Thrombosis. *Stroke*. **24** (4), (1993).
9. Röttger, C. et al. A new model of reversible sinus sagittalis superior thrombosis in the rat: magnetic resonance imaging changes. *Neurosurgery*. **57** (3), 573-580; discussion 573-580 (2005).
10. Chen, C. et al. Photothrombosis combined with thrombin injection establishes a rat model of cerebral venous sinus thrombosis. *Neuroscience*. **306**, 39-49 (2015).
11. Yang, H., Meng, Z., Zhang, C., Zhang, P., Wang, Q. Establishing a new rat model of central venous sinus thrombosis and analyzing its pathophysiological and apoptotic changes. *Journal of Neuroscience Methods*. **203** (1), 130-135 (2012).
12. Longa, E. Z., Weinstein, P. R., Carlson, S., Cummins, R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*. **20** (1), 84-91 (1989).
13. Fluri, F., Schuhmann, M. K., Kleinschnitz, C. Animal models of ischemic stroke and their application in clinical research. *Drug Design, Development and Therapy*. **9**, 3445-3454 (2015).
14. Wang, E. et al. Mapping tissue pH in an experimental model of acute stroke – Determination of graded regional tissue pH changes with non-invasive quantitative amide proton transfer MRI. *Neuroimage*. **191**, (2019).
15. Liu, C. et al. Identification of Vigilin as a Potential Ischemia Biomarker by Brain Slice-Based Systematic Evolution of Ligands by Exponential Enrichment. *Analytical Chemistry*. **91** (10), 6675-6681 (2019).





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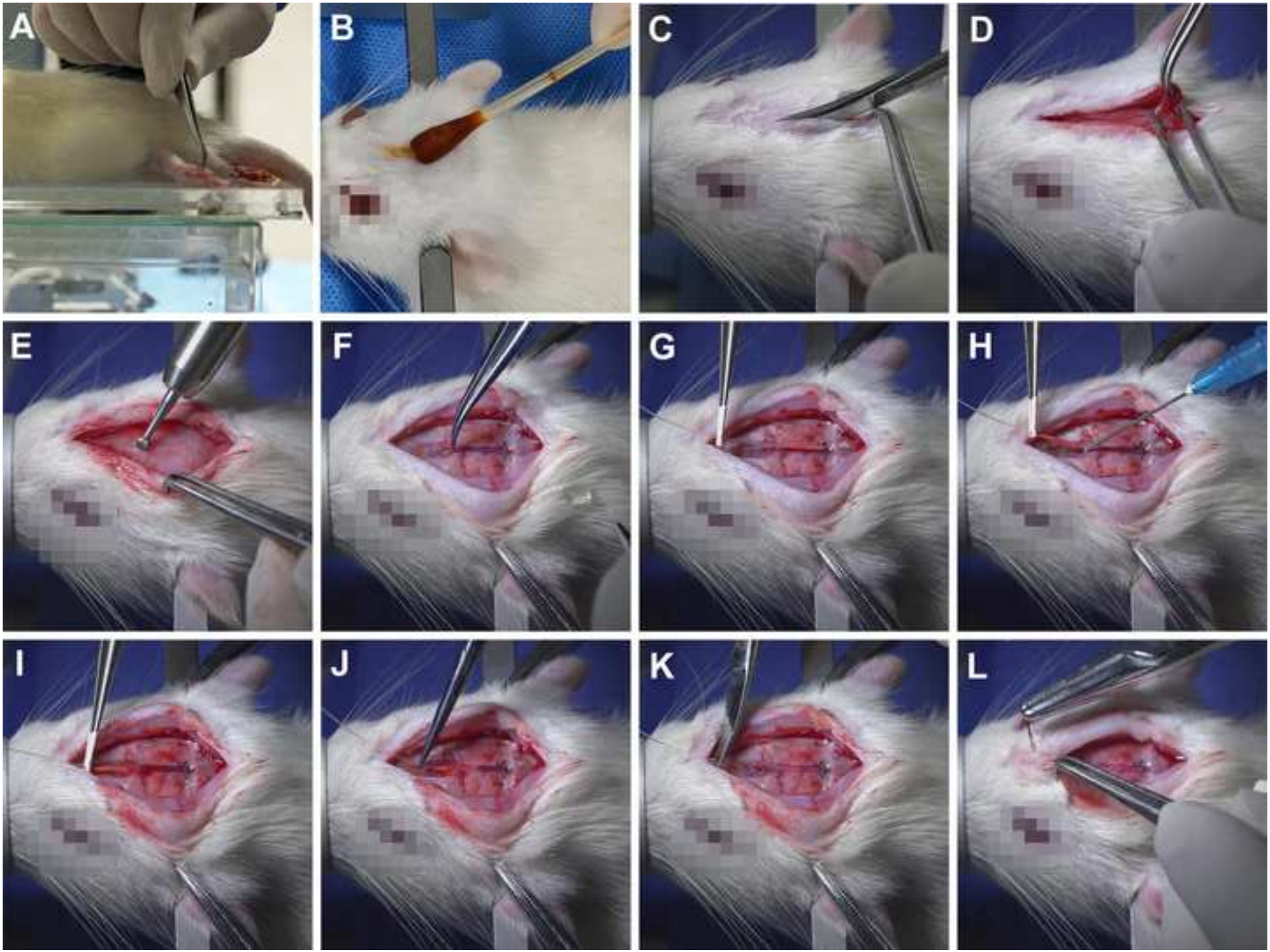

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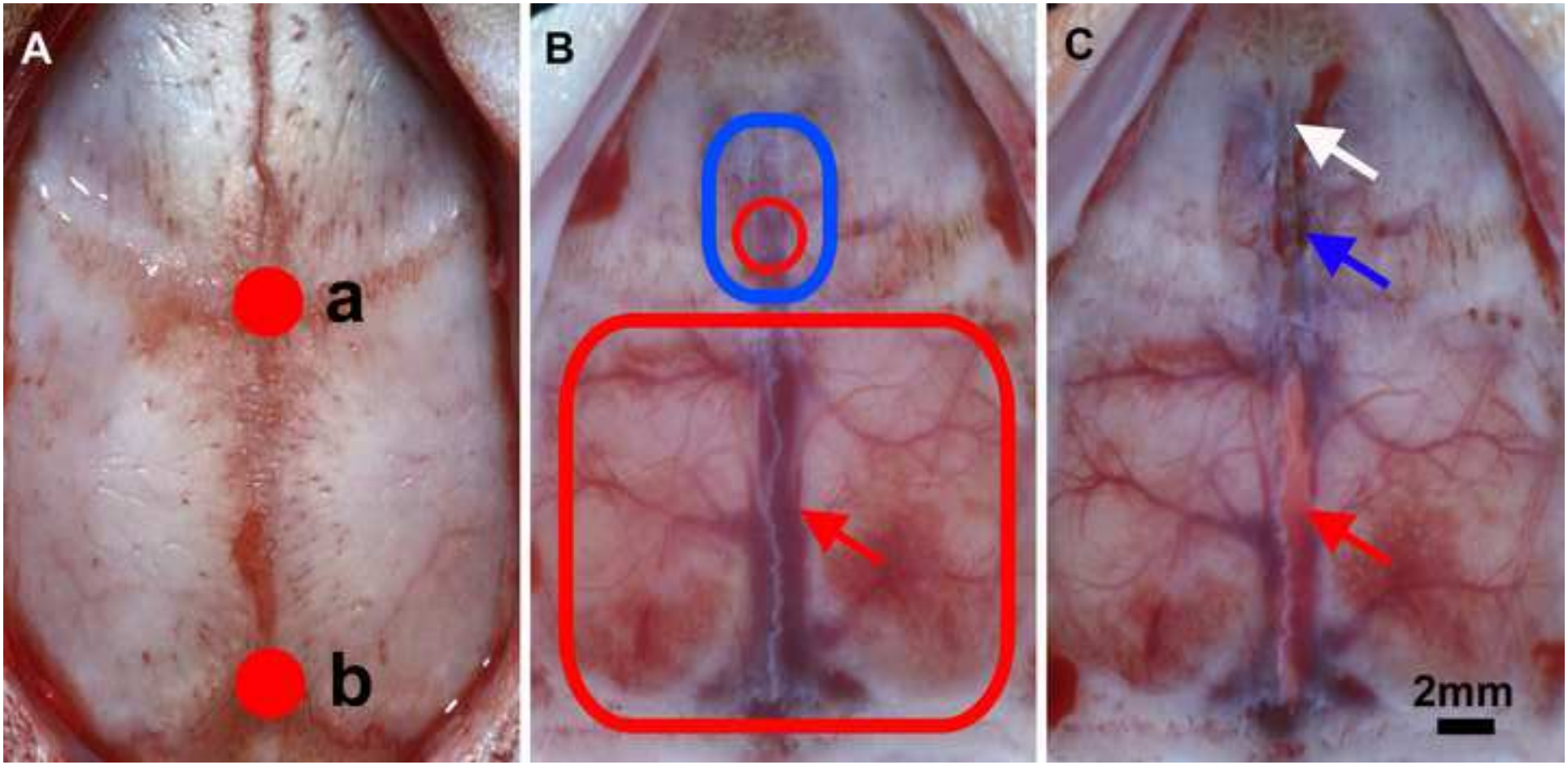

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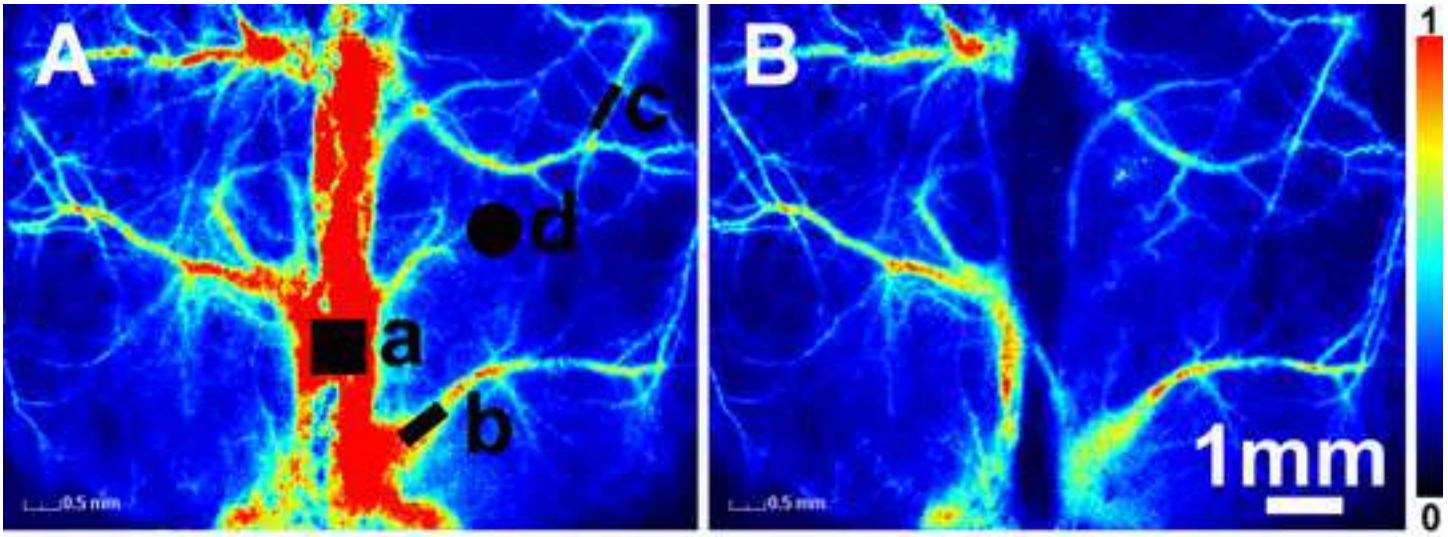

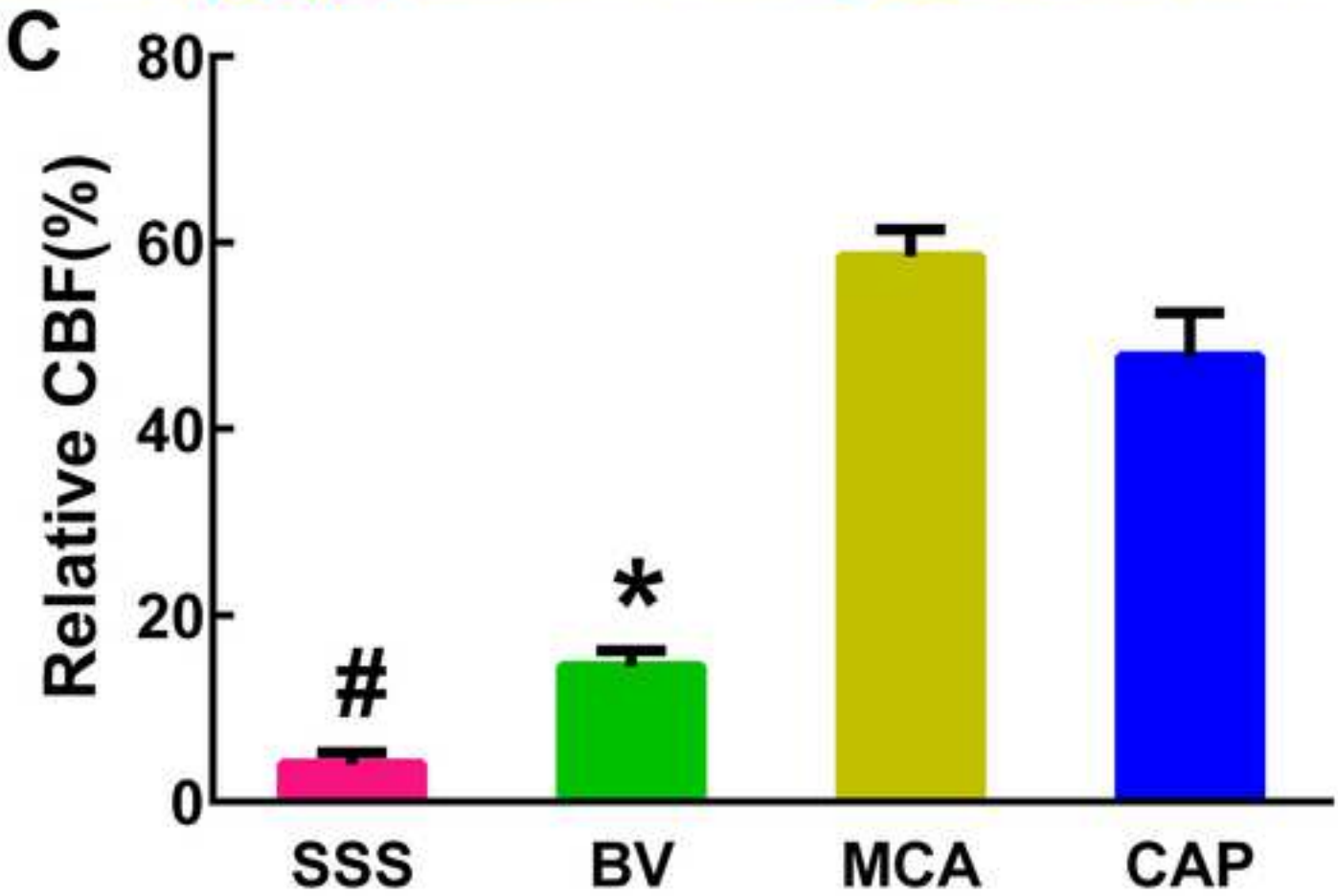

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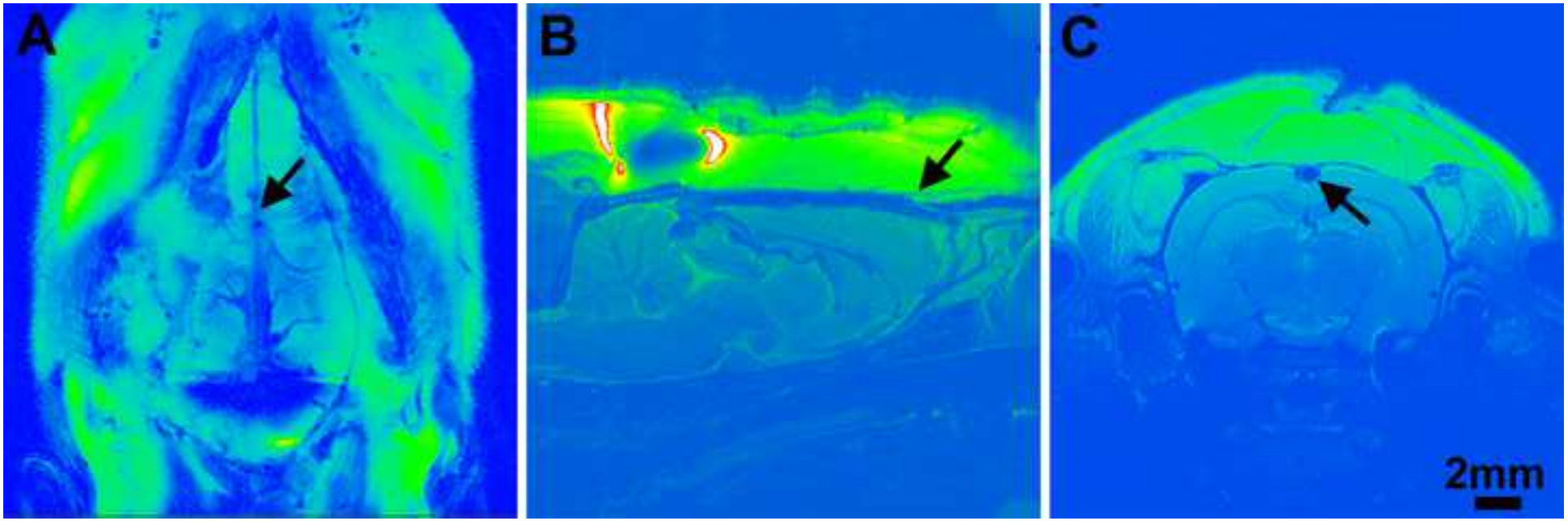

CVST animal model
simple SSS-ligation model
SSS internal-injection-accelerator model
ferric-chloride-induced SSS thrombosis model
photochemical-induced SSS thrombosis model
self-made embolism-occlusion model

limitations
cerebral cortex damage, permanently occluded
cerebral cortex damage, recanalize spontaneously, ischemic time and location inaccurate
cerebral cortex damage, recanalize spontaneously
cerebral cortex damage, recanalize spontaneously
cerebral cortex damage, permanently occluded, complicated operations

Table of Materials

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
2 mL syringe	Becton,Dickinson and Company	301940	
brain stereotaxic instrument	Shenzhen RWD Life Technology Co., Ltd	68025	
dissecting microscope	Wuhan SIM Opto-technology Co.	SIM BFI-HR PRO	
high-speed skull drill	Shenzhen RWD Life Technology Co., Ltd	78046	
laser-speckle blood-flow imaging system	Wuhan SIM Opto-technology Co.	SIM BFI-HR PRO	
needle holder	Shenzhen RWD Life Technology Co., Ltd	F31022-12	
needle thread	Shenzhen RWD Life Technology Co., Ltd	F33303-08	
scissors	Shenzhen RWD Life Technology Co., Ltd	S13029-14	
silica gel	Heraeus Kulzer	302785	
small animal anesthesia machine	Shenzhen RWD Life Technology Co., Ltd	R540	
small-animal MRI	Bruker Medical GmbH	Biospec 94/30 USR	
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Response: We have finished. The video has been broken up into three chapters.

8. Please present the figures of the written manuscript in the video as well. This should be in the representative results section of the video.
Response: We have finished.

9. Remove the shot of the anesthesia from the video.
Response: We have finished.

10. Blur or block out the animals face as much as possible.
Response: We have finished.

Please upload a high-resolution video here:
https://www.dropbox.com/request/j0fgfX11W1oXrQftbUnS?oref=e

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

In this manuscript, Jiang et al. reported a novel Sprague-Dawley (SD) rat model of superior sagittal sinus (SSS) thrombosis via a thread-embolization method. The authors have also shown the stability and reliability of the established model. The study is interesting and has acceptable levels of new data. However, there are few minor issues to be addressed before this manuscript can be accepted for publications.

Minor Concerns:

a. As CVST accounts for only 0.5-1.0% of all causes of stroke, it would be ideal to provide the mortality rate of CVST induced stroke, which will highlight the importance of work.
Response: We have finished.

b. Line 85-86 "Owing to many limitations of clinical research, animal models have irreplaceable advantages in terms of basic and translational research." Please provide examples of these limitations.
Response: Compared with animal experiment, the limitation of clinical research is very obvious, and it does not need to be described too much.

c. Line 91-99 highlights several methods to develop CVST animal models, however limitation of each model was not highlighted. It will be better to add a table highlighting the advantages and limitations of these techniques.
Response: We have added a table highlighting the limitations of these techniques.

d. Minor typographical errors should be rectified.
Response: We have finished.

e. Details of figure 3A and 5A are not presented in the text. Please update them or remove the figure the sections (if not necessary). Also, update the information about figure 5A in the figure legends.
Response: We have finished.

f. Unify the font of figure legend 1.
Response: We have finished.

g. Kindly provide the full form of the abbreviations used in the figure legends.
Response: We have finished.

Reviewer #2:
Manuscript Summary:
The authors established a novel and effective animal model of CVST, which has vital scientific significance for the diagnosis and treatment of CVST

Minor Concerns:
1. However, due to the abundant collateral circulation in rats, we wonder whether this thread-occlusion model can cause bridging vein thrombosis and corresponding cerebral infarction. If possible, once the thread plug is inserted, how long can it cause bridging vein thrombosis?
Response: In our previous study, due to the abundant collateral circulation in rats, this thread-occlusion model only cause cerebral cortical edema. Bridging vein thrombosis is the further research content in our laboratory.

2. What are the main neurological symptoms of rats after successful modeling?
Response: No obvious neurological symptoms were found in our previous study. But, Pathological changes of the SSS parasagittal cortex were observed by HE staining. Changes in the cellular and perivascular structure suggested the occurrence of cytotoxic edema and vasogenic edema，neovascularization appeared beside the SSS, which may be related to the compensation of collateral circulation. This is worthy of our in-depth study.

3. When puncturing the head end of SSS with a syringe, it will inevitably cause bleeding and make it difficult to insert

the thread plug. Can the surgical process be further optimized?

Response: The further optimization of the surgical process needs continuous training.

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Title: Establishment of a Rat Model of Superior Sagittal-Sinus Occlusion via a Thread-Embolism Method

URL: https://www.jove.com/v/62118/title?status=a64124k

Were animals used humanely and was the appropriate anesthesia or analgesia provided for potentially painful procedures?

Please provide additional comment, if necessary.

Response: Procedures involving animal subjects have been approved by the Medical Norms and Ethics Committee of Wenzhou Medical University and are in accordance with the China legislation on the use and care of laboratory animals.

#	Time in the video	comment	Change in video required Yes/No	Change in text is sufficient Yes/No	Suggested Changes
Example	2:20 – 2:34	Name of drug used for anesthesia is not mentioned	No	Yes	
1	Beginning and end of video	In my version, voice does not match lip movements	Yes **We've re-recorded the video**	No	
2	1:30	Sterile ophthalmic eye lubricant not applied – this should be done to protect the corneas from drying during anesthesia	Yes **We've re-recorded the video as suggested**	Yes **It has been revised in the text**	Apply eye lubricant before rat is put on stereotaxic frame
3	1:49	Insufficient skin prep for surgery – a single wipe with isopropyl alcohol was the only thing used.	Yes **Skin prep with 5% povidone iodine alternating three times with 75% ethanol.**	It is stated in the text the skin should be "sterilize d" three times with	Skin prep with povidone iodine (or equivalent skin disinfectant) alternating 3 times with isopropyl alcohol

				iodophor (lines 141-142), but this is not done in the video . **It has been revised in the text**	
4	1:35	Fur removal performed at the same location where surgery will be done – fur removal should be done at another location other than the place where surgery will be performed. Fur removal procedure is very unusual – removed with scissors and not clippers	Yes **We've re-recorded the video as suggested**	Yes **It has been revised in the text**	Fur removal And skin prep should be performed away from the location where surgery will be performed
5	~5:42	Does the animal go right from surgery into the MRI or is there a recovery period? This is unclear	**The animal goes right from surgery into the MRI.**		
6		No mention of administration of analgesia. Is the animal euthanized immediately after surgery and MRI; therefore, never recovering from surgery? Line 197 in the text suggests that the animal was	Yes **We explained it in the video.**	Yes **SD rats were euthanized by intraperitoneal injection of excessive pentobar**	Analgesia and description of post-operative care for this model should be included

		awake before being anesthetized for MRI scanning		**bital after the detection .**	

1. Please be specific in your comments. If possible, divide your comments into 2 categories:
   a) Absolutely not acceptable - for serious errors and deviations from the animal research standards.
   a. In my opinion as a veterinary surgeon, comments 3, 4 and 6 would qualify as absolutely not acceptable.
2.
   b) Improvement requires - for minor deviations, missing parts, etc....
   a. Comments 1, 2 and 5 would require improvement

   For each comment, please specify if the changes in video are required, or if only changes in the complementary text are necessary. **Obviously, changes in the video are more difficult so it is important to note if changes in the text are sufficient.** Please use the chart below to provide details on each issue (replace examples listed):