

# A Novel Rat Model for Comprehensive Microvascular Training of End-to-End, End-to-Side, and Side-to-Side Anastomoses

Xiaoliang Yin, MD<sup>1,2</sup>  Gengfan Ye, MD<sup>1,2</sup> Jun Lu, MD<sup>1</sup> Lijun Wang, MD<sup>1</sup> Peng Qi, MD<sup>1</sup>  
Haifeng Wang, MD<sup>1,2</sup> Junjie Wang, MD<sup>1</sup> Shen Hu, MD<sup>1</sup> Ximeng Yang, MD<sup>1</sup> Kunpeng Chen, MD<sup>1</sup>  
Daming Wang, MD<sup>1,2</sup>

<sup>1</sup> Department of Neurosurgery, Beijing Hospital, National Center of Gerontology, Dong Dan, Beijing, China

<sup>2</sup> Graduate School of Peking Union Medical College, Dongcheng, Beijing, China

Address for correspondence Daming Wang, MD, Beijing Hospital, National Center of Gerontology, No.1 Da Hua Road, Dong Dan, Beijing 100730, P. R. China (e-mail: dm.wang@bjhmoh.cn).

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## Abstract

**Background** End-to-end, end-to-side, and side-to-side microvascular anastomoses are the main types of vascular bypass grafting used in microsurgery and neurosurgery. Currently, there has been no animal model available for practicing all three anastomoses in one operation. The aim of this study was to develop a novel animal model that utilizes the rat abdominal aorta (AA), common iliac arteries (CIAs), and the median sacral artery (MSA) for practicing these three types of anastomosis.

**Methods** Eight adult Sprague–Dawley rats were anesthetized and then laparotomized. The AA, MSA, and bilateral CIAs were exposed and separated from the surrounding tissues. The length and diameter of each artery were measured. The relatively long segment of the AA without major branches was selected to perform end-to-end anastomosis. One side of the CIAs (or AA) and MSA were used for end-to-side anastomosis. The bilateral CIAs were applied to a side-to-side and another end-to-side anastomosis.

**Results** Anatomical dissection of the AA, CIAs, and MSA was successfully performed on eight Sprague–Dawley rats; four arterial-to-arterial anastomoses were possible for each animal. The AA trunk between the left renal artery and right iliolumbar arteries was  $15.60 \pm 0.76$  mm in length,  $1.59 \pm 0.15$  mm in diameter, for an end-to-end anastomosis. The left CIA was  $1.06 \pm 0.08$  mm in diameter, for an end-to-side anastomosis with the right CIA. The MSA was  $0.78 \pm 0.07$  mm in diameter, for another end-to-side anastomosis with the right CIA or AA. After finishing end-to-side anastomosis in the proximal part of bilateral CIAs, the distal portion was juxtaposed for an average length of  $5.6 \pm 0.25$  mm, for a side-to-side anastomosis.

**Conclusion** This model can comprehensively and effectively simulate anastomosis used in revascularization procedures and can provide more opportunities for surgical education, which may lead to more routine use in microvascular anastomosis training.

## Keywords

- ▶ bypass
- ▶ anastomosis training
- ▶ animal model

Xiaoliang Yin's ORCID is <http://orcid.org/0000-0002-2079-0340>.

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Microvascular anastomosis has wide applications in the fields of microsurgery and neurosurgery. In addition to end-to-end and end-to-side anastomosis, which are routinely performed by microsurgeons,<sup>1,2</sup> side-to-side anastomosis is also used by neurosurgeons to treat cerebral ischemia or complex cerebral aneurysms via the intracranial arteries.<sup>3,4</sup> However, to date, there has been no animal model that enables the practice of all three types of anastomoses at one time. Therefore, we explored how to use the abdominal aorta (AA), common iliac arteries (CIAs), and median sacral artery (MSA) of adult rats to develop a model for systematic microvascular anastomosis training.

## Methods

### Experimental Animal

The animals involved were eight healthy male adult Sprague–Dawley rats at  $12 \pm 1.2$  weeks old with an average body weight of  $263 \pm 15.3$  g. The rats were housed in separate cages in the animal room with a 12-hour light/dark cycle and unrestricted access to food and water, except for a 12-hour preoperative fasting period. The Animal Ethics Committee of Beijing Hospital approved the animal experiment.

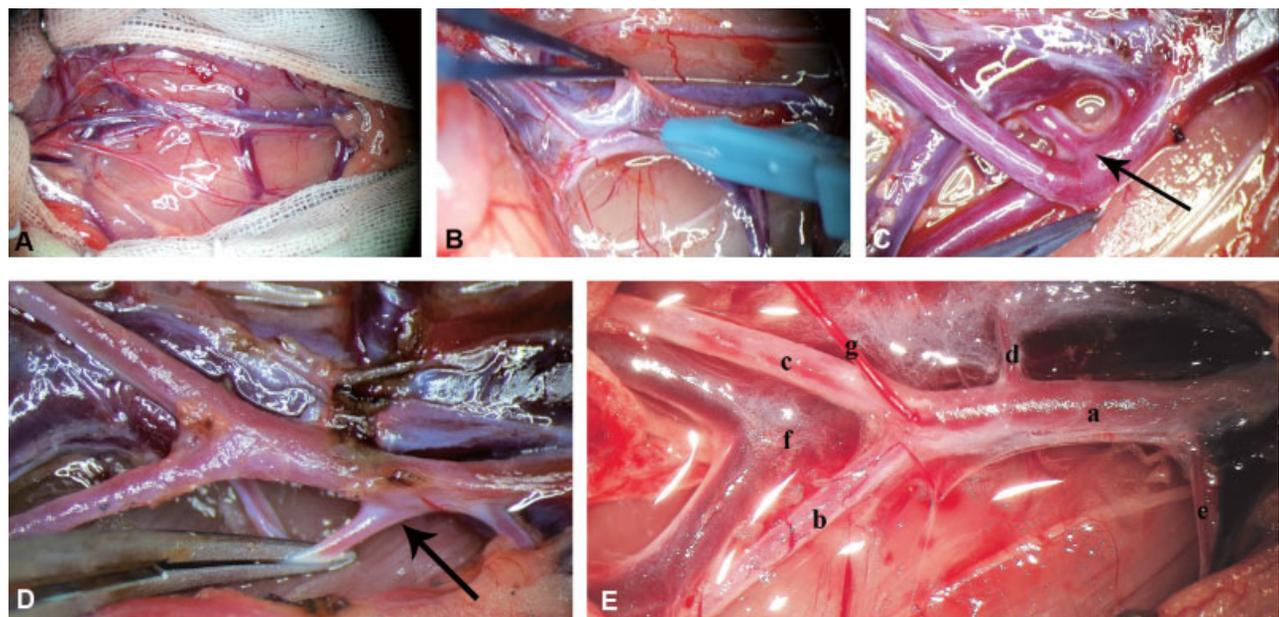
### Gross Anatomy

All procedures were performed under general anesthesia using an intraperitoneal injection of 40 mg/kg pentobarbital and 0.01 mg atropine sulfate. The rat was fastened in the supine position on a dissecting board. The abdomen was sterilized and a skin incision from the xiphoid process to the symphysis pubis was made along the midline. Subcutaneous

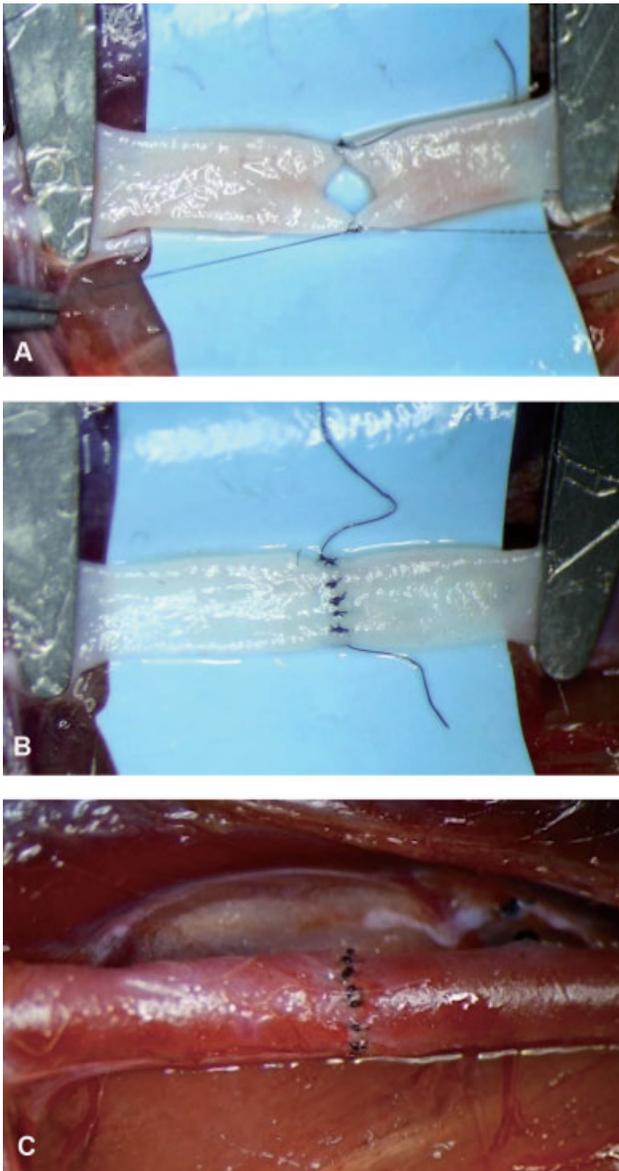
dissection was completed to expose the abdominal wall muscles, and whole layers of the abdominal wall were cut along the linea alba. Then, we used retractors to open the abdominal wall and fully expose the abdominal visceral organs. The intraabdominal organs were moved extraperitoneally, wrapped in isotonic saline-soaked gauze, and pushed to the right side of the abdominal wall to facilitate exposure of the AA and reduce fluid loss.

### Microsurgical Dissection

Retroperitoneal fat was visible under the microscope (Möller-Wedel GmbH & Co. KG, Germany; ▶Fig. 1A). Gentle blunt dissection using two cotton swabs enabled the safe removal of the retroperitoneal fat off the aorta and efficient exposure of the CIAs and its adjacent inferior vena cava (IVC). A pair of microscopic tweezers were used to gently clamp the connective tissues between the IVC and the AA, the interface that must be strictly adhered to during the dissection process; we then moved the tissues to the right side. We used the tip of a vascular dissector (Ruifeng; Bohaikangyuan Medical Co. Ltd., China) to make a fine sharp cut on the side of the AA to leave more tissues to the side of the IVA (▶Fig. 1B). This process, which needs to be performed with caution, can be performed in two directions: (1) on the distal aorta toward the aortic bifurcation in a proximal-to-distal direction and (2) on the CIAs toward the aortic bifurcation in a distal-to-proximal direction.<sup>5</sup> Finally, the dissection focused on aortic bifurcation. The MSA may originate precisely from the dorsal of aortic bifurcation (▶Fig. 1C) or slightly toward the cranial side, from the distal segment of AA (▶Fig. 1D). The above method was used to separate the MSA and move it



**Fig. 1** Microsurgical dissection. (A) Retroperitoneal fat was visible under microscope. (B) The connective tissue between the AA and IVC (white opaque) was the interface that must be strictly followed during the dissection process. (C) The MSA (arrow) may originate from the dorsal of the abdominal aorta bifurcation. (D) The MSA (arrow) can also originate from the distal segment of abdominal aorta. (E) Anatomy of rat abdominal aorta and common iliac artery: (a) is the AA, (b and c) are the bilateral CIAs, (d and e) are the right and left lumbar artery, (f) is the IVC, and (g) is the inferior mesenteric artery. AA, abdominal aorta; CIAs, common iliac arteries; IVC, inferior vena cava; MSA, median sacral artery.



**Fig. 2** End-to-end anastomosis. (A) After transecting the AA and trimming the adventitia, two stay stitches were placed at the 0 and 6 o'clock position. (B) The anterior wall was first sutured using a simple interrupted or spiral-interrupted technique. (C) After suturing the posterior wall and releasing the vessel clamp, there was no obvious stenosis in the anastomosis, and the distal artery was well-filled. AA, abdominal aorta.

downwards to the area where it penetrates the lumbosacral muscles. It is important to note that during the dissection process, small arterial and venous branches should be ligated or coagulated; the IVC should be carefully protected to reduce bleeding. In this way, the AA, bilateral CIAs, and MSA can be safely isolated from the adjacent veins (►Fig. 1C–E).

After isolation, we coated the vessels with a small piece of cotton impregnated with 1% lidocaine for 3 minutes to relieve a possible vasospasm. Then, the outer diameter of each vessel was measured using a hand-held electronic caliper (0.02 mm accuracy). The vascular outer diameter was deemed to be the diameter of the blood vessel.

## Microanastomosis Procedures and Steps

### 1. End-to-end anastomosis

Usually, the segment of the rat AA between the left renal artery and the right lumbar artery has no other branches. An end-to-end anastomosis was performed on this segment using a simple interrupted or spiral-interrupted technique as previously described<sup>6</sup> (►Fig. 2A–C).

### 2. Double-barrel end-to-side anastomosis

Anatomically, bilateral CIAs are well-suited for end-to-side anastomosis. The left CIA was ligated with 9-0 nylon thread at its point of origin and was clamped with an aneurysm clip at its distal end (►Fig. 3A). The anastomosis should be located in the proximal third segment of the right CIA. A longitudinal arteriotomy was performed, and a fish-mouth incision of the left CIA stump was performed successively. Although Hall<sup>7</sup> introduced a method of exposing and suturing the posterior wall by flipping the blood vessels, this technique may cause excessive tension and trauma, thus the posterior-wall-first (PWF) technique is preferable.<sup>8</sup> After finalizing the heel and toe stitches (►Fig. 3B), the posterior wall suture line was completed (►Fig. 3C and D), and then the anterior wall suture line was completed using a continuous suturing technique (►Fig. 3E).

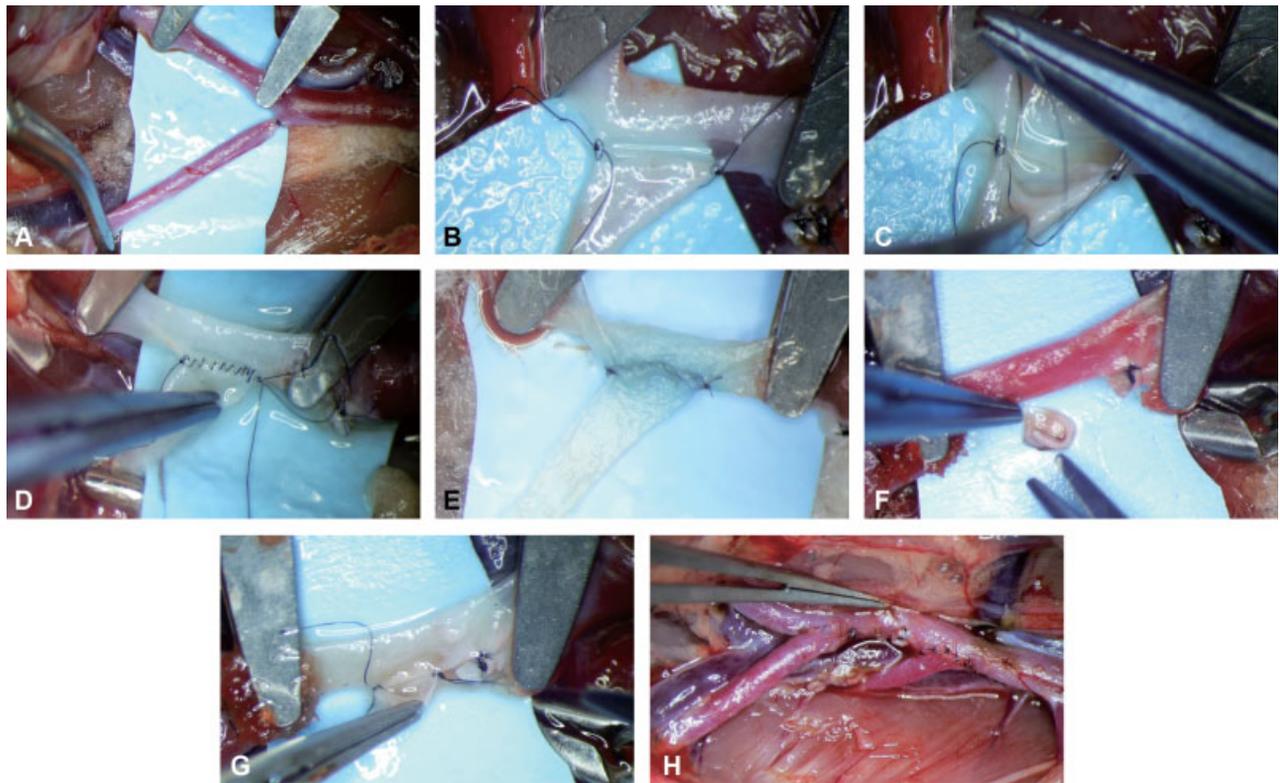
After end-to-side anastomosis of the bilateral CIAs, the MSA was easily revealed below the stump of the left CIA. Due to the different origin position of the MSA, the position of the second end-to-side anastomosis was placed in the proximal segment of the right CIA or distal part of the AA, and a fish-mouthing of the MSA stump was performed (►Fig. 3F). After finalizing the heel and toe stitches (►Fig. 3G), the deep suture line was completed using the PWF technique. Then, we ran the long remnant thread of the heel stitch along the anterior wall using the continuous or spiral-interrupted technique<sup>6</sup> (►Fig. 3H).

### 3. Side-to-side anastomosis

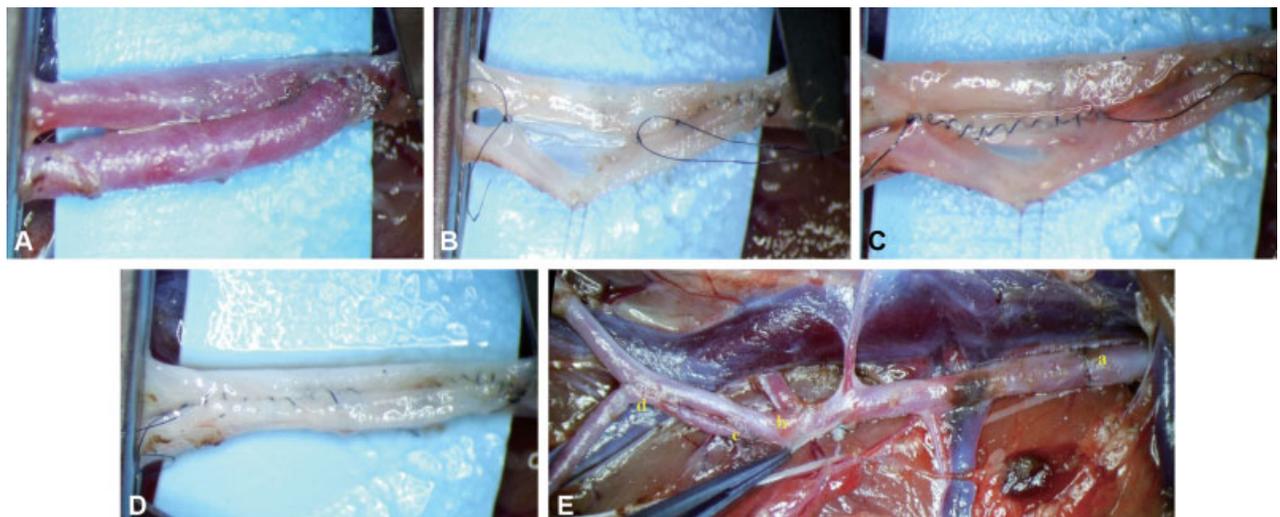
After finishing end-to-side anastomosis of the bilateral CIAs using the above method, there was still sufficient length of their distal segments for a side-to-side anastomosis. At the distal side of the end-to-side anastomosis, the bilateral CIAs were juxtaposed for an average length of  $5.6 \pm 0.25$  mm and clamped together with an aneurysm clip; another aneurysm clip clamped the distal AA (►Fig. 4A). Then, longitudinal arteriotomies were made on the lateral walls of the bilateral CIAs for a length three times the arterial diameter and stay stitches were placed at the 3 and 9 o'clock positions (►Fig. 4B). Using the PWF technique to suture the posterior wall (►Fig. 4C), we finished closure of the anterior wall with the continuous suturing technique (►Fig. 4D). One hour after the procedures, all anastomoses were patent (►Fig. 4E).

### 4. Patency test

One hour and 7 days (the second observation) after the procedures, the patency of the vessels was verified by an empty-and-refill test and a Doppler's ultrasound probe.



**Fig. 3** Double-barrel end-to-side anastomosis. (A) The left CIA clamped with an aneurysm clip at its distal point, ligated at its point of origin with a 9-0 nylon thread, an approximator applied to the right CIA. (B) Heel and toe stitches placed to approximate left CIA onto right CIA. (C and D) Suturing the closed posterior walls with outside-in at the left CIA and inside-out at the right CIA (PWF technique). (E) The long remnant thread of the heel stitch ran along the anterior wall using the continuous suture technique. (F) Fish-mouthing of the MSA stump. (G) Longitudinal arteriotomy was made at the proximal part of the CIA or distal part of the AA, and the heel and toe stitches were finished. (H) Finishing the double-barrel end-to-side anastomosis and releasing the vessel clamp, the arteries were patent. AA, abdominal aorta; CIAs, common iliac arteries; MSA, median sacral artery; PWF, posterior-wall-first.



**Fig. 4** Side-to-side anastomosis using bilateral CIAs and the panorama after all procedures. (A) The distal bilateral CIAs were juxtaposed and clamped together with an aneurysm clip. (B) Longitudinal arteriotomies were made on the lateral walls, and the stay stitches were placed at the 3 and 9 o'clock positions. (C) Using the PWF technique to suture the posterior wall. (D) Closing the anterior wall with the continuous suturing technique. (E) The panorama after all procedures: (a) is the end-to-end anastomosis of AA, (b) is the end-to-side anastomosis of right CIA and MSA, (c) is the end-to-side anastomosis of bilateral CIAs, (d) is the side-to-side anastomosis of bilateral CIAs. AA, abdominal aorta; CIAs, common iliac arteries; MSA, median sacral artery; PWF, posterior-wall-first.

## Results

All eight animals survived the procedure. The AA trunk between the left renal and right ilio-lumbar arteries was  $15.60 \pm 0.76$  mm in length,  $1.59 \pm 0.15$  mm in diameter, and supplied eight end-to-side anastomosis procedures.

The diameter and length of the CIA were  $1.06 \pm 0.08$  and  $8.45 \pm 0.50$  mm on the left side and  $1.11 \pm 0.10$  and  $8.36 \pm 0.75$  mm on the right side, respectively. Through dissection, it was found that the MSA from six rats originated from the AA bifurcation and the MSA from two rats originated from the distal part of the AA. The diameter of the MSA was  $0.78 \pm 0.07$  mm, with  $3.46 \pm 0.08$  mm in length. Eight rats enabled 16 end-to-side anastomosis procedures: eight cases by bilateral CIAs and eight cases by right CIAs (or AAs) and MSAs. Thus, these formed the “double-barrel” end-to-side anastomosis.

After finishing the end-to-side anastomosis in the proximal part of the bilateral CIAs, their distal parts could be juxtaposed for an average length of  $5.6 \pm 0.25$  mm, for a side-to-side anastomosis. Eight rats were used for eight side-to-side anastomosis procedures.

Following completion of the anastomoses, the animals were allowed to recover from the anesthesia. The vessels were patent in 100% of cases 1 hour after the procedure; 7 days later, all anastomoses remained patent, resulting in an overall patency rate of 100%.

## Discussion

Mastering vascular anastomosis is a long-term process that requires systematic training in the laboratory before it can be used skillfully in the operating room. Since end-to-end anastomosis is a relatively simple technique, most of the microsurgical training courses begin anastomosis training from end-to-end, and progress to end-to-side, or side-to-side, or from vessels with large diameters to smaller diameters.<sup>9–11</sup> Compared with other rat models that use the tail artery, carotid artery, or femoral artery, the diameter of the AA trunk is larger ( $1.59 \pm 0.15$  mm), and is a favorable caliber for those individuals who are at the beginning stages of anastomosis training with an animal model.<sup>5,10</sup>

Compared with end-to-end anastomosis, end-to-side is more difficult and is widely used by neurosurgeons and microsurgeons.<sup>1,2,12–14</sup> End-to-side anastomosis models have been reported in previous studies, such as in the unilateral common carotid artery to carotid external vein,<sup>15</sup> femoral artery to femoral vein,<sup>16,17</sup> and bilateral carotid arteries.<sup>18</sup> Nevertheless, the above models all have deficiencies. For instance, the venous models have the inherent shortcoming of not simulating arterial to arterial anastomosis scenarios. The result of the models of artery to vein anastomosis is the formation of arteriovenous fistula. The bilateral common carotid arteries underwent anastomosis that required temporary blocking. If this process takes too long then brain ischemia may occur, and the transfer of one common carotid artery to the contralateral side may result in poor postoperative blood flow.<sup>18</sup> The animal model

we developed contains several features that are well-suited for end-to-side anastomosis training. First, anatomically, the bilateral CIAs are near each other, the diameters of both sides are similar, and after being completely freed, the average length of the left CIA can reach 8.45 mm and the right side can reach 8.36 mm. During the anastomosis procedures, we shifted the left common iliac artery stump distally to the proximal third segment of the right CIA. This is equivalent to changing the hypotenuse of a triangle to a right-angled edge to ensure it remains tension-free during the procedure. Second, the MSA may originate from the dorsal of the aortic bifurcation or from the distal part of the AA. As the left CIA was severed at its point of origin and shifted to join the right CIA, the MSA blockage was relieved, and it became relatively easy to isolate the MSA. If the MSA originates from the bifurcation (due to the shift of the left CIA) this leaves room to perform another end-to-side anastomosis. Third, because the diameter of the MSA is smaller than that of the right CIA or AA, a fish-mouth incision can be made to enlarge an aperture area of the anastomosis and increase its patency. In summary, each rat could be used for two end-to-side anastomosis procedures, one case by bilateral CIAs and another case by right CIA (or AA) and MSA. This kind of “double-barrel” end-to-side anastomosis can provide more educational opportunities for surgical trainees.

In neurosurgery, side-to-side anastomosis is primarily used for the cerebral revascularization of intracranial arteries or to treat complex intracranial aneurysms.<sup>3,4</sup> The existing literature on side-to-side anastomotic models mainly addresses the femoral artery and femoral vein,<sup>19</sup> as well as the internal and external carotid arteries.<sup>20</sup> A shortcoming of the above models is the thin wall of the vein and formation of arteriovenous fistula or ischemia of the vital organs. In our model, although a case of end-to-side anastomosis was performed at the proximal side of the bilateral CIAs, an average vascular length of  $5.60 \pm 0.25$  mm was enough at the distal side for a side-to-side anastomosis. It should be noted that the position of the arteriotomies should be selected on the adjacent side walls of the two vessels to reduce any pulling of the vessel wall during suturing; the length of the vascular wall incision should be three times the diameter of the artery. Since the vessels cannot be inverted, it is still necessary to suture the posterior wall first. The advantage of using this method is that the entire anastomosis can be completed without upturning the blood vessels. Therefore, when the intracranial deep and narrow space vessels are anastomosed, the above operating techniques are more applicable.

Our bypass animal model achieved a 100% patency rate which is important for rat survival. In addition to the correct anastomosis methods, the anastomosis position in our model minimizes the change in the original anatomy by only shifting the left CIA and MSA slightly from their points of origin to the caudal aspect with no excessive pulling force when bilateral CIAs are juxtaposed for side-to-side anastomosis. This tension-free state is critical for ensuring the long-term patency of the anastomosed blood vessel.

## Conclusion

In conclusion, the animal model we have established has the following advantages: (1) each rat provides three types and four pure arterial anastomosis operations, (2) an optimized position of the anastomosis to maximize the retention of the original arterial anatomy, and (3) a high patency rate to ensure survival of the animals. Our experimental rat model can comprehensively and effectively simulate the three main anastomoses that are used in revascularization procedures which may warrant more routine use in microvascular anastomosis training.

### Conflict of Interest

None declared.

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