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ORIGINAL ARTICLE

A simple and novel technique for training in microvascular suturing in a rat model

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Summary *Background:* Though microvascular clamps are widely used for anastomosis training, there still have several shortcomings, including the bulging, expensiveness and unavailability due to sterilization. The aim of this study is to introduce a simple and novel microvascular training model without use of microvascular clamps.
Methods: Femoral vessels of Sprague Dawley rats training model were used to evaluate the usefulness of 4-0 silk as a slipknot for performing arterio-arterial and veno-venous microvascular anastomoses. A total of 12 Sprague Dawley rats were randomly assigned to either slipknot group or vascular clamp group. We also assess other endpoints, including ischemic time, patency rate, and clinical features. An additional histological study was performed to compare their immediate traumatic effects on vessel wall.
Results: There was no ischemic change or congestive sign in the lower limb after microvascular anastomosis. The total warm ischemic time for the vascular anastomosis was not significantly different. We performed the patency test immediately after microvascular anastomosis and one week after surgery. No intraoperative vascular bleeding was found during these procedures and no thrombosis occurred postoperatively. The histologic damages to occluded area were not significantly different in both groups.
Conclusion: We demonstrate a microsurgical suture technique performed without any vascular clamp on a rat model. This rat model was designed for training in the technique of microvascular anastomosis. Compared with microvascular clamps, silk slipknot is a cheap, easily available, less space-occupying technique while performing microvascular anastomoses training.

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This preliminary study provides a simple and effective alternative method for microvascular anastomosis training.

1. Introduction

Microvascular anastomosis remains a technically difficult procedure and a vital training in surgical skills. The Sprague Dawley (SD) rat is an excellent model for microsurgical training.[1] Surgeons learn to perform end-to-end or end-to-side surgery on the femoral artery or vein, even other advanced skills. However, when performing anastomosis, stopping the blood flow is extremely important for a clear operation field.

Vascular clamps, including double and single clamps, are often used during microvascular anastomosis. A vascular clamp provides good approximation, a tension-free anastomosis, and ability to rotation of the vessel from front to back by holding it at both ends.[2] However, the use of vascular clamps presents several complications. This study reports a microsurgical suture technique performed without using a vascular clamp.

Silk, a non-absorbable material, is used for surgery and is characterized by high friction, which is useful for ligating vessels during surgery. We attempted using 4-0 silk as a slipknot for occluding the blood flow temporarily while practicing anastomosis in an animal model. Our aim was to develop a simple and novel method for microvascular surgical training using an animal model.

2. Material & method

We analyzed single-surgeon experiences of using 4-0 silk during microvascular surgery between July and October 2017. We designed an experimental group and a control group to demonstrate that the result of using a slipknot is not inferior to that of using a traditional vessel clamp. Both groups included 6 SD rats separately performing artery-arterial and veno-venous end to end anastomosis simultaneously. The SD rats were healthy 12 weeks old with a body weight of 300—400 g. The only difference between the groups was the tool used for hemostasis, i.e., silk was used as a slipknot in the experimental group, whereas a traditional vessel clamp was used in the control group. We recorded the ischemic time in each group and used a *t*-test for data analyses.

The groin area of the rat was surgically prepared using standard methods, and the femoral vessels were exposed, clamped using a slipknot or a clamp, transected, and then anastomosed using 10-0 nylon with interrupted one-way-up sutures. All the basic procedures were performed on the femoral artery and vein. The procedures were carried out at a midrange magnification (×25). The interrupted 10-0 suturing was performed on the end-to-end anastomoses using the rat model.

For clamping the vessel in the experimental group, we used 4-0 silk to cross the vessel and let the short end of the silk above the vessel and the long end below the vessel. Encircle the micro forceps and grasp the long end proximal part and then tighten it. The slipknot can be easily loosened by just grasping both the short end and the long end with two forceps simultaneously and pulling in the opposite direction and parallel to the vessel (Fig. 1). In the control group, the physicians used a microsurgical vessel clamp on the vessel for stopping the blood flow.

We checked the vessel conditions immediately after the anastomosis. If no ischemic nor congestive change of rat limbs is noted, we will reopen the wound to do the patency test again one week later. The patency test is performed as the following: with one pair of forceps, occlude the vessel distal to anastomosis. With a second pair of forceps, empty a short length of vessel distal to the first pair. Then, holding the second pair of forceps closed, release the proximal pair and see if the emptied length of vessel refills.[3]

Both vascular clamps and silk slipknots may cause endothelial injury due to their induced pressure and clamping force. To investigate the effects of 4-O silk slipknots on the vascular damage of rat femoral vessels, an additional histological study was performed in two groups. Group A: vascular clamp was applied on femoral artery and vein for 60 min; group B: 4-O silk slipknots was applied on femoral artery and vein for 60 min. At the end of 60 min, segments occluded with vascular clamp or 4-O silk slipknots were excised in all rats and endothelial structures were evaluated histopathologically (total 24 segments).

The specimens were fixed in 10% buffered formalin. The morphological changes were studied after staining the paraffin sections with Haematoxylin and Eosin. The extent of damage to the layers of the vascular walls were assessed and compared by light microscopy.

3. Result

The adequate tension and pressure when tying the slipknot could be obtained easily for the beginners. No leakages were observed and patency was regularly demonstrated by testing via emptying and refilling. The ischemic time was found to be similar in both groups (41 $\pm$ 2.4 min in the experimental group and 40.2 $\pm$ 1.6 min in the control group) ($p = 0.53$; Table 1). There was no ischemic change or congestive sign in the lower limb after microvascular anastomosis in both groups. We performed the patency test immediately after microvascular anastomosis and one week after surgery. There is strong evidence that maintaining adequate blood flow in the legs after surgery in both groups. The patency test was positive in all the rats of both groups. Fig. 2 shows an image of the anastomosis.

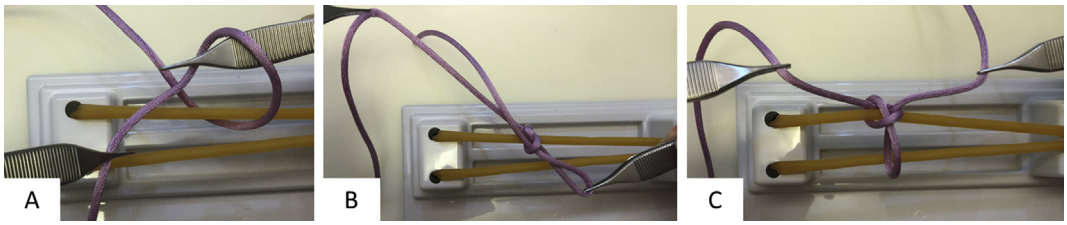

Figure 1 How to tie a silk slipknot and how to unbend is easily feasible when doing microsurgery training.

Table 1 The ischemic time in both group.

Experiment	Silk group (experiment group) Ischemic time (min) N = 6	Clamp group (control group) Ischemic time (min) N = 6	p vaule
1	45	38	
2	43	40	
3	40	41	
4	38	43	
5	41	40	
6	39	39	
Mean (min)	41 ± 2.4	40.2 ± 1.6	P = 0.53

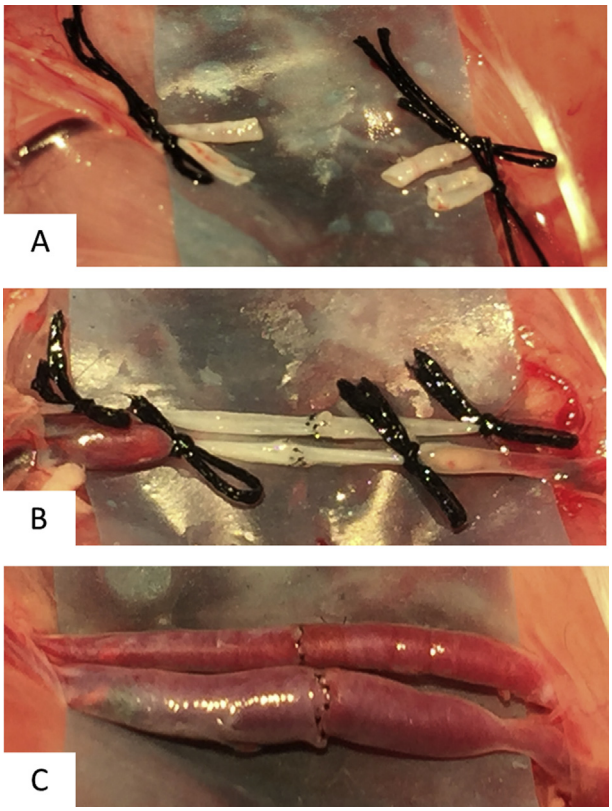

Figure 2 The AA & VV end to end anastomosis was performed with silk slipknot.

The artery wall injuries observed by light microscopy were classified according to previous schemes described.[4,5] In both groups, light microscopy analysis identified mild endothelial injury of arterial segments. Local vacuolization of some smooth muscle cells were observed in some specimens (Fig. 3A,B). Normal cellular sequencing and structure were determined in all veins segments (Fig. 3C,D). The results suggest that histologic damages to occluded area are not significantly different in both groups. The use of 4-O silk slipknot during microvascular anastomosis demonstrated a result not inferior to vascular clamp group.

4. Discussion

Though vascular clamps are widely used during microvascular repair and for performing anastomosis, some disadvantages still exist, including bulging and occupying large areas of the operation field, which interfere with the physician's work. In addition, clamps might be loosen due to elastic fatigue and cause oozing in the operation field. Vessel clamps are also expensive and require sterilization after use. Moreover, surgeons might require several clamps in some situations, such as when performing an interposition vein graft. In general, investigators often use clamp appliers for precise clamping position which makes another economic cost in an animal laboratory. Actually, making a slipknot is much easier to interrupt a rat vessel compared to vascular clamp if clamp applier is unavailable. Some vessel clamps get magnetic material and will be attractive to needle holder, micro forceps which will interfere anastomosis.

Compared with traditional clamps, silk slipknots are inexpensive, convenient, sterilized at any time, and easily available. Silk slipknots can be used even in a limited operation field, which is not possible with clamps, and hence provide more working space. Because of more and more restrictions involved in animal use, such as the need for a sterile environment and the increasing financial constraints of training tools, there is a need for low-cost equipment for physicians for microsurgery training. Silk slipknots can meet the need for low-cost on surgical tools and are simple, especially feasible when both vessel end gaps are too long to perform primary anastomosis and in need of a vein graft (Fig. 4). In addition, some laboratories even lack the availability of single or double clamps. Besides, silk slipknots are disposable and, therefore, eliminate the concern of cleansing and disinfection (Table 2). Interestingly, there is no issue of the occurrence of loosening currently, and they also result in better occlusion of blood flow without any leakage. It is common to use expired vascular clamps obtained from the operating room for training purposes but these clamps may be too loosen to occlude the blood flow completely due to elastic fatigue. It

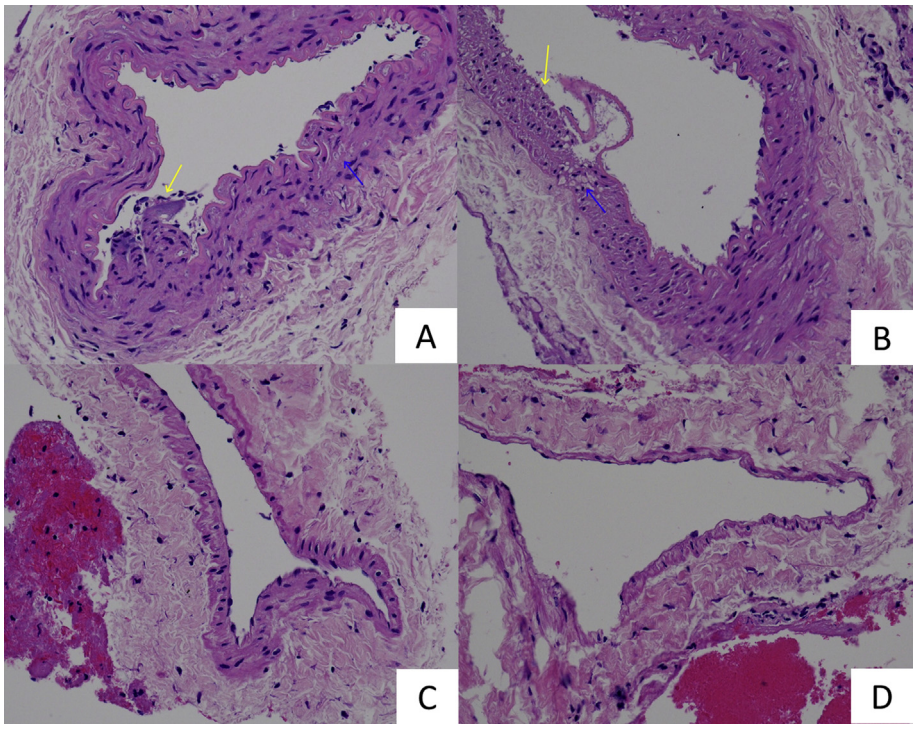

Figure 3 A: Arterial segment occluded with 4-O silk slipknots was excised. Figure 3B: Arterial segment occluded with vascular clamp was excised. Figure 3C: Venous segment occluded with 4-O silk slipknots was excised. Figure 3D: Venous segment occluded with vascular clamp was excised. Both 3A and 3B showed mild vascular injury of femoral artery. There were local endothelial loss, detached internal elastic lamina (yellow arrow) and local vacuolar formations of muscle cells (blue arrow). Both 3C and 3D showed normal vascular endothelium of femoral vein. The histologic damages to occluded area were not significantly different in both groups.

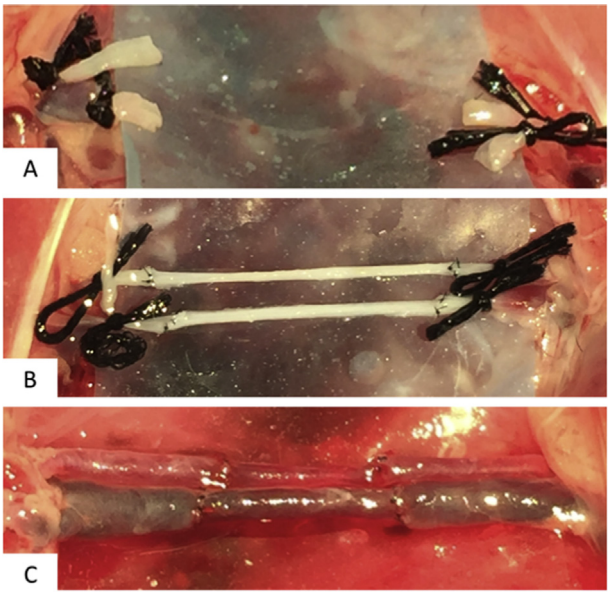

Figure 4 Long gap in interposition venous graft practice is especially suitable for slip slipknot.

also depends on the numbers of expired vascular clamps in different training center. Therefore, silk slipknots are a good options in some situations such as unavailability of a vascular clamp, need of waiting for sterilization, need for several clamps, and a limited space for microvascular anastomosis. Complication of microvascular anastomosis, such as thrombus or intima tear, could still occur even with traditional vascular clamp. On the basis of our experience, we believe that the use of 4-O silk slipknot during microvascular anastomosis does not lead to more complication compared to vascular clamp group.

Table 2 Comparison between silk slipknot and traditional vessel clamp.

Comparison	Silk slipknot	Traditional vessel clamp
Cost	Cheap	Expensive
Space occupied	No	Yes
Loosening	No	Yes
Displacement	No	Yes
Disinfection	Sterile any time	Need disinfection
Disposable	Yes	No
Skill	Easy	Easy
Long gap use	Ok	Use single clamp
Applier	No need	Need
Magnetic	No	Yes

Nevertheless, there are still limitations in this study. One major limitation of our study is that we cannot standardize how tight the knots are tied. Tension and pressure of 4-O slipknot may be not constant even created by the same surgeon. The purpose of this article is to demonstrate a simple, and safe alternative method for microsurgical suture training. The adequate tension and pressure when

tying the slipknot could be obtained easily for the beginners. The least force when tying is just enough to occlude the blood flow not interfering the operation field, and the strongest force is not to make the silk or vessel severance. The diameter of the rat's femoral vessel is around 0.5—1.0 mm; it is easy to interrupt blood flow by the 4-o silk slipknots.

Actually, the tension and pressure of blood vessels also varied from different vascular clamps. It depends on the preparation of vessel exposure and elastic fatigue of vascular clamps. This rat model was designed for training in the technique of microvascular anastomosis. We determined whether the microvascular anastomosis was patent or totally occluded by double forceps patency test. It was performed immediately after the microvascular anastomosis and one week after the operation.

Although various pressure of the slipknot may lead to different blood vessels damage, we still tried to compare its traumatic effects on vessel wall with that resulting from vascular clamps. The closing forces generated by vascular clamps correlated positively with the extent of artery wall injury. Although the minimum level of force that arrest blood flow by the 4-O silk slipknot could not be standardized, we used histological examination to compare vessel wall damage in both groups. Effective occlusion of vessels could be performed by the 4-O silk slipknots and mild injury on vessel wall was confirmed. There was no significant difference in vessel wall damage between two groups.

The other disadvantage of slipknot is that, unlike double clamps, without making both vessel ends approximation. Nonetheless, appropriate judgment of tension between both vessel ends when doing anastomosis should be more essential for a beginner before cutting a vessel. If the distance between both ends is too long to complete tension free anastomosis, it is inevitable to use vein graft. Besides, it is also necessary to well prepare vessel exposure before anastomosis like trimming off the surrounding soft tissue. By practicing anastomosis with slipknot technique, residents should also learn how to approximate two ends under tension-free consideration.

5. Conclusion

Here we present a simple and convenient rat model designed for training in the technique of microvascular anastomosis. This study examined the clinical features after a microvascular anastomosis of rat femoral vessels using 4-0 silk slipknot techniques. The adequate tension and pressure when tying the slipknot could be obtained easily for the determinations.

The purpose of this article is to demonstrate a microsurgical suture training performed without any vascular clamp on a rat model. Their immediate traumatic effects on vessel wall may be similar to that resulting from vascular clamps. Although we do not plan to apply it on human being, it may be worth performing further studies for the percentage reduction of the vessel lumen area in the future. This preliminary investigation provides an alternative method for microvascular anastomosis training.

Disclosure

None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.asjsur.2018.05.005.

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