Microvascular Anastomosis of Submillimeter Vessels—A Training Model in Rats

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Abstract The advent of supermicrosurgery, which allows the anastomosis and dissection of small-caliber vessels ranging from 0.5 to 0.8 mm in diameter, has led to the development of new reconstructive operations. Lymphaticovenous anastomosis, fingertip replantation and perforator flap surgery all require the supermicrosurgical techniques. In this paper, a rat model is described for the training of anastomosis of submillimeter vessels. Sprague-Dawley rats with the weight of 300 to 500 g were used as the training models. The gender, weight and size of the femoral vessels of 20 rats were recorded. The segment of the femoral vessel which was lying on the ventral muscle group of the hind limb and distal to the origin of the superficial inferior epigastric vessels was used for the practice of microvascular anastomosis. In this study, there were 13 male and 7 female rats. The mean weight of the 20 rats was 395.6 g. The mean diameters of the femoral artery and femoral vein were 0.54 mm and 0.56 mm respectively. The consistent size and anatomy of the femoral vessel make it a suitable training model for microvascular anastomosis of submillimeter vessels.

Keywords Supermicrosurgery · Submillimeter · Microvascular · Anastomosis · Training · Animal · Rat · Model

Introduction

Supermicrosurgery is a major advance in modern reconstructive microsurgery. The term ‘supermicrosurgery’ was coined by Koshima et al., which refers to the technique of handling and anastomosing vessels with a size of 0.3–0.8 mm [1, 2]. This technique is crucial in operations like lymphaticovenous anastomosis and fingertip replantation. In perforator flap surgery, with the skills of supermicrosurgery, it becomes unnecessary to trace and divide the proximal part of the pedicle for larger vessel size for anastomosis, the muscle of the perforator flap and its blood supply can then be preserved. The donor site morbidity can therefore be reduced.

The techniques that are used in microsurgery for large-caliber vessels are not entirely the same as those for submillimeter vessels. Training is necessary for supermicrosurgery. A rat training model for submillimeter vessel anastomosis is described in the following article.

Method

Sprague-Dawley rats with the weight around 300–500 g were used as the training model. Twenty rats were dissected for the training purpose. The weights and diameters of the femoral vessels of the 20 rats were measured. All the rats were handled according to the animal research guidelines devised by the author’s institution. Anesthesia was achieved by intraperitoneal infusion of sodium pentobarbital with a dose of 40 mg/kg. Boosters of anesthetic solution were given to the animal during the operation if an increase in

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respiratory rate or heart rate was observed. After the dissection and anastomosis, the rats were euthanized via intraperitoneal sodium pentobarbital overdose (100 to 150 mg/kg).

To start the operation, the rat was placed in a supine position after the administration of anesthesia. The four limbs of the rat were mounted on the operation table with tapes. The region of the groin and hind limb was shaved. The skin was prepared with chlorhexadine solution. A longitudinal incision was then made from the groin down to the hind limb.

After the skin incision, an operating microscope (magnification power of $15\times$ to $18\times$) was used for the vessel dissection and anastomosis. The femoral artery, vein and nerve were first visualized. The superficial inferior epigastric (SIE) vessels were readily identified by retracting the skin flap superiorly. In this model, it was not necessary to trace the origin of the SIE vessels and the common femoral vessels. The superior border of the hind limb ventral muscle group was identified. The measurements of the femoral vessels were made at this level (Fig. 1). The diameter of the vessel was measured with a stainless steel ruler (Stainless steel ruler model 58698, Shinwa Measuring Tools Corp.). The calibration of the ruler was to the nearest 0.05 mm.

The segment of the femoral vessel which was overlying the ventral muscle group and distal to the SIE vessels origin was used for the microvascular anastomosis. The dissection skills could be practiced by skeletonising the nerve, artery and vein. The nerve was located laterally whilst the vein was located medially. The artery was located deep to both the vein and nerve (Figs. 2 and 3). Prior to the division of the vessel, the adventitia of the proposed division site, i.e. the later anastomosis site, was removed completely. The vessel was denuded until a transparent appearance was obtained. Topical antispasmodic solution, e.g. 2 % lignocaine or

Fig. 1 The left groin region of a rat. The neurovascular bundle (*single arrow*) always travels along the surface of the ventral muscle group (*asterisk*). The superficial inferior epigastric pedicle (*double arrow*) is readily visualized by retracting the skin flap superiorly. The superior border of the ventral muscle group is marked with the dotted line. The measurements of the femoral vessels were made at this level

Fig. 2 The segment of the femoral vessels which overlies the ventral muscle group was used for the practice of microvascular anastomosis. This figure shows the three structures after skeletonization, femoral vein; femoral artery and femoral nerve (from *left to right*)

papaverine, was applied in order to relieve the vessel spasm after the removal of adventitia.

After the preparation of the division site, single microvascular clamps were applied at both the distal and proximal ends of the vessel. The vessel was then divided for anastomosis. The author used 11-0 or 12-0 nylon sutures with 50 or 65 μm needles for anastomosis. Six stitches usually sufficed to secure an anastomosis.

After the completion of the anastomosis, the patency of the anastomosis was then tested with refill test. The vessels could also be divided at the site distal to the anastomosis in order to check for the blood flow (Fig. 4). The rat was then euthanized with the abovementioned manner.

Results

Measurements were made in 13 male rats and 7 female rats. The mean weight of the 20 rats was 395.6 g (range, 326 to 472 g). All of the measurements of the femoral vessels were

Fig. 3 The diameter of the femoral vessels was measured with a stainless steel ruler. The diameters of the femoral vein (*left*) and femoral artery (*right*) were 0.5 mm and 0.55 mm respectively

Fig. 4 An example of a successful 0.45 mm femoral artery anastomosis (*circle*). (On the *right*) After the completion of anastomosis, the distal end of the femoral artery was cut to check for blood flow. The patency of the anastomosis was also checked by probing the vessel with a pair of fine forceps

less than 1 mm. The mean diameter of the femoral artery was 0.54 mm (range, 0.45 to 0.60 mm). The mean diameter of the femoral vein was 0.56 mm (range, 0.45 to 0.65 mm). The details of the measurements were listed in Table 1.

Discussion

The advent of supermicrosurgery has brought reconstructive surgery into another stage. Operations like lymphaticovenous anastomosis; perforator flap reconstruction and

fingertip replantation require the supermicrosurgical skills. Lymphaticovenous anastomosis is now a well-established treatment for lymphedema. It involves the making of anastomoses between subdermal lymphatic vessels and venules. The sizes of these subdermal lymphatic and venules are less than 1 mm. The anastomoses then act as new channels for the lymphatic fluid to flow to the venous system bypassing the proximal lymphatic obstruction. In the field of perforator flap surgery, apart from the reduced donor site morbidity, perforator-to-perforator anastomosis is also made possible with supermicrosurgery. The perforator flap can be anastomosed to the perforator vessels at the recipient site, which reduces the operation time for vessel dissection. The main trunk of the recipient site vessels can also be preserved.

As the skills in handling submillimeter vessels are not entirely the same as those with large-caliber vessels, surgeons who are familiar with large-caliber vessel anastomosis may not perform equally well in the abovementioned operations. The skills of submillimeter vessels anastomosis can be obtained through training.

Various animal models have been described for the training of supermicrosurgery. These models include free groin flap and free abdominal flap in rats. The rat groin flap has always been a popular model in microvascular surgery. It has been utilized extensively for the study of flap physiology, pharmacological studies and transplantation experiments [3–5]. Since 1980s, the rat free groin flap, which is based on the SIE vessels, has been described as a training model for anastomosis of small-caliber vessel [6–8]. The documented diameters of the SIE vessels were 0.3 to 0.8 mm. Ozkan et al. described a free abdominal perforator flap model. In this model, the blood supply was based on the deep epigastric vessels. As it was a musculocutaneous perforator flap model, the flap elevation required intramuscular dissection of the blood vessel. The flap was anastomosed with the SIE vessels and inset at the groin region. The diameters of the donor vessels were 0.3–0.5 mm [9]. In these flap models, the anesthesia was reversed and the rat was kept for the observation of flap viability.

In this paper, an alternative training model is suggested. It is not meant to replace the abovementioned flap models. Instead, it aims to serve as a simple training model which is solely for the practice of microvascular anastomosis, as it does not require any intramuscular dissection of blood vessels or flap elevation.

The author believes that the femoral vessel of 300–500 g Sprague-Dawley rat is a valid training model as the diameter of the femoral vessel was less than 1 mm for all rats in this study. This model also has two major advantages. Firstly, the preparation and dissection of the femoral vessels are simple. The femoral neurovascular bundle always lies underneath the dermis and travels along the surface of the ventral muscle group of the hind limb. There is no need for any intramuscular

Table 1 The weights and the diameters of femoral vessels of 20 rats

	Weight (grams)	Diameter of femoral artery (mm)	Diameter of femoral vein (mm)
1	326	0.50	0.45
2	329	0.60	0.60
3	332	0.50	0.55
4	342	0.60	0.60
5	345	0.50	0.50
6	356	0.55	0.55
7	368	0.45	0.50
8	378	0.60	0.60
9	387	0.50	0.50
10	397	0.55	0.65
11	401	0.55	0.60
12	410	0.45	0.50
13	412	0.50	0.55
14	428	0.50	0.50
15	435	0.55	0.55
16	436	0.55	0.60
17	438	0.55	0.60
18	451	0.60	0.65
19	468	0.60	0.55
20	472	0.55	0.55
mean	395.6	0.54	0.56

dissection of blood vessels. In addition, it is also not necessary to trace the common femoral vessels and the origin of the SIE or deep epigastric vessels as in the flap models. The ease of vessel preparation is especially helpful for surgeons who just want to practice the skills of microvascular anastomosis without tedious flap dissection. Secondly, as the rat is sacrificed after the dissection, this model is convenient for centres that do not have the resources to keep the animal for flap observation.

The technical details of anastomosis are not discussed in this paper. However, the author would like to stress a point that a clear visualization of vessel lumen, especially for venous anastomosis, is an important factor which determines the success of an anastomosis. And in the author's point of view, this is the main difference in technique between large-caliber vessel anastomosis and submillimeter vessel anastomosis.

To clearly visualize the vessel lumen during anastomosis, it is worthwhile to remove all the adventitia of the proposed anastomosis site prior to the division of the vessel. If the adventitia is not cleared before the vessel division, the vessel lumen often retracts inside the skirt of adventitia. This makes the identification of the lumen, and hence the anastomosis, very difficult. Extra effort is required in order to remove the adventitia. The dissection also possesses the risk of vessel wall injury.

Conclusion

In this article, a training model for microvascular anastomosis of submillimeter vessel is described. The consistent size and anatomy of the femoral vessels allow very easy and

quick dissection of vessels, which is particularly useful for surgeons who want intensive training of submillimeter vessels anastomosis.

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