Original Article

A Novel Basic Training Laboratory Model on Live Subjects for Supermicrosurgery: Mouse Femoral Artery, Vein and Nerve

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ABSTRACT

BACKGROUND
Increasing success rates of supermicrosurgery operations have increased the importance of developing the ideal training model for super-microsurgery. Working on the model is very important for increasing and continuing microsurgery and supermicrosurgery skills. We aimed to present a standardized, simple and easy to access live training model for supermicrosurgery.

METHODS
The experiment was performed in the University of Health Sciences, Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, IDEA, Istanbul, Turkey in 2020. Twelve BALB/c male albino mice weighing 20-45 gr were used in the study. Unilateral femoral artery, vein and nerve diameters of mice were measured. Anastomosis was performed on the bilateral femoral vessels. The surgical procedure times were also recorded.

RESULTS
The mean weight of the mice was 36.6 ± 6.09 gr, the length was 15.10 ± 1.10 cm. The mean external diameter of the femoral artery, vein and nerves were 0.31 ± 0.34 mm, 0.48 ± 0.70 mm, 0.38 ± 0.43 mm, respectively. The mean preparation time of neurovascular structures for anastomosis was 15.75 ± 1.54 min, mean femoral artery and vein anastomosis time was 24.91±1.72 and 33.16±1.74 min, respectively. Vascular patency was detected as 100% after all vascular anastomosis procedures.

CONCLUSION
Mice femoral neurovascular structures are similar to rats in terms of basic morphology, and they are small enough for super-microsurgery education model. Dissection of mice femoral bundles are easy to perform. In terms of training models, anesthetic requirement and laboratory costs are less for mice, and handling them is much easier compared to rats; thus, making them especially suitable for basic supermicrosurgery training courses.

KEYWORDS
Femoral vessels; Mouse; Supermicrosurgery; Training model

Please cite this paper as:
Bas S, Sızmaz M, Aydın AC, Karsıdag S. A Novel Basic Training Laboratory Model on Live Subjects for Supermicrosurgery: Mouse Femoral Artery, Vein and Nerve. World J Plast Surg. 2022;11(1):44-50.
doi: 10.52547/wjps.11.1.44

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Received: 22 Mar 2021
Accepted: 10 Jul 2021

INTRODUCTION

Microsurgical procedures took their place in plastic surgery with the development of microscope and surgical equipment that were suitable for microsurgery. Daniel and Taylor reported first free flap in 1973. Following this groundbreaking improvement, microsurgery developed

exponentially and became an indispensable part of reconstruction steps[1]. Over time, with the definition of the angiosome and with publishing the first free perforator flap, a new era in reconstruction started. Perforator flap concept became very popular among reconstructive microsurgeons[2,3]. In addition, they developed new physiological procedures in lymphatic surgery such as lymphaticovenicular anastomosis (LVA) and free lymph node transfer. These surgical procedures became possible with the developments in microscope and surgical instrument technologies that allow reconstructive microsurgeons to perform surgery on very small vessels[4,5].

All procedures performed for repairing neurovascular structures between 0.3 and 0.8 mm are called super-microsurgery[6]. Super-microsurgical procedures include fingertip replantations, LVA, perforator-to-perforator flaps and vascularized nerve grafts[7]. With the help of super-microsurgery, the surgeon can benefit from new potential donor areas and reach better functional and aesthetic results. Less donor area morbidity is provided with technical developments especially in perforator flap concept.

Working on a surgical model is very important for gaining microsurgery and supermicrosurgery skills. With training models, basic skills such as how to use a microscope, eye and hand coordination under magnification, how to perform non-traumatic surgery, how to handle a tissue gently and how to perform anastomosis on sub-millimetric vessels can be gained. A candidate microsurgeon can learn how to position him/herself on microscope with these training models. Among the models used, there are living, non-living, biological and non-biological materials[8].

We aimed to present a standardized, simple and affordable live training model for supermicrosurgery by measuring the external diameters of mouse femoral neurovascular structures.

MATERIALS AND METHODS

After approval of animal experiments local Ethics Committee (University of Health Sciences, Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, IDEA; 2020/02), the study was conducted in the animal laboratory of the same institution. The study was conducted according to the Declaration of Helsinki. Twelve BALB/c male albino mice weighing 25-45 gr were used in the experiment (Figure 1A). Surgical procedures were performed by same surgeon.

Fig. 1: A) Balb / C male albino mouse. B) Longitudinal skin incision in the inguinal region.

Unilateral femoral artery, vein and nerve diameters of mice were measured. Anastomosis was performed on the bilateral femoral vessels. Furthermore, surgical procedure times were recorded. Mice were kept under 24-hour light-dark cycle with 12 h of light and 12 h of darkness in a 22-24 °C temperature-controlled room. They were fed with standard ad libitum rodent food and water.

General anesthesia was provided by intraperitoneal administration of 50 mg/kg Ketamine (Ketalar; Pfizer, New York, NY) and 5 mg/kg Xylazine (Rompun 2%; Bayer, Leverkusen, Germany). The surgical procedure was performed under microscope (S100/OPMI pico; Carl Zeiss Meditec AG, Jena, Germany) with a standard microsurgery set. Super-microsurgical interventions were recorded and documented with photographs. Dissection of neurovascular structures and ligation of vascular branches of main vessels were done under 6x to 10x magnification while anastomoses were performed under 25x magnification. With a longitudinal skin incision in the inguinal region that was approximately two cm, skin and subcutaneous tissue were passed. Inguinal fat pad was put aside with a gentle maneuver (Figure 1B). The femoral neurovascular bundle was reached by meticulous dissection. The femoral neurovascular bundle was revealed with a meticulous dissection. The femoral sheath was opened, and femoral artery, vein and nerve were exposed (Figure 2A, 2B). The superficial circumflex iliac artery and vein were ligated with 10-0 nylon suture for preventing unwanted bleeding (Ethilon® nylon suture, ©Ethicon US, LLC). Between the inguinal ligament and inferior epigastric artery, a double microvascular clamp was placed to the femoral artery, and the artery was transected between the legs of the clamp. Adventitiectomy was performed to the vessel ends. The vessels were dilated with the dilator. Vessel lumens were irrigated with 5000 IU/ml sodium heparin (Pfizer, West Ryde, NSW, Australia) solution following the mechanical dilation. Two percent lidocaine (Aritmal® 2%, 100 mg/5 ml Osel Ilaclari, Sanayi ve Ticaret AS., Beykoz, Istanbul, Turkey) was used to prevent vascular spasm. 6-0 polypropylene suture was placed intraluminally and used as a guide (Figure 2B). Femoral artery was repaired end to end with 11-0 nylon suture (Ethilon® nylon suture, ©Ethicon US, LLC). Same procedures were executed to both femoral artery and vein at adjacent anatomic locations. Vascular patencies were evaluated by inspection and milking test. Both anastomoses and coaptations were sutured with four to six stitches (Figure 2C). One week later, the femoral vessels were re-dissected through the

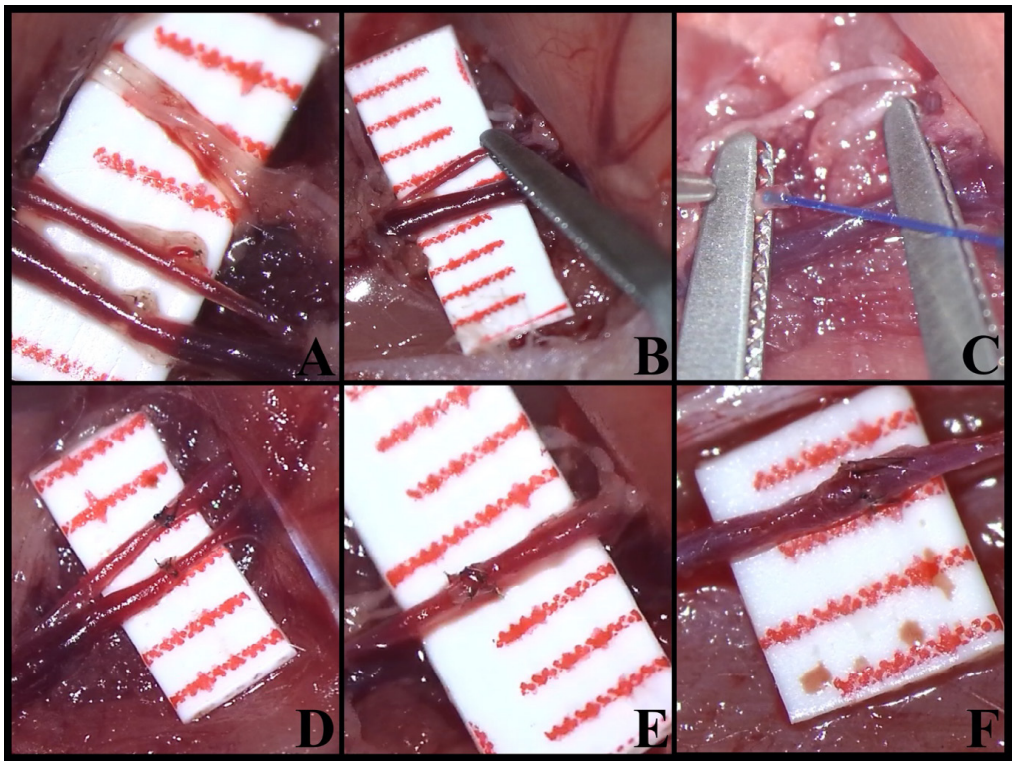

Fig. 2: A, B) Mouse femoral artery, vein and nerve. B) 6-0 polypropylene suture was placed intraluminally and used as a guide. C) Early postoperative femoral artery and vein anastomosis view. D) Postoperative first week femoral artery anastomosis view. E) Postoperative first week vein anastomosis view.

Table 1: Physical properties of mice

Mouse	Artery (mm)	Vein (mm)	Nerve (mm)	Weight (g)	Lenght (cm)
1	0.31	0.41	0.37	42	16.5
2	0.29	0.55	0.35	34	15.0
3	0.37	0.58	0.47	45	16.0
4	0.33	0.46	0.43	42	16.0
5	0.36	0.57	0.41	44	16.7
6	0.28	0.46	0.36	32	14.0
7	0.32	0.35	0.31	25	13.5
8	0.25	0.52	0.37	36	14.0
9	0.29	0.43	0.34	36	14.5
10	0.32	0.54	0.36	34	15.0
11	0.35	0.46	0.40	30	14.0
12	0.31	0.44	0.39	40	16.0
Mean ± SD	0.31 ± 0.34	0.48 ± 0.70	0.38 ± 0.43	36.6 ± 6.09	15.10 ± 1.10

Table 2: Statistical relationship the artery, vein and nerve diameters to weight and length of mice.

	Artery diameters	Vein diameters	Nerve diameters
	p	*p*	*p*
Weight	0.230	0.351	0.219
Lenght	0.555	0.115	0.298

same incision (Figure 2D). Anastomotic patents were evaluated by applying the Acland's patency test (Figure 2E, 2F). After surgery, animals were euthanized with high-dose anesthesia

Digital photographs were evaluated with Photoshop CC 2019 ver. 20.0.8 (Adobe Systems, Inc., San Jose, CA) software program to measure diameters of neurovascular structures in millimeters (mm). SPSS 26.0 for Windows program (Chicago, IL, USA) was used for statistical analysis. In descriptive statistics, minimum, maximum, average and standard deviation were given for numerical variables. Shapiro-Wilk test was used to assess if numeric variables were normally distributed in groups. The artery, vein and nerve diameters to weight and length values were assessed with Chi-Squared test. Statistical significance was accepted at $P<0.05$.

RESULTS

The mean weight of the mice was 36.6 ± 6.09 gr, the length was 15.10 ± 1.10 cm. The mean external diameter of the femoral artery, vein and nerves were 0.31 ± 0.34 mm, 0.48 ± 0.70 mm, 0.38 ± 0.43 mm, respectively (Table 1). There was no statistical difference in the artery, vein and nerve diameters of mice to weight and length of mice (Table 2). Mean preparation time of neurovascular structures for anastomosis was 15.75±1.54 min, mean femoral artery and vein anastomosis time was 24.91 ± 1.72 and 33.16 ± 1.74 min, respectively. Vascular patency was 100% in all vessels after anastomosis, and no thrombosis was detected in the early period. Thrombosis was detected in 2 arteries and 3 veins in the postoperative 1st week. After 7 d, the patency rate for the femoral artery and vein was 75% and 90%, respectively.

DISCUSSION

Microsurgery training generally starts with nonbiological models. With the progression of the trainee, the education continues with nonliving-biological models and then with living-biological models[9]. Similar models have also been defined for supermicrosurgery training. They are divided into two categories; roughly simple and advanced. Examples of simple training models are silicone tubes and nonliving biological materials. Nevertheless, advanced training models are generally rat models[10]. The simplest model is the silicone tubes that do not require any dissection. Direct anastomosis can be practiced on silicone tube models. They are cheap, easy to acquire, store and clean[11]. Due to these advantages, they are usually preferred at the initial stage. Nonliving chicken model, which is another

model used frequently at initial stages, provides progression in dissection skills. As it is a biological model, it contributes to tissue handling ability. It is cheap and easy to obtain. Moreover, when compared to live models, it does not require ethical committee approval. Animal laboratory conditions and anesthesia are not needed[12]. However, it is insufficient in evaluating anastomosis patency. As it is not a living organism, trainee cannot learn how to deal with reflex responses such as vasospasm.

Supermicrosurgery compared to microsurgery, has some drawbacks other than working on smaller vessels. Lymphatic vessels are thinner and more translucent than other vessels. In lymphedema patients, lymphatic vessels become much thinner due to loss of smooth muscle in the early stages of the disease. However, in the advanced stages of the disease, lymphatic vessels become thicker and more fragile due to fibrosis. In LVA surgery, it is important to anastomose lymphatic vessels to subdermal veins that are smaller than 0.5 mm in diameter because anastomosis to larger veins causes retrograde flow due to pressure gradient[12-14]. Since perforator-to-perforator flaps are usually harvested with a short pedicle, anastomosis is performed in a narrow area, as in the fingertip replantations. Single fascicular nerve transfers and fascicular turnover flaps require meticulous dissection and atraumatic supermicrosurgery technique[6]. Although fascicular turnover flaps have been reported in a limited number of studies in the literature, they are promising especially in cases with short nerve gap[15,16]. All these supermicrosurgical procedures require advanced experience and practice. For this reason, it is very important to gain skills on living models similar to human tissue for the education of future supermicrosurgeons.

Ozkan et al. described perforator-to-perforator flap model in rats. Deep inferior epigastric artery and vein were used as recipient vessels for abdominal-based perforator flap in 2006[17]. Intramuscular dissection and anastomosis of 0.3-0.5 mm vessels with advanced supermicrosurgical skills showed the perfection of the study. A new model was defined in rats by raising the superficial inferior epigastric artery flap and anastomosing it to the same artery and vein. Its advantages such as not requiring intramuscular dissection, having no side branches, being simple and having short operation time are emphasized[18]. Besides, in the literature, there are rat face and penis allotransplantation models that require high level of knowledge, skills and experience for advanced supermicrosurgery. These studies require knowledge on immune tolerance as well[19,20].

In recent years, the first rat model was described for LVA. Mean external diameter of the lymphatic vessels was 0.24 ± 0.057 mm, and the veins was 0.37 ± 0.146 mm[14]. Lymphatics are smaller and subcutaneous fat layer is thinner in rats compared to humans; thus, dissection is performed more easily than in lymphedema patients. This is reported as the disadvantage of the model. However, the described model is the most similar to humans as a live LVA model. Bas et al comparing end-to-end and one-way-up anastomosis techniques on saphenous artery (mean external diameter, 0.273 ± 0.03 mm) and great saphenous vein (mean external diameter, 0.291± 0.02 mm) in rats. It has been proposed as a practical model for supermicrosurgery courses[10]. However, there may be anatomic variations according to the rat interstrain difference. The external diameters of superficial epigastric and common thoracic vessels were less than 0.8 mm in rats, and the long thoracic-common thoracic pedicle was approximately 1 cm longer than the superficial epigastric pedicle. Thus, they have presented vessel samples that are small in diameters and that have long pedicle for free flap and supermicrosurgery practice[21]. The mean external diameter of the femoral artery of the mice in this study was 0.31±0.34 mm, the femoral vein was 0.48±0.70 mm. Mouse femoral vessel diameters are similar to vessel diameters in rat models defined above for supermicrosurgery practice. In addition, according to our own experience, femoral veins of mice were more fragile than superficial inferior epigastric veins of rat's event though both vessels have similar diameters.

At the first experimental study in rats, 8 mm defects of marginal mandibular nerve were repaired with fascicular turn over nerve flaps in 2009[22]. The turnover flap was presented similar histopathological features with the autologous nerve graft. Following 12 wk of healing period, muscle movements were observed. Mouse femoral nerve diameters were measured for creating new training model in the same study. The average femoral nerve external diameter of mice was 0.38±0.43 mm. Experimental nerve regeneration studies are generally performed on the sciatic and femoral nerves in rats[23]. Nerve healing occurs in a shorter time in mice than in rats[24]. For this reason, we think that the mouse femoral nerve may be a new

model for turnover flaps and supermicrosurgery practice compared to rats.

Our knowledge, microsurgery studies in mice have been reported in a very limited number. The first study about vessel anastomosis was reported in mice. It was performed anostomosis on 48 saphenous arteries with diameter about 0.2 mm and discussed difficulties of the method[25]. Cooley et al. did autologous replantation with free groin flap in IRC mice (n=12). The mean weight of the mice was 30.3 gr, average femoral artery and vein diameters were 0.4 mm and 0.8 mm, respectively. In the same study, they performed syngeneic transplantation in BALB/c mice (n=18). Diameters of arteries and veins were 0.2-0.3 mm and 0.3-0.6 mm, respectively[26]. In this study, BALB/c mice were used, similar vessel diameters were determined and no anatomic variation other than the difference of diameter of vessels was found.

Mice are preferred experimental animals that often used in biomedical researches, immunology and genetics. Rats and mice are rodents that share same family and subfamily. Their physical properties, feeding and housing conditions are similar. Mice are more docile and easier to handle. Since they are smaller than rats, their usage as experimental animals is much more affordable in terms of both housing and anesthetic needs.

This study and the mice model for supermicrosurgery education have some limitations. First of all, femoral nerve healing and functional outcomes were not evaluated. Furthermore, the most important handicap of the mouse model is working in a small and narrow area. This situation is compelling for beginner trainees. However, this difficulty is a good simulation for real supermicrosurgical procedures. Moreover, mouse model is more expensive compared to tube models and nonliving-biological models. It requires ethical committee approval, animal laboratory, housing, and maintenance conditions.

Dissection to reach femoral neurovascular structures in mice is simple. Every microsurgeon that had practice on rats is familiar with the anatomy which is very similar to that of mice. Consequently, trainees had no difficulty during dissection of mice femoral neurovascular structures. In addition, the absence of variations in basic anatomy; the absence of muscle dissection for the exposition of vessels and nerves ensure energy and time for anastomosis and coaptation. As the trainee does not lose time and energy for the preparation of vessels and nerves, he/she can concentrate better on suturing.

CONCLUSION

Mice's femoral neurovascular structures are similar to rats in terms of basic morphology, easily dissected and small enough for supermicrosurgery training. In addition, compared to rats, anesthetic medications and laboratory costs are less expensive for mice because of the weight of the rodents, and they are easier to handle; thus, making them especially suitable for supermicrosurgery training courses in which many fellows participate.

ACKNOWLEDGEMENTS

There was no financial support for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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