Original Article

Preparation of animal model of chicken for lymphatic anastomosis technique training

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Background: To discuss the preparation of a simple and practical animal model for lymphatic anastomosis technology training. At present, there is no widely used animal model for training lymphatic anastomosis.

Methods: The neck of chicken is long enough, and there are abundant lymphatic lymph nodes. The caliber of lymphatic vessels in the neck of chicken is similar to the caliber of lymphatic vessels in human limbs, so multi-stage anastomosis practice can be carried out. The lymphatic vessels of chicken are easier to stain and fully exposed, which meets the requirements of lymphatic vein anastomosis model. This model allows for training of end-to-end anastomosis (LVA), end-to-side anastomosis, and lymph node vein anastomosis under microscope to be carried out. In this study, the trainees were divided into six groups, with five animals in each group. After 14–28 days of training, the average training was 21 days. Data were statistically analyzed by SPSS15.

Results: Obvious and clear lymphatic staining could be obtained after animal modeling, and the success rate of dissecting and staining lymphatic vessels under microscope could reach 100%. After training, the trainees could perform the anastomosis of lymphatic vessel and vein and lymph node vein under ultra-microscopic conditions, and the immediate patency rate of the anastomosis of the trainees can reach 80% during the examination.

Conclusions: This animal model can be used for effective lymphatic vein anastomosis training, which is a simple, practical, and effective microscopic lymphatic anastomosis technology training model.

Keywords: Animal model; lymphatic vein anastomosis; lymphatic staining; technology training

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Introduction

Breast cancer is the most common cancer worldwide, and the leading cause of cancer-related deaths globally (1). For women who undergo breast cancer surgery, one in five will have lymphedema of the upper limbs after lymphadenectomy (2). In addition to increasing limb edema, limb lymphedema also affects the regulation of lymphatic-related immune responses, leading to skin and soft tissue inflammation. This increases the risk of patients with erysipelas and seriously affects their quality of life.

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The general standard procedure for radical mastectomy is to remove all lymph nodes in the tumor area, although sentinel lymph node biopsy and selective lymph node dissection are used after improvement. However, there is still a 4–10% incidence of lymphedema after radical mastectomy (3). Lymphedema of the lower limbs can also easily lead to lymphedema of the lower limbs, with about 20% of patients developing lymphedema after inguinal lymphadenectomy (4). First proposed in 1962 was the idea that it is possible to establish an anastomosis between lymphatic vessels and veins, and shunt lymph to the venous system in advance to treat the possibility of chronic lymphedema (5). It was not until 1976 that O'Brien *et al.* submitted a clinical report that confirmed the clinical effectiveness of this method in human patients, which was a major breakthrough in the field of lymphatic microsurgery (6). Since then, with the promotion of super microsurgical anastomosis technology, 0.3–0.8 mm vascular anastomosis technology has matured, and the reliability of lymphatic venous anastomosis has also been enhanced. The use of lymphatic vessels with a diameter of 0.5–0.7 mm and subdermal veins with a diameter of 0.7–1.0 mm for postoperative follow-up results of lymphatic and venous anastomosis is good, and is gradually recognized as an effective method for the treatment of chronic lymphedema (7,8). In recent decades, autologous lymph node transplantation, lymphatic venous anastomosis, and other lymphatic surgeries have attracted attention with increased clinical application, but most microsurgeons have no experience in finding and anastomosing lymphatic vessels and veins. In the surgical lymphatic anastomosis technique, Super microscopy Training is the basic technique for clinical lymphedema treatment.

Limb lymphedema is a refractory disease that involves a variety of technical requirements, which cannot be solved by anatomical training. Therefore, the establishment of an animal training model for lymphatic anastomosis is key to the treatment and promotion of secondary lymphedema. It is reported in the literature that the first stable animal model of lymphedema was established by Olszewski *et al.* in 1968 in dogs (9). They established the first reliable and reproducible model of acquired lymphedema through an innovative circumferential resection method and double-layer cutting of the superficial and deep lymphatic vessels. Later, Das *et al.* (10) combined radiation irradiation on this basis in 1981 to obtain a more reliable lymphedema model, which can extend the incidence of lymphedema to approximately 100%; however, this requires the cooperation of radiologists. Thereafter, this method of circumferential removal of lymphatic vessels was applied to rabbit ears (11,12) and rodent hindlimb (13,14) models. Some scholars have also constructed rodent tail models (15).

However, animal models for training in lymphatic microscopy technique still exhibit the following shortcomings. The dog model is the closest to the human body, but it is difficult to capture and raise, and is costly and difficult to operate. Rabbit ear tissue is extremely thin and layered; thus, it is not easy to find lymphatic vessels, and the animals are prone to infection and necrosis after surgery. Although it is easy to construct a rat animal model, this is mainly an acute lymphedema model, and it is difficult to obtain a more reliable model of chronic lymphedema. Also, it is difficult to perform anastomosis of the small blood vessels and lymphatic vessels for training purposes. There are still no recognized widely used animal models for lymphatic anastomosis training. Chicken has been proposed as anastomotic model for vascular anastomosis (16).

In this study, we analyzed and improved on the current animal model of lymphedema, and proposed the idea of using an experimental chicken neck lymphatic venous anastomosis model. To obtain an anastomotic patency rate of 80%, the trainers were trained for 2–4 weeks, and we achieved reliable lymphatic venous anastomosis training results. The training had the characteristics of short training period, low cost, and easy construction of training venues. The animal model has the advantages of being relatively reliable and stable, low cost, and simple to construct.

In this group of animal models, methylene blue dye was used to obtain clear lymphatic imaging in the experimental area through our staining method, which created favorable conditions for anastomosis technical training. This type of training enables trainees to master good lymphatic staining and lymphatic anastomosis techniques in a short period of time.

We present the following article in accordance with the ARRIVE reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-22-113/rc).

Methods

Experimental animals

This experimental animal research project was performed under a project license (No. 2021623) granted by the Ethics Committee of Zhejiang Provincial People's Hospital and in accordance with Chinese guidelines on animal care

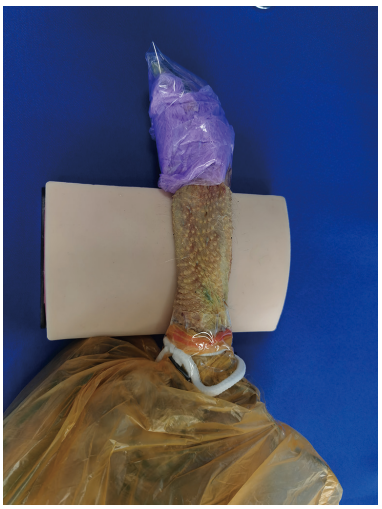

Figure 1 Preparation of animals before training.

and use. Thirty specific pathogen free (SPF) male white leghorn chickens (provided by Guangzhou Laidemeng Biotechnology Co., Ltd). The chickens were 2–3 months old and weighed 1,800–2,500 g. Before the experiment, the experimental animals were kept in an air-conditioned temperature-controlled room (24–25 ℃) to ensure stable temperature and humidity, clean environment, and good ventilation. They were fed routinely in the laboratory under standard conditions for 1 week.

Main materials for experiment

The following materials were used: Methylene blue injection (2 mL/20 mg, Jichuan Pharmaceutical Group Co., Ltd. Room 602, Floor 6, Building 9, No.88 Keyuan South Road, High-tech Zone, Chengdu); 100 g/L sodium sulfide; physiological saline; 75% medical alcohol; povidone iodine; microsurgery instrument microscope (ZEISS OPMI Vario S88 Germany); disposable cutting knife (Bohai Kangyuan Company Zhongguancun Science and Technology Park, Tongzhou District, Beijing); fixed plate; needle holder; micro scissors; scalpel; thread scissors; nylon monofilament 11-0/12-0 suture needle (Shanghai Pudong Jinhuan Medical Supplies Co., Ltd., China); experimental cage (provided by the Animal Experiment Center of Zhejiang Provincial People's Hospital, China); medical skin preparation knife (Yangzhou Huawei Company, Hanjiang Middle Road, Hanjiang District, Yangzhou City, Jiangsu Province); and digital vernier caliper (Sanfeng Group, Japan).

Grouping

According to the training requirements, the trainees were divided into six groups, each with two people (one in charge of the knife and one assistant), take turns to other and repeated exercises. Each group randomly selected five white leghorn chickens for preparation and micromanipulation training (provided by the Zhejiang Province, provided by Animal Experiment Center of People's Hospital).

Preoperative preparation and staining of experimental animals

Healthy experimental animals were prepared prior to the operation. Using 2 mL of methylene blue injection (Melan stain) was injected into the heads and necks of the animal for staining. After staining, the neck of the animal was ligated to fill the experimental area, and 5 mL of air was injected through the eyeball into the animal's skull. After being sacrificed, 10 mL of sodium sulfide was used to dehair the neck of the chicken. Subsequently, the head and body of the chicken were sealed and wrapped with plastic bags and taped. The animal was then placed on a test bench and sterilized with povidone iodine for use (*Figure 1*)

Lymph separation and anastomosis under microscope

The skin and subcutaneous fascial tissue were incised with an 8 cm longitudinal incision using the No. 11 surgical blade. The skin and subcutaneous tissue were separated using a 2-0 silk thread at multiple points, and the lymphatic vessels and lymph nodes stained with methylene blue were found along the deep layer of external jugular vein. Lymphatic vessels and lymph nodes were stained with methylene blue. The lymphatic vessels, lymph nodes, and fat internal veins were dissected and separated under a 16-fold microscope. The 11-0/12-0 lines were used to perform lymphatic vessel-venous end-to-end anastomosis, end-to-side anastomosis, and lymph node-venous anastomosis surgical techniques (*Figure 2*).

Design of lymphatic vein anastomosis

Lymphatic venous anastomosis (LVA) refers to an end-to-end or end-to-side anastomosis between the distal end of the lymphatic vessel and the proximal end of a venous tube with a similar diameter.

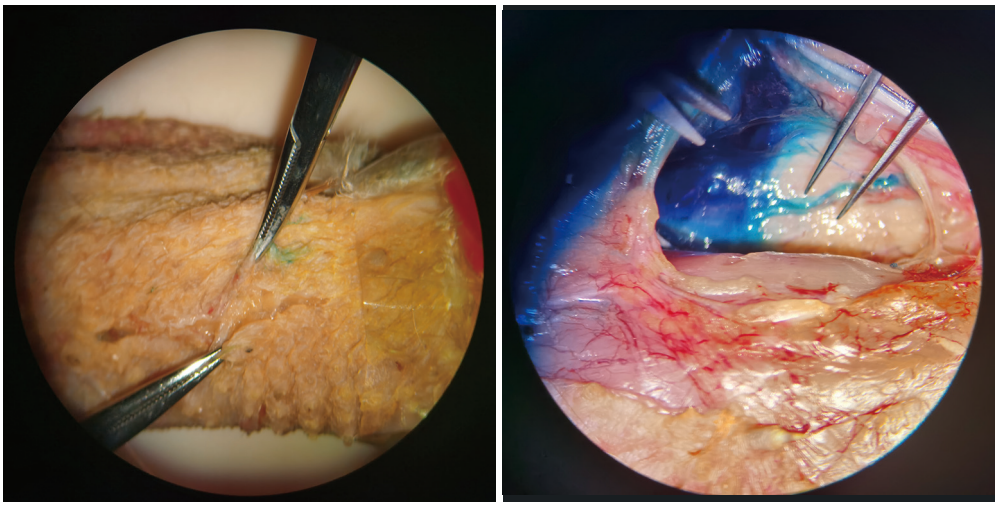

Figure 2 Exposure of training sites and lymphatic vessels.

The specific operations of the experimental design were as follows:

(I) End-to-end anastomosis of lymphatic vessels and veins involved dissection to reveal stained lymphatic vessels and adjacent veins of similar caliber. After adjusting the tension of the anastomosis, the lymphatic and venous vessels were cut off under a 16-fold microscope. The proximal ends of the lymphatic vessels were left untreated, while microscopic ligation was applied to the distal end to stop the bleeding. After measuring the calibers of the veins and lymphatic vessels with digital vernier calipers, the lymphatic vessels and venous orifices were trimmed at the anastomosis, and the needled 11-0 or 12-0 microanastomosis line was used at the first position and sutured at 6 o'clock. The number of anastomosis needles was fixed with three stitches at 2 o'clock and 10 o'clock according to the diameter of the lymphatic vessel (diameter of 0.2 mm and below), and the four-needle anastomosis method was used at the 12, 3, and 9 o'clock position for lymphatic vessels with a diameter above 0.2 mm. fixed. The margins and stitch lengths were required to be even, with slight outward turning and knotting.

(II) End-to-side anastomosis of lymphatic and venous tubes should be adopted when the diameters of the lymphatic and venous tubes exposed by anatomical examination are large. The venous tube should be cut with a microslice knife in the direction of the venous tube towards the lymphatic tube wall 0.3 mm. For this, the 6 o'clock to position was used and the posterior wall was sutured first. Subsequently, different anastomoses were performed based on the diameter of the lymphatic vessel: lymphatic vessel diameters 0.2 mm and below were sutured at 2 o'clock and 10 o'clock, respectively, using a three-needle end-to-side anastomosis; however, when the diameter of the lymphatic vessel was >0.2 mm, four-needle anastomosis at 12 o'clock, 3 o'clock, and 9 o'clock was adopted.

Design of lymph node venous anastomosis

The lymph nodes and adjacent venous tubes in the stained animal tissue were dissected and separated under a 16-fold microscope, according to the diameter of the venous tube. Venous tubes ≤0.3 mm were ligated at the distal end and anastomosed at the proximal end. 1/4–1/3 of the lymph node tissue was removed from the periphery, and the proximal orifice of the venous tube, and the wound surface of the lymph node were used for four-needle anastomoses. The 11-0 or 12-0 microdissection with needle was used to anastomose the wound surface of the lymph node and the proximal orifice of the vein tube, and the four-needle method was used at the position of 12 3, 6 and 9 o'clock, respectively. When the diameter of vein tube was >0.3 mm, the wall of vein tube opposite to lymph node was cut with micro scalpel. The 11-0 or 12-0 micro anastomosis line with needle was used for end-to-side anastomosis at 0, 3, 6, and 9 o'clock, respectively (*Figure 3*).

Training and observation

Following anastomosis, the vascular patency experiment method was used to observe the patency of the anastomosis and promptly record the abnormal situation. Anastomotic patency test: (I) The lymphatic squeeze test was used to check whether the blood in the venous vessel was moved; and (II) the venous squeeze test was used to observe whether the blood in the venous tube passed through the anastomosis. The patency of lymphatic vessels and veins was evaluated through multiple tests, and the assistant surgeon exchanged and repeatedly trained the anastomosis technique (*Figure 4*).

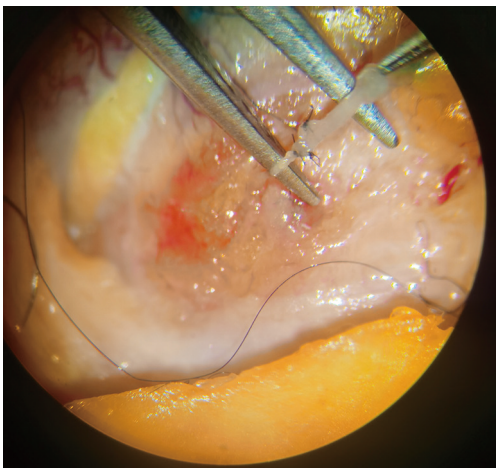

Figure 3 Patency degree after anastomosis.

Statistical analysis

Training Data were statistically analyzed by SPSS15, The analysis of anastomosis training data accords with the training law of microsurgery and the training curve of microanastomosis.

Results

General observation

The primary training requirements were as follows: (I) Proficiency in animal handling, methylene blue injection staining, execution, skin preparation, etc.; and (II) familiar with fixed anatomy, searching for lymphatic vessels, lymph nodes, and veins. The lymphatic vessels and veins were initially anastomosed 20 times.

The advanced training requirements were as follows: (I) Proficiency in finding lymph vessels, lymph nodes, and corresponding veins under the microscope; and (II) proficiency in performing end-to-end anastomosis, end-to-side anastomosis, and lymph node-venous anastomosis. After accumulating the number of anastomoses, the anastomotic stoma was unobstructed and counted 60 times.

Training effect evaluation

The learning curve of microsurgical lymphatic and venous anastomosis was determined according to the operation time and the number of unobstructed anastomoses (*Figure 5*).

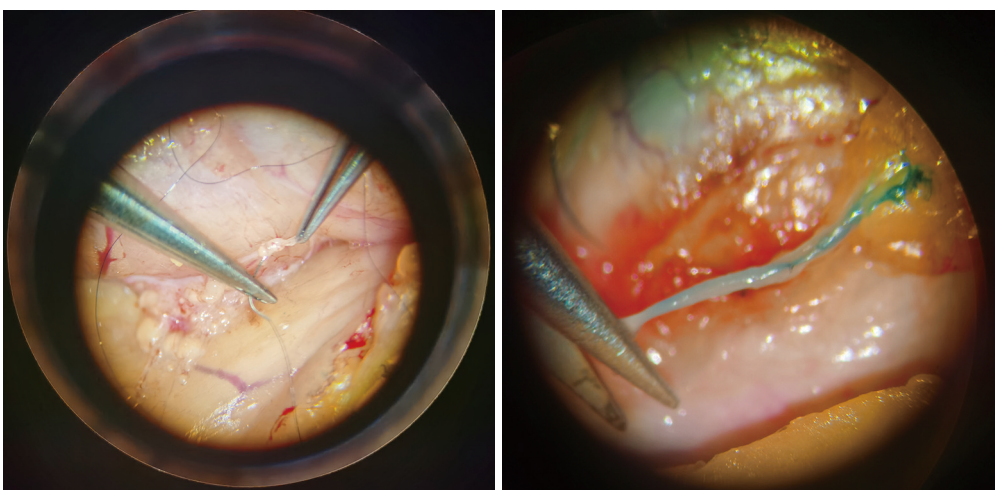

Figure 4 Lymphatic venous anastomosis.

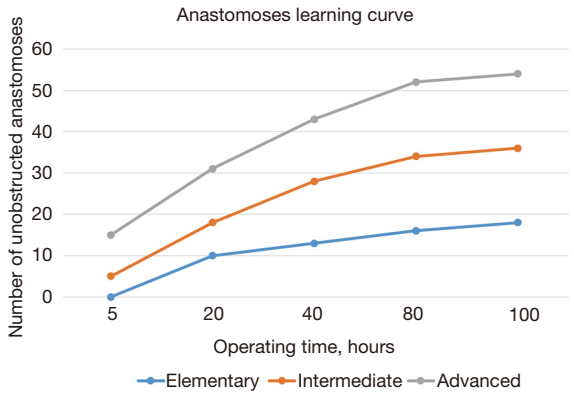

Figure 5 Number of lymphatic and venous anastomosis, anastomotic learning time.

Discussion and conclusions

With autologous transplantation, lymph node lymph vein anastomosis and other lymphatic operation over the past few decades the increase of the clinical application and widely attention, but most of the microsurgery doctors did not skilled fluorescent tracer technique and the anastomosis of lymphatic and venous experience, as the super microsurgical lymph vein anastomosis of the training of the promotion, will push for clinical treatment of lymphedema.

The animal model of chicken lymphatic anastomosis technique training is a more suitable animal model for microsurgical lymphatic anastomosis training. On the one hand, its advantages include reliability and stability, low cost, simple production, which can be used as a very close simulation of clinical lymphatic venous anastomosis. On the other hand, lymphatic vein technology training can play a positive role in microsurgery, and super microsurgical anastomosis technology offers certain advantages, so as to provide better clinical treatment for chronic lymphedema and vascular anastomosis patients.

In the preparation of animal models in this group, methylene blue staining was used to obtain clear lymphatic vessel development in the experimental area through intracranial staining. Meanwhile, the staining pollution in the experimental area was relatively light, which created favorable conditions for the training of lymphatic vein anastomosis technology

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Footnote

Reporting Checklist: Both authors have completed the ARRIVE reporting checklist. Available at https://gs.amegroups.com/article/view/10.21037/gs-22-113/rc

Data Sharing Statement: Available at https://gs.amegroups.com/article/view/10.21037/gs-22-113/dss

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://gs.amegroups.com/article/view/10.21037/gs-22-113/coif). Both authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This experimental animal research project was performed under a project license (No. 2021623) granted by the Ethics Committee of Zhejiang Provincial People's Hospital and in accordance with Chinese guidelines on animal care and use.

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