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Step training in a rat model for complex aneurysmal vascular microsurgery

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Abstract: *Introduction*: Microsurgery training is a key step for the young neurosurgeons. Both in vascular and peripheral nerve pathology, microsurgical techniques are useful tools for the proper treatment. Many training models have been described, including ex vivo (chicken wings) and in vivo (rat, rabbit) ones. Complex microsurgery training include termino-terminal vessel anastomosis and nerve repair. The aim of this study was to describe a reproducible complex microsurgery training model in rats. *Materials and methods*: The experimental animals were Brown Norway male rats between 10-16 weeks (average 13) and weighing between 250-400g (average 320g). We performed n=10 rat hind limb replantations. The surgical steps and preoperative management are carefully described. We evaluated the vascular patency by clinical assessment-color, temperature, capillary refill. The rats were daily inspected for any signs of infections. The nerve regeneration was assessed by foot print method. *Results*: There were no case of vascular compromise or autophagia. All rats had long term survival (>90 days). The nerve regeneration was clinically completed at 6 months postoperative. The mean operative time was 183 minutes, and ischemia time was 25 minutes.

Key words: Microsurgery, training, replantation

Introduction

With the increasing use of microsurgical techniques in neurosurgery, regular laboratory training has become essential. Microneurosurgical operations differ from other surgery. Longer operative time, narrow

and deep-seated operative corridors, hand-eye coordination, fine manipulation, and physiologic tremor present special problems. Proper understanding of visual feedback, control of physiologic tremor, better instrument design, and development of surgical skills with better precision is important for optimal surgical results. Sufficient clinical case volume or opportunity during routine operative hours may not be available in the beginning for young neurosurgeons and microsurgical training using various models can enable them to gain experience.

Training models using deep-seated and narrow operative corridors, drilling, knot-tying technique, and anastomosis using fine sutures under high magnification can be practiced for skill improvement. Training laboratory and simulation modules can be useful for resident training and skill acquisition. The knowledge of ergonomics, proper training, observing hand movements of skillful surgeons, and the use of operative videos can improve skill (1). The aim of this article is to describe the surgical steps in limb replantation in a rat model as complex microsurgical training.

Material and methods

The study was done in the microsurgery laboratory in the Emergency Clinical Hospital in Bucharest. The experimental animals were Brown Norway male rats between 10-16 weeks (average 13) and weighing between 250-400g (average 320g). We performed n=10 rat hind limb replantations. The surgical steps and preoperative management are carefully described. We evaluated the vascular patency by clinical assessment-color, temperature, capillary refill. The rats were daily inspected for any signs of infections. The nerve regeneration was assessed by foot print method. For anesthesia the animals were placed into the Induction Chamber with anesthetic gas inflow. The rats were anesthetised with Xylazine (0,02 ml) and Ketamine (0,01ml). Monitoring throughout anesthesia was done using the tail pinch reflex, respiratory rate, heart/pulse rate and tissue color. The whole operation is performed under aseptic conditions and antibiotic prophylaxis was administered using Clavulox 0.1 ml/100 g. s.c. at the commencement of the operation. Fluid loss during the operation was compensated with 6-7 ml 0.9% Sodium Chloride Solution given by intraperitoneal injection. No vasodilatant or anticoagulation drugs were used except for the limb perfusion.

Surgical procedure

A circumferential skin incision of the hind limb was made at the mid-thigh level (Figure 1). The inguinal fat flap with pedicle a. and v. epigastrica superficialis and sensory nerve branch from n. saphenus was sharply dissected and after isolation of the pedicle flap was reflected distally. The saphenus nerve was prepared and transected proximally at the level of its branching from n. femoralis. The femoral artery and vein were then identified and skeletonized (Figure 2). The femoral artery was clamped with a double microvascular clamps and transected. The femoral vein was clamped and transected. Perfusion washout was performed with 4oC cold heparinized

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solution (1500 UI of Heparin in 500 ml of 10% Dextran 40 Intravenous Infusion BP in 0.9% Sodium Chloride Intravenous Infusion). The thigh muscles were sharply cut approximately 1 cm distally from the level of the femoral nerve branching into the muscular branches and n. saphenus. This muscle dissection must be performed very carefully as it is close to the branching of the femoral artery and vein into the saphenous and popliteal vessels. During muscle dissection the sciatic nerve was solicitously protected when it emerged between thigh adductors and m. quadratus femoris on the one side and m. biceps femoris on the opposite side (Figure 3). This nerve was isolated and cut proximally. Skeletonisation of the middle third of the femoral bone was done. The femur was then divided in the middle using a bone saw. After amputation, muscles, bone, vessels and nerves of the limb were meticulously washed down with 0.9% Sodium Chloride Solution (Figure 4). For the replantation, first, femoral bone fixation was achieved by a combined technique using intramedullary rod made from a 19-gauge stainless steel needle and osteosuture using 28 SWG monofilament stainless steel suture wire. Muscles were then sutured with 4/0 nylon interrupted stitches with emphasis on precise adaptation of functional muscle groups. An epiperineural suture of the sciatic nerve was performed an-block either from the anterior or posterior access under the operating microscope using 10/0 nylon before muscle suture completion (Figure 5). Femoral vessels were washout with 0.9% Sodium Chloride Solution and the ends were precisely trimmed before anastomosis. Revascularisation started with vein suturing. Both vessels were sutured under the microscope using 10/0 nylon single stitches (Figure 6). Clamps were removed first from the artery and after a few seconds, when femoral vein began to expand with blood, the clamps were removed from the vein. Firstly the distal vein clamp was taken away to allow dilatation of the vein anastomosis and then the proximal clamp was removed. The initial bleeding from both anastomoses stopped after a few seconds of gentle compression with a wet gauze swab.

After restoration of the blood flow in the reconnected vessels the suturing of n. saphenus was done under the microscope using the same technique as for the sciatic nerve. An ample washout of the wound with 0.9% Sodium Chloride Solution was performed and the replantation was completed by skin closure with running 4/0 nylon sutures. Note it is important to include subcutaneous fat into this suturing to prevent bleeding from large vessels in the subcutaneous fat mainly in the hypogastric and inguinal area. After completion of the surgery, the skin suture was wiped down with Povidone-Iodine 10% Solution and no wound covering or limb splint was used.

The animals after awakening from anesthesia were put in a clean, dry, warmed box on a folded towel in a quiet post-operative room (30-32oC) away from strong light. Immediately post recovery the animals were housed with a companion and allowed free access to food and water. No collar or other special device was used to prevent self-mutilation.

Figure 1 - skin incision

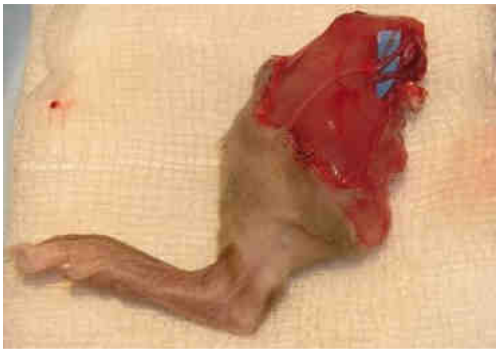

Figure 4 - limb ready for replantation

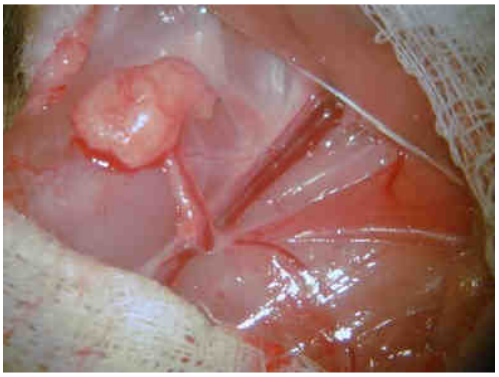

Figure 2 - femoral vessel dissection

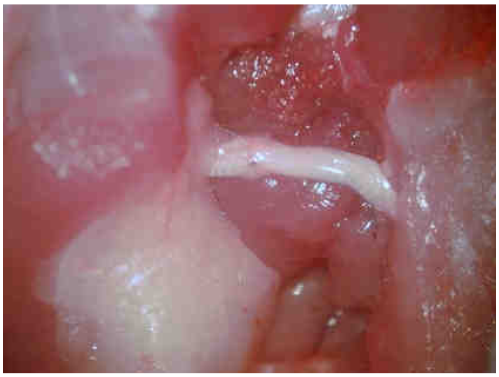

Figure 5 - epineural sciatic nerve repair

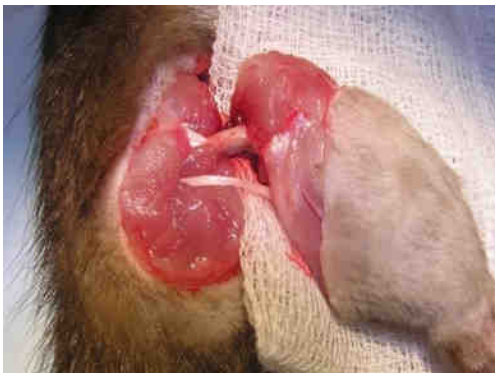

Figure 3 - sciatic nerve identification

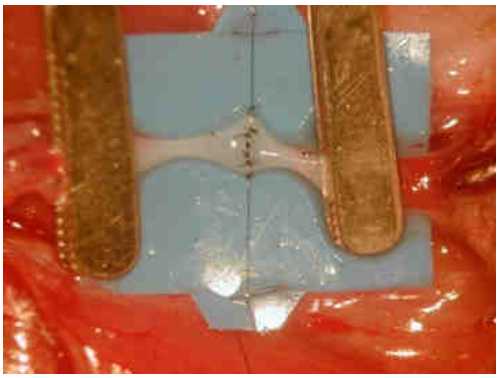

Figure 6 - arterial anastomosis

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Results

There were no case of vascular compromise or autophagia. All rats had long term survival (>90 days). The nerve regeneration was clinically completed at 6 months postoperative. The mean operative time was 183 minutes, and ischemia time was 25 minutes.

Discussions

While microvascular suturing technique was the province of neurosurgeons a couple of decades ago, nowadays almost every surgical specialty uses this technique for its one pathology. Many programs have integrated training in microvascular anastomosis techniques in their curricula. The surgeons are either trained at their own institution, if this happens to have a microvascular animal laboratory, or they are sent to centers that conduct microsuturing courses.

Materials that are initially used for learning microsurgical suturing and knot techniques are latex (2) and silicon tubules (2), (3), (4). However, the ‘‘feel’’ of manipulating viable biological tissue is achieved only in small animal laboratories (4) (5). The available models for training microvascular anastomosis techniques may be classified into three categories: (1) the nonbiological and nonfunctional, (2) the biological and nonfunctional, and (3) the vital small animal model, which demands investment and maintenance (6). Each of these models has its advantages and disadvantages, which are very well-known. Recently Abouod et al. proposed a model of a perfused human cadaver head for training neurosurgical procedures (7). The success of a microvascular suture, irrespective of the technique, can only be proved through surviving replanted parts in chronic experiments, e.g., tail and limb replantation or organ transplantation in rats (5), (8), (9) (10), (11), (12), (13).

Since 1968s when Komatsu and Tamai reported first successful digit replantation with repair of micro blood vessels, the laboratory microsurgical training on rats become popular model in microvascular surgery.

Having in mind that microsurgery is a precise surgical skill that requires an extensive training period of time and creates a simulated surgical environment that allows gifted surgeon to make and recognize mistakes in microsurgery techniques and thus shifts any related risks of the early training period from the real case on the operating room to the lab allowing in this way achieving a high level of skill acquisition.

The classical training schemes in microsurgery lab experimental on the rat model was to take surgeon through a series of microsurgical maneuvers that would develop his skills utilized the femoral artery and vein, the epigastric artery and vein, and the sciatic nerve working at mid –to high-range magnification.

Sun Lee's manual fifty years ago describing microsurgery techniques in the rat to respond the aspirations of the surgeons of that time, the surgical field is again in need of further microsurgical training interventions with the advent of new horizons in microsurgery that will and be applied in vascular microvascular surgery (14).

They are many papers and studies that presents the utility and beneficiates of the classical rat model of microsurgery training centres and the new prospects that this versatile and not suchexpansive training model offers. It is motivated and supported the idea that practice is key to microsurgery skill maintenance and improvement, at least at a trainee level where a laboratory based surgical skills can significantly improve skill.

The widespread use of microsurgery in numerous surgical fields has increased the need for basic microsurgical training outside of the operating room. This stage is basic and the most important; the aim is to develop the fundamental skills of using microsurgical instruments under a magnified field. The minimum requirements would be an operating microscope, microsurgical instruments, suture materials and non-living models.

Advances in microsurgery continue to be based on the experimental animal models.

Ilie et al. organized microsurgical models into five main groups: 1) Basic manipulation, movement, and orientation in the microscopic field. 2) Knot placement/tying principles, apposition of edges, non-dominant hand usage, and deformable volumes. 3) Three-dimensional models/completing the anastomosis. 4) The real tissue experience (15).

The distinctive emphasis that microsurgery places on advanced technical skills gives it particular challenges for training. Currently, there is no universal system by which surgical skills are assessed, but generally there are considered to be three broad categories: cognitive/clinical skills, technical skills, and social/interactive skills (16). The distribution of these skills is debated and depends upon the specialty and the particular operation being undertaken (17).

As in any other domain more so in microsurgery the past and everything go well in this field must be remembered, updated and taken as a landmark. The limited time, resources and opportunities to practice microsurgical technique in clinical settings, along with the serious consequences of failure, have led to the establishment of microsurgical training courses. Reviews of microsurgical training centers worldwide shows that basic microsurgery courses range in duration and intensity from 20 to 1,950 hours. On average (18), a basic microsurgical training course lasts 40 hours (5 days) costing $1,500 (USD). So it is worth remembering the rat model in microsurgery education and development of this practice along other training courses: Paris School of Surgery (Paris, France), Northwick Park workshops (London, UK), and Columbia University (New York, USA).

The Paris School of Surgery is the longest serving out of eighteen training courses on the record to date in France, all of which are either basic 'certificate' courses or advanced university 'diploma' courses. The training lab was established in 1976 by Dr. Alain Gilbert, Gisèle Amichot, and Josette Legagneux as instructors. Currently, the course is directed by Professor Alain Masquelet, the course with two parts: basic and advanced teaches about 60-70 surgeons per year (19).

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In the United Kingdom (working within Home Office guidelines for the use of animals), basic microsurgery workshops were established at Northwick Park Institute of Biomedical Research in 1979 by Professor Colin Green and Sandra Shurey (20).

At Columbia University (CU), the Microsurgery Research and Training Lab was established in the early 1980s by Dr. Harold M Dick in the Department of Orthopedic Surgery, and now it is under the leadership of Dr. Melvin P. Rosenwasser and Dr. Yelena Akelina, training over 150 surgeons every year, from 12 specialties and more than 45 countries.

All of the courses emphasise the importance of the right attitude and psychology set in achieving a successful outcome in microsurgery. Classical exercises were developed to take surgeons through a series of microsurgical maneuvers that would develop their skill (21).

Vascular microsurgery training, maybe more than others surgical fields, across the world is very heterogeneous. Ex-vivo prosthetic models such as the latex glove, silicone sheets and tubing can be for some level a common starting point in microsurgical training, followed by a variety of non-living animal models, such as the turkey coronary artery, and chicken wing artery, the advantages of non-living and prosthetic models are: portability, minimal maintenance over a favorable shelf life, and with no biological hazards or regulations for their use- but they may not be as realistic as living models.

Recently the most commonly used animal model in microsurgical courses is the rat. Microvascular end-to-end and end-to-side anastomoses are common to most basic models, with half the practice on larger arteries (>1 mm diameter) prior to practice on smaller arteries (≤1 mm diameter). In vivo vascular microsurgery models offers an opportunity for reviewing the functional results of anastomoses while taking account that nerve models demand a different dissection and tissue handling.

Amputated rat limbs storage by wet and cold (4°C) showed no macroscopic changes or weight increase rate. Limbs preserved by wet and cold appeared to have insufficient blood circulation, causing limb necrosis within 3 days of surgery. The current method of preserving amputated limbs involves simply wrapping them with saline-moistened gauze and then cooling them on ice (22).

This method is basically similar to Allen's, which was reported more than 70 years ago (23).New methods modified of preserving muscle tissues have not been developed for a long time.

At this moment replantation it is considered to be successful not only when there is vascular circulation to the amputated limb but also when limb function is restored. Some animal experiments have evaluated functional recovery of ischemia–reperfusion limbs. Song et al. (24) reported good functional recovery of rat limb allografts after the immediate limb transplantation by using a cutaneous reaction test, walking track analysis, and electrophysiological evaluation.

Tsuji et al (25) performed an electrophysiological study after rat limb transplantation in several ischemia periods and found that the distal motor latencies of the sciatic nerve at 3 weeks increased with increasing limb ischemia time. The results may depend on not only muscle function recovery but also sciatic nerve regeneration.

Preservation solutions can protect muscle function and morphology in ischemia-reperfusion limbs and improve recipient survival rates after transplantation of long-term-preserved limbs (26). Replantation of major extremities after long periods of ischemia can lead to viable replants in many cases, but functional restoration is often poor owing to fibrosis of the muscle (27).

Intermittent blood flow provided a much better outcome than continuous ischemia for rat limb preservation at room temperature.

Only a short period of time (on average 10-min) perfusion every 4 hour appeared to result in insufficient blood supply, but was nonetheless effective in preserving the limb (28). It is postulate that as in health under normal physiologic conditions, there is a safety margin of oxygen delivered to the tissues (29).

Having this as information, we revised a series of limb preservation studies with oxygen carriers. Autologous blood is often used in such experiments but the clinical utility of this approach is limited (30).

In systematic review done by Ghanem et al to identify randomized control trials looking for educational and training interventions that objectively improved microsurgical skill acquisition, although there is significant paucity in the literature reviewed to support microsurgical course and training practices, simulated training on fidelity models in microsurgery is an effective intervention that leads to acquisition and refining of transferable skills and improved technical performance (31).

Sutherland et al, looked at 30 randomized control trials, all of methods of delivering surgical education, such as computer simulation, video simulation, and physical models against classic predominant current teaching summarizing that computer and video simulation did not significantly enhance training but that model and cadaveric training showed promise expectation (32).

Sturm et al. carried a systematic review comparing whether skills acquired in simulation training were transferable to the operating room in the surgical field, they concluded that on the whole simulation training does transfer to the operative setting and is a safe and effective means for adjunct surgical education particularly in novice trainees, as it helps eliminate part of the steep learning curve, and improve visuo-spatial awareness (33). Szalay et al. showed that novice surgeons had to perform between 40 and 48 vessel anastomoses to achieve 100% patency on live rats two weeks after the procedure (34).

Zhaowei Zhu et al used showed that limbs rats with a minimal amount of muscle tissue can be successfully replanted. Because of its complexity, cryopreservation of muscle tissue is challenging and injury during the cryopreservation process and from reperfusion results in swelling and elimination of blood flow (35). In our study, the mean

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ischemia time was acceptable and no cryopreservation was done, only a moist gauze was applied over the limb to avoid tissue desiccation.

Hin-Lun Liu reveled that consistent size and anatomy of the femoral vessels in a rat model allow very easy and quick dissection of vessels, which is particularly useful for surgeons who want intensive training of submillimeter vessels anastomosis (36). In our study we performed the microvascular arterial anastomosis in a classical method on femoral artery with a mean diameter of 0.5 mm.

Conclusions

We successfully descried step by step the surgical procedure of a limb replantation in a rat model. Several options of training and assessing microanastomosis have been described previously. Methods of assessment included long-term evaluation of patency of the micro-anastomosis performed in animals models (37), as well as structured video assessment by expert microsurgeons (38) (39). The use of these training models and assessment modalities validated improvement while training (40).

Performing a complex operation such as a limb replantation is a highly demanding procedure for the young neurosurgeon. Important operative skills as microvascular arterial and venous anastomosis are trained, useful tools in daily clinical work.

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