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Learning Curve in Hemifacial Transplantation in Rats

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Rezumat

Curba de învățare a transplantului de hemifață la șobolani

Introducere: Scopul acestui studiu este de a evidenția curba de învățare a transplantului de hemifață la șobolani prin compararea a doi operatori: studentul la medicină cu cunoștințe de bază de microchirurgie și un microchirurg cu experiență.
Materiale și Metodă: S-a efectuat un număr total de 15 transplanturi de hemifață de către cei doi operatori, între șobolani Brown Norway folosiți ca donatori și Wistar folosiți ca primitori: microchirurgul experimentat (grupul II, n=5) și studentul la medicină (grupul III, n=10). Au fost comparate timpul de ischemie caldă și durata totală a intervenției chirurgicale. Toți șobolanii au primit tratament imunosupresor - monoterapie cu ciclosporină A timp de 30 de zile. Rezultatele au fost prelucrate din punct de vedere statistic folosind Microsoft Excel®.
Rezultate: Durata transplantuluie efectuat de către microchirurgul cu experiență a fost inițial de 420 minute și a scăzut după cele 5 intervenții chirurgicale până la 330 minute cu un interval de încredere (probabilitate 95%) de 382±37.9 minute, iar timpul de ischemie caldă a scăzut de la 140 minute la 50 minute, intervalul de încredere fiind de 90±33.52 minute. După transplant șobolanii au fost tratați cu ciclosporină A și monitorizați timp de 30 de zile. Studentul la medicină a avut tendința să realizeze aproximativ aceleași rezultate, după nouă transplanturi efectuate durata totală a intervenției chirurgicale ajungând de la 660 minute la 330 minute, iar timpul cald de ischemie de la 190 minute la 60 minute. Intervalul de încredere (95%) în ceea ce privește durata totală a intervenției chirurgicale a fost de 467±80.66 minute, iar pentru timpul de ischemie caldă a fost de 133.5±31.44 min. Majoritatea șobolanilor (n=11) au supraviețuit în ambele grupuri transplantate (grupurile II și III) de către microchirurg și de către student. Analizând curbele de învățare în funcție de cei doi parametri (durata totală a intervenției chirurgicale și timpul de ischemie caldă) și de rată de supraviețuire nu se constată diferențe din punct de vedere statistic ($p > 0.05$).
Concluzii: Modelul transplantului de hemifață la șobolani reprezintă o metodă folositoare în vederea pregătirii de aplicații experimentale și clinice pentru transplantul de față. Reprezintă o metodă bună pentru pregătirea tinerilor specialiști în vederea viitoarelor transplanturi. Trebuie reținut faptul că studentul la medicină avea cunostințe anterioare de microchirurgie, iar curba de învățare a fost aplicată exclusiv pe acest tip de intervenție chirurgicală. Chiar și un tânar specialist în microchirurgie poate efectua o asemenea intervenție complexă după o perioadă adecvată de pregătire (în studiul nostru după 9 transplanturi consecutive efectuate) în aceeași manieră și cu aceleași rezultate precum ale unui microchirurg cu experiență. Administrarea ciclosporinei A în monoterapie a avut rezultate bune din punct de vedere al imunosupresiei în transplantul șobolanilor pe durata efectuării studiului (30 zile).

Cuvinte cheie: transplant facial la șobolani, educație, curba de învățare, transplant de țesuturi compozite, alografă compozită vascularizată

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Abstract

Introduction: The aim of this study was to emphasize the learning curve of hemifacial transplantation in rats by comparison between 2 operators: medical student trained in basic microsurgery and an experienced microsurgeon.

Materials and Methods: A total number of 15 hemifacial transplants between Brown Norway as donors and Wistar as receiver rats were performed by two operators: experienced microsurgeon (group II, n=5) and the medical student (group III, n=10). Warm ischemia time and operative time were used as instrument for comparison. All the rats received immunosuppressive treatment with cyclosporine A in monotherapy for 30 days. Results were processed statistically using Microsoft Excel.

Results: Transplantation procedure duration time performed by experienced microsurgeon began from 420 min and decreased to 330 min after 5 transplantations, with confidence interval (95% probability) 382±37.9 min and the warm ischemia time decreased from 140 min to 50 min, confidence interval of the warm ischemia time being 90±33.52 min. After transplantation the rats were treated with cyclosporine A and monitored for 30 days. Medical student tended to equalize the operative time and warm ischemia time, approximately, after 9 transplantations, from 660 min to 330 min and warm ischemia time from 190 min to 60 min. The confidence interval (95%) of the procedure by duration of the surgery was 467± 80.66 min and 133.5±31.44 min for the warm ischemia time. Most of the rats (n=11) survived in both transplanted groups (group II and group III) performed by microsurgeon and student. By analyzing learning curves using two parameters (operative time and warm ischemia time) and survival rates no statistically significant difference was found ($p > 0.05$).

Conclusion: Hemifacial transplantation model in rats is a useful tool for preparing experimental and clinical application of the facial transplantation. It is a good model for training young specialists for future transplantation surgery. It is important to notice that the medical student had previous experience in microsurgery and the learning curve was applied only for this specific procedure. Even young specialists in microsurgery could perform such a complex procedure after an appropriate training period (in our study after 9 consecutive transplantations) in the same fashion and with the same results as an experienced microsurgeon. Usage of cyclosporine A as monotherapy gave good immunosuppression results in rats' transplantations for the studied duration of time (30 days).

Key words: facial transplantation in rats, education, learning curve, composite tissue allotransplatation, vascularized composite allograft

Introduction

At this point vascularized composite allotransplantation is a clinical reality. The experience gained from the first VCA until the last facial transplant in humans puts in question the problem of training specialists for such complex transplantation procedures. Microsurgery is a "must know" technique in many surgical fields and, due to frequent usage of small animal models in scientific research it is on high demand. It is a known fact that there is a lack of young doctors that master this technique due to specificity of specialties training (usually during late years of residency, and training in a manner of supra specializations). It was shown that facial transplantation in humans and animals is feasible, despite the fact that ethical problems correlated with such procedures are not yet solved. The incorporation of the face as an allograft is worthy mostly because the face is considered an organ of social and psychological importance and facial deformities cause functional and social isolation (1,2,3). The main difference between VCA and solid organ transplantation (SOT) consists in the existence of a flap of different types of tissues, from which the most allogenic structure is the skin, and then the subcutaneous tissue, fat, muscles, tendons, cartilage, bone, bone marrow, vessels and nerves that form a mix of immunogenic structures that induce a powerful immunologic response, challenging to control. Despite all of these problems multiple cases of successful VCAs can be cited and these achievements open new perspectives in reconstructive surgery.

In this study we extended the training of students from basic microsurgery to an advanced procedure - hemifacial transplantation, the model that was proposed by Demir & all (4,5,6) in order to test the hypothesis if a young microsurgeon could perform a complex procedure in the same fashion and with the same outcome as an experienced microsurgeon. This was performed by comparison in performing the hemifacial transplantation in rats by two operators - a trained microsurgeon and a medical student, previously trained in basic microsurgery. By this we intend to emphasize the learning curve and the potential applicability as a training procedure of this model. At the same time our goal was to gain familiarity with the immunological side of the transplantation (very few residents in plastic surgery are familiar with transplantation procedures) and achievement of immunological tolerance with cyclosporine A in monotherapy. The facial VCA that we transplanted was composed of skin, loose tissue, vessels, fascia, muscle and ear cartilage transferred from Brown Norway to Wistar rat.

Materials and Methods

All the animals in this study were used according to the European Convention on Animal Protection and Laboratory Animal Care guidelines (Directive 609/1986), as well as the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, published by the National Institute of Health. This study was performed in the Microsurgical Laboratory of the Central Emergency Hospital of Bucharest between January and September 2009. The animals were kept in a warm - light source condition. The fur on the head and neck regions was shaved. The skin was cleaned with povidone-iodine 2% solution. We used antibiotic as prophylaxis against infections before the operation and 2 days

after (potassium penicillin 100000UI/kg) administered intramuscular twice a day and Kanamycin ophthalmic ointment topically 2 days after the operations. In order to rehydrate we used saline solution (5 ml, subcutaneously every 4 hours) the first day after operation. Harvested flap was perfused with a cold (4 o C at a height of 135 cm above) heparin solution (1500 UI of Heparin in 500 ml saline solution). After the operation every rat was caged separately under standard conditions. Postoperative analgesia was performed with Meglumin Flunixin (2.5 mg/kg IM) in the first two days after the transplant. Water and food was administered ad libitum. In this study we used Brown Norway and Wistar adult rats, average weight 350 g from the National Research and Development for Microbiology and Immunology Institute "Cantacuzino", Bucharest. Rats were kept under the cycle of 12 hours day-night. Transplantations were performed under magnification of the OPMI® Pico Lab (Carl Zeiss, Gottingen, Germany). A total number of 15 transplants of hemiface were performed against major histocompatibility barriers between Wistar as receiver and Brown Norway rats as donor.

Experimental groups

A total number of 39 rats (20 Wistar and 19 Brown Norway) were used in this study. Rats were divided in 3 experimental groups:

- Group I (5 Wistar and 4 Brown Norway) was studied for the anatomical features and simulations of transplantations by both operators (experienced microsurgeon and medical student).
- Group II (5 Wistar and 5 Brown Norway) a total number of five hemifacial transplants were performed by the experienced microsurgeon (DZ) and treated consequently with standard immunosuppressive therapy - cyclosporine A. The operative time and warm ischemia time were measured.
- Group III (10 Wistar and 10 Brown Norway) a total number of ten hemifacial transplants were performed by a medical student (MC), previously trained in basic microsurgery (>200 hours of practice and courses attended). All the rats received standard immunosuppressive therapy - cyclosporine A. The operative time and warm ischemia time were measured.

Rat anesthesia

Standard anesthesia was performed with ketamine (Calypsol® - 10 ml with 500 mg substance) and Xylazine (Rompun® 2%) in proportion 2:1 (7). Administration pathway of the anesthesia was intramuscular.

Operative technique for the donor rat (the harvesting of the hemifacial flap)

In this study we used an adaptation of the harvesting method proposed by Demir & all (4,5,6). Head and neck of the rat were previously shaved and incision lines were drawn on the left hemiface of the Brown Norway rat. The skin was disinfected with povidone-iodine 2%. Upper and lower eyelids were incised circularly; they were not included in the flap. The skin was incised in the neck region; subcutaneous tissue was dissected until visualizing the external jugular vein. The dissection continued with separation of the external jugular vein from the loose tissue toward the bifurcation into anterior and posterior facial veins. For better visibility and ease of access to profound tissues, after the ligaturing superior thyroid artery and vascular branches of the anterior facial vein, left hemithyroidectomy was performed. Skin incisions continued on the upper part of the skull to the nose, deep towards the periosteum. The flap was detached from the dorsal-anterior side. The semicircular incision made in the perioral area was deepened and levator labii superioris and dilatator naris muscles were cut, vascular branches were ligated and facial artery was visualized. It was included in the flap with the masseteric fascia. Dissection continued toward the ear cartilage, next the dissection was redirected towards the common artery, and the sternocleidomastoidian muscle was excised, common carotid was evidenced and freed from vascular tissues upward. Posterior belly of digastric muscle was transected and freed from its insertion on the hyoid bone; and also the omohyoid muscle was transected. The great horn of the hyoid bone was resected with care, in order not to damage the lingual artery that could cause quick death due to bleeding of the donor rat. Dissection continued exposing all the branches of the common carotid artery, the glossopharyngeal nerve was excised (it crosses the external carotid artery anteriorly). Dissection continued towards the ear, leaving the posterior auricular artery and posterior facial vein in the flap. In the retro-auricular region the internal maxillary vein and its main branch that is drained by the pterygoid plexus was ligated and transected. In the posterior neck region after sectioning of platysma muscle and levator auricle longus, the flap was detached, preserving the posterior auricular vessels. Facial artery, temporal artery, posterior auricular artery and external jugular vein with its branches anterior and posterior facial veins were left in the flap; all the other vessels were ligated and transected (internal carotid, superior thyroidal, ascending pharyngeal, lingual, ascending palatine and internal maxillary arteries). The external ear canal was detached at the osteo-cartilaginous junction and was included in the flap. At the end, the common artery and external jugular vein were divided, and the vascular pedicle of the flap was created. The flap was perfused with heparin saline solution until clear venous outflow was obtained. The donor rat was euthanatized.

Operative technique for the recipient rat

The skin on the left side of the recipient rat was detached as described in (4) superficially on the same patterns as the harvested flap. External jugular vein was isolated and prepared for the anastomosis. Sternocleidomastoidian muscle was transected in order to have large access to the common carotid artery that was prepared for the anastomosis by freeing it from the perivascular tissues located lower than the bifurcation. Vagus and phrenic nerves were left intact. After the rat was prepared, the transplanted flap was attached with several sutures

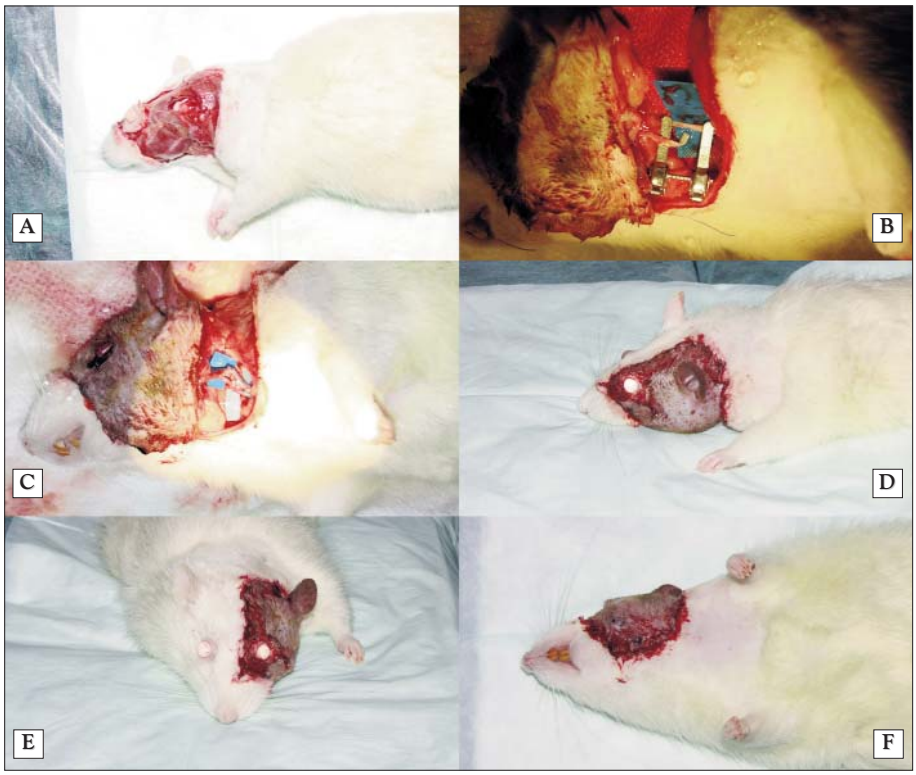


Figure 1. *Operative technique for the recipient rat:* ***(A)*** *the recipient site;* ***(B)*** *end to side carotid arthery anastomosis;* ***(C)*** *end-to-end external jugular vein anastomosis;* ***(D-F)*** *immediate postoperative aspect*

to the recipients' resected area and end-to-end anastomosis of the external jugular vein of the recipient and donor was performed. Next, end-to-side anastomosis of the common carotid artery of the donor flap and the common carotid artery of the recipient was performed. (*Fig. 1A,B,C,D,E,F, 2*) Anastomoses were executed under microscope magnification using Nylon 10-0. The ear canal was closed using Vicryl 5-0 and the skin using Nylon 5-0. After the transplantation the recipient rat received 5 ml of saline solution subcutaneous to compensate the liquid loss (4,5,6).

Immunosuppression end-to-side

Groups number II and III that received hemifacial allografts followed a treatment with cyclosporine A in mono-therapy. It was administered in the beginning with the day 0 in dose of 16 mg/kg until day 7; afterwards the dose was progressively reduced to 2 mg/kg and was maintained at this level.

Clinical evaluation

After the operation, general health and weight of the rats was monitored daily in order to emphasize clinical signs of rejection (erythema, edema, hair loss, desquamation, ulceration and progressive atrophy of the flap, and also the signs of infection or obstruction of the flap vessels).

Statistical analysis

All the data were processed statistically using SPSS program (16^{th} edition) and Microsoft Excel. Paired t test was used in order to compare the groups of transplants. A value of $p < 0.05$ was set as statistically significant.

Figure 2. *One week postop aspect*

Results

Group I

All the rats from this group were carefully dissected and their anatomical structures were identified; photos were made for future recall of the anatomy. Vascular structures were identified and difficult moments of the dissection were emphasized. Anesthetic protocol was tested.

Group II

Five hemifacial transplantations were performed in this group from Brown Norway rats to Wistar rats by the experienced microsurgeon (DZ). Two transplants were necessary in order to reduce the operative time and warm

ischemia time, so at the 5th transplant the operative time was 330 min and warm ischemia time 50 min. The mean duration of the surgery was 382±37.9 (Confidence interval with 95% probability) and the warm ischemia time was 90± 33.52min. All the rats were treated with cyclosporine A and 4 of them survived (80% success) for 4 weeks.

Group III

In this group ten hemifacial transplantations were performed from Brown Norway rats to Wistar by the beginner microsurgeon – medical student (MC). First transplants required a long period of time 660-600 min, about 11 hours, but after 5 transplants the student was able to achieve operative time and warm ischemia time close to those of the experienced microsurgeon. The mean duration of the surgery was 467±80.66 min and the warm ischemia time was 133.5± 31.44 min. In this group all the rats were treated with cyclosporine A for a 4 week period, four rats died (40%), primarily from long time during anesthesia, operative, immunological-stress and infection, 6 rats survived (70% success rate) with good flap condition.

Comparison between Group 2 (n=5) and Group 3 (n=10)

For the ease of comparison we separated group 3 (n=10) into the first 5 operations and the last ones. This way we formed 3 pairs for comparison, by two criteria's, operative time and warm ischemia time. We found no significant difference between the last 5 cases of group 3 and group 2, but there were differences between the first and last 5 in group 3 ($p > 0.05$); we used 2 tailed distributions for paired T-test for this comparison between groups. (*Fig. 3-6*)

Discussions

With every transplant performed, a tendency for the operative time and warm ischemia time to decrease at both operators was noticed and after approximately 9 transplants they tend to equalize. (*Table 1*)

We used in this study the model of hemiface transplantation proposed by Demir & all (4) because of its complexity and combination of skills necessary for it to be performed successfully (flap harvesting, microsurgical anastomosis). The operative duration cited (4) is 3 hours for this procedure; we achieved at best 5.5 hours, more training will probably help us achieve the same duration. Starkes & all (8,9,10) have shown that operative time is a good estimate of the individual's motor skill, however it should not be considered a goal in itself, but a "natural by-product of the result of skill and practice". The ultimate test of successful anastomosis is the vascular patency in the first week. Also, the first week is important for infection and

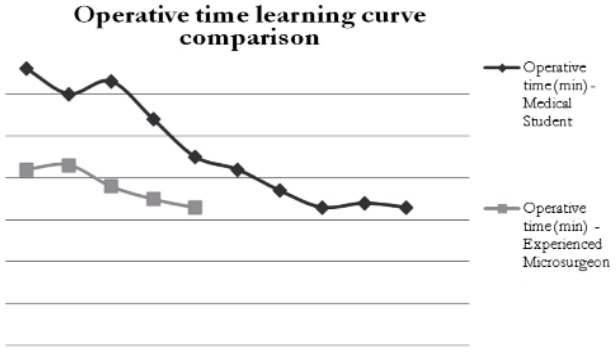
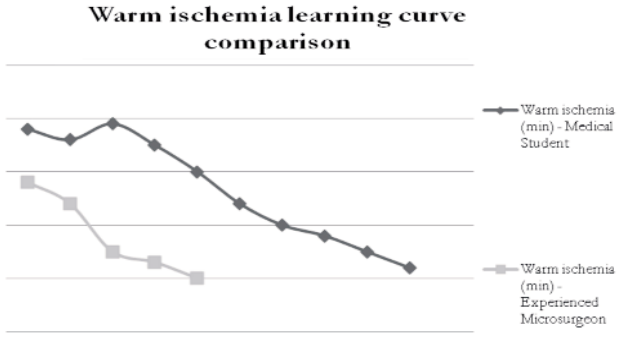


Figure 3.



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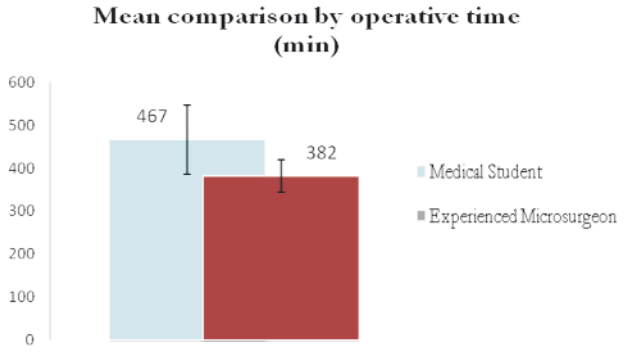

Figure 5.

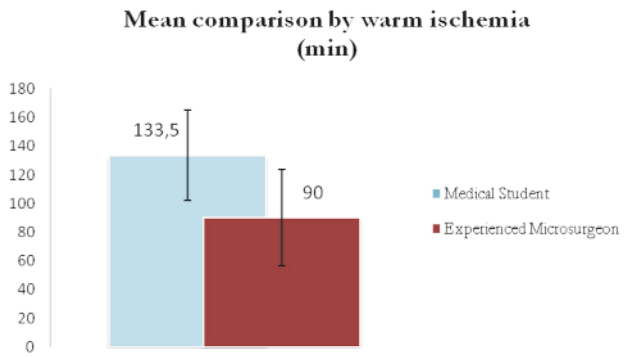

Figure 6.

Table 1.

	1	2	3	4	5	6	7	8	9	10	CI (95%)	T test	
Warm ischemia time	Student	190	180	195	175	150	120	100	90	75	60	133,5±31.4	0.092
	Microsurgeon	140	120	75	65	50	-	-	-	-	-	90±33.5	
Operative time	Student	660	600	630	540	450	420	370	330	340	330	467±80.7	0.08
	Microsurgeon	420	430	380	350	330	-	-	-	-	-	382±37.9	

acute rejection, in this way survival of the animal in our opinion being the best marker of surgical performance. For the comparison of survival rates we used Fisher's exact test, because the total number of observations was less than 20. The survival rates are comparable, and no statistically significant difference was found ($p > 0.05$).

The results of this study are not a surprise for us, our previous study (11) having shown that residents in microsurgery after a period of training could attain the same results as experienced microsurgeons.

Performance of this procedure by only two operators could imply the bios of selection, so more studies should be conducted in order to set a clear conclusion, but it is a challenging procedure and it is hard to find available candidates to be trained and to perform it. However, we believe that it is worth continuing research in this area, because if we were to compare it to the training of young athletes in motor activities, training of young specialists in microsurgery (beginning with medical school) is recommended (12).

Our previous study confirms that an experienced microsurgeon learns new techniques/procedures or recovers after a brake (warm up decrement-typically seen from the beginning) quicker than a beginner microsurgeon (11).

Conclusions

Facial transplantation represents a new surgical procedure that has been in the attention of the medical world and mass-media for the last years. This procedure generated a lot of controversies especially of ethic nature, but the results of the first cases revealed an unexpected functional success that was known worldwide. The experimental research must continue in order to extend the applicability of this concept on a larger scale.

The model chosen by us in order to familiarize with this domain of facial transplantation is a difficult one, due to its microsurgical complexity and duration. This implies experience in microsurgical procedures, in dissection and reconstruction of fine structures and also knowledge in immunology and immunosuppression of the transplant.

As in every other new procedure, there is a training period and also a learning curve, and after you pass this step, the transplant can be performed relatively safe.

Immunosuppression with cyclosporine A in monotherapy did not present any problems, protection against rejection was obtained in most of the cases.

Anesthesia is very important in realizing such a long procedure.

There is a learning curve of this procedure. After 9 transplantations, the duration of the surgery becomes to equal with the one that is performed by an experienced microsurgeon. After 9 transplants the warm ischemia time becomes equal with the one performed by experienced microsurgeon. It is important to mention that the student that performed the transplantations had previous experience in experimental microsurgery and the learning curve was applied only for this specific transplantation model.

We would like to emphasize the importance of training in microsurgical techniques in preparing the complex experimental model (knowledge of anatomy, dissection and simulation of entire procedure). This allows a student that possesses necessary knowledge and skills in basic microsurgery to achieve grasp of this procedure after a period of training.

The experimental model of facial transplantation is a mandatory and essential step in preparing clinical application of facial transplantation in humans.

Acknowledgments

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