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A new composite midface allotransplantation model with sensory and motor reinnervation

Fatih Zor,[1] Mehmet Bozkurt,[1] Dileep Nair[2] and Maria Siemionow[1]

1 Department of Plastic and Reconstructive Surgery, Cleveland, OH, USA
2 Department of Neurology, Cleveland Clinic, Cleveland, OH, USA

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Correspondence
Maria Siemionow MD PhD DSc, Department of Plastic Surgery, Cleveland Clinic, 9500 Euclid Avenue, A-60, Cleveland, OH 44195, USA. Tel.: 216 445-2405; fax: 216 444-9419; e-mail: siemiom@ccf.org

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Summary

In this study, we extended application of face transplantation model in rat by incorporation of vascularized premaxilla, and nose with infraorbital and facial nerves for evaluation of allotransplanted sensory and motor nerve functional recovery. In group I ($n = 3$) the dissection technique is studied. In group II ($n = 5$) isotransplantations were performed. In group III ($n = 5$) allotransplantations were performed under Cyclosporin A monotherapy. Grafts; composed of nose, lower lip, and premaxilla; were dissected. Infraorbital nerve and facial nerve were included into the transplant. A heterotopic transplantation was performed to inguinal region of recipient. Nerve coaptations were performed between infraorbital-sapheneous nerve and facial-femoral nerve. CT scan, somatosensory-evoked potential testing (SSEP), motor-evoked potential testing (MEP), and microangiography were used for evaluation. All transplants survived indefinitely over 100 days. Microangiography showed preserved vascularization of the graft. Computed tomography revealed vital premaxillary bone segments. SSEP and MEP confirmed recovery of motor and sensory functions and latencies reached 67% of normal infraorbital nerve value and 70% of normal facial nerve value at 100 days post-transplant. We have introduced new midface transplant model of composite midface allograft with sensory and motor units. In this model, motor and sensory functional recovery was confirmed at 100 days post-transplant.

Introduction

Reconstruction of composite defects of the central face including functional and esthetic units such as nose is always challenging for the reconstructive surgeon. As a prominent and defining feature of the face, nose is a complex structure composed of skin, mucosa, cartilage, muscular and subcutaneous tissues, and bone. For this reason, defects of multiple subunits of the face, including nose and lips, are difficult to recreate using conventional autologous reconstructions. Introduction of composite tissue allotransplantation (CTA) opens new reconstructive options for such challenging defects of central face [1–3].

Most experimental studies on face transplantation were tested in the rat model [4,5]. Surgical viability [6,7], tolerance induction [6,8,9] technical aspects of the full-face and the hemifacial transplants [6,8] and facial subunit transplants [10] with long-term survival have been reported. Most of the studies on CTA are focused on the immunologic aspects of transplants and prevention of allograft rejection [11]. There are only a few studies testing functional (motor and sensory) recovery following CTA [12,13]. The aim of this study was to extend the application of our face transplantation model in the rat by incorporation of vascularized premaxilla, and nose with infraorbital and facial nerves for evaluation of functional recovery of allotransplanted sensory

and motor nerves in this new model of midface transplantation.

Material and methods

Animals and animal groups

Eight- to 10-week-old inbred rats were used for transplants (Harlan, Indianapolis, IN, USA). In group I (anatomic study group, $n = 3$), Lewis ($RT1^1$) rats were used for anatomical dissections of the midface flap for becoming familiar with the anatomy, perform microangiographic studies and to develop the midface transplant model in rat. In group II (isograft transplantation group) five isograft transplantations were performed between Lewis ($RT1^1$) rats. In the third group (allograft transplantation group) five allogenic transplantations was performed across the major histocompatibility barrier between Lewis-Brown Norway (LBN, $RT1^{1+n}$) donors and Lewis ($RT1^1$) recipient rats.

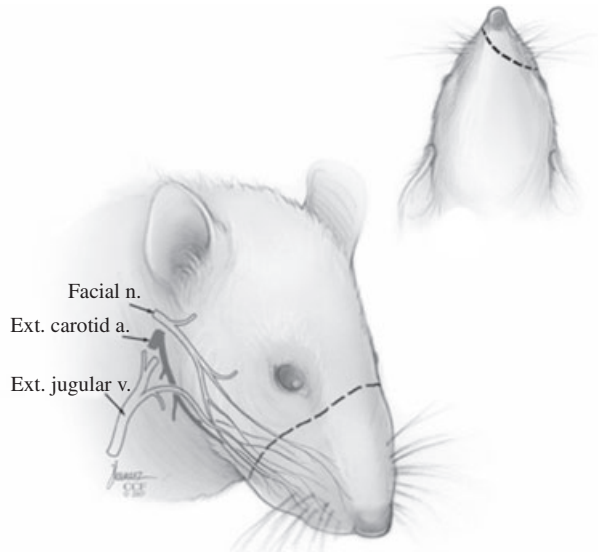

Figure 1 Illustration showing the design of composite midface graft.

Surgical technique

All operations were performed under sterile conditions. Anesthesia was induced with sodium pentobarbital (50 mg/kg), which was administered intraperitoneally, and maintained with dose of 10 mg/kg/h. Dissections and microvascular anastomoses, and nerve coaptations were performed under operating microscope magnification (Zeiss OP-MI 6 SD; Carl Zeiss, Goettingen, Germany).

Preparation of the donor

Harvesting technique of the composite osteocutaneous midface graft with motor and sensory nerves including their target organs as units. Grafts composed of nose, lower lip, masseter muscle and premaxilla; were dissected based on the same vascular pedicle of common carotid artery and external jugular vein (Fig. 1). A vertical midline skin incision was made from the anterior neck to the lower lip. The submandibular gland was excised after ligation of the glandular branches of facial artery and vein. External jugular vein and its two main branches, the anterior and posterior facial veins, were dissected and preserved. The sternocleidomastoid muscle was detached from its sternoclavicular and mastoid insertion and excised to expose the common carotid artery and its main branches, the external and internal carotid arteries. The anterior and posterior bellies of the digastric muscle were excised, the omohyoid muscle was transected and greater horn of the hyoid bone was excised for better visualization of external carotid artery and its branches. Internal carotid artery, superior thyroid artery, ascending pharyngeal artery, ascending palatine artery, superficial temporal artery, posterior auricular artery, lingual artery and internal maxillary artery in the flap were ligated and transected. Only anterior facial artery was preserved and included into the vascular pedicle in this model.

The surgical plane of dissection was kept below the masseter muscle. The flap was elevated from anterior midline incision at the neck to lateral and anterior directions including the masseter muscle and the right hemi lower lip. The masseter muscle was included into the graft to avoid iatrogenic damage to the branches of facial nerve during dissection. The midface transplant included whole nose and premaxillary bone segment with hard palate and teeth.

The facial nerve was transected at the stylo-mastoid foramen and infraorbital nerve was transected at the level of infraorbital fissure and both nerves were included into the midface graft. The dissection was then carried around the nose, upper lip, right hemi lower lip and right mystacial pad. Finally, periosteum was incised and premaxillary bone was transected transversely using a burr. The composite midface graft was raised on the common carotid artery and external jugular vein (Figs 2 and 3).

Preparation of the recipient

For humanitarian reason of major complications and for animal survival, a heterotopic transplantation was performed to the inguinal region of the recipient rat. Skin incision was made at left inguinal region of the recipient rat medial to the inguinal crease. Under an operating microscope magnification, the femoral vessels were

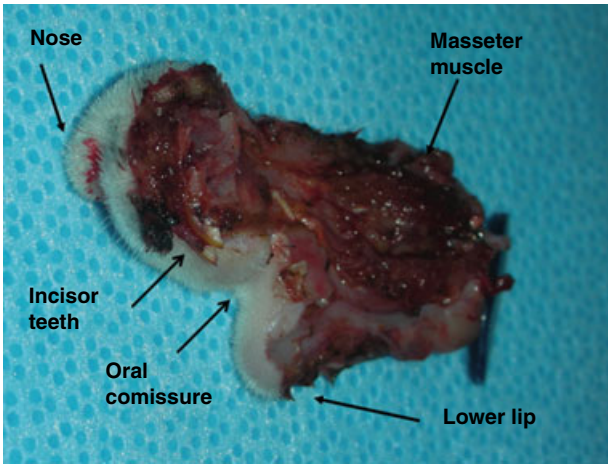

Figure 2 Composite midface graft before transplantation. Masseter muscle, incisor teeth, nose, lower lip and oral commissure are shown.

exposed and dissected from the inguinal ligament and prepared for microsurgical anastomosis. Femoral nerve and saphenous nerve were transected and prepared for nerve coaptation.

Transplantation procedure of the composite midface graft

The donor graft was placed in the recipient's inguinal region and was fixed with few stay sutures before vessel anastomoses were performed. Venous anastomosis was performed first using standard end-to-end microsurgical technique between the external jugular vein of the donor and femoral vein of the recipient. Next, end-to-end anastomosis was performed between common carotid artery of the recipient and femoral artery of the donor under operating microscope magnification using 10/0 nylon

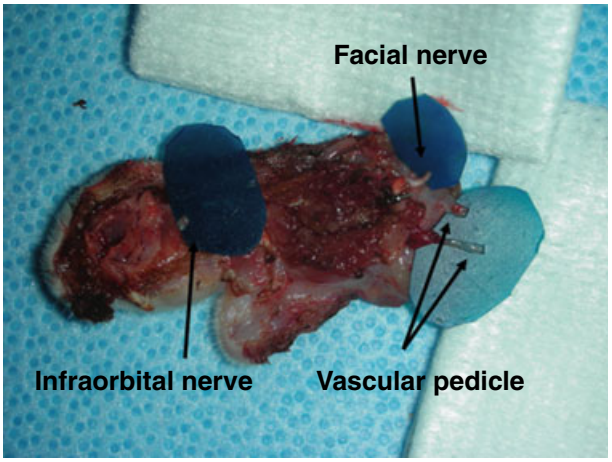

Figure 3 Neurovascular pedicle of the graft before transplantation.

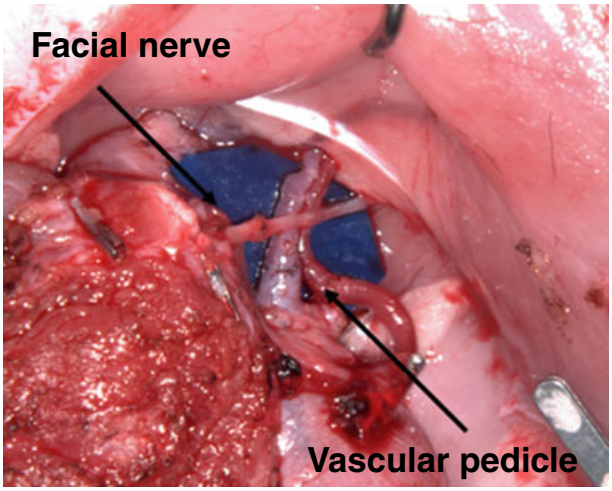

Figure 4 End-to-end anastomoses of common carotid artery and external jugular vein to femoral vessels.

sutures. The clamps were released and ischemia time was kept under 45 min (Fig. 4). Following perfusion of the graft, standard epineural neurorraphies were performed between infraorbital nerve of donor and sapheneous nerve of the recipient and between facial nerve of the donor and femoral nerve of the recipient with 10/0 nylon sutures (Fig. 5). To maximize the period of animals' survival, we have reduced surgery time of recipient rat to total of 120 min, minimized blood loss and maintained meticulous fluid resuscitation. Also, during the final skin closure, we avoided mucosa embedment into the inguinal region of the rat. Mucous collections from the nasal mucosa were drained daily for the first week at which time they subsided.

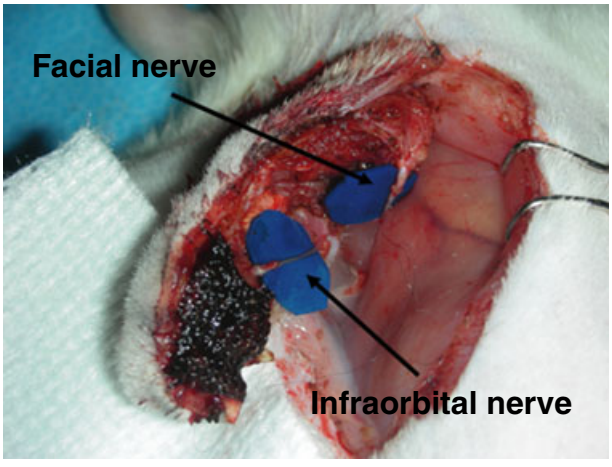

Figure 5 Nerve coaptations: facial nerve to femoral nerve and infraorbital nerve to saphenous nerve.

Immunosuppression protocol

After transplantation, animals in the allograft treatment group (group III) received cyclosporin A (CsA) monotherapy, tapered from 16 mg/kg/day to 2 mg/kg/day over 21 days, and maintained at this level over the entire follow-up.

Clinical evaluation and assessment methods

Animals were evaluated in the immediate postoperative period for graft loss, arterial or venous compromise, hematoma or seroma formation and general health. Return of motor function was evaluated by observation of the return of movement to the mystacial pad. In addition, allografts were monitored for clinical signs of rejection including erythema, edema, desquamation, and necrosis.

Microangiography: Microangiography was performed to confirm preservation of vascular territories, using technique described by Rees *et al.* [14]. Briefly, intra-arterial infusion of lead oxide-gelatin mixture was performed via catheterization of the common carotid artery. Then the composite midface allograft specimen was harvested and underwent radiography with a soft X-ray machine (Mammo Diagnost UC, Philips, Hamburg, Germany) at the settings of 22 kV and 5 mAs.

Spiral computed tomography: To confirm normal premaxillar bone structure and a lack of bone absorption or necrosis over time, four rats (two from each group) were evaluated at 100 days post-transplant by spiral computed tomography.

Somatosensory-evoked potential (SSEP): Somatosensory-evoked potential analysis was performed at 100 days post-transplant for the evaluation of the sensory recovery of the composite midface allotransplant, while the rat was under pentobarbital anesthesia. A Nihon Kohden Neuropack MEB-2200 evoked potential electromyograms (EMG) machine (Tokyo, Japan) was used for testing. Band pass filter of 30–1500 Hz was used and the grain was set at 3000. Stimulus duration was 200 μs, stimulus intensity was at motor threshold, stimulus frequency of 2.7 per second and each response was replicated at least once. A display of 100-ms window was used. The two stimulating electrodes were placed on the mystacial pad of the composite midface flap. The ground electrode was placed into subcutaneous plane of the tail. Next a midline scalp incision of 2 cm in length was performed on the sagittal suture and the parasagittal regions of the parietal bones were exposed. Two burr holes were drilled on both sides of the sagittal suture and the recording electrodes were placed through these holes over the dura of the parietal cortex. The cortical responses were recorded with each average consisting of 300 trials. The waveform morphology consisted of a series of negative and positive potentials in the SSEP measurements. An initial negative wave (N1) was followed by a positive waveform (P1) and a second negative waveform (N2) in a characteristic waveform pattern. Because the P1 and N2 waveforms are the most robust and consistent potentials, these latencies were used to compare sensory recovery between different treatment groups.

Motor-Evoked potentials (MEPs): For MEPs, anodal stimulation was applied epidurally to both sides of motor cortex. An electrode was inserted to the abdominal muscles and was used for reference. The stimulating intensity was carried at 18 mA of constant current, duration 80 μs. Anodal stimulation was performed from right motor cortex and MEPs were recorded using needle electrodes inserted into facial animation muscles of midface graft transplanted to left inguinal region of the recipient rat. The recording reference electrode was inserted 1 cm distal to the active electrode. A band pass filter 100–5000 Hz was used. For each response, peak-to-peak amplitude (μV) at a latency of 5–20 ms post stimulus was calculated.

Results

In group I, three composite midface grafts were harvested and preservation of the vascular network was confirmed by microangiography. The facial artery was found to be well-perfused and all vascular territories were intact (Fig. 6).

A total of 10 composite midface transplantations (five isografts and five allografts) were performed in groups II and III. Transplantation procedure required an average of 4 h to complete and the ischemia time was approximately 45 min. All transplant recipients are alive and still under observation (120, 123, 112, 121, 135 and 142 days).

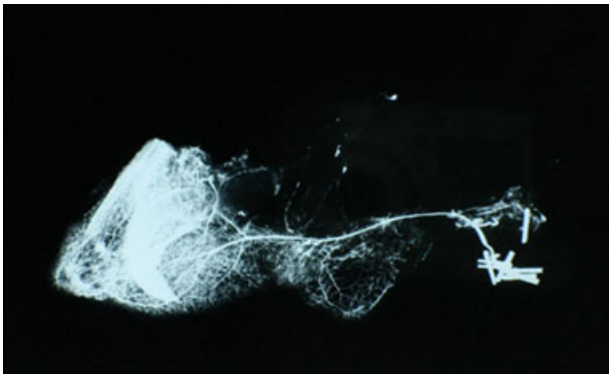

Figure 6 Microangiography of the composite midface graft showing intact vascular territories of the entire graft.

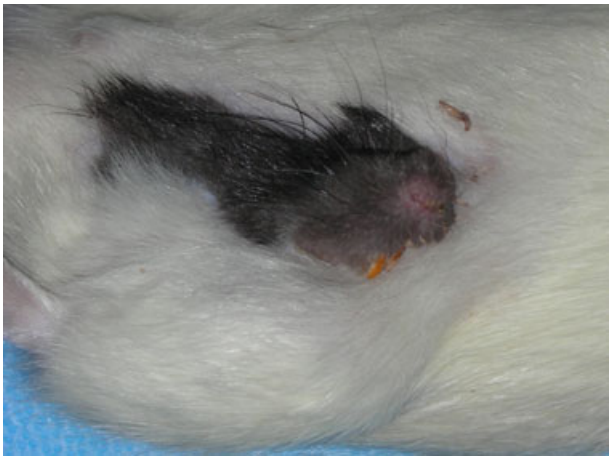

Figure 7 Composite midface allograft transplantation at 100 days post-transplant.

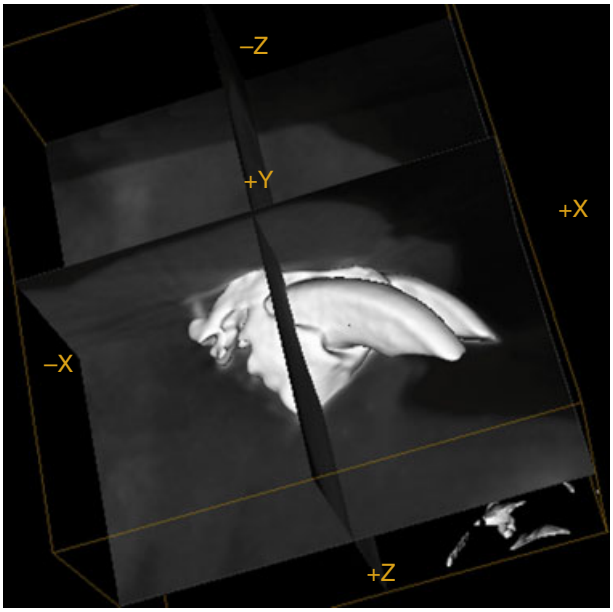

Figure 8 Computed tomography of composite midface allograft, showing persistence of premaxillary bone at 100 days post-transplant.

Follow-up and survival

All transplanted grafts in group II and III survived indefinitely (Fig. 7). We have not observed any vascular compromise in any of the transplants. Successful midface transplantation was accomplished in all 10 animals, with 100% graft survival over 100 days. All animals tolerated surgery well and returned to their normal activities first day after transplantation. The body weights were stable, and we did not observe any sign of infection. Clinically, all grafts were pink and pliable during the entire observation period. The incisors continued to grow; teeth buds, bone, cartilage, and mucosa remained intact. Motor recovery was observed at 21 days post-transplant in both iso- and allograft groups and was confirmed by the movement of the mystical pad. New hair growth was observed within 20–25 days post-transplant. No self mutilation of the flap was observed. Although it has been known that bone atrophies could be expected resulting from lack of muscle tone, as in this heterotopic as well as nonfunctional models, premaxillary component of the midface graft could be easily palpated in animals at all time-points after transplantation.

Computed tomography of composite midface flap showed persistence of normal premaxillary bone (Fig. 8). The quality of bone density of the isografts and the allografts were similar.

Somatosensory-evoked potential evaluation tests confirmed that at day 100 post-transplant, stimulation of the mystacial pad of the transplanted composite midface graft revealed cortical responses recorded in the somatosensory cortex of the recipient rat. In group III (allotransplantation group), mean latency times (P1–N1) of the normal saphenous nerve and normal infraorbital nerve were 16.4 ± 0.8 ms and 9.3 ± 0.6 ms respectively. Mean latency time for the sensory nerve of the midface graft (saphenous nerve to infraorbital nerve) was found to be 23.1 ± 1.5 ms. We have also observed clinically evasive behavior and defense reactions when the transplanted whiskers were pulled. These confirmed the successful afferent innervation of the midface allograft.

Motor movements of the mystacial pad began as early as 20 days post-transplant and recovery progressed during the entire follow-up. MEP tests at 100 days post-transplant confirmed the motor reinnervation of the transplanted grafts by recordable MEPs. In allotransplantation group (III), mean amplitudes of normal facial and femoral nerves were 2.9 ± 0.3 mV and 3.8 ± 0.7 mV respectively. Mean amplitudes of the motor nerve of the midface graft was found to be 2.2 ± 0.5 mV. Results of the electrophysiological studies (SSEP and MEP) are summarized in Table 1.

Discussion

Reconstruction of the composite defects of the perioral and nasal area continues to be a challenging task in plastic surgery. Face transplantation provides the best tissue to reconstruct 'the like' with 'the like' and allows complying with Gillies' principles. The successful outcome of CTAs such as the face, hand, and larynx revealed that allotransplantation of facial subunits may be an alternative reconstructive option in the near future. Face

Table 1. Electrophysiological evaluation in groups II and III at 100 days postoperative.

	SSEP						MEP					
	Saphenous nerve*		Infraorbital nerve*		Saphenous to infraorbital nerve		Femoral nerve*		Facial nerve*		Femoral to facial nerve	
	Lat	Amp	Lat	Amp	Lat	Amp	Lat	Amp	Lat	Amp	Lat	Amp
Group II (n = 5)	15.2 ± 0.5	1.3 ± 0.3	9.4 ± 0.3	2.7 ± 0.6	22.4 ± 1.7	1.4 ± 0.5	13.9 ± 0.9	3.6 ± 0.7	10.2 ± 0.9	3.3 ± 0.4	23.1 ± 1.5	2.0 ± 0.8
Group III (n = 5)	16.4 ± 0.8	1.4 ± 0.2	9.3 ± 0.6	2.6 ± 0.3	23.1 ± 1.5	1.2 ± 0.4	14.7 ± 0.9	3.8 ± 0.7	9.6 ± 0.5	2.9 ± 0.3	21.8 ± 1.3	2.2 ± 0.5

SSEP, somatosensory-evoked potential; MEP, motor-evoked potential; Lat, mean latency in ms; Amp: mean amplitude in mV.
The values of latencies and amplitudes are presented as mean ± SD.
*Recorded from contralateral side.

transplantation includes multi-functional, sensorimotor subunits. Thus, sensory and motor recovery of these facial subunits has paramount importance for the final clinical outcome. The evidence of recovery of facial function after face transplantation in animal models will support expected functional outcomes in humans. There is however limited access to the functional facial transplantation models. We have introduced a face transplantation model in rats and established anatomic considerations and immunological protocols; however, these models were not functional models [6,7].

In this study, we have introduced a new functional midface transplantation model in rat by incorporation of the bone segment of vascularized premaxilla, and sensory and motor units of the midface for quantitative evaluation of functional recovery. In 2005, Ulusal *et al.* [10] described auricle transplantation model with sensory reinnervation. They evaluated sensory recovery by reaction of animals to the pain stimulus induced by pinching. Later, in 2008, Landin *et al.* [15] described transplantation model of functional facial subunit. They reported on allotransplantation model, which included branches of facial nerve and infraorbital nerve to mystacial pad and evaluated neurophysiological and histological recovery of the graft [12]. Sensory evaluation was performed only by pulling rat whiskers and motor evaluation was performed by electroneurograms of the facial nerve and EMG of the mystacial muscles. Washington *et al.* [13] reported a study on hemifacial transplant model evaluating motor and sensory recovery using nerve conduction test and cortical test respectively. Our model differs from both of these studies as we have performed quantitative evaluation of both sensory and motor recovery. Sensory recovery was monitored by SSEP recordings. To the best of our knowledge, this is the first study reporting quantitative sensory recovery in facial CTA. The anatomical studies on rat mystacial pad showed that it consists of external and internal muscles. The internal muscles serve as piloerector muscles. They are small muscles and their origin and insertion are both at the soft tissue level. The external muscles are larger and their origins are at the maxilla and the insertions are in the skin [16]. In contrast to models described by Landin and Washington [13,15], which contained only soft tissue components, in our midface transplant model we have included bone segment of premaxilla, which is the origin of the external muscles of the mystacial pad. By including premaxilla and by preserving the origin of the extrinsic muscles of the mystacial pad, we were able to measure motor recovery of the extrinsic muscles. We believe that, these measurements give more accurate estimation of graft recovery when compared with recordings taken only from small intrinsic muscles.

Nose, along with mystacial region, is an important facial subunit in rats. It not only contains the sensory and motor units but also several tissues such as bone, mucosa, skin, and teeth. The main sensory nerve of nose and mystacial pad in rats is the infraorbital nerve and motor innervation is provided through the facial nerve and its branches [16,17]. In our model, nose and mystacial pad, including facial nerve and infraorbital nerves, were harvested as a composite midfacial graft based on common carotid artery and external jugular vein. Our model is not technically challenging and presents low complication rate. This model will be applicable for studies of central face component transfers, including functional subunits. As this model includes skin, mucosa, teeth, nose, maxillary components with motor and sensory reinnervation, it makes it an appealing transplant model for both functional outcome and immunological studies [18]. In CTA, each tissue has different relative antigenicity and presents different healing pattern. The isotransplantation may be considered simply as the functioning free flap. However, in allotransplantation model, the functional recovery is much more complex, as skin which contains the sensory nerve endings is the most allogenic tissue prone to graft rejection. As a result, the overall functional outcome is a combination of both allogenic nerve healing and response of allotransplanted sensory-motor units within the skin component of the graft. The functional outcome in our study has demonstrated both the recovery of the allogenic nerve, as well as the restoration of function of the allotransplanted end-organs, neuromuscular junctions and somatic sensory nerve endings.

The long-term (over 100 days) survival with functional recovery was achieved in this composite midface allograft under low-dose CsA monotherapy and also confirmed the feasibility of SSEP and MEP recordings for the assessment of allograft functional recovery.

As is the case with every experimental model, this midface transplant model in rat has its limitation. Rat models of face transplantation are difficult to manage as nose, lips and eyelids must be preserved in the transplant recipient. As they provide critical functions such as breathing, feeding and blinking and they have to be preserved in order to keep the animals alive. For this reason, in this study, we have performed a heterotopic transplantation of the midface graft to the inguinal region of the recipient rat. Femoral neurovascular bundle and saphenous nerve were used for vessel and nerve repairs. This allowed for graft monitoring without scarifying vital functional units of the recipient rat face.

The monitoring electrodes were placed between locations of the sciatic nerve end-organs and the mystacial pad and epidural recordings were performed. Once the model is well established, we are planning multifocal recordings of rat homunculus that could be recorded to understand whether the graft was reintegrated into the facial area or the implantation site.

Several immunosuppressive agents are used in experimental face transplantation models. CsA alone or in combination with different antibodies is the most frequently used agent to prevent rejection. There are several studies demonstrating that tacrolimus enhances nerve regeneration and accelerates functional return following nerve repair [12,19,20]. Landin *et al.* [12] performed facial allograft transplantation with motor nerve coaptation and they used tacrolimus as immunosuppressive agent. In current study of midface allograft model, we used our established immunotherapy protocol of tapered CsA monotherapy, which was tested in our previous models of face transplants. However, we are considering using tacrolimus in the future studies once the model is established.

Our clinical observations showed that motor and sensory recovery started at 21 days post-transplant. Under light ketamine anesthesia, we observed movements in the mystacial pad and defense reactions when the whiskers were pulled. As the animals were under anesthesia, it was most likely an intrinsic reflex rather than a central response. To evaluate sensory and motor recovery, SSEP and MEP were used respectively. At 100 days post-transplant, SSEP and MEP tests revealed that sensory and motor recovery reached 67% of normal latency values for infraorbital nerve and 70% for facial nerve latency values. This is close to clinical results of nerve recovery after limb replantations or hand transplantations [21,22].

An ideal reconstructive procedure should replace the missing tissues and restore motor and sensory functions. Composite osseomusculocutaneous midface allograft transplantation in clinical practice could provide several advantages over currently available reconstructive procedures. It provides option for single-stage reconstruction of the central part of the face including functional subunits allowing for sensory and motor recovery. Our results are encouraging, and potential clinical applications will be rewarding for patients with complex midfacial deformities.

Authorship

FZ and DN: research design, performance of the research, writing of the paper, and data analysis. MB: research design. MS: research design, writing of the paper, and data analysis.

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References

1. Gander B, Brown CS, Vasilic D, *et al.* Composite tissue allotransplantation of the hand and face: a new frontier in transplant and reconstructive surgery. *Transpl Int* 2006; **19**: 868.
2. Petit F, Minns AB, Dubernard JM, Hettiaratchy S, Lee WP. Composite tissue allotransplantation and reconstructive surgery: first clinical applications. *Ann Surg* 2003; **237**: 19.
3. Gordon CR, Nazzal J, Lozano-Calderan SA, *et al.* From experimental rat hindlimb to clinical face composite tissue allotransplantation: historical background and current status. *Microsurgery* 2006; **26**: 566.
4. Nasir S, Klimczak A, Sonmez E, Bozkurt M, Gibson S, Siemionow M. New composite tissue allograft model of vascularized bone marrow transplant: the iliac osteomyocutaneous flap. *Transpl Int* 2010; **23**: 90.
5. Chang KP, Huang SH, Lin CL, Chang LL, Lin SD, Lai CS. An alternative model of composite tissue allotransplantation: groin-thigh flap. *Transpl Int* 2008; **21**: 564.
6. Siemionow M, Ulusal BG, Ulusal AE, *et al.* Functional tolerance following face transplantation in the rat. *Transplantation* 2003; **75**: 1607.
7. Ulusal BG, Ulusal AE, Ozmen S, *et al.* A new composite facial and scalp transplantation model in rats. *Plast Reconstr Surg* 2003; **112**: 1302.
8. Siemionow M, Bozkurt M, Kulahci Y. Current status of composite tissue allotransplantation. *Handchir Mikrochir Plast Chir* 2007; **39**: 145.
9. Siemionow M, Klimczak A. Advances in the development of experimental composite tissue transplantation models. *Transpl Int* 2010; **23**: 2.
10. Ulusal AE, Ulusal BG, Hung L-M, *et al.* Establishing a composite auricle allotransplantation model in rats: introduction to transplantation of facial subunits. *Plast Reconstr Surg* 2005; **116**: 811.
11. Horner BM, Randolph MA, Huang CA, Butler PE. Skin tolerance: in search of the Holy Grail. *Transpl Int* 2008; **21**: 101.
12. Landin L, Cavadas PC, Gonzalez E, Rodriguez JC, Caballero A. Functional outcome after facial allograft transplantation in rats. *J Plast Reconstr Aesthet Surg* 2008; **61**: 1034.
13. Washington KM, Solari MG, Sacks JM, *et al.* A model for functional recovery and cortical reintegration after hemifacial composite tissue allotransplantation. *Plast Reconstr Surg* 2009; **123**: 26S.
14. Rees MJ, Taylor GI. A simplified lead oxide cadaver injection technique. *Plast Reconstr Surg* 1986; **77**: 141.
15. Landin L, Cavadas PC. The Mystacial pad flap A functional facial flap. *Ann Plast Surg* 2005; **56**: 107.
16. Josef Dörfl. The musculature of the mystical vibrissae of the white mouse. *J Anat* 1982; **135**: 147.
17. Josef Dörfle. The innervation of mystacial region of the white mouse: a topograhical study. *J Anat* 1985; **142**: 173.
18. Lee WP, Yaremchuk MJ, Pan YC, Randolph MA, Tan CM, Weiland AJ. Relative antigenicity of components of a vascularized limb allograft. *Plast Reconstr Surg* 1991; **87**: 401.
19. Gold BG, Katoh K, Storm-Dickerson T. The immunosuppressant tacrolimus increases the rate of axonal regeneration in rat sciatic nerve. *J Neurosci* 1995; **15**: 7509.
20. Jost SC, Doolabh VB, Mackinnon SE, *et al.* Acceleration of peripheral nerve regeneration following tacrolimus administration. *Restor Neurol Neurosci* 2000; **17**: 39.
21. Machetanz J, Röricht S, Gress S, Schaff J, Bischoff C. Evaluation of clinical, electrophysiologic and computed tomographic parameters in replanted hands. *Arch Phys Med Rehabil* 2001; **82**: 353.
22. Kvernmo HD, Gorantla VS, Gonzalez RN, Breidenbach WC. Hand transplantation A future clinical option? *Acta Orthop* 2005; **76**: 14.