Face transplantation in rats. Reproducibility of the experimental model in Brazil[1]

Fábio de Freitas Busnardo[I], Pedro Soler Coltro[II], Marcelo Vitoriano Olivan[III], Guilherme Cardinali Barreiro[III], Rachel Rossine Baptista[IV], Marcus Castro Ferreira[V], Rolf Gemperli[VI]

DOI: http://dx.doi.org/10.1590/S0102-86502014000800009

[I]PhD, Surgeon-in-Chief, Department of Surgery, Plastic Surgery Division, Faculty of Medicine, University of Sao Paulo (USP), Brazil. Conception and design of the study, critical revision, final approval of the version to be published.
[II]Fellow PhD degree, Postgraduate Program in Surgical Clinic, Department of Surgery, Plastic Surgery Division, Faculty of Medicine, USP, Sao Paulo-SP, Brazil. Acquisition of data, manuscript preparation.
[III]Fellow PhD degree, Postgraduate Program in Surgical Clinic, Department of Surgery, Plastic Surgery Division, Faculty of Medicine, USP, Sao Paulo-SP, Brazil. Acquisition of data.
[IV]Resident, Department of Surgery, Plastic Surgery Division, Faculty of Medicine, USP, Sao Paulo-SP, Brazil. Acquisition of data.
[V]Full Professor, Department of Surgery, Plastic Surgery Division, Faculty of Medicine, USP, Sao Paulo-SP, Brazil. Scientific and intellectual content of the study, critical revision.
[VI]Associate Professor, Head, Department of Surgery, Plastic Surgery Division, Faculty of Medicine, USP, Sao Paulo-SP, Brazil. Scientific and intellectual content of the study, critical revision, final approval of the version to be published.

ABSTRACT

PURPOSE: To investigate the reproducibility of the experimental model of face allotransplantation in rats in Brazil.

METHODS: Eighteen rats were operated, nine-nine donors recipients. Animals underwent transplantation of the left hemiface, with periorbital and scalp. Transplants were made from donor Wistar rats to recipients Lewis rats. Flaps were based on the common carotid artery and the external jugular vein of the donor animal and the anastomosis in the recipient area was performed in common carotid artery (end-to-side) and in external jugular vein (end-to-end).

RESULTS: Of the nine recipient animals operated, six survived and three progressed to death in the first days after surgery (survival rate = 67%). The mean time of the procedure was 252 minutes and the mean time of flap ischemia was 95 minutes. The five surviving animals were sacrificed at 14 days, in good general condition and without signs of tissue rejection.

CONCLUSIONS: The experimental model of face allotransplantation in rats is reproducible in our midst. Duration of surgery, time of flap ischemia, animal survival rate and complications observed were similar to those described in the literature.

Key words: Models, Animal. Facial Transplantation. Rats.

Introduction

Some facial defects are complex, with large proportions, and may occur due to traumatic injuries, severe burns or extensive oncologic resections, leading to an extensive tissue loss involving skin, soft tissue (subcutaneous tissue, muscles, vessels and nerves), osteocartilaginous structures and facial functional subunits (nose lips and eyelids)[1-3]. This leads to three-dimensional defects with a difficult reconstruction, that require multiple surgical procedures[1,4,5]. Reconstructive surgery of the face aims to restore anatomical normality to obtain satisfactory aesthetic and functional results[2,6].

Recently, a new approach to the treatment of complex facial defects has been proposed: the face transplantation. It consists of an allogenic flap formed by heterogeneous tissues of the face such as skin, muscle, bone and other supplied by a pedicle. This procedure has become a viable option, with many successful cases around the world[1,4,7,8]. The applicability of composite tissue allotransplantation has been made possible by the establishment of microsurgical techniques, the recent advancement of immunosuppression regimes, by anatomical cadaver studies and development of experimental animal models[9-18].

Most research on face transplants have been performed in experimental models, which use various animals, especially rats. In 2003, it was described the first experimental model of face transplant in rats (the face of the donor animal to the face of the recipient animal)[19]. Since then, several other models have been proposed, with emphasis on the use of only a hemiface in order to simplify the procedure and make it faster, without prejudice to studies of immunosuppressive and tolerance induction[20].

The aim of this study was to test the reproducibility of the experimental model of face allotransplantation in rats in Brazil.

Methods

Study conducted at the Plastic Surgery and Microsurgery Laboratory, Division of Plastic Surgery, Faculty of Medicine, University of Sao Paulo, Brazil. All animals were treated strictly following the principles of Brazilian laws 9.605/98 and 11.794/08, the Ethical Principles in Animal Experimentation of CONCEA (National Council for the Control of Animal Experimentation) and the Principles for Research Involving Animals (Geneva, 1985). This study received previous approval of the local Ethics Committee (302/10).

Eighteen rats were operated (weight 300-350g), nine-nine donors recipients. Anesthesia was induced with intraperitoneal pentobarbital sodium and dissections and vascular anastomoses were performed with an optical microscope Zeiss x40 magnification, using mononylon 10-0. After the procedure, receptors rats remained in the vivarium and received analgesia with buprenorphine in the first two days.

Animals underwent transplantation of the left hemiface, with periorbital and scalp. The transplants were made from donor Wistar rats to recipients Lewis rats. Flaps were based on the common carotid artery and the external jugular vein of the donor animal and the anastomosis in the recipient area was performed in common carotid artery (end-to-side) and in external jugular vein (end-to-end).

Immunosuppression was adopted to prevent acute rejection, using cyclosporin A (16 mg/kg/day) for the first 14 days, when the animals were sacrificed by lethal intraperitoneal dose of pentobarbital (100 mg/kg).

Statistical analysis was performed using Microsoft® Excel® for Mac 2011 version 14.3.9.

Surgical technique for donor animal

First, a left cervical incision 1.0 cm above the sternum was made, later continuing 1.0 cm caudal to the ear level, another elliptical incision was made around the eyes and the last taken 2.0 cm caudal to the tip of the nose continuing perorally 1.0 cm above the commissure and the lower lip (Figure 1). With the animal in supine position, the external jugular vein and its branches were identified, and the glossopharyngeal nerve was removed to expose the common carotid artery and its branches.

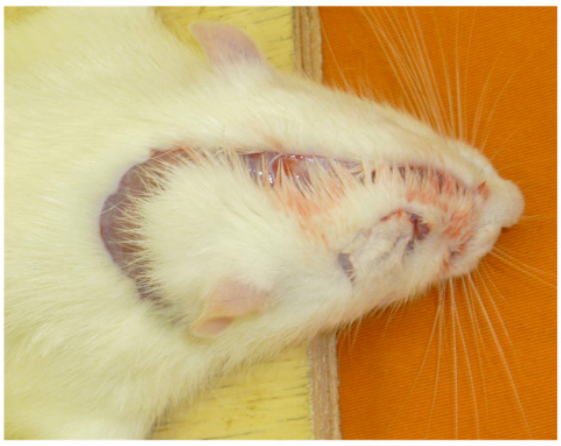

FIGURE 1 - Hemiface incisions in the donor animal.

After that, with the animal in lateral position, the posterior auricular artery and vein were dissected and the ear was included in the flap with the release of its external cartilaginous canal. Then, with the animal in the prone position, the flap was dissected in the subgaleal plane and around the periorbital and perioronasal incision, being connected to the internal maxillary artery.

The flap was then completely released from all adjacent tissues. After ligature of the internal carotid artery and cervical branches of the external carotid artery, common carotid artery and jugular vein were separated, creating the vascular pedicle of the donor flap (Figures 2 and 3).

FIGURE 2 - Outside hemifacial flap.

FIGURE 3 - Inside hemifacial flap, with artery and vein holded by forceps.

Surgical technique for recipient animal

The same donor animal incisions were made on the recipient rat to create a defect on the face and scalp. The periorbital region and perioronasal tissues were preserved. The external jugular vein was identified as the recipient vein and prepared for end-to-end anastomosis as well as the common carotid artery was identified as the recipient artery and prepared for end-to-side anastomosis.

Results

Of the nine recipient animals operated, six survived and three progressed to death in the first days after surgery, with an animal survival rate of 67% (Table 1). Most complications were observed in the first four days after the procedure, as partial or total flap necrosis and poor clinical status. Two animals evolved with a little partial flap necrosis, with good recovery, surviving until they were sacrificed.

TABLE 1 - Data of the nine operated animals.

Rat	Recipient Surgery (minutes)	Flap Ischemia (minutes)	Survival (days)	Complications
1	285	120	1	death
2	260	105	3	total flap necrosis and death
3	245	95	14	parcial flap necrosis
4	270	110	14	none
5	225	80	14	none
6	250	90	14	parcial flap necrosis
7	215	85	4	poor clinical status and death
8	260	80	14	none
9	255	95	14	none

The mean time of the recipient transplantation procedure was 252 minutes and the mean time of flap ischemia was 95 minutes (Table 2). The six surviving animals were sacrificed at 14 days, in good general condition and without signs of tissue rejection (Figures 4 and 5).

TABLE 2 - Statistical analysis of data.

	Recipient Surgery (minutes)	Flap Ischemia (minutes)
Mean	251.66	95.55
Standard Deviation	21.50	13.79
Median	255	95

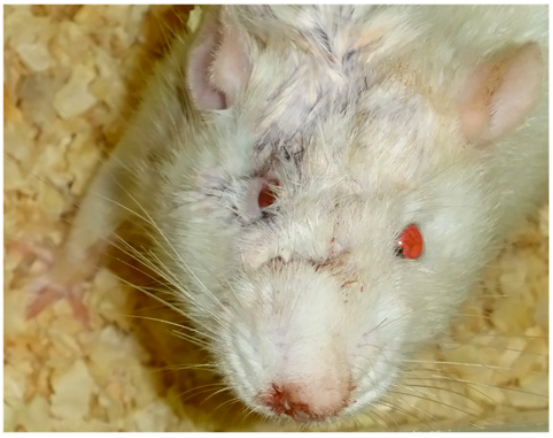

FIGURE 4 - Anterior view. Postoperative, with flap in good condition.

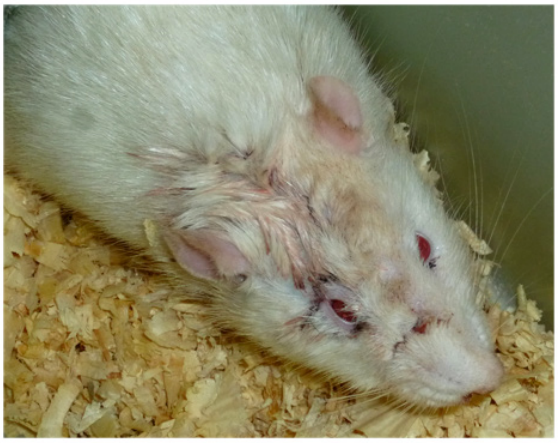

FIGURE 5 - Superior view. Postoperative, with flap in good conditions.

Discussion

Most research on face transplants have been performed in experimental models, which use various animals such as rats, pigs, dogs and primates[21-25]. These models are suitable because the facial circulation of these animals is provided mainly by branches of the external carotid artery and jugular vein, as in humans. Among them, rats are commonly used.

Many of these models have been described and developed in recent years, mostly by Siemionow *et al*.[19], in the United States. The development of an experimental model requires a certain learning curve and is followed by high animal mortality due to the complexity of this procedure. The use of experimental models of allotransplants has enabled the development of surgical techniques and immunosuppressive protocols, contributing to the future adoption of this method as an alternative therapy for complex facial defects[26-30].

In 2003, the first experimental model of face transplantation in rats was described by Siemionow *et al*.[19], as an orthotopic facial allotransplant. The donor rat was of Lewis Brown Norway strain (dark hair) and the transplanted segment included the periorbital, malar, forehead and scalp, based on the common carotid artery and external jugular vein. The recipient rat was of Lewis strain (light hair) and the flap vessels anastomosis were performed in the external carotid artery and the facial vein. The difference in color in the coat of these animals aimed to show the contrast of dark hair in flap donor rat with a light coat of the recipient rat.

Since then, several other models have been proposed, with inclusion of facial segments as mandible, maxilla, tongue, etc. Among these, we highlight the use of only rat hemifac instead of fulface, in order to simplify the procedure and to make it faster, without prejudice to the studies of immunosuppressive and tolerance induction[20]. As the facial transplant is a very complex procedure and have a high animal mortality rate, other studies have correlated the sites of vascular anastomoses with the rat survival rate, pointint the sites that have been most favorable[31].

In 2004, Demir *et al*.[20] proposed transplanting only the hemiface mouse. In eighteen transplanted animals, the mean operating time was 180 minutes and the mean time of flap ischemia was 80 minutes, achieving survival rates of 100% of the animals, and no major complications in the postoperative period. In 2006, Yazici *et al*.[32] described the transplantation of hemiface with rat calvaria, and the mean time of flap ischemia was 60 minutes, and all the seven operated animals survived. These two articles came from the group headed by Siemionow, in Cleveland, United States. They are pioneer in experimental models of face transplants, and they have a great experience and a long learnig curve.

In 2012, Sucher *et al*.[33] developed a novel surgical technique with which to perform hemiface transplantation in mice. The surgical procedure was performed with a success rate of 78% and the mean operating time was 150 minutes for the recipient animal.

In 2013, Climov *et al*.[34] studied the learning curve of hemifacial transplantation in rats by comparison between two operators: medical student trained in basic microsurgery (10 rats) and an experienced microsurgeon (five rats). Transplantation procedure duration mean time (donor plus recipient) was 930 minutes to the microsurgeon and 470 minutes to the medical student. Flap ischemia mean time was 90 minutes to the microsurgeon and 133 minutes to the medical student. The global survival rate was 73% of 15 animals.

In our study, we only performed transplantation of rat hemiface, because it is technically simpler and it is associated with lower mortality rates. Except by the Cleveland group, which has a long learning curve and very good outcomes, our results were similar to those described in the literature, such as duration of surgery (252 minutes), time of flap ischemia (95 minutes), survival rate (66%) and complications observed.

As future perspectives, this experimental model can serve as a basis for establishing experimental protocols of vascularized composite facial allotransplantation tissues. Thus, we intend to collaborate with the performing of a human face transplant in Brazil.

Conclusions

The experimental model of face allotransplantation in rats is reproducible in our midst. Duration of surgery, time of flap ischemia, animal survival rate and complications observed were similar to those described in the literature.

References

1. Siemionow MZ, Kulahci Y, Bozkurt M. Composite tissue allotransplantation. Plast Reconstr Surg. 2009 Dec;124(6 Suppl):e327-39. doi: 10.1097/PRS.0b013e3181bf8413.
2. Wallace CG, Wei FC. The current status, evolution and future of facial reconstruction. Chang Gung Med J. 2008 Sep-Oct;31(5):441-9. PMID: 19097590.
3. Siemionow M, Sonmez E. Face as an organ. Ann Plast Surg. 2008 Sep;61(3):345-52. doi: 10.1097/SAP.0b013e3181844ea3.
4. Siemionow M, Papay F, Alam D, Bernard S, Djohan R, Gordon C, Hendrickson M, Lohman R, Eghtesad B, Coffman K, Kodish E, Paradis C, Avery R, Fung J. Near-total human face transplantation for a severely disfigured patient in the USA. Lancet. 2009 Jul 18;374(9685):203-9. doi: 10.1016/S0140-6736(09)61155-7.
5. Siemionow M, Unal S, Agaoglu G, Sari A. A cadaver study in preparation for facial allograft transplantation in humans: part I. What are alternative sources for total facial defect coverage? Plast Reconstr Surg. 2006 Mar;117(3):864-72; discussion 873-5. PMID: 16525277.
6. Park EE, Genden EM. Facial transplantation: the next frontier in head and neck reconstruction. Facial Plast Surg Clin North Am. 2009 May;17(2):271-7. doi: 10.1016/j.fsc.2009.01.007.
7. Hui-Chou HG, Nam AJ, Rodriguez ED. Clinical facial composite tissue allotransplantation: a review of the first four global experiences and future implications. Plast Reconstr Surg. 2010 Feb;125(2):538-46. doi: 10.1097/PRS.0b013e3181c722a8.
8. Lengelé BG. Current concepts and future challenges in facial transplantation. Clin Plast Surg. 2009 Jul;36(3):507-21. doi: 10.1016/j.cps.2009.02.006.
9. Ravindra KV, Wu S, McKinney M, Xu H, Ildstad ST. Composite tissue allotransplantation: current challenges. Transplant Proc. 2009 Nov;41(9):3519-28. doi: 10.1016/j.transproceed.2009.08.052.
10. Pomahac B, Lengele B, Ridgway EB, Matros E, Andrews BT, Cooper JS, Kutz R, Pribaz JJ. Vascular considerations in composite midfacial allotransplantation. Plast Reconstr Surg. 2010 Feb;125(2):517-22. doi: 10.1097/PRS.0b013e3181c82e6f.
11. Banks ND, Hui-Chou HG, Tripathi S, Collins BJ, Stanwix MG, Nam AJ, Rodriguez ED. An anatomical study of external carotid artery vascular territories in face and midface flaps for transplantation. Plast Reconstr Surg. 2009 Jun;123(6):1677-87. doi: 10.1097/PRS.0b013e3181a3f3ae.
12. Manna F, Guarneri GF, Re Camilot MD, Parodi PC. An easy and cheap way of staining the arterial supply of the face: a preclinical study of visualization of facial vascular territories in human cadavers. J Craniomaxillofac Surg. 2010 Apr;38(3):211-3. doi: 10.1016/j.jcms.2009.04.004. Epub 2009 May 17.
13. Meningaud JP, Paraskevas A, Ingallina F, Bouhana E, Lantieri L. Face transplant graft procurement: a preclinical and clinical study. Plast Reconstr Surg. 2008 Nov;122(5):1383-9. doi: 10.1097/PRS.0b013e3181882146.
14. Ulusal BG, Ulusal AE, Ozmen S, Zins J, Siemionow M. A new composite facial and scalp transplantation model in rats. Plast Reconstr Surg. 2003 Oct;112(5):1302-11. PMID: 14504514.
15. Yazici I, Cavusoglu T, Comert A, Vargel I, Cavusoglu M, Tekdemir I, Siemionow M. Maxilla allograft for transplantation: an anatomical study. Ann Plast Surg. 2008 Jul;61(1):105-13. doi: 10.1097/SAP.0b013e318095a7a1.
16. Kuo YR, Shih HS, Lin CC, Huang CC, Yang JC, Wu WS, Goto S, Chen CL, Lee WP. Swine hemi-facial composite tissue allotransplantation: a model to study immune rejection. J Surg Res. 2009 May 15;153(2):268-73. doi: 10.1016/j.jss.2008.03.050.
17. Follmar KE, Baccarani A, Das RR, Mukundan S, Levin LS, Erdmann D, Marcus JR. Osteocutaneous face transplantation. J Plast Reconstr Aesthet Surg. 2008;61(5):518-24. doi: 10.1016/j.bjps.2007.12.014.
18. Siemionow M, Agaoglu G, Unal S. A cadaver study in preparation for facial allograft transplantation in humans: part II. Mock facial transplantation. Plast Reconstr Surg. 2006 Mar;117(3):876-85; discussion 886-8. PMID: 16525279.
19. Siemionow M, Gozel-Ulusal B, Engin Ulusal A, Ozmen S, Izycki D, Zins JE. Functional tolerance following face transplantation in the rat. Transplantation. 2003 May 15;75(9):1607-9. PMID: 12792528.
20. Demir Y, Ozmen S, Klimczak A, Mukherjee AL, Siemionow M. Tolerance induction in composite facial allograft transplantation in the rat model. Plast Reconstr Surg. 2004 Dec;114(7):1790-801. PMID: 15577350.
21. Washington KM, Solari MG, Sacks JM, Horibe EK, Unadkat JV, Carvell GE, Simons DJ, Lee WP. A model for functional recovery and cortical reintegration after hemifacial composite tissue allotransplantation. Plast Reconstr Surg. 2009 Feb;123(2 Suppl):26S-33S. doi: 10.1097/PRS.0b013e318191bca2.
22. Kuo YR, Shih HS, Lin CC, Huang CC, Yang JC, Wu WS, Goto S, Chen CL, Lee WP. Swine hemi-facial composite tissue allotransplantation: a model to study immune rejection. J Surg Res. 2009 May 15;153(2):268-73. doi: 10.1016/j.jss.2008.03.050.
23. Landin L, Cavadas PC, Gonzalez E, Rodriguez JC, Caballero A. Functional outcome after facial allograft transplantation in rats. J Plast Reconstr Aesthet Surg. 2008 Sep;61(9):1034-43. doi: 10.1016/j.bjps.2007.12.084.
24. Silverman RP, Banks ND, Detolla LJ, Shipley ST, Panda A, Sanchez RA, Azimzadeh AM, Pierson RN 3rd, Wang D, Rodriguez ED, Holton LH 3rd, Bartlett ST. A heterotopic primate model for facial composite tissue transplantation. Ann Plast Surg. 2008 Feb;60(2):209-16. doi: 10.1097/SAP.0b013e318061b792.
25. Shengwu Z, Qingfeng L, Hao J, Banich J, Kaiding F, Benson C, Huiyong W, Danning Z, Bing G, Qinxiu L, Lujia T, Tao Z, Yuping L, Tisheng Z. Developing a canine model of composite facial/scalp allograft transplantation. Ann Plast Surg. 2007 Aug;59(2):185-94. PMID: 17667414.
26. Zor F, Bozkurt M, Nair D, Siemionow M. A new composite midface allotransplantation model with sensory and motor reinnervation. Transplant Int. 2010 Jun;23(6):649-56. doi: 10.1111/j.1432-2277.2009.01032.x.
27. Kulahci Y, Siemionow M. A new composite hemiface/mandible/tongue transplantation model in rats. Ann Plast Surg. 2010 Jan;64(1):114-21. doi: 10.1097/SAP.0b013e3181a20cca.
28. Landin L, Cavadas PC, Gonzalez E, Caballero-Hidalgo A, Rodriguez-Perez JC. Sensorimotor recovery after partial facial (mystacial pad) transplantation in rats. Ann Plast Surg. 2009 Oct;63(4):428-35. doi: 10.1097/SAP.0b013e31819031ef.
29. Ulusal BG, Ulusal AE, Wei FC. Long-term outcomes of composite auricle as a neurosensorial facial subunit allotransplant. Ann Plast Surg. 2009 Mar;62(3):311-6. doi: 10.1097/SAP.0b013e31817e9cd3.
30. Siemionow M, Bozkurt M, Kulahci Y. Current status of composite tissue allotransplantation. Handchir Mikrochir Plast Chir. 2007 Jun;39(3):145-55. PMID: 17602375.
31. Unal S, Agaoglu G, Zins J, Siemionow M. New surgical approach in facial transplantation extends survival of allograft recipientes. Ann Plast Surg. 2005 Sep;55(3):297-303. PMID: 16106171.
32. Yazici I, Unal S, Siemionow M. Composite hemiface/calvaria transplantation model in rats. Plast Reconstr Surg. 2006 Nov;118(6):1321-7. PMID: 17051102.
33. Sucher R, Lin CH, Oberhuber R, Kern B, Zheng XX, Zelger B, Pratschke J, Schneeberger S, Lee WP, Brandacher G. Hemiface allotransplantation in the mouse. Plast Reconstr Surg. 2012 Apr;129(4):867-70. doi: 10.1097/PRS.0b013e3182450aff.

34. Climov M, Maciuceanu Zarnescu MB, Stefanescu A, Zamfirescu D, Lascar I. Learning curve in hemifacial transplantation in rats. Chirurgia (Bucur). 2013 Mar-Apr;108(2):234-40. PMID: 23618574.

Correspondence:
Pedro Soler Coltro
Alameda Jauaperi, 943/172
04523-014 São Paulo – SP Brasil
Tel.: (55 11)99190-5832
pscoltro@hotmail.com

Received: Mar 10, 2014
Review: May 12, 2014
Accepted: Jun 11, 2014
Conflict of interest: none
Financial source: none

[1]Research performed at Laboratory of Plastic Surgery and Microsurgery, Plastic Surgery Division, Faculty of Medicine, University of Sao Paulo (USP), Brazil.