

Hemiface Allotransplantation in the Mouse

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Summary: Rat models of experimental face transplantation have been widely used to study vascularized composite tissue allotransplantation. Because the mouse represents a superior species for vascularized composite tissue allotransplantation research, the authors developed a novel surgical technique with which to perform hemiface transplantation in mice. BALB/c hemifacial grafts were transplanted into BALB/c (group 1) or C57BL/6 (group 2) recipients ($n = 6$ per group). Myocutaneous hemiface grafts including a vascular pedicle consisting of the common carotid artery and the external jugular vein were retrieved using superfine microsurgical instruments. The graft was transplanted orthotopically and revascularized using the recipient common carotid artery and external jugular vein for anastomosis applying a non-suture cuff technique. After an initial learning curve, the surgical procedure was performed with a constant and high success rate (78 percent). Operating time was comparable in all groups and lasted 120 ± 15 minutes for the donor and 150 ± 12 minutes for the recipient. All syngeneic grafts survived long term (>100 days). Allograft rejection in group 2 occurred within 14 ± 2 days. Hematoxylin and eosin stains of syngeneic grafts revealed unaltered muscle and skin histology. Allogeneic grafts gradually showed distinct rejection patterns progressing with time and similar to those observed after human face transplantation. This is the first description of a mouse hemiface allotransplantation model. The microsurgically demanding procedure may be used to investigate basic immunology and rejection and to address questions related to nerve regeneration in reconstructive face transplantation. (*Plast. Reconstr. Surg.* 129: 867, 2012.)

Orthotopic face transplantation in rats has been the most developed experimental animal model in the past decade.¹ For basic immunology research, however, the mouse would be a most suitable model because of the presence of genetically defined inbred transgenic and knockout strains and the availability of monoclonal antibodies and molecular probes. In this article, we describe the first supermicrosurgical technique of hemiface transplantation in mice.

MATERIALS AND METHODS

Hemiface grafts from BALB/c mice (Jackson Laboratories, Bar Harbor, Me.) were orthotopically transplanted into either syngeneic BALB/c (group 1) or allogeneic C57BL/6 (group 2) recipients ($n = 6$ per group) using xylazine (5 mg/kg body weight) and ketamine (100 mg/kg body weight) for anesthesia and heparin (30 units) for anticoagulation. Custodiol (Köhler Chemie, Ben-

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sheim, Germany) solution (4°C) was used for preservation and cold storage.

Donor Operation

The hemiface was retrieved using a similar technique as described previously in the rat.² The common carotid artery together with the external jugular vein served as the vascular pedicle of the graft (Fig. 1, *above*).

Recipient Operation and Hemiface Transplantation

To enable orthotopic transplantation, the recipient's native hemiface had to be removed to make room for the graft. Both the external jugular vein and the common carotid artery were isolated for vascular anastomoses. Revascularization was achieved using a non-suture cuff technique.³ The

basic principles of this technique involve pulling the recipient's external jugular vein and common carotid artery through a circular cuff (Fig. 1, *below, left*). The vessels are then folded over the cuff to expose the endothelial surface and secured with a circumferential 10-0-nylon ligature. For anastomosis, donor vessels are then inserted into the recipient's external jugular vein and common carotid artery (Figs. 1, *below, right* and 2). (See Video, Supplemental Digital Content 1, which demonstrates murine hemiface transplantation showing three-dimensional reconstruction of vascular anastomoses, <http://links.lww.com/PRS/A469>.)

Histology

For histology, processed muscle and skin tissue samples were stained with hematoxylin and eosin.

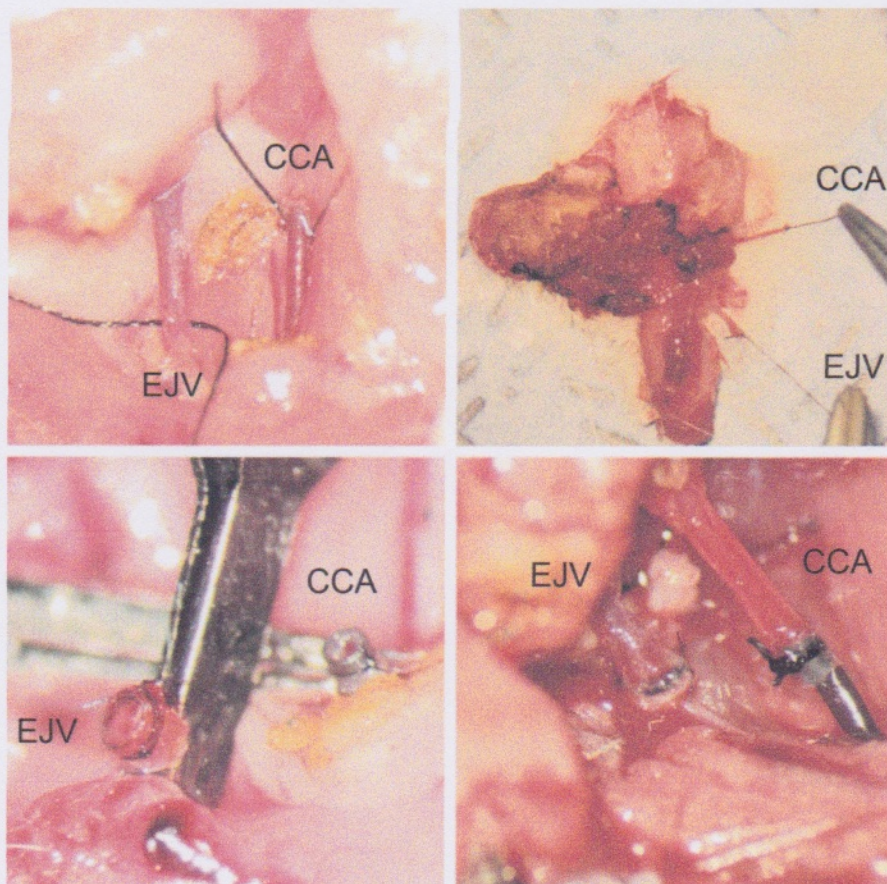


Fig. 1. Intraoperative photographs show the external jugular vein (EJV) and common carotid artery (CCA) at the donor site (*above*). For anastomosis, the recipient's external jugular vein and common carotid artery were assembled with a circular cuff (*below, left*). Donor vessels were then inserted into the recipient's external jugular vein and common carotid artery and the final anastomosis was secured with a 10-0 nylon tie (*below, right*).

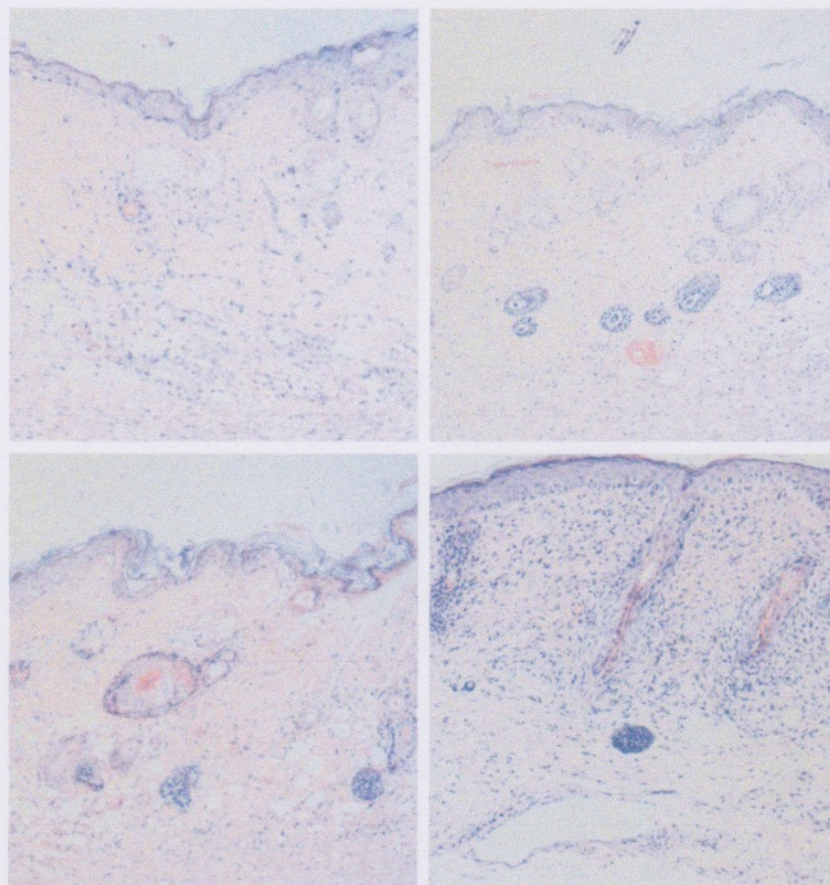


Fig. 2. Skin hematoxylin and eosin histologic images of murine hemiface allografts showing (above, left) mild perivascular infiltration with no involvement of the overlying epidermis (grade 1); (above, right) moderate perivascular infiltration with and without epidermal and adnexal involvement (grade 2); (below, left) severe inflammation and epidermal involvement with epithelial apoptosis, dyskeratosis, and/or keratinolysis (grade 3); and (below, right) necrotizing acute rejection (grade 4).



Video. Supplemental Digital Content 1 demonstrates murine hemiface transplantation showing three-dimensional reconstruction of vascular anastomoses, <http://links.lww.com/PRS/A469>.

Slides were scored according to the Banff 2007 working classification of skin containing composite tissue allograft abnormality.⁴

Statistical Analysis

Results are expressed as mean \pm SD. Statistical analysis was performed with GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, Calif.). When two groups were compared, a two-tailed *t* test was used. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Surgery

A total of six mice underwent transplantation in each group, with an overall success rate of 78

percent. The donor operation lasted 120 ± 15 minutes and the recipient operation including transplantation could be completed in 150 ± 12 minutes. No self-mutilation was observed.

Histology

In the allogeneic group, histopathologic evaluation revealed changes consistent with acute cell-mediated rejection resembling what has been described in human composite tissue allograft rejection. Syngeneic grafts did not show any histopathologic changes.

DISCUSSION

For the past decade, small animal face transplantation models aiming to test functional outcome and long-term survival were almost exclusively developed and pioneered by Siemionow et al. in the Microsurgery Laboratory of the Cleveland Clinic. Although the rat face transplantation model is suitable for functional and immunologic assessment, the mouse represents the most widely used species in biological and basic immunologic research. Based on our previous experience with the cuff technique for arterial and venous anastomoses in various solid organ and vascularized composite tissue allotransplantation models⁵ and most recently with murine hind-limb transplantation,³ we were able to modify and adapt this method for the current study to successfully revascularize and transplant a murine hemiface. This hemiface transplant model is the first mouse myocutaneous vascularized composite allotransplantation model

consisting of facial skin, muscle, and the ear. In contrast to our previously described murine hind-limb transplant model, the hemiface model is not permissive for the study of donor-specific stem cells, because the hemiface graft does not contain bone as a vascularized source of hematopoietic stem cells. However, we believe that this model is ideally suited for studies in immunology in addition to ischemia-reperfusion injury and nerve regeneration in reconstructive face transplantation.

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REFERENCES

1. Ulusal BG, Ulusal AE, Ozmen S, Zins JE, Siemionow MZ. A new composite facial and scalp transplantation model in rats. *Plast Reconstr Surg*. 2003;112:1302–1311.
2. Siemionow M, Gozel-Ulusal B, Engin Ulusal A, Ozmen S, Izycki D, Zins JE. Functional tolerance following face transplantation in the rat. *Transplantation* 2003;75:1607–1609.
3. Sucher R, Lin CH, Zanoun R, et al. Mouse hind limb transplantation: A new composite tissue allotransplantation model using nonsuture supermicrosurgery. *Transplantation* 2010;90:1374–1380.
4. Cendales LC, Kanitakis J, Schneeberger S, et al. The Banff 2007 working classification of skin-containing composite tissue allograft pathology. *Am J Transplant*. 2008;8:1396–1400.
5. Sucher R, Oberhuber R, Rumberg G, et al. A rapid vascular anastomosis technique for hind-limb transplantation in rats. *Plast Reconstr Surg*. 2010;126:869–874.