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A murine model of orthotopic periorbital subunit transplantation

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ABSTRACT

Background: Conventional reconstructive methods fail to achieve satisfactory results in total eyelid defect cases. Vascularized composite tissue allotransplantation might provide both good appearance and function for these patients. We developed an orthotopic periorbital transplantation model in rats to facilitate further experimentation in this field.

Methods: In anatomical studies, the vascular distribution to and innervation of the periorbital unit were identified and recorded. Then, according to the anatomical studies, eight orthotopic transplantations and two transplantations with pedicle ligation were performed. The posterior facial vein and the external carotid artery were selected as the graft pedicles, while the temporal and upper zygomatic facial nerves were used for graft innervation. All transplanted eyelids were assessed daily. Micro-CT scanning and hematoxylin and eosin staining of the grafts were performed 60 days after the operation.

Results: In total, 90% of recipients tolerated the operation well. All grafts without pedicle ligation survived, and new hair growth was observed. The position of the eyelid was maintained, and eyelid function was partially restored. In the recipients with graft pedicle ligation, the grafts became necrotic and mummified within four to five days. Micro-CT of the surviving grafts showed a good blood supply, and histological staining revealed normal morphology.

Conclusions: A periorbital subunit orthotopic transplantation model was established, which might facilitate future eyelid allotransplantation-related experimentation.

1. Introduction

Severe injury to the face may cause loss of the eyelid, which may be associated with a poor cosmetic appearance, chronic discomfort, corneal ulceration, and subsequent loss of vision. Because of the unique position, stability, motion, sensitivity, and appearance of the eyelid, conventional reconstructive methods fail to replicate either the esthetic appearance or the function of the eyelid [1].

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Vascularized composite tissue allotransplantation (VCA) provides an alternative to conventional soft tissue reconstruction. VCA allows a graft to be precisely tailored to the missing tissues, without a donor defect. Total face and midface allotransplantations have both yielded encouraging outcomes in the past decade [2–4]. These successes provide important references for the allotransplantation of even more delicate structures, such as the finger or eyelid. However, before the clinical application of periorbital subunit allotransplantation, more experiments will be needed to determine the impact of ischemia, immunosuppressive agents, and deinnervation effects on both the graft and the recipient.

Murine models are commonly used in VCA research because of their ease of manipulation, affordability and relatively fixed genetic background. To date, various murine models have been established [5–9]. The present study aimed to develop a periorbital subunit transplantation model in rats.

2. Materials and methods

2.1. Animals, experimental design, and anesthesia

A cohort of 25 inbred male Lewis rats (weight 250–275 g) were used for anatomical studies ($n = 5$) or as transplant donors or recipients ($n = 20$). The animals were purchased from Vital River (Beijing, China). In total, ten graft anatomical studies and ten transplantation procedures were performed, including eight orthotopic transplantations and two orthotopic allotransplantations with graft pedicle ligation.

All experimental animals were anesthetized with isoflurane inhalation (dose: 3% for induction anesthesia; 2% for maintenance anesthesia). After graft harvesting or red latex perfusion, donor rats and rats for the anatomical studies were euthanized with an overdose of 5% isoflurane, and their death was confirmed by cervical dislocation. For analgesia, recipient rats also received a subcutaneous injection of meloxicam (2 mg/kg) before and after the operation, including daily injections for the first 4 days after the operation. The study protocol was approved by the institutional animal care and use committee of Shanghai Jiaotong University School of Medicine.

2.2. Anatomical studies

The red latex perfusion procedure has been described in detail in a previous publication [10]. In brief, the heart of each rat was located after cutting through the thoracic wall. A cotton swab was used to expose the aorta by pulling the right atrium laterally. A 24 g catheter was placed into the heart and advanced forward along the direction of the aorta. A vent was cut in the right atrium to allow fluid to exit. Then, 250 units (0.25 ml of 1000 μ/ml) heparin, 400 ml saline, and 30 ml red latex were perfused, in that order. The rat was maintained at 4 °C overnight to allow the latex to polymerize. On the next day, through an incision along the mid-line of the cervical area, the external cervical vein and common aorta were exposed and traced. The vascular distribution to the periorbital unit was then identified and recorded. Innervation of the graft was also recorded (Fig. 1).

2.3. Surgical procedures

2.3.1. Graft harvesting

For graft harvesting, a circular periorbital incision, for which the medial margin was the medial orbital rim, the upper and lower margins were 2–3 mm away from the orbital rim, and the lateral margin was the approximate midpoint between the orbital rim and the ear root, was made in the donor rat. Two additional horizontal, cervical incisions in the face and one paramedian incision in the neck region were made for graft pedicle dissection (Fig. 2A).

In the anterior neck region, the platysma muscle, loose areolar tissue, several superficial lymph nodes, and the submandibular gland were removed to expose the external jugular vein and its posterior facial branch, while the other branches (i.e., the anterior facial vein, cephalic vein, posterior external jugular vein, and superficial cervical vein) were coagulated. Next, the posterior bellies of the digastric muscles, the stylohyoid muscle, the omohyoid muscles, the glossopharyngeal nerve, and the greater horn of the hyoid bone were removed to expose the common carotid artery and its main branches. Subsequently, the superior thyroid artery, ascending pharyngeal artery, lingual artery, branch to the submaxillary gland, external maxillary artery, and internal carotid artery were ligated.

The animal was then positioned laterally, and the anterior and posterior auricular vessels were ligated, while the temporal vessels were preserved. Next, the animal was placed in the prone position. The graft was undermined superiorly in the subgaleal plane to the orbital margin, where the orbital septum, along with the orbital periosteum, was detached from the orbital rim before the orbital cavity was exposed (Fig. 2B). The preaponeurotic fat was peeled off to expose the levator palpebrae superioris. The levator palpebrae superioris and its underlying mucosa were then cut near the equator of the eyeball. Medially and inferiorly, the graft was also elevated to the subperiosteal level, the orbital septum was detached from the orbital rim, and the mucosa was cut near the equator of the eyeball level. At the lateral part of the graft, a small piece of temporal muscle was elevated as a muscular cuff around the pedicle vessels (the infraorbital vessels and the frontal branches of the temporal vessels). At the cheek of the donor, the temporal branch and zygomatic branch of the facial nerve were identified and dissected along the graft. The whole eyelid graft was then completely peeled off, except for the pedicle of the graft, leaving the external vein and common carotid artery connected (Fig. 2C). Finally, the pedicle vessels were severed at the neck area (Fig. 2D). The grafts were draped with saline-soaked gauze, placed in a 50-ml centrifuge tube and stored at 4 °C until transplantation.

2.3.2. Preparation of recipient bed and graft insetting

In recipient rats, a circular incision similar to that made in the donor rat was created. In this manner, a total eyelid defect model was created (Fig. 2F). Specifically, the levator palpebrae superioris was severed right above the tarsus, leaving enough intact levator aponeurosis for functional reconstruction. In the neck region, the external jugular vein was exposed and prepared as the recipient vein, while the common carotid artery was prepared as the recipient artery.