Review Article

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Experimental Craniofacial Composite Tissue Allotransplantation (CCTA) Models in Rats

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Abstract

Rats are the most frequently used animals in **composite tissue allotransplantation (CTA)** studies and most craniofacial composite tissue transplantation models are described in rats. During the last 10 years, a total of 8 different **craniofacial composite tissue allotransplantation (CCTA)** models have designed and developed in rats. These models include full face/scalp transplants, hemiface transplants, composite hemiface/calvarium transplants, rat maxilla allotransplants, composite osteomusculocutaneous hemiface/mandible/tongue flap transplants, composite midface allotransplants, total osteocutaneous hemifacial allotransplantation model and composite face and eyeball allotransplant model with optic nerve.

All these models provide a basic and scientific foundation for future success in CTA in the clinical setting. This review presents different experimental models of CCTA in rats which are relevant to observed in clinical scenario cases of severe facial deformities.

Key words: *Composite tissue allotransplantation, experimental models, craniofacial composite tissue allotransplantation models, rat, face transplantation*

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Introduction

Various techniques have been described for treatment of severe facial defects however both the functional and aesthetic outcomes of these conventional reconstructive procedures are not satisfactory [1-4]. The first successful clinical hand and face transplantations have changed traditional "reconstructive surgery" to the novel approach of "restorative surgery". Despite growing number of CTA cases reported worldwide, there are still many questions to be answered specifically regarding tissue immunogenicity and chronic graft rejection [5-10]. In order to address these problems, continuation of research using craniofacial transplant experimental models are needed. Up to date various models

of craniofacial transplantation have been described in rats . Herein, we present these different experimental models of craniofacial transplantation.

Craniofacial Transplantation Models in Rats

In 2003, Ulusal et al. described and published the first full face transplantation model in rat [11]. In the following years 8 additional CCTA models have been developed. Authors have designed these models to make them relevant to different types of facial trauma observed in clinical practice [11-23]. We have divided craniofacial transplant models into three major categories: Soft tissue transplantation models, soft tissue and bone transplantation models and functional unit transplantation models.

I. Soft Tissue Transplantation Models:

1. Full Face/Scalp Transplant Model:

In 2003 Ulusal et al. have described the first face/scalp allotransplantation model in the rat. The transplantation was performed between LBN (RT1l+n) donors and Lewis (RT11) recipients across major histocompatibility complex (MHC) barrier [11,12].

The allograft was harvested based on the bilateral common carotid artery and external jugular vein of the donor rat and was composed of all facial skin, scalp, and bilateral ears. Periorbital structures and nose were excluded from the allograft. A similar facial/scalp defect including facial skin, scalp, and external ear structures was created in the recipient rat. The facial nerves and muscles, and the perioral and the periorbital regions, were preserved to avoid functional deficits that could interfere with animal feeding, breathing, and eye closure. Next, the common carotid arteries and external jugular veins of the recipient rat were prepared for anastomosis. Arterial anastomoses were performed either to the common carotid arteries in end-to-side fashion or to the external carotid arteries of recipients in end-to-end fashion. Standard end-to-end venous anastomoses then were performed, connecting the external jugular and anterior facial veins [11].

In this model, cyclosporine A (CsA) monotherapy was used as immunosuppressive protocol which was given at a dose of 16 mg/kg/day during first week post-transplant and was tapered to 2 mg/kg/day over 4 weeks, and was maintained at this level during the entire follow-up period of over 200 days. Later, to improve the survival of facial/scalp allograft recipients, we have introduced a new approach by modifying the arterial anastomoses in the recipient. The single (unilateral) common carotid artery of the recipient was used to vascularise the entire transplanted facial/scalp flap reducing time of transplantation and animal survival [13].

The same model was also introduced in fully MHC mismatched model between the ACI (RT1a) donors and Lewis (RT11) recipients. Under the same immunosuppresion protocol of CsA monotherapy over 180 days of facial/scalp allograft transplant survival was achieved [13].

2. Hemiface/Scalp Transplant Model:

The average time for full face transplantation ranged between 6–7 hours. In order to shorten the surgery time and reduce morbidity and mortality related to long lasting procedure and brain ischemia time in full face/scalp transplant model, Demir et al. have introduced a hemifacial allograft transplant model which is technically less challenging when compared with the full facial/scalp model. Hemifacial allograft transplants were performed between both the semi-allogeneic LBN (RT11+n) and fully allogeneic ACI (RT1a) donors and Lewis (RT11) recipients [14].

Using the same facial dissection approach as described for full face/scalp transplant model, the unilateral hemifacial allograft was harvested including the external ear and scalp, based on the common carotid artery and external jugular vein. In the recipient, skin defect was created to accommodate allograft transplant. The arterial and venous anastomoses were performed to the common carotid artery (end-to-side) and to the external jugular vein (end-to-end), respectively. The same CsA monotherapy immunosuppressive protocol was used and long term survival was achieved in both the semiallogeneic transplants (400 days) and fully allogeneic transplants (300 days) [14,15]

II. Soft Tissue and Bone Transplantation Models:

1. Composite Hemiface/Calvarium Transplantation Model:

In order to extend the application of face/scalp allotransplantation model, Yazici et al have incorporated into hemifacial allograft a vascularized calvarial bone,

introducing new composite hemiface/calvarium transplantation model. The allograft was based on the same vascular pedicle of common carotid artery/external jugular vein and transplantation was performed across MHC barrier between LBN (RT11+n) and LEW (RT11) rats [16].

In this model, dissection of hemiface was performed according to previously described face transplant models. Composite hemifacial/scalp flaps including the external ear and scalp, based on the common carotid artery and external jugular vein, were harvested from the donors.

During facial graft dissection, temporoparietal bone was included into the allograft as a model testing new treatment options for extensive craniomaxillofacial deformities with large bone defects [16].

Following harvest, the allograft was transplanted to the matching facial defects in the recipient rats. The arterial anastomosis was performed to the common carotid artery (end-to-side) and venous anastomosis was performed to the external jugular (end-to-end). The calvarial component of the composite allograft was placed on the de-epithelialized surface of the recipient rat face, above the facial musculature, and no bony fixation was performed [16].

CsA monotherapy was used as immunosuppressive protocol. Evaluation of the allograft was performed with angiography, daily inspection, computed tomography (CT) scan, and bone histology. No signs of rejection and no allograft loss were noted at 220 days posttransplantation. Intact vascular supply was demonstrated on graft angiography. CT scans taken at days 14, 30, and 100 revealed normal bones without resorption. Histological evaluation revealed viable bone at 7, 30, 63 and 100 days post-transplantation. This new osteomusculocutaneous hemiface/calvarium flap model may serve as a new surgical tool testing technical, immunological and functional aspects of coverage of large bone and soft-tissue defects of craniofacial deformities in one surgical procedure of allotransplantation [16].

2. Maxilla Allotransplantation Model:

Maxilla allotransplantation model was developed to test the effects of vascularized maxilla allotransplantation on composite maxillary substructures. Transplantations were performed across the MHC barrier between LBN donor and LEW recipient rats under CsA monotherapy. Allograft dissection was performed along the maxillary Le-Fort II osteotomy lines based on the common carotid artery and external jugular vein. As an orthotopic transplantation was not technically feasible in small animal model, a heterotopic transplantation was performed to the inguinal region of the recipient rat. Vascular anastomoses were performed between unilateral common carotid artery, external jugular vein and femoral vessels [17].

Evaluation of the transplanted allograft was performed by daily inspection, computerized tomography, flow cytometry, angiography, and histology. Allografts survived up to 105 days without signs of rejection. The maxillary incisor teeth continued to grow. A high level of donor-specific chimerism for T-cell and B-cell lineages was achieved and maintained over entire post-transplant period. Histological evaluation revealed that tooth buds, bone, cartilage, and mucosa remained intact. This model introduced feasibility of testing bone and teeth growth and contribution of vascularized bone to donor chimerism [17].

3. Composite Osteomusculocutaneous Hemiface/Mandible/Tongue-Flap Model:

Composite osteomusculocutaneous hemiface/mandible/tongue allograft transplant was developed to extend the application of the face/scalp transplantation model in the rat by incorporation of the vascularized mandible, masseter, and tongue to test feasibility of this model as a new reconstructive option for extensive head and neck deformities with large soft- and bone-tissue defects [18-20]. Hemimandibular bone, masseter muscle, tongue, and hemifacial flaps were dissected on the same pedicle of the external carotid artery and jugular vein and were transplanted to the donor inguinal region. The feasibility of composite osteomusculocutaneous hemiface/mandible/tongue transplantations was tested both in isotransplantation and allotransplantation models. The allotransplantation model was performed across the MHC barrier between LBN donors and LEW recipients [18-20].

A heterotopic transplantation was performed to the inguinal region of the recipient rat. Under magnification of operating microscope and using microsurgical instruments and techniques, the common carotid ar-

tery and external jugular vein of the graft were anastomosed in end-to-end fashion with 10-0 sutures to the femoral artery and vein, respectively. All allogeneic transplant recipients received our established protocol of CsA monotherapy [18-20]..

Evaluation was performed by daily inspection, angiography computerized tomography and histology. Isograft transplants survived indefinitely. All hemiface/mandible/tongue allotransplants survived over 100 days posttransplant. Flap angiography demonstrated an intact vascular supply to both the soft and bony tissues of the allograft. No signs of rejection and no graft loss were noted. Both the CT scan and bone histology confirmed viable bone components of the composite allografts. Viability of the tongue was confirmed by both the inspection and histology. Histological evaluation revealed presence of viable bone marrow cells within the transplanted mandible. Donor-specific chimerism was confirmed by flow cytometry by presence of donor T cells (2.7%, CD4/RT1n, 1.2% CD8/RT1n) and B cells (11.5%, CD45RA/RT1n) at day 100 posttransplant [18-20].

4. Total Osteocutaneous Hemifacial Allotransplantation Model:

Recently, a total osteocutaneous hemiface flap model was developed in order to extend the application of the face/scalp transplantation model in the rat, as a new reconstructive option for extensive head and neck deformities with large soft tissue and bone defects. This model included all hemifacial structures such as vascularized nose, premaxilla, eyelids, and upper and lower lips, external ear and facial skin. Common carotid artery and external jugular vein served as composite allograft pedicle as described in other facial transplantation models. Transplantations were performed between Lewis-Brown Norway (LBN, RT11+n) donors and Lewis (RT11). The composite facial allograft was harvested in subplatysmal and sub-SMAS plane and all hemifacial tissues and subunits including the nose, premaxilla, mystacial pad, external ear, scalp, and periorbital structures were included into the graft [21].

A heterotopic transplantation was planned and the allografts were transplanted to the inguinal regions of the recipient rat. End-to-end anastomoses were performed between the common carotid artery/femoral artery and the external jugular vein/femoral vein. All allogeneic transplant recipients received our established protocol of CsA monotherapy, which was given at a dose of 16 mg/kg/day for the first week posttransplant, and was next tapered to 2 mg/kg/day over 4 week period , and maintained at this level during the entire follow-up [21].

Evaluation was performed with macroscopic evaluation, angiography, computerized tomography and flow cytometry. All hemiface/nose allotransplants survived over 100 days posttransplant. There were no signs of allograft rejection. Graft angiography revealed an intact vascular supply to both the soft and bony tissues of the facial allograft. CT scan demonstrated a viable premaxillary bone at 100 days posttransplant. Flow cytometry analysis revealed presence of donor-specific chimerism at day 100 posttransplant. Fluorescent immunostaining of donor showed presence of the MHC Class I cells in the recipient's skin, lymph node, and liver at 150 days posttransplant [21].

Histological examination revealed intact nasal and oral mucosa, nasal septal cartilage, and tooth structures. Histology of the eyelid demonstrated integrity of the eyelid components of the composite facial allograft [21].

III. Functional Units Transplantation Models

1. Composite Midface Transplant Model with Sensory and Motor Neuromuscular Units:

To test feasibility of functional facial unit transplantation Zor et al. have developed a new rat model of composite midface allograft transplant with sensory and motor neuromuscular units by incorporation of vascularized premaxilla, mystacial pad, and nose with infraorbital and facial nerves. This model tested functional recovery of transplanted sensory and motor nerves following midface transplantation. Functional allotransplantations were performed in both the isogeneic and allogenic models across MHC barrier between the LBN donors and LEW recipients [22].

In this model, midfacial structures including nose, premaxillary bone segment, mystacial pad, masseter muscle, and lower lip were harvested on the same pedicle of the common carotid artery and external jugular vein. Out of the branches of external carotid artery, only facial artery was preserved as the pedicle of the al-

lograft [22].

In contrast to previously described facial transplant models where subplatysmal dissection was performed, in this model the surgical plane of dissection was below the masseter muscle in order to avoid iatrogenic damage of the facial nerve branches during dissection. The facial nerve and the infraorbital nerve were included into the composite midface allograft [22].

Since orthotopic transplant was not feasible in small animal model due to survival problems in such extensive and complex transplant model a heterotopic transplantation was planned. The composite midface allografts with sensory and motor units were transplanted to the donor inguinal region and vascular anastomoses were performed between the vascular pedicle of the facial graft and femoral vessels. Next, standard epineural neurorrhaphies were performed between the infraorbital nerve of the donor and saphenous nerve of the recipient and between facial nerve of the donor and femoral nerve of the recipient. All allogeneic transplant recipients received immunosuppression protocol of CsA monotherapy [22].

Recovery of motor function was evaluated by observation of the return of movement to the mystacial pad. Sensory recovery was observed clinically by evasive behavior and defense reactions when the transplanted whiskers were pulled. Somatosensory evoked potentials (SSEP) and motor evoked potentials (MEP) were applied to evaluate the sensory and motor recovery, respectively [22].

Successful allograft transplantation was accomplished in all animals, with 100% flap survival rate over 100 days. Clinically, all grafts were pink and pliable during the entire observation period. The incisors continued to grow; tooth buds, bone, cartilage, and mucosa remained intact. Motor recovery was observed at 21 days posttransplant and was confirmed by the movement of the mystacial pad. Clinically evasive behavior and defense reactions were observed when transplanted whiskers were pulled. Computed tomography of composite nose flap showed vital bony premaxilla. On 100 days posttransplant, 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 [22].

This model for the first time allows for evaluation of functional recovery of the sensory and motor nerves following midface allotransplantation [22].

2. Composite Face and Eyeball Allotransplant Model with Optic Nerve:

Recently, another functional face transplantation model was developed, composed of face and eyeball including the optic nerve. Composite face/eyeball transplantations were performed between Sprague-Dawley rats. The composite face/eyeball allograft was composed of facial skin, auricle, eyeball and periorbital soft tissues including optic nerve [23].

The allograft was harvested based on the common carotid artery and external jugular vein. All orbital contents including the periorbital fat tissue and muscles were included into the flap. The optic nerve was included in the allograft. The harvested allograft was transplanted to the anterior neck of the recipient rat. Common carotid artery and external jugular vein of the recipient rat were prepared for anastomosis. Standard microsurgical arterial anastomosis was performed to the common carotid artery (end-to-side) and venous anastomosis was performed to the external jugular veins of the recipient rat in the end-to-end fashion. For nerve coaptation, the great auricular nerve of the recipient rat was prepared and transected. Next, nerve coaptation was performed between the great auricular nerve of the recipient rat and the optic nerve of the donor allograft using standard epineural technique with 10-0 sutures. Following allograft inset, a total tarsorrhaphy was performed with 8-0 sutures in order to protect the cornea of the transplanted eyeball [23].

All transplant recipients received immunosuppression protocol of CsA monotherapy. The post-transplant evaluation was performed by daily inspection, MRI and histopathology. All transplants survived indefinitely. However, the eyeball lost its brightness in early posttransplant period with a minimal decrease in its volume. The volumetric analysis of the eyeball was performed at the first and 30th day posttransplant by MRI and indicated 35% of eyeball volume loss; the histological evaluation of nerve sections showed severe degeneration with no evidence of regeneration. There was presence of lymphocytic infiltration of skin, retina, periorbital fat tissue and periorbital muscles. This

experimental model provided allowed for evaluation of optic nerve regeneration and the effect of allotransplantation on composite facial tissues including orbital content [23].

Conclusions

We present the experimental models of CCTA. Functional and aesthetic outcome following application of conventional reconstructive procedures or prosthetic materials is not satisfactory, especially in patients with severe deformities and disabilities. Since the first successful hand transplantation in France in1998, CTA transplantation has gained a great deal of interest in the field of plastic surgery. Up to date, more than 70 CTA transplants including hand, face, larynx, knee, abdominal wall and lower extremities have been performed worldwide. There is no doubt that CTA transplantation will improve patients' functional and aesthetic outcomes, however this is at the expense of different types of complications and morbidities including serious infections, organ toxicity, and malignancies due to the need for lifelong immunosuppression to support CTA survival. In addition the ethical, social, and psychological issues and debates will continue as long as lifelong immunosuppression support will be needed. Thus, it is obvious that future experimental models of different CTA transplants should be developed for to test the feasibility, safety and immunological as well as functional outcomes of CTA transplants.

Conflict of interest statement

The authors do not declare any conflict of interest or financial support in this study.

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