Labial Salivary Gland Transplantation for Severe Dry Eye in a Rhesus Monkey Model

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PURPOSE. To evaluate the effectiveness of autologous labial salivary gland with labial mucous membrane graft in a rhesus monkey model with severe dry eye.

METHODS. Eight eyes of eight rhesus monkeys with severe dry eye were included. Four eyes underwent autologous labial salivary gland and mucous membrane graft (group 1) and four eyes served as controls (group 2). The ocular surface was evaluated before and after transplantation surgery (at 1, 4, 8, 12, and 24 weeks). Conjunctival impression cytology was performed before and 24 weeks after transplantation. Finally, a histological analysis of the cornea, conjunctiva, and transplanted grafts was performed.

RESULTS. At inclusion (n = 8) the mean Schirmer test was 1.31 ± 0.53 mm, the mean fluorescein score was 4.7 ± 1.65, and the mean lissamine green staining was 4.38 ± 0.48. After transplantation, a significant increase in tear secretion was observed with the mean Schirmer test results in group 2 significantly higher than those observed for group 1 at all time points (P < 0.05). Similarly, fluorescein and lissamine green scores were significantly lower in group 2 than in group 1 at all time points after transplantation (P < 0.05). Impression cytology specimens showed severe conjunctival squamous metaplasia without goblet cells in both groups. Under light microscopy, no significant difference was observed between the cornea and the conjunctiva of the two groups.

CONCLUSIONS. Labial salivary gland transplantation provided a basal secretion of tears and improved ocular surface staining scores during the first 3 months in a severe rhesus monkey model of dry eye. However, this was not accompanied by major improvement of ocular surface tissues.

Keywords: dry eye, transplantation, labial salivary glands, rhesus monkey
Patients with severe dry eye experience permanent symptoms and show major ocular surface alterations despite the frequent use of unpreserved tear substitutes or ointments, lacrimal punctum occlusion, or anti-inflammatory eye drops.¹⁻³ Severe complications of the ocular surface may occur such as keratinization and corneal neovascularization, which in turn may cause loss of vision.⁴,⁵ Ophthalmologists have tried several methods to substitute for natural lubrication in eyes with severe dry eye disease.⁶⁻⁸ Except for artificial devices, transplantation of significant amounts of actively fluid-secreting tissue is the only option available to provide a sufficient volume of tears.⁹ The composition of saliva and tears was found to be fairly similar in their complexity as well as in several specific parameters.¹⁰ They contain large amounts of immunoglobulins, albumin, growth factors, lipids, and mucins, all of which are also present in tears. However, saliva also includes enzymes such as amylase, but due to their substrate specificity, they have not been found to be deleterious for the ocular surface.¹⁰ Microvascular autologous transplantation of submandibular glands with implantation of Wharton’s duct into the upper conjunctival fornix was first introduced by Murube-del-Castillo in 1986¹¹ to treat severe dry eye and was further evaluated in several studies.¹²⁻¹⁶ This procedure could offer a permanent autologous source of tears with the basal secretion of a transplanted revascularized but denervated submandibular gland in order to overcome gustatory epiphora. However, the surgical procedure is complex, requiring different specialized surgeons and close postoperative follow-up with frequent reintervention.¹⁷,¹⁸ An inappropriate tear secretion has also been observed in response to physical activity or chewing.¹⁹ More recently, autologous labial gland transplantation has been reported for severe dry eye patients.¹⁰,²⁰⁻²⁶ These studies showed relief from dry eye symptoms, a decreased frequency of instillation of artificial tears, higher Schirmer test results, and improved fluorescein breakup time. The benefits over other gland transplantation techniques include easy access, ample availability of labial glands, and virtually no rejection.¹⁰,²⁰⁻²⁶ Consequently, the transplantation of minor salivary glands could be a promising new treatment option for severe cases of dry eye.

Considering that little is known about the survival of glands, the characteristics of the salivary tear film and the benefit for the ocular surface, the aim of the present study was to evaluate autologous labial salivary gland transplantation effectiveness in a model of severe dry eye in rhesus monkeys.
knowledge, this is the first study to evaluate this procedure in a severe dry eye monkey model with a control group and using objective dry eye tests.

MATERIALS AND METHODS

Animals and Surgical Procedures

Eight male rhesus monkeys (aged 3–5 years and weighing 5.5–6.0 kg) were used for the study. All eight monkeys were purchased from the Academy of Military Medical Sciences in Beijing, China. Animal housing and handling were done by the Capital Medical University Laboratory Animal Center (Beijing, China). This study was carried out in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines of the Animal Experimental Committee of Capital Medical University. The animals underwent slit-lamp examination before surgery. Only animals with a normal anterior segment and ocular surface were included in the study. Only the right eyes of the monkeys were used in this study; the left eyes were not treated.

Our group previously reported the development of a rhesus monkey severe dry eye model. Briefly, in the right eye, the main lacrimal gland and nictitating membrane were completely removed, and 50% trichloroacetic acid was applied on the conjunctiva to induce severe dry eye conditions. The severity of dry eye was confirmed by ocular surface tests performed on every animal 12 weeks after surgery (Dry Eye Workshop criteria). After 12 weeks, dry eyes (i.e., right eyes) were randomly divided into two groups: group 1 (n = 4), in which rhesus monkeys remained untreated as a control group, and group 2 (n = 4), where rhesus monkeys underwent autologous labial salivary gland transplantation.

Monkeys were first anesthetized through an intramuscular injection of 0.2 mL/kg of ketamine hydrochloride (Shanghai no. 1 Biochemical & Pharmaceutical Co. Ltd., Shanghai, China). The skin around the right eye was shaved and wiped with 2% iodine tincture (Shuang Qiao Pharmaceutical Company, Beijing, China), and the oral cavity sterilized with 0.05% chlorhexidine. Lidocaine hydrochloride 2% (Yi Min Pharmaceutical Co. Ltd., Beijing, China) was injected under the conjunctiva and mucosa to provide local anesthesia. Two labial mucosa grafts containing labial salivary glands, measuring 8 mm in diameter, were obtained from the lower lip, above the orbicularis oris muscle. The presence of salivary glands in labial mucosa grafts was histologically identified in a previous study by our group. This labial wound was not sutured to allow for second-intention healing. Both the upper and lower eyelids were inverted using a Desmarres retractor, and the fornical-temporal conjunctiva was incised over 10 mm. One labial graft was then accommodated into the superior conjunctival fornices and one graft in the inferior fornices of each eye (two grafts for each eye), with the glands facing the muscle and sutured at the conjunctiva using Vicryl 8.0 (Johnson & Johnson, Somerville, MA, USA). Tobramycin and dexamethasone eye ointment (Tobradex; Alcon, Fort Worth, TX, USA) was applied once in the conjunctival cul-de-sac of the operated eyes immediately after transplantation.

Ocular Surface Tests

Ocular surface tests were performed on both eyes during the same examination time—before (12 weeks after inducing severe dry eye) and 1, 4, 8, 12, and 24 weeks after transplantation in the following order: first the fluorescein test, then lissamine green staining and last the Schirmer-1 test. Fluorescein corneal staining consisted of wetting a fluorescein strip (Tianjin Jingming New Technology Development Co.,
Ltd., Tianjin, China) with isotonic sodium chloride solution and gently applying it to the lower conjunctiva. After several blinks, slit-lamp examination was performed with a cobalt blue filter to visualize the areas of fluorescein staining. The cornea was divided into four quadrants and the intensity of staining was assessed using the following grades: 0, no staining; 1, few separate punctate stainings; 2, many separate punctate stainings; 3, confluent punctate staining or presence of filament.28 Then a lissamine green strip (Hub Pharmaceuticals, LLC, Cucamonga, CA, USA) was wetted with isotonic sodium chloride solution and gently applied to the lower conjunctiva. The intensity of staining was analyzed in the cornea and the conjunctiva using the Van Bijsterveld scoring system.29,30 After a 10-minute washout, the Schirmer test strip (Tianjin Jingming New Technology Development Co., Ltd.) was placed in the lower conjunctival fornix at the junction of the middle and lateral thirds of the lower eyelid margin. The extent of wetting was measured after 5 minutes with no anesthesia.

### Conjunctival Impression Cytology

Conjunctival impression cytology specimens were collected using a 5-mm round cellulose acetate filter (Toyo, Roshi Kaisha, Ltd., Tokyo, Japan). Impression cytology specimens were collected 2 mm from the limbus. After 5 seconds, filters were gently removed to collect a homogeneous population of superficial conjunctival cells and then fixed with 95% alcohol. The specimens were then stained with periodic acid–Schiff (PAS).31 After staining, the goblet cells were counted under a light microscope (Olympus, CH-2, Tokyo, Japan) with a high-power-field ×40 objective, and the conjunctival epithelium morphology was graded according to the Nelson classification.31 Ten randomly chosen areas (220 × 162 μm, 0.036 mm²) around the center of the filter were evaluated; the results of the goblet cell count and grading represented the mean value of these 10 areas.

### Histopathologic Examination

The animals were killed at the end of the experiment (at 24 weeks). The upper and lower eyelid, bulbar conjunctiva, and cornea were obtained from both treated and control eyes. Tissue samples were fixed in 10% formalin. After dehydration, the specimens were embedded in paraffin, cross-sectioned, and stained with hematoxylin-eosin (HE). The sections were observed under an optical light microscope (Olympus CH-2).

### Statistical Analysis

Schirmer tests were analyzed preoperatively and postoperatively using repeated analysis of variance. A Kruskal-Wallis test was used to compare the ocular surface staining scores in the two groups at each postoperative time point. The statistical significance of the goblet cell density was evaluated among both operated and control eyes using a 1-way analysis of variance. Statistical analysis was carried out using SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). P < 0.05 was considered significant.

### RESULTS

The wound in the lower lip spontaneously recovered after 4 weeks in all eyes. Swelling of the lids could be observed within 4 weeks. Healing of the surgical wounds was uneventful without infection, necrosis, entropium, or ectropium in either the eyelids or lips.

### Slit-Lamp Examination

In the eyes receiving the transplant (group 2), foamy and viscous secretions were found on the ocular surface attached with filaments (Fig. 1A). Spumescent secretions were observed in the inner canthus. The upper eyelid grafts were slightly exposed for examination. The lower eyelid grafts had clear boundaries until week 12 without visible sutures (Fig. 1B),

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**FIGURE 2.** Comparison of the Schirmer tests in both groups. In the untreated group (group 1), the Schirmer tests was consistently below 2 mm at 5 minutes. In the transplantation group (group 2), the Schirmer test was maximum at the 1st week, then decreased gradually to approximately 2 to 4 mm. There was a statistically significant difference in tear secretion between the two groups. (P < 0.05).

**FIGURE 3.** Slit-lamp photographs of fluorescein staining test. (A) In untreated eyes, abnormal fluorescein staining was obviously detected. (B) In transplantation eyes, fluorescein staining was much milder with filaments attached at the 12th week.
then the boundaries gradually became invisible. After 24 weeks, the four eyes with grafts showed the following characteristics: in the upper eyelids, three grafts were unidentifiable due to severe scarring and one appeared contracted (Fig. 1C); in the lower eyelids, only one kept the original size and the other three contracted to various degrees (Fig. 1D).

**Ocular Surface Tests**

Before transplantation and after inducing dry eye, the mean Schirmer test was $1.31 \pm 0.53$ mm (range, 0.5–2.0 mm) in the right eyes (dry eyes) and $27.13 \pm 2.1$ mm (range, 25–31 mm) in the left eyes (control eyes without dry eye). One week after labial salivary gland transplantation, a substantial increase in aqueous tear secretion was observed, and the mean Schirmer test increased from $1.38 \pm 0.48$ mm to $8.25 \pm 4.75$ mm in group 2, with a maximum of 15 mm in one eye (range, 5–15 mm). Then tear secretion decreased gradually from week 12 to the end of the experiment. After 24 weeks, in group 2 the mean Schirmer test reached $3.0 \pm 0.82$. Tear secretion in group 1 (right eyes without graft) was not modified during follow-up, and at 24 weeks the mean Schirmer test was $0.63 \pm 0.48$ mm. The mean Schirmer test in group 2 was significantly higher than that observed for group 1 at all time points ($P < 0.05$) (Fig. 2).

Similarly, before transplantation and after inducing dry eye, the mean fluorescein score was $4.7 \pm 1.65$ (range, 3–6) and result of the mean lissamine green staining was $4.38 \pm 0.48$ (range, 4–5) in the right eyes. In contrast, no abnormal staining was observed in the left eyes throughout the duration of the study. During the 24 weeks of follow-up after

**TABLE. Summary of Published References of Minor Salivary Transplantation**

<table>
<thead>
<tr>
<th>Author and Published Year</th>
<th>No. of Patients (Eyes)</th>
<th>Causes</th>
<th>Graft Site and Size, mm</th>
<th>Receptor Site (Conjunctival Fornix)</th>
<th>Examinations</th>
<th>Follow-up Time (Month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murube 1998</td>
<td>6 (6)</td>
<td>Sjögren syndrome = 5; cicatricial pemphigoid = 1; Stevens-Johnson = 1; Seventh nerve palsy = 1</td>
<td>Lower lip = 5; Upper lip = 1</td>
<td>Upper and lower</td>
<td>ST, BUT</td>
<td>11 (6–24)</td>
</tr>
<tr>
<td>Guerrissi 2004</td>
<td>1 (1)</td>
<td>Sjögren syndrome</td>
<td>Upper gingival-buccal groove; 15×5</td>
<td>Lower</td>
<td>ST, BUT, FUATS, Biopsy</td>
<td>3</td>
</tr>
<tr>
<td>Soares 2005</td>
<td>21 (37)</td>
<td>Severe dry eye (associated with other surgical procedures in 27 eyes)</td>
<td>Lower lip</td>
<td>Upper or lower</td>
<td>BCVA, FUATS</td>
<td>≤46</td>
</tr>
<tr>
<td>Geerling 2008</td>
<td>17 (NDA)</td>
<td>Alkali burn; Congenital alacrimia; Others</td>
<td>Lower lip; 25×10</td>
<td>Upper and lower</td>
<td>ST, BUT, Biopsy</td>
<td>18–36</td>
</tr>
<tr>
<td>Diane 2010</td>
<td>14 (14)</td>
<td>Stevens-Johnson = 10; Chemical burns = 4</td>
<td>Lower lip; 15×15</td>
<td>Upper</td>
<td>BCVA, ST, BUT</td>
<td>14 (6–27)</td>
</tr>
<tr>
<td>Sant’Anna 2012</td>
<td>19 (19)</td>
<td>Stevens-Johnson</td>
<td>Lower lip</td>
<td>Upper and lower</td>
<td>BCVA, ST, Q</td>
<td>6</td>
</tr>
<tr>
<td>Luo 2013</td>
<td>8 (8)</td>
<td>Stevens-Johnson = 6; Others = 2</td>
<td>Lower lip; 25×20</td>
<td>Upper and lower</td>
<td>BCVA, ST, Q</td>
<td>6</td>
</tr>
<tr>
<td>Wakamatsu 2017</td>
<td>4 (4)</td>
<td>Stevens-Johnson</td>
<td>NDA</td>
<td>NDA</td>
<td>BCVA, ST Biopsy</td>
<td>52.75 (1–120)</td>
</tr>
</tbody>
</table>

BCVA, best-corrected visual acuity; ST, Schirmer test; BUT, tear film break-up time; FUATS, frequency of use artificial tear substitution; Q, questionnaire; NDA, no data available.

![Figure 4](image-url)  
**Figure 4.** Slip-lamp photographs of lissamine green test. (A) In untreated eyes, abnormal lissamine green staining was obviously detected. (B) In transplantation eyes, a lissamine green staining was milder at the 12th week.
transplantation, slit-lamp examination always revealed abnormal fluorescein and lissamine green staining in both groups. However, fluorescein and lissamine green scores were significantly lower in group 2 than in group 1 at all time points (Figs. 3–5). After 24 weeks, the mean fluorescein score was 6.25 ± 0.96 in group 1, ranging from 5 to 7 mm, and 2.75 ± 0.96 in group 2, ranging from 2 to 4 mm ($P < 0.05$). The mean lissamine green score was 5.25 ± 0.96 in group 1, ranging from 4 to 6 mm, and 3.0 ± 0.0 in group 2 ($P < 0.05$) (Figs. 3–5).

**Conjunctival Impression Cytology**

In the left eyes, the impression cytology specimens showed many small round conjunctival epithelial cells, and fewer large oval goblet cells (315.43 ± 88.2 cells/mm²). Epithelial cells had a large nucleus with a nucleocytoplasmic ratio of approximately 1/1.3, and goblet cells had a strongly PAS-positive cytoplasm. All normal eyes had a Nelson classification grading between 0 and 1. Before autologous labial salivary gland transplantation, the impression cytology dry eye specimens (right eyes) showed conjunctival squamous metaplasia and a lower number of goblet cells. No goblet cells were found on impression cytology in the right eyes of both groups at the sampling site. All the Nelson classification grading scores reached grade 3. Twenty-four weeks after transplantation, conjunctival epithelial cells were distributed sparsely with fusiform-shaped epithelial cells in dry eye eyes in both groups. The nucleocytoplasmic ratio was higher than 1/10 with a high number of pyknotic cells in both groups. Conjunctival squamous metaplasia was severe and goblet cells remained invisible, with a mean Nelson classification grading of 3 in both groups with no difference observed. Numerous purple-red substances from salivary secretion were observed in group 2 (Fig. 6).

**Histopathological Examination**

Twenty-four weeks after surgery, the conjunctival epithelium showed squamous metaplasia with an irregular epithelium thickness, loss of stratification, smaller homogeneous cell size and staining, and an obvious loss of goblet cells with infiltration of inflammatory cells in both groups. Sparse goblet cells were observed only in the conjunctival fornix. There was hyperplasia of the corneal epithelium with signs of hyperkeratosis. Neovascularization was observed in a disorganized corneal stroma. Furthermore, conjunctival blood vessel vasodilation was observed.

In comparison to normal labial salivary glands, the density of the alveolar glands in the grafts drastically decreased, with abnormal structure and lymphocyte infiltration. Most graft tissues showed numerous fibroblasts and scar tissue (Fig. 7).

**Discussion**

Minor oral salivary glands are present in large numbers in the labial, buccal, and palatal mucosa, and make up approximately half of the baseline secretion of saliva in humans. Labial salivary glands can be transplanted with the overlying mucosa as a complex graft to the posterior lamella of eyelids with the
intention of increasing ocular surface lubrication and reducing discomfort and pain in dry eyes. Transplantation of minor salivary glands has been reported to be a promising new treatment option for severe dry eye patients. In the literature, a total of 106 eyes of 90 patients underwent minor salivary transplantation for severe dry eye. The results of these studies are gathered in the Table. Although this procedure was described to be effective and simple to perform with minimal surgical risks, it is still not commonly used.

Our experimental results on a rhesus monkey model of severe dry eye were different from the above-mentioned clinical reports, in which the transplanted glands maintained considerable secretion from the early stage to 3 months. In the present study, within the first 12 weeks after transplantation, an obvious increase in the quantity of tears was observed and foam-like secretions resembling saliva, suggesting an exocrine activity of the grafted salivary glands, were observed at the level of the ocular surface. This was associated with decreased staining scores in group 2, indicating that the transplanted labial glands retained a secreting function in the early stage after surgery. Since numerous purple-red substances (PAS staining) that could only come from transplanted labial glands were observed and the sutures were absorbable and disappeared within 4 weeks, the increase in tear secretion (Schirmer test) was not explained by inflammatory secretions. Moreover, as previously published, only very few ocular surface secretory glands could be present in the present severe dry eye model.

Nevertheless, from week 12, the quantity of tears in group 2 decreased regularly to eventually be close to the level of untreated group 1 at 24 weeks. Due to graft scarring, labial acinar disappeared and the graft lost its secretion function, rendering the procedure ineffective. These results differed substantially from previous reports (Table) as well as the recent study by Wakamatsu et al. on patients with severe dry eye, which showed functional salivary glands several years after the transplantation surgery. The difference between monkeys and humans, in the density and secretory function of labial salivary glands, the microenvironment and the blood supply to the ocular surface could also partially explain these results.

Although rhesus monkeys have a larger oral cavity than humans, the density of labial glands is much sparser in monkeys than in humans. Consequently, the graft tissues in the present study were probably smaller with fewer glands than what was analyzed in previous studies in humans. Unfortunately, the size of labial grafts could not be larger in our experiment, considering rhesus monkey’s eyes. Moreover, in previous reports, there were many differences in the etiology of dry eye, the methods used to evaluate the ocular surface and tear secretion as well as postoperative treatments.

Although tear secretion measured by the Schirmer test rapidly decreased after 12 weeks, the ocular surface staining scores remained stable in group 2, still lower than that in group 1. With PAS staining, numerous purple-red substances coming from transplanted labial glands were observed in group 2 at the end of the experiment, suggesting that a certain secreting function was maintained. Although the watery secretion detected by Schirmer tests almost disappeared, acinar from the grafted glands might still secrete glycoprotein. This was demonstrated recently by immuno-histochemistry on biopsies of minor salivary gland grafts in

![Histologic examination of the cornea, conjunctiva, and graft at the 24th week in group 2 (transplantation) (HE, A–C, magnification ×200). A The conjunctival epithelium showed squamous metaplasia with an obvious loss of goblet cells, inflammatory cell infiltration, and vasodilation. B There was hyperplasia of corneal epithelium with foot processes. New blood vessels were detected in the disorganized corneal stroma. C Decreased alveolar gland tissue with abnormal structure (horizontal arrow), lymphocyte infiltration, and obvious fibroblasts and scar tissue in the transplanted grafts. The vertical arrowhead points to sebaceous glands of the eyelid. D Normal labial gland acinar of rhesus monkey (HE, ×400).](image)
human indicating local production of proteins, enzymes, and mucins even several years after surgery.\textsuperscript{26} Despite these differences in corneal and conjunctival staining grading scores throughout the study, no difference between the two groups was highlighted on impression cytology grading scores. Moreover, this was in accordance with the results of histopathologic examination. After 24 weeks, no histological difference between the two groups was observed in the ocular surface tissues. The properties of saliva, which might be less effective than tears in protecting the ocular surface, might explain these results.

Clinically, only one of the grafts (1/8) remained at its original size until the end, and the others presented different degrees of atrophy or even disappeared. The histopathologic examination results showed that the amount of glandular tissue drastically decreased and the grafts were surrounded by large numbers of fibroblasts and scar tissue. Since they were autologous grafts, it is highly unlikely that this stemmed from graft rejection, and a nonspecific inflammatory reaction was probably responsible for tissue destruction. Severe dry eye is drastically decreased and the grafts were surrounded by large numbers of fibroblasts and scar tissue. Since they were autologous grafts, it is highly unlikely that this stemmed from graft rejection, and a nonspecific inflammatory reaction was probably responsible for tissue destruction. Severe dry eye is undoubtedly accompanied by inflammation of the ocular surface.\textsuperscript{35,36} Similar inflammatory cell infiltration and acinus atrophy were also found in previous clinical studies on minor salivary gland grafts.\textsuperscript{23} Moreover, as previously published, the animal model of severe dry eye used herein is also associated with severe inflammatory changes of the ocular surface,\textsuperscript{27} which also might explain the rapid destruction of the transplanted glands.

Labial salivary glands with mucous membrane grafting was partly effective in a rhesus monkey model of severe dry eye at the early stage, but the efficacy of fluid production rapidly decreased during follow-up. However, despite improvement of the ocular surface staining score, no differences could be observed at the level of histological tissues within transplanted eyes. Further studies should investigate the way to maintain long-term secretion function of the labial gland after grafting, to prevent transplanted graft scar formation with anti-inflammatory eye drops, and finally to obtain sufficient fluid production to prevent conjunctival and corneal changes and consequently to alleviate symptoms and improve dry eye patients’ quality of life.

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**References**