Ocular Surface Management in Corneal Transplantation, a Review

Kazuo Tsubota*

*Departments of Ophthalmology,
Tokyo Dental College, Chiba, and Keio University School of Medicine, Tokyo, Japan

Purpose: To discuss the importance of ocular surface management in corneal transplantation, especially in limbal transplantation.

Methods: Since the corneal epithelium is not completely recovered after corneal transplantation, meticulous attention should be paid to the ocular surface to prevent infection and rejection, which are the major causes of corneal transplantation failure. Preoperative evaluations of the ocular surface should be carried out, followed by appropriate surgical procedures, depending on the condition of each patient. Vigorous immunosuppressive measures should be taken after surgery.

Results: In both case reports presented in this study, each patient underwent successful surgery and his condition was controlled by medication suited to his needs.

Conclusions: For those patients with stem cell deficiency, limbal transplantation, possibly with the use of autologous serum drops, should be considered to reconstruct and maintain the ocular surface. Ocular surface management is necessary for the success of corneal transplantation, especially for limbal transplantation. **Jpn J Ophthalmol 1999;43:502–508** © 1999 Japanese Ophthalmological Society

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Ocular surface management is the key to a successful corneal transplant. Infections and rejection arise from the corneal epithelium, and can be initiated by as little as a single eyelash pointing inward. Furthermore, recent research has shown that corneal epithelial stem cells exist only in the limbal area,[1,2] so that a simple corneal transplantation, which replaces the central cornea, cannot replenish the stem cell supply. Limbal transplantation is necessary in the case of depleted or dysfunctional stem cells, and represents a major breakthrough in ocular surface reconstruction conditions that may affect the type and course of transplantation including allergic conjunctivitis, decreased corneal sensitivity secondary to diabetic epitheliopathy or other pathology, and dry eye.[3,4] Tears provide chemicals that are essential for maintaining a healthy ocular surface epithelium, including vitamins and growth factors and other cytokines.[5–7] There is new evidence that dry eye may be divided into two types, with and without reflex tearing,[8–10] with the latter frequently representing a significant challenge in transplant patients.

Preoperative Evaluation of Ocular Surface in Corneal Transplantation

Some ocular surface disorders are easy to detect, such as entropion, symblepharon, meibomianitis, or typical dry eye, whereas others are subclinical, yet may still complicate corneal transplantation. Meticulous preoperative evaluation of the ocular surface is thus critical to a successful postoperative course. The simplest and most important examination of the ocular surface involves staining with rose bengal and fluorescein, which can reveal otherwise subtle corneal or conjunctival epithelial problems (Figures 1A, 1B). We use a micropipette to instill 2 μL of a preservative-free combination of 1% rose bengal and 1% fluorescein.[11]
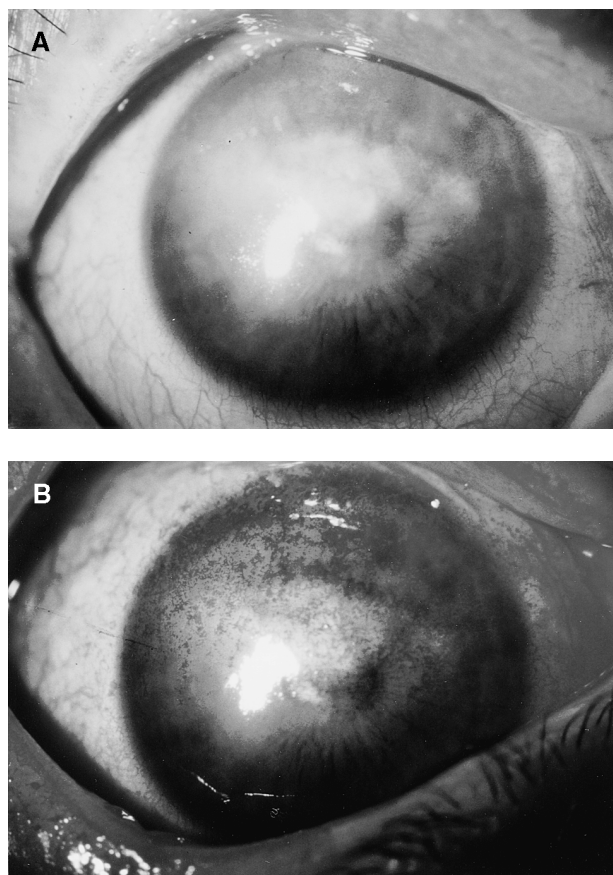
Figure 1. Ocular surface examination with and without vital staining with 2 μL 1% rose bengal and 1% fluorescein. (**A**) Slit-lamp examination of ocular surface before penetrating keratoplasty (PK). No apparent abnormalities without vital staining. (**B**) Slit-lamp examination of ocular surface before PK with vital staining. Note staining of both corneal and conjunctival epithelium.

The small dye volume means that the eye does not have to be washed out with saline, and also avoids the pain and cell damage that can be caused by rose bengal toxicity. Fluorescein staining indicates epithelial cell damage or breakdown of cell–cell junctions, whereas rose bengal staining indicates epithelial cells not adequately covered by mucin.[12] Fluorescein staining thus suggests stem cell dysfunction, whereas rose bengal staining can indicate dry eye.

Impression cytology is the definitive means of evaluating the ocular surface. We use the criteria of Tseng,[13] especially to diagnose "conjunctivalization of corneal epithelium" and "squamous metaplasia resulting from dry eye," which are indicated by goblet cells on the cornea and the lack of goblet cells on the conjunctiva, respectively.

The simple Schirmer test does not provide enough information to adequately evaluate tear dynamics. The tear clearance rate (TCR) and degree of reflex tearing are more important factors. TCR is measured by instilling a 10-μL drop of 0.5% fluorescein and 0.4% oxybuprocaine hydrochloride into the conjunctival sac.[14] A standard Schirmer test strip is then inserted for 5 minutes. The length of the wet portion is measured and the intensity of its staining is compared with nine standard dilution colors ranging from 1 (low TCR) to 1/256 (high TCR) (Figure 2). [15] Patients with a low TCR should use preservative-free eye drops to avoid epithelial problems from preservatives lingering in the conjunctival sac. The new measure of tear dynamics, the tear function index (TFI), is calculated by dividing the result of the Schirmer test with anesthesia by the TCR, and reflects both tear production and drainage.[14] We have determined a cutoff of 96 for TFI, below which we diagnose dry eye; our threshold for severe dry eye is
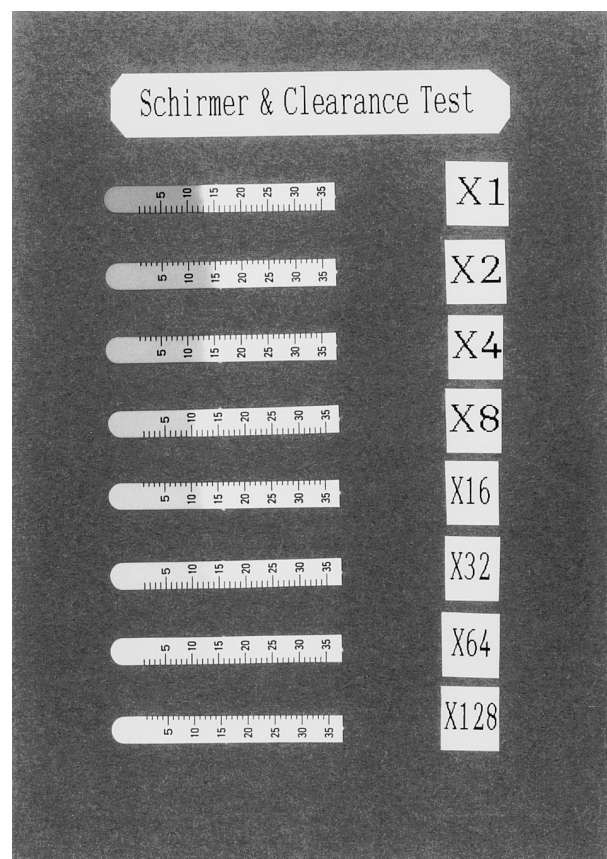

Figure 2. Standard color plate of tear clearance rate. Reprinted with permission from Br J Ophthalmol (1995; 79:1042–5), Copyright © 1995, BMJ Publishing Group.

a TFI of 34. As an example, a Schirmer test result of 15 mm is apparently normal, but if the patient's TCR is only 1/2, the TFI is $15/(1/2) = 30$. The wet portion of this patient's Schirmer paper would reflect only the pooling of the anesthesia drops in the conjunctival sac.

A low Schirmer test result may mean inadequate lacrimal gland function, or may reflect insufficient stimulus of the lacrimal gland.[8,9] The Schirmer test with nasal stimulation can yield important information in such cases. Briefly, patients are screened with the regular Schirmer test without topical anesthesia for 5 minutes. Then, a small cotton swab (8 cm long, 3.5 mm wide at the tip) is inserted into the nasal cavity, slightly upward and parallel to the lateral wall (Figure 3). The Schirmer paper is then placed in the conjunctival sac for 5 minutes, as in the ordinary Schirmer test, while the swab is kept in place. The wet part of the strip is measured as in the regular Schirmer test. When the wet portion is at least 10 mm, the patient is considered to have normal reflex tears, and would be expected to have proper postoperative wound healing of the ocular surface epithelium resulting from an adequate supply of growth factors and other cytokines and nutrients in reflex tears. If the wet portion is less than 10 mm, the decreased reflex tears may predispose the patient to epithelial breakdown or chronic epithelial defect.

Management of Ocular Surface Conditions Before Surgery

We aggressively manage any abnormalities in the ocular surface epithelium or tear dynamics. Most of the conditions are subclinical states where no clinical abnormalities are manifested sometimes because they are masked by such primary diseases as corneal scar or herpes keratitis, but are nevertheless important to detect and treat.

Inflammation

Inflammation should be minimized both pre- and postoperatively. Direct mechanical trauma, such as caused by entropion or trichiasis, should be corrected, the latter sometimes by electrolysis. If infection is suspected, the ocular surface is cultured and appropriate antibiotic therapy is begun. Meibomianitis is treated with topical and/or oral tetracycline to control the associated blepharitis. Prophylactic topical or oral acyclovir is recommended for at least 2–3 months after surgery to prevent recurrence in patients with herpes keratitis.

Dry Eye

Preservative-free artificial tears should be used for lubrication. Protective glasses, sometimes with side

Figure 3. Schirmer test with nasal stimulation. Note wet Schirmer paper on stimulated side. Reprinted with permission from J Rheumatol (1996;23:313–20), Copyright © 1996, The Journal of Rheumatology.

panels containing moist inserts, are a good method to prevent desiccation and can also keep foreign bodies from getting into the conjunctival sac.[16,17] Punctal occlusion can decrease tear drainage, thereby increasing the nutrient requirements for proper wound healing and epithelial health.[18] Inflammatory cytokines and small foreign bodies may also accumulate following punctal occlusion, so frequent flushing with physiologic saline or artificial tears is necessary. Punctal occlusion may be inadequate to treat dry eye associated with Sjögren's syndrome with no reflex tearing, in which case drops made from autologous serum may be beneficial.[5,19] Serum contains epidermal growth factor, vitamin A, transforming growth factor (TGF)-β, and other components that are present in natural but not in ordinary artificial tears.[6,10]

Corneal Epithelial Stem Cell Dysfunction

Puangsricharern and Tseng[20] reported a list of conditions involving total loss of corneal stem cells, including chemical burn, Stevens-Johnson syndrome, multiple surgeries or cryotherapies of the limbal region, contact lens–induced keratopathy, and severe microbial keratitis. Although these may be easy to diagnose, chronic inflammation and corneal vascularization can be difficult to interpret. We always perform impression cytology of the corneal epithelium to look for goblet cells. Such cells represent "conjunctivalization," and indicate local or total stem cell dysfunction. It is not clear yet whether stem cell dysfunction is permanent or transient. We try to control any inflammation associated with dry eye, following the patient for at least 3 months. If stem cell function does not recover, then a corneal stem cell transplant is considered.

Allergic Conjunctivitis

Allergic conjunctivitis, especially a severe form such as vernal keratoconjunctivitis, must be aggressively managed; topical steroids or cyclosporine are the treatment of choice. Patients with keratoconus tend to have papilla formation of the upper tarsal conjunctiva and should be examined very carefully.

Diabetic Epitheliopathy

Elevated glucose can adversely affect corneal innervation and the epithelium. Sensation is decreased, causing a slower blink rate. Epithelial proliferation is slowed, with weaker attachment to the basement membrane and abnormal cellular morphology. Corneal epitheliopathy often develops in diabetic patients, especially after the stress of surgery and the resulting delayed wound healing. It was originally reported after vitrectomy, but may follow any ocular surgery. In Japan, we have obtained good results treating diabetic epitheliopathy with an oral aldose reductase inhibitor (ARI; Kinedac, Ono, Osaka). A topical ARI is still under investigation.[21–23]

Decreased Corneal Sensation

Abundant innervation of the cornea is necessary not only for sensation, but also to supply neurotransmitters such as acetylcholine and substance P, the latter essential for proper epithelial wound healing. When sensation decreases, the blink rate, tear production, and epithelial mucin expression are also decreased, resulting in dry eye. Neurotrophic keratitis is the most severe condition involving decreased corneal sensation. Chikama et al[24] recently reported that neurotrophic keratitis is partially caused by the depletion of neural peptides such as substance P. Since it has been recently confirmed that autologous serum contains these neurotransmitters, it makes sense to use autologous serum for treating conditions involving decreased corneal sensation. Although there is no cure for the underlying problem, we have recently obtained good results in treating decreased corneal sensation after penetrating keratoplasty, using drops developed from autologous serum.[25]

Limbal Transplantation for Ocular Surface Reconstruction

Autograft limbal transplantation is performed in patients with unilateral corneal stem cell dysfunction, such as unilateral chemical or thermal burn.[26] This has the advantage of facilitating epithelial recovery without requiring immunosuppressive measures after surgery. However, allograft transplantation must be considered in any patients with bilateral pathology.

When necessary, we perform penetrating keratoplasty, extracapsular cataract extraction, anterior segment reconstruction, and intraocular lens implantation (triple procedure) followed by limbal transplantation.[27]

The central donor cornea is trephined to the appropriate diameter for penetrating keratoplasty. First, the scleral portion is removed from the rim with microscissors, while the epithelium is preserved as much as possible. The stroma of the rim is removed from the epithelial portion with scissors, so that the limbal tissue consists of only Bowman's layer, a small amount of stromal tissue, and corneal and limbal epithelium. These two pieces are placed

on the recipient limbus with 10-0 nylon sutures. At the end of the surgery, we administer a subconjunctival injection of 10 mg of gentamicin and 2 mg of dexamethasone. We then cover the eye with a soft contact lens until the corneal epithelium has healed.

Pre- and Postoperative Management

Oral cyclosporine A (10 mg/kg) is prescribed 2 days before the limbal transplantation surgery, and continued for 1 to 3 months after surgery to maintain a serum level of 100–150 ng/mL, and then tapered. Intravenous dexamethasone (8 mg) is given for the first 3 days, and then tapered within 12 days. Topical eye drops include antibiotics 5 times a day (Tarivit; Santen, Osaka), 0.1 % dexamethasone 5 times a day (betamethasone; Santen), and 0.05% cyclosporine diluted in 4% α-cyclodextran 5 times a day. We make the cyclosporine drops in our laboratory using cyclosporine A powder. The topical antibiotics and dexamethasone are tapered when the intraocular pressure is elevated, but are otherwise continued. The cyclosporine drops are continued indefinitely in all patients.[27]

Case Report

A 73-year-old man had suffered for more than 10 years from significant visual loss resulting from an acid burn. Sight in the fellow eye was lost entirely from a perforating injury. The cornea of the right eye was vascularized and opaque, with visual acuity of hand motion. Impression cytology revealed goblet cells on the corneal epithelium. The conjunctiva appeared normal and tear function was good, with a TFI of 152. Limbal transplantation was performed with triple procedure. The visual acuity recovered to 0.2 the day after surgery and to 0.3 a month later. The cornea was stable for more than 3 months with no staining, nor were any goblet cells seen on the cornea by impression cytology. Specular microscopy showed regular cell morphology, with no elongated or abnormally large epithelial cells.[28,29] The patient experienced the typical endothelial-type rejection 3 months after surgery, and it was successfully controlled by medication. The corneal epithelium was stable for 2 years after surgery.

Ocular Surface Reconstruction for Severe Cicatricial Disease

Simple allograft limbal transplantation is not successful in patients with severe cicatricial disease, such as Stevens-Johnson syndrome or ocular cicatri-

cial pemphigoid (OCP).[30,31] Patients often have chronic inflammation, entropion, symblepharon, and dry eye. Comprehensive surgical and medical strategies are needed for these patients. To control the dry eye, we combine lateral tarsorrhaphy with frequent use of serum-derived drops and wearing of protective glasses with moist inserts and side panels.[16,17,32] These procedures keep the ocular surface moist and provide necessary tear proteins despite the total loss of endogenous tears. To replenish the corneal stem cells, we combine limbal allograft and partial amniotic membrane transplants, which results in a stable, quiet epithelium.[33] To control the inflammation of OCP, we remove all the inflamed ocular surface epithelium and submucosal fibrous tissue down to the surface of the sclera. We also give oral diaminodiphenylsulfone (DAP, dapsone) and cyclosporine A,[34] which suppress both the underlying disease and rejection of the limbal graft.

Surgical Goals

Surgical goals are: (1) removal of the inflamed ocular surface epithelium and submucosal fibrous tissue, (2) substrate replacement by amniotic membrane, (3) ocular surface stem cell replacement by limbal allograft transplant, and (4) partial tarsorrhaphy to prevent desiccation and control trichiasis.[32]

Removal of inflamed ocular surface epithelium and submucosal tissue. The conjunctival or dermal epithelium covering the cornea is removed and the symblepharon is dissected. Conjunctival epithelium with fibrous tissue is completely removed to free the eye. When the symblepharon is so severe that there is no conjunctival sac, we dissect the bulbar and palpebral conjunctiva to create a new conjunctival sac.

Amniotic membrane transplantation. Sterile fetal membrane is obtained from a cesarean-section patient, who has been screened for HIV, HCV, and HBV. The fetal membrane is cut into 5 cm × 5 cm pieces, and stored in a freezer at −80°C for 2 weeks before surgery. After defrosting the tissue, we remove the amniotic membrane from the fetal membrane and place it on the eye, with the basement membrane on the outside. The membrane is sutured to the eye with eight 10-0 nylon sutures. Although we try to cover as much of the ocular surface as possible, the palpebral conjunctiva is not covered by the amniotic membrane.

Limbal transplantation. The procedure is described in the previous section. An autograft or allograft lim-

bal transplantation is performed, with or without corneal transplantation.

Tarsorrhaphy. After the amniotic membrane and limbal transplantation, we do a lateral tarsorrhaphy to prevent desiccation. The upper and lower lid margins are cut with microscissors and sutured with 4-0 silk. Small portions of the inner lid are sutured together so that the lid is slightly everted, alleviating entropion with trichiasis. A silicone sponge bed is used for the 4-0 nylon sutures to protect the skin. The sutures are removed after 1 month, by which time a permanent lateral tarsorrhaphy is formed.

Postoperative Management

Vigorous immunosuppressive measures are an integral part of our technique. We prescribe oral cyclosporine A (10 mg/kg) 1 day before the surgery and continue for at least 1 month afterward, trying to maintain a serum cyclosporine trough of 100–150 ng/mL. When the trough is greater than 150 ng/mL, or kidney and/or liver function is abnormal (creatinine > 1.3 mg/dL or twice the preoperative value, blood urea nitrogen > twice the preoperative value, serum glutamic-oxaloacetic transaminase > 40 IU/L, or serum glutamic-pyruvic transaminase > 40 IU/L), the dose is reduced to 5 mg/kg. In patients who can tolerate it, we continue low dose oral cyclosporine (75 mg/day).

We start topical cyclosporine A dissolved in α-cyclodextron (0.05%, 5 times a day) 1 day after the surgery and continue it indefinitely. We give intravenous steroids (8 mg dexamethasone) for the first 3 days postoperatively and taper over the next 9 days. We use topical 0.1% dexamethasone and 0.3% ofloxacin (Tarivit; Santen) 5 times a day for the first 3 months and taper over the next 3 months to 2 times a day.

Because OCP patients have severe dry eye, proper wound healing is not expected without supplementary tears. Meticulous prevention of ocular surface desiccation is critical to successful ocular surface reconstruction. A total of 40 mL of blood is obtained by venipuncture and centrifuged for 5 minutes at $2,800 \times g$. The serum is carefully separated in a sterile manner and put into a colored bottle. Since vitamin A is easily degraded by light, patients are instructed to keep the bottle in a dark and cool place. They are told to apply the serum drops every 15 minutes, in addition to the frequent use of preservative-free artificial tears, highly viscous methylcellulose 4 times a day, and the wearing of special dry eye glasses. These glasses maintain the humidity level around the eye at 60%–80%, while the methylcellulose covers the ocular surface effectively.

Case Report

A 37-year-old woman experienced severe Stevens-Johnson syndrome 7 years previously and gradually developed unilateral cicatricial keratoconjunctivitis. The left cornea became opaque and keratinized with visual acuity of hand motion at 30 cm (Figure 4A).[35] Tear production, including reflex tears, was completely lost.[8] We performed corneal limbal allograft and amniotic membrane transplantation and tarsorrhaphy on October 13, 1994, followed by the use of autologous serum every 15 minutes. The corneal epithelium was reconstructed in 2 weeks and visual acuity recovered. During the observation period, the Schirmer test yielded 0 mm even with nasal stimula-
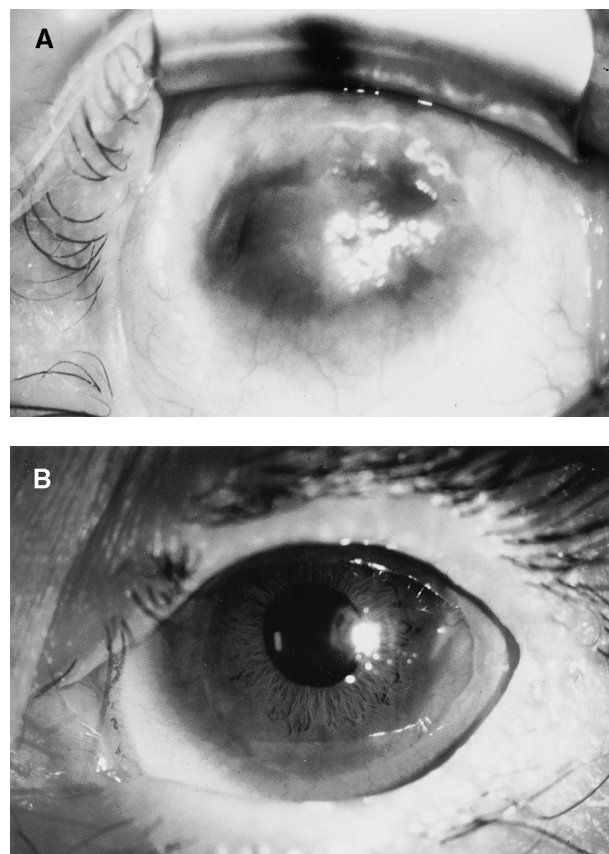
Figure 4. Ocular surface reconstruction in 37-year-old woman with Stevens-Johnson syndrome. (**A**) Preoperative photograph. Note keratinized conjunctivalization on cornea. (**B**) Postoperative slit-lamp photograph (18 months after surgery). Reprinted with permission from Lancet (1996;348:123), Copyright © 1996, The Lancet, Ltd.

tion, therefore we continued to prescribe the autologous serum drops 10 times a day. Low dose cyclosporine A (75 mg/day) was maintained indefinitely. There were no systemic side effects. The corneal epithelium remained clear, without any keratinization or vessel invasion, with a final visual acuity of 20/30 with −1.25 D of astigmatism (Figure 4B).[35–37] The patient still had a clear epithelium at the last visit on March 1999.

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