

Original Article

Combined penetrating keratoplasty and keratolimbal allograft transplantation in comparison with corneoscleral transplantation in the treatment of severe eye burns

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ABSTRACT

Purpose: The aim of this study is to compare the therapeutic outcomes between penetrating keratoplasty (PK) combined with keratolimbal allograft (KLAL) transplantation and corneoscleral transplantation in patients with severe corneal burns.

Methods: Thirty-eight patients (39 eyes) diagnosed severe corneal burns in stable status with vascularization and corneal opacity were included. We performed combined PK with KLAL transplantation in 23 eyes (group A) and corneoscleral transplantation in 16 eyes (group B). The main outcome measures were postoperative complications and long-term visual acuity and rejection.

Results: The incidence of postoperative complications (corneal epithelium defect, hyphaema and hypotony) in group A were obviously less than those in group B. Fifteen eyes (65%) in group A and four eyes (25%) in group B had best-corrected visual acuity of >0.05 at 24 months ($P=0.022$). Limbal stem cell rejection occurred in eleven grafts (48%) in group A and eight grafts (50%) in group B ($P=1.000$). Nine grafts (39%) in group A and 12 grafts (54%) in group B had endothelial rejection ($P=0.049$).

Conclusions: PK combined with KLAL transplantation may reduce the risk of postoperative complications. Long-term prognosis appears better than corneoscleral transplantation in the treatment of severe eye burns.

Key words: eye burn, keratolimbal allograft, penetrating keratoplasty, rejection.

INTRODUCTION

Corneal limbal stem cells (LSCs) concentrate at limbus and maintain homeostasis of corneal epithelium. Usually both cornea and LSC are destroyed in eyes with severe chemical or thermal burns. If LSCs are partially or totally depleted, persistent epithelial defects and corneal vascularization may occur.^{1–4} However, it remains difficult to control corneal limbal stem cell deficiency (LSCD) and corneal opacity simultaneously.

Surgical approaches for corneal chemical or thermal burns include penetrating keratoplasty (PK), total lamellar keratoplasty (LK) with limbus, keratolimbal allograft (KLAL) transplantation and corneoscleral transplantation. However, PK has been proven ineffective for the long-term treatment of this disorder because it does not address the LSCD.^{5–8} Total LK with limbus fails to resume corneal transparency despite the effective treatment of LSCD. KLAL transplantation is used for ocular surface reconstruction in eyes with severe LSCD, providing the recipient with corneal LSC from donor eyes,^{9–11} but it also could not control corneal opacity. Corneoscleral transplantation can solve these two problems and is widely used to treat severe eye burns. However, the incidence of rejection is high, many postoperative complications may occur,¹² and the long-term prognosis is unsatisfactory. Simultaneous PK combined with KLAL transplantation was first reported by Shimazaki *et al.*¹³ They investigated the incidence and prognosis of immunologic rejection of the central graft after the surgery but did not mention complications. Whether PK combined with KLAL transplantation is better than corneoscleral transplantation remains unknown. In this study, we evaluated the role of PK combined with KLAL transplantation in the treatment of severe eye burns and compared the therapeutic outcomes with corneoscleral transplantation.

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METHODS

Protocol

Clinical protocols and investigational surgical consent forms were approved by the Institutional Review Board of Shandong Eye Institute, and enrolment of patients with corneal burns in stable stages was initiated in 1999. Indications and patient selection details were as follows. Patients were binocular burned, with corneal vascularization of more than three quadrants and total corneal opacity of >5 mm. Preoperative best-corrected visual acuity (BCVA) was <0.05. Diagnosis of LSCD was confirmed by impression cytology.¹⁴ Patients with one of the following conditions were excluded from this study: lagophthalmos, intraocular pressure <10 mmHg or >21 mmHg, no light perception, no wave shape in visually evoked potential examinations or suggested optic nerve atrophy by B-scan, or suggested extensive conglutination in anterior chamber angle by ultrasound biomicroscopy. Patients with tear secretion of less than 5 mm in basic Schirmer's test were given Schirmer's tests 1 and 2, and those still with tear secretion of less than 5 mm were also excluded.

A retrospective study was conducted on thirty-eight consecutive patients (39 eyes) with severe corneal burns. Comparable severity in all aspects was randomly assigned to either of the surgical procedures. There were 33 male and 5 female patients. Surgery was performed by two surgeons in our group (Shi W and Xie L). Twenty-three eyes in 22 patients received PK combined with KLAL transplantation (group A), and 16 eyes in 16 patients received corneoscleral transplantation (group B). Indications for surgery included acid burns (16 patients), alkali burns (14 patients) and thermal burns (8 patients). Twenty-eight eyes had total corneal vascularization, and eleven had corneal vascularization of more than three quadrants. Total corneal opacity was observed in 20 eyes, and the diameter of corneal opacity was >5 mm in the other.

PK combined with KLAL transplantation

Penetrating keratoplasty combined with KLAL transplantation was performed as previously described with a few modifications.¹³ A total limbal peritomy was made to rescue donor LSC at the conjunctival edge. Oversized grafts with 2–3 mm scleral rims were prepared as donor grafts and stored in Optisol corneal medium for not more than 3 days before used. During the PK, fibrous tissue was extensively excised from the ocular surface, and conjunctiva was preserved as much as possible. Central cornea was excised using a Hessburg–Barron trephine with a diameter of 7.25–7.50 mm and was then removed and replaced with donor corneal button (7.50–7.75 mm). The donor graft was sutured by 10-0 nylon interrupted sutures. After the PK, we manually dissected the remaining ring of the same donor to remove excess tissue surrounding the limbal area. After the limbal graft was fixed onto the host eye with 10-0 nylon sutures, the recipient conjunctiva was sutured onto the corneo-

scleral limbus. At the end of the surgery, amniotic membrane was covered on the corneal surface and fixed with 10-0 nylon suture (Figs 1,2).

Corneoscleral transplantation

Corneoscleral transplantation was performed as previously described.¹² Fibrous tissue was adequately freed from the ocular surface, and conjunctiva was preserved as much as possible. After full exposure of the anterior sclera, a trephine of 11–12 mm was used to mark the marginal of the cornea and cut off the whole cornea. The donor graft (prepared like those used in group A) was sutured to the recipient scleral bed using interrupted 10-0 nylon sutures (Fig. 2). Finally, the recipient conjunctiva was sutured onto the donor corneoscleral limbus.⁷ Amniotic membrane transplantation was performed in the same way.

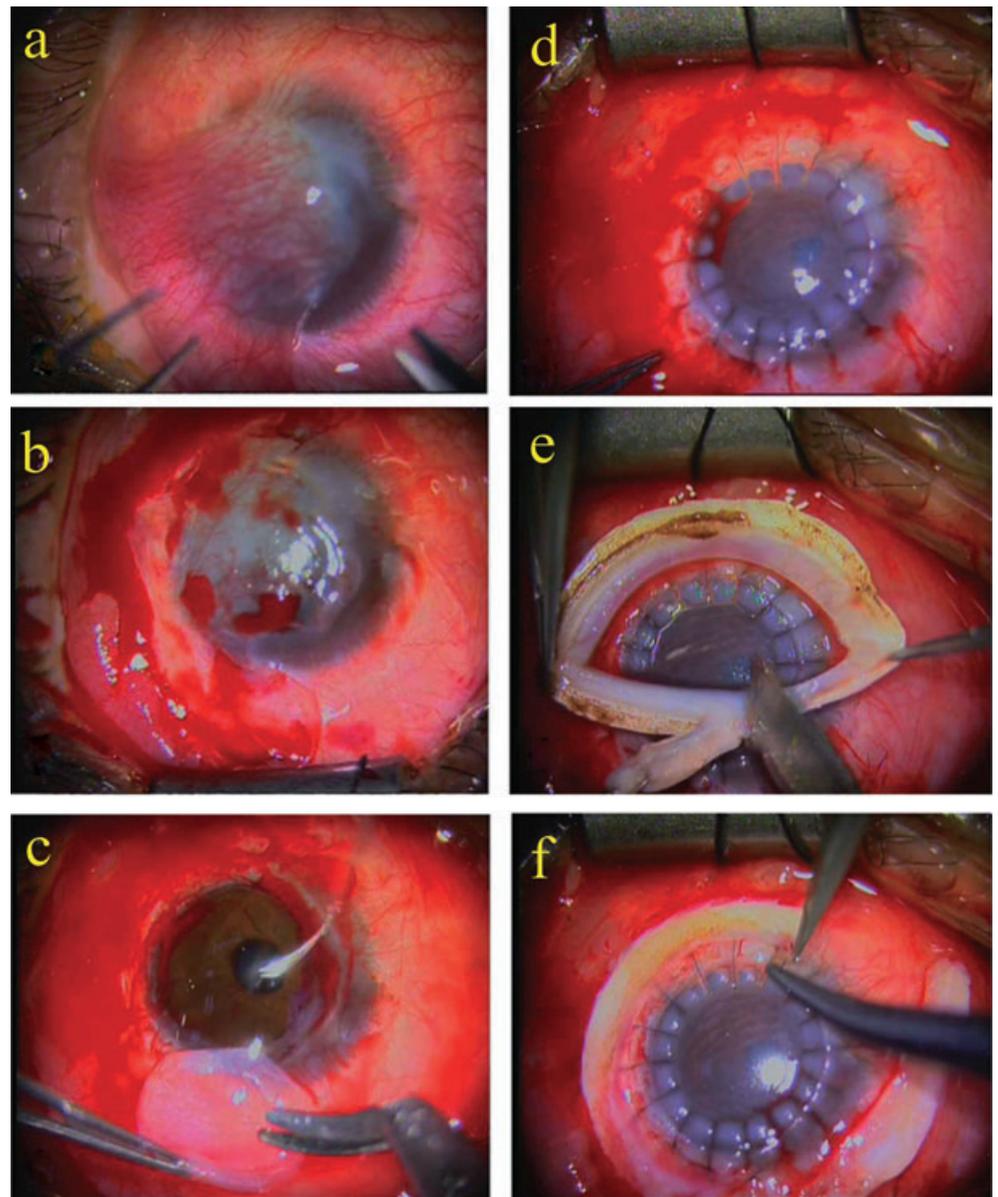
Postoperative treatment and follow up

After the surgery, local and systematic immunosuppression was performed in all patients. Each patient was given 5 mg/kg of dexamethasone daily, which was tapered within approximately 8 weeks. Oral cyclosporine A (CsA) was started from 4–6 mg/kg daily for 1 week, maintained 3–4 mg/kg daily for 6 months and tapered to 2–3 mg/kg daily for at least 1 year. Topical tobramycin and dexamethasone eye drops (Alcon, Fort Worth, TX, USA) were used 4 times every day for the first 3 months, tapered to three times for the next 3 months and 1–2 times for the next 6 months. Tobramycin and dexamethasone ophthalmic ointment was administered at night for 3 months and tapered to two times weekly for 1 year. CsA 0.5% eye drops were given four times per day all along. The patients were observed daily for the first week after surgery, weekly for the next 2 months, and monthly thereafter. Postoperative complications, including corneal epithelial defects, hyphaema, hypotony and graft rejection, were recorded. Visual acuity was measured and compared at 6, 12 and 24 months postoperatively.

If corneal epithelial defects occurred, the patients were required to cover both eyes for 3 days with tobramycin and dexamethasone ointment and vitamin A ointment. Then autologous blood serum eye drops or recombinant human epidermal growth factor eye drops were used topically four times per day to facilitate the healing of epithelium. Epithelial defects were considered persistent if they lasted more than 2 weeks,¹⁵ and permanent tarsorrhaphy was performed if persistent corneal epithelial defects occurred.

Clinical grading of hyphaema was as follows: grade 1, layered blood occupying less than one-third of the anterior chamber; grade 2, blood filling one-third to one half of the anterior chamber; grade 3, layered blood filling one half to less than total of the anterior chamber, and grade 4, total clotted blood. Patients with hyphaema early after surgery were given haemostatic drugs and required to rest in bed

Figure 1. (a) Corneal vascularization after severe corneal burns. (b) Total corneal opacity after the fibrous tissue is extensively excised. (c) Central corneal button is excised with corneal scissors. (d) Penetrating keratoplasty is performed. (e) Annular keratolimbic allograft is prepared with a round-bladed knife. (f) Keratolimbic allograft transplantation is performed after penetrating keratoplasty.



with both eyes covered. Irrigation of anterior chamber was performed when hyphaema was \geq grade 2 and not absorbed within 3–5 days.

Hypotony was defined as an intraocular pressure <8 mmHg.¹⁶ When ocular hypotony occurred, fluorescence staining was made. If Seidel test was positive, resuturing was performed; if not, no additional operation was performed.

Limbic stem cell rejection^{1,17,18} was judged from symptoms of pain, photophobia and lacrimation, obvious sector or circular limbal congestion and oedema with subconjunctival haemorrhage, epithelial oedema and epithelial defect or epithelial rejection line (Fig. 3). The criteria of endothelial rejection^{19–22} were as follows: circumcorneal injection, an anterior chamber reaction, keratic precipitates present on the graft, an endothelial rejection line and graft oedema. When immune rejection occurred, systemic cortisol (2 mg/kg)

was given intravenously for 3 days and changed to oral prednisone of 0.5 mg/kg daily for up to 1 month. Topical tobramycin and dexamethasone eye drops and CsA 0.5% eye drops were also used four times a day.

Statistical analysis

Data were analysed using SPSS 10.0 software. Chi-square test/Fisher's exact test was used wherever applicable. A *P*-value of <0.05 was considered statistically significant.

RESULTS

The follow up ranged from 24 to 38 months, and the mean was 32.0 and 32.5 months in groups A and B, respectively ($P = 0.710$) (Table 1). At the final follow up, clear grafts were achieved in 13 eyes (56.5%); ten eyes (43.5%) had a BCVA

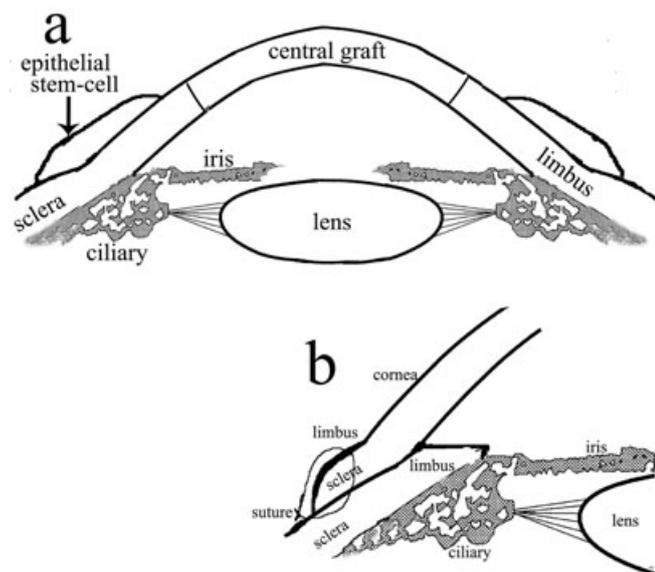


Figure 2. Positioning of graft in relation to angle structures. (a) Penetrating keratoplasty combined with keratolimbal allograft transplantation. (b) Corneoscleral transplantation.

of 6/60 or better in group A. In group B, clear grafts were achieved in 3 eyes (18.8%), and only one eye (6.2%) had a BCVA of 6/60 or better.

Corneal epithelial defects occurred in two eyes (9%) in group A and six eyes (38%) in group B ($P = 0.045$). One eye in group A and three in group B healed after tobramycin and dexamethasone ointment patches; the other required permanent tarsorrhaphy.

Three eyes (13%) in group A and eight eyes (50%) in group B had hyphaema ($P = 0.027$), among which six (three in group A and two in group B) were cured with conservative therapy. Five eyes in group B with hyphaema of \geq grade 2 were treated with anterior chamber irrigation.

Ocular hypotony occurred in one eye (4%) in group A and six eyes (38%) in group B within one month after surgery ($P = 0.013$). Seidel test was positive in one eye in group B, which was resutured. The other eyes with negative Seidel test were left untreated. All hypotony became normal, except that one eye in group B eventually atrophied. There was no sign of secondary glaucoma in the two groups during the follow up.

Twenty-two eyes (96%) in group A and 14 eyes (88%) in group B got BCVA of 6/120 or better at 6 months. The difference was not significant in the rate of freedom from blindness ($P = 0.557$). Twenty eyes (87%) in group A and six eyes (38%) in group B got BCVA of 6/120 or better at 12 months ($P = 0.002$). Fifteen eyes (65%) in group A and four eyes (25%) in group B got BCVA of 6/120 or better at 24 months ($P = 0.022$).

Limbal stem cell rejection occurred in eleven grafts (48%) in group A at 1, 3 (two eyes), 4, 7 (three eyes), 12 (two eyes), 15 and 28 months after surgery and in eight grafts (50%) in group B at 1, 3 (two eyes), 7 (two eyes), 10, 12 and 18

months. The difference between the two groups was not significant ($P = 1.000$). Immunosuppressant treatment failed to reverse the LSC rejection episode in 3/23 (13%) eyes in group A, and failed in 3/16 (19%) patients in group B ($P = 0.674$).

Nine grafts (39%) in group A suffered endothelial rejection, which occurred at 1, 6, 7 (two eyes), 8, 12, 15, 18 and 36 months after surgery, respectively. The rejection episode in group A was easy to control and 88.9% of endothelial rejection was reversible. Sixteen eyes (69.6%) maintained clear grafts at the last follow up (Fig. 4). Twelve grafts (75%) with endothelial rejection in group B occurred at 1 (two eyes), 5, 6 (three eyes), 10, 12 (two eyes), 18 (two eyes) and 20 months. The difference was significant ($P = 0.049$). The rejection episode in group B was not easy to control, and 66.7% of endothelial rejection was reversible. Four eyes (25.0%) developed corneal oedema and opacity at the last follow up (Fig. 5).

DISCUSSION

Different surgical techniques have been used for patients with various severe levels of corneal burns. Corneal epithelial reconstruction with *ex vivo* expanded limbal cells is a potential tool in ocular surface reconstruction.²³ Our group performed this kind of surgery for LSCD patients. The clinical results were good in patients with mild corneal burns but poor in severe cases. All patients in this study had full thickness corneal opacity and damaged endothelium. Deep anterior lamellar keratoplasty could not control corneal opacity successfully. Therefore, we performed PK combined with KLAL transplantation in these patients and made a comparison with corneoscleral transplantation.

In the current series, corneal epithelial defects, hyphaema and postoperative hypotony in group A were obviously less than those in group B. The stem cells of the corneal epithelium are located at the limbus and ultimately responsible for the renewal and regeneration of the entire corneal epithelium under normal circumstances.^{1,2} In group B, there was excessive excision and impairment in corneoscleral transplantation. It was suggested in previous study that sub-basal plexus reduction at every level of the transplanted cornea were apparent even up to 40 years after corneal transplantation. The nerve regeneration in large grafts was perhaps more slowly than that in small grafts. The corneal sensitivity and healthy blink reflex in large donors may reduce significantly and result in the higher incidence of corneal epithelial defects. There are abundant vessels at limbus. When corneal tissue is completely excised, it is prone to bleeding. The edge-to-edge inosculation in group A produced low risk of hyphaema. On the contrary, the anterior structure of the eye was wildly excised, and the inosculation was not close-knit in group B. Ocular hypotony may occur owing to the leakage.¹²

The incidence of endothelial rejection in group A was less than that in group B, and the difference was significant. The reason may be the different mechanisms between the two



Figure 3. (a) Total corneal opacity and vascularization after severe acid burns. (b) Slit-lamp examination shows limbal stem cell rejection. (c) The graft is clear at 12 months after penetrating keratoplasty combined with keratolimbal allograft transplantation.

Table 1. Combined penetrating keratoplasty and keratolimbal allograft transplantation in comparison with corneoscleral transplantation for the treatment of severe corneal burns (number of eyes, %)

Group	No.	Complications			Free from blindness			Rejection	
		Epithelial defects	Hyphaema	Hypotony	6 months	12 months	24 months	Limbal stem cell	Endothelium
A	23	2 (9%)	3 (13%)	1 (4%)	22 (96%)	20 (87%)	15 (65%)	11 (48%)	9 (39%)
B	16	6 (38%)	8 (50%)	6 (38%)	14 (88%)	6 (38%)	4 (25%)	8 (50%)	12 (75%)
<i>P</i> -value		0.045	0.027	0.013	0.557	0.002	0.022	1.000	0.049

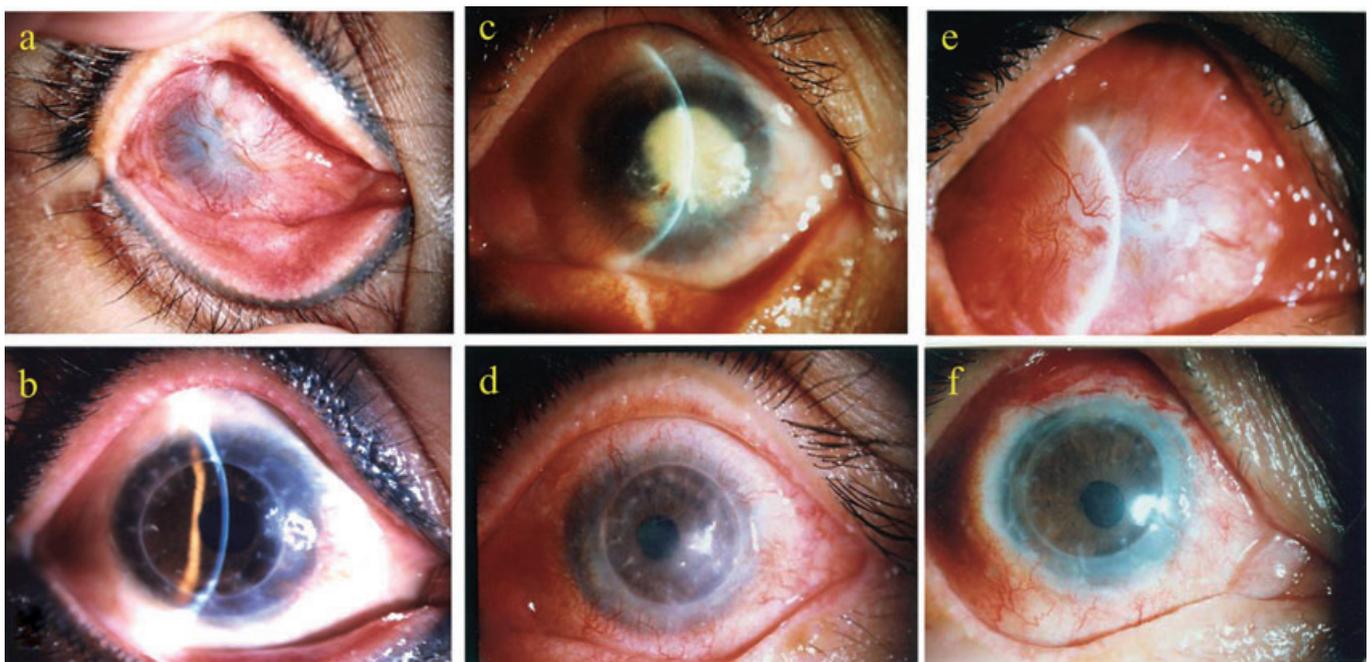


Figure 4. The eyes before and after penetrating keratoplasty combined with keratolimbal allograft transplantation. (a) Alkali burn and (b) 36 months after surgery. (c) Acid burn and (d) 24 months after surgery. (e) Thermal burn and (f) 24 months after surgery.

groups. First, lymphocytes can reach the central graft and endothelium through conjunctival vessels and possibly move to the corneal endothelium through aqueous and ciliary vessels when endothelial rejection occurs.^{13,24} The central graft was associated with the limbus, and the LSC rejection was prone to induce endothelial rejection in group B.

However, central graft was separated from the limbal graft in group A, which can prevent the occurrence of endothelial rejection. Shimmura *et al.*²⁵ reported that one-piece LK was prone to neovascularization. These vessels may be responsible for the graft rejection in these cases, which may have been directed towards donor stromal tissue. Therefore, we

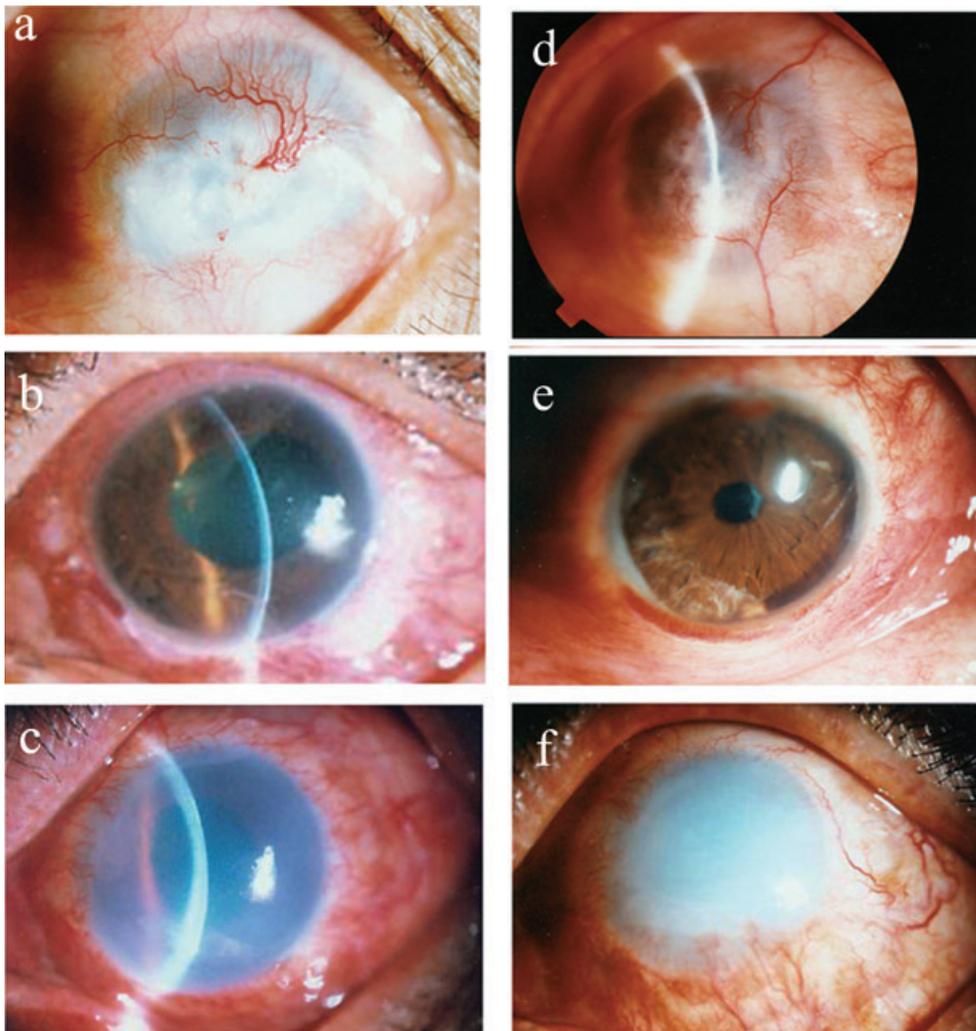


Figure 5. The eyes before and after corneoscleral transplantation. (a) Total corneal opacity and vascularization after severe alkali burns. (b) The graft is clear at 6 months after corneoscleral transplantation. (c) The graft becomes oedema after endothelial rejection 15 months after corneoscleral transplantation. (d) Total corneal opacity and vascularization after severe acid burns. (e) The graft is clear at 12 months after corneoscleral transplantation. (f) The graft becomes opacity 24 months after corneoscleral transplantation.

conclude that the presence of peripheral corneal tissue in the two-piece approach may act as a physical barrier against vessels and immune cells invading the corneal stroma. Second, corneoscleral transplantation is of high-risk keratoplasty with oversized graft. The rejection rate increases with the graft area. The area of allogenic endothelium in group A was smaller than group B. Hence, the intension of endothelial rejection in group A was lower than group B.

The rate of LSC rejection was higher than endothelial rejection in group A, which was attributed to the fact that more blood vessels, lymphatic cells and Langerhans cells are contained in the corneal limbus than in the centre. Limbal grafts become more susceptible to immunologic reactions. The rate of endothelial rejection was higher than LSC rejection in group B, and the rejection was not easy to control. This is perhaps associated with the large number of antigens in large donor grafts, because the previous study found that reduction of the antigens in number can significantly reduce the rejection rate.²⁶ Clinically, we also observed that the endothelial rejection was intensive and not easy to control, and the cornea graft can become oedematous and opaque in 1–2 days. Moreover, the incidence of endothelial rejection in

group A was similar to that in high-risk corneal transplantation (31–62%).^{27,28} It was also reported that the incidence rate of LSC rejection was similar to single KLAL transplantation (42.9–64.0%).^{1,29} It is suggested that PK combined with KLAL transplantation does not increase the incidence of endothelial rejection and LSC rejection.

Ilari and Daya³⁰ and Solomon *et al.*³¹ also performed KLAL with or without PK for LSCD patients, showing that the overall long-term success rate was less than 50% when oral CsA was used. Our current results in the KLAL with PK group seem better which may be attributed to the difference in surgical indications, exclusion criteria and immunosuppressive regimens used. The previous studies included patients with Steven–Johnson syndrome whose tear film and ocular surface were unstable, and the clinical results were very unfavourable, which affected the overall success rate. Patients with severe tear film and abnormal eyelid were excluded in our study, and artificial tear was used after the surgery, which help avoid significantly unstable ocular surface. Systemic and topical CsA were used in all cases in our group, and this possibly decreased chronic low-grade rejection and prolonged graft survival time. Holland *et al.*³²

also reported that the patients receiving systemic immunosuppression had a greater likelihood of achieving ocular surface stability and improving visual acuity.

In conclusion, PK combined with KLAL transplantation may be a better choice for the treatment of severe eye burns for it can effectively reduce the risk of postoperative complications and endothelial rejection and improve the long-term rate of visual recovery.

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