Early Results of Penetrating Keratoplasty After Cultivated Limbal Epithelium Transplantation

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Objective: To describe the early results of penetrating keratoplasty (PKP) in patients who had previously undergone cultivated limbal epithelium transplantation.

Methods: Medical records of patients with limbal stem cell deficiency due to chemical burns who underwent PKP after cultivated limbal epithelium transplantation were reviewed for demographics, primary etiology, type of limbal transplantation, ocular surface stability, visual acuity, graft clarity, and complications. Histopathologic features of the recipient corneal buttons were studied with special attention to epithelial status.

Results: Of the 125 patients with limbal stem cell deficiency treated with cultivated limbal epithelium transplantation, 15 underwent PKP at a mean interval of 7 months (range, 2-12 months) following cultivated limbal epithelium transplantation (autologous, n=11; allogenic, n=4). All 4 patients treated with allogenic cultivated limbal epithelium transplantation were undergoing immunosuppressive therapy. Fourteen (93%) of the 15 eyes had a successful corneal graft with a stable corneal epithelium. Preoperative best-corrected visual acuity was less than 20/200 in 14 of the 15 eyes. At a mean ± SD follow-up of 8.3 ± 5.0 months after PKP, the best-corrected visual acuity was more than 20/60 in 8 eyes, 20/200 to 20/60 in 5 eyes, and less than 20/200 in 2 eyes. Three of the 15 eyes experienced corneal allograft rejection, which was managed successfully. One eye with graft rejection also had glaucoma. None of the limbal epithelial allografts showed signs of rejection.

Conclusions: Early results of PKP following cultivated limbal epithelium transplantation are favorable when performed after stabilizing the ocular surface. Adequate immunosuppression is essential for allogenic cultivated limbal epithelium transplantation to avoid rejection. Corneal allografts can separately reject the limbal allografts.


S EVERE OCULAR SURFACE DISORDERS with limbal stem cell deficiency (LSCD) require a complex approach of multiple surgical procedures, such as limbal transplantation and penetrating keratoplasty (PKP), for final visual rehabilitation.1,2 The outcome of PKP in these cases is reportedly poor.3,4 Penetrating keratoplasty performed in an eye with LSCD carries a high risk of rejection4-6 and non-rejection-related failures. Use of systemic cyclosporine to prevent rejection in these high-risk grafts has been discussed at length by many authors,3,6 but considering the significant adverse effects6,7 and high cost involved with only a marginal improvement in the outcome,6,8 the role of systemic cyclosporine in these high-risk grafts is debatable. The poor results due to non-rejection-related failure, such as persistent epithelial defect, could be attributed to the transfer of only the transient amplifying cells onto the central corneal surface after PKP. The transient amplifying cells, which have a limited life span and limited proliferative potential, fail to provide a stable epithelial surface to these grafts, necessitating the combination with a limbal transplantation procedure for better results. However, results of various limbal transplantation procedures combined with PKP are also not encouraging.10,12 Severely affected patients with unilateral LSCD require a large area of the limbus from contralateral normal eyes, and bilateral cases require similar tissues from the donor. Both of these procedures carry a risk of LSCD at the donor site.13 To avoid this potential complication, cultivated limbal epithelium transplantation is a better choice in these cases.14 Because cultivated limbal epithelium transplantation is a relatively new technique, there are no reports of the outcome of PKP following this procedure. We report herein the early results of PKP after cultivated limbal epithelium transplantation.

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During the study period (May 2001 to September 2002), 125 cultivated limbal epithelium transplantation procedures were performed at L. V Prasad Eye Institute, Hyderabad, India, on eyes with a diagnosis of LSCD, of which 15 eyes of 15 patients underwent PKP after the cultivated limbal epithelium transplantation. The medical records of patients who underwent PKP after cultivated limbal epithelium transplantation were reviewed for demographics, primary etiology, previous surgical procedures, preoperative and postoperative best-corrected visual acuity, type of cultivated limbal epithelium transplantation, complications, and final outcome. Histopathologic reports of recipient corneal buttons were also reviewed. Clinical photographs of the patients were studied with special attention to any abnormalities in the limbal region, vascular engorgement, conjunctival staining, epithelial defect in the limbal area, and conjunctivalization after the rejection episodes.

SURGICAL TECHNIQUES

Our surgical techniques for cultivated limbal epithelium transplantation have previously been reported. In brief, following approval from the Institute Ethics Committee, prior informed consent was obtained from the patients or guardians. Limbal biopsy was performed on the healthy contralateral eye or a healthy area of the same eye in cases of autologous transplantation and from the donor eye in cases of allogenic transplantation. The procedure included careful dissection of a 3 × 3-mm piece of conjunctival epithelium with 1 mm into clear corneal stromal tissue at the limbus. The conjunctiva was excised just behind the pigmented line (palisades of Vogt), and the limbal tissue that contained epithelial cells and a part of the corneal stroma was excised. Both tissues were transported in human amniotic membrane. The cells were cultured using human amniotic membrane. The recipient cornea was excised using a disposable handheld trephine, with 0.5 mm of graft-host disparity. The graft was secured by 10-0 nylon inter-

rupted sutures (extra sutures were placed if necessary) with knots buried on the donor side. The recipient corneal button was sent for histopathologic examination, and special attention was paid to the epithelial status, epithelial stratification, and residual human amniotic membrane. Lensectomy, anterior vitrectomy, and intraocular lens insertion were performed, depending on the clinical situation in each case. At the end of the surgery, a subconjunctival injection of dexamethasone sodium phosphate (4 mg/mL) and gentamicin sulfate (20 mg/mL) was given.

IMMUNOSUPPRESSION

Systemic immunosuppressants were administered to all patients with allogenic limbal grafts after adequate counseling regarding the adverse reactions. Baseline hematologic investigations and hepatic and renal parameters were obtained, and these parameters were reassessed every 4 to 6 weeks. Our routine immunosuppression protocol is to start cyclosporine therapy systemically in a dosage of 5 to 7 mg/kg for 48 hours before surgery, along with methylprednisolone, 1 g intravenously, for the first 3 consecutive postoperative days. During the postoperative period, cyclosporine was tapered to the maintenance dosage of 1.5 to 2 mg/kg for 4 to 8 weeks, with diltiazem hydrochloride, 90 mg, added as an adjunct to cyclosporine to reduce the cost and increase serum levels of cyclosporine. Diltiazem also decreases the dose required to achieve immunosuppression and thus decreases the cost of the treatment. Diltiazem by its antihypertensive effect helps to control hypertension, which is the most common systemic adverse effect of the cyclosporine. Use of immunosuppressants is being continued in all of these patients. Both patients with allogenic-cultivated limbal epithelium transplantation received systemic prednisolone acetate, 1 mg/kg, which was tapered on a weekly basis to the maintenance dosage of 5 mg/d.

When rejection developed, patients were treated with frequent topical corticosteroids. Patients who underwent allogenic-cultivated limbal epithelium transplantation received systemic corticosteroids with continuing systemic immunosuppressants.

PATIENT FOLLOW-UP

Following cultivated limbal epithelium transplantation, all patients were treated with 1% prednisolone acetate eye drops 8 times a day tapered to once a day in 5 to 6 weeks and 0.3% ciprofloxacin hydrochloride eye drops 4 times a day for 1 week. Use of the 0.3% ciprofloxacin eye drops was continued if there were any epithelial defects or until the bandage contact lens was used. We used to apply a bandage contact lens postoperatively, but we have recently stopped this because we believe that it is not required. The patients who underwent allogenic-cultivated limbal epithelium transplantation were treated with 1% prednisolone eye drops 2 times hourly, which was tapered to once a day at 6 months, and these patients also received immunosuppressants as described herein. The patients were seen on postoperative day 1, week 1, week 2, week 3, and monthly thereafter. Each examination included a complete history, notation of new ocular or systemic symptoms, a complete evaluation of the recipient and donor sites, and notation of any signs of neovas-
keratoplasty; PL, perception of light; PR, projection of rays.

A total of 15 eyes of 15 patients underwent cultivated limbal epithelium transplantation and 8.3±5.0 months after PKP. All 15 eyes underwent PKP 2 to 12 months (mean, 7 months) after cultivated limbal epithelium transplantation. The patients ranged in age from 3 to 36 years (mean±SD, 20.3±9.9 years), and 11 were male and 4 female. In all 15 eyes the origin of LSCD was chemical burns, of which 11 were alkali burns, 3 were acid burns, and 1 was due to an unknown chemical (Table). Six (40%) of the 15 eyes had a history of surgery in the form of allogenic bone marrow transplantation in 3 eyes (20%), PKP in 2 eyes (13%), and limbal transplantation in 1 eye (7%).

Eight (53%) of the 15 eyes had symblephara, ranging from the 2- to 10-o’clock hours. Fourteen (93%) of the 15 eyes had total LSCD with 360° loss of limbal pali-
sades of Vogt and 360° conjunctivalization, whereas 1 (7%) of 15 had partial LSCD with loss of limbal pali-
sades of Vogt of 120° and pannus localized to that area (Table).

Eleven of the 15 eyes were autografts, of which 9 were from the contralateral normal eyes and 2 were from the unaffected area of the same eye. Three of the 15 were living related allografts, and 1 was a nonrelated allograft. All of the eyes underwent cultivated limbal epithelium transplantation except 3 eyes, in which a co-cultivated (limbal and conjunctival) epithelium transplantation was performed (Table). All 4 patients with limbal allografts underwent immunosuppression with cyclosporine and systemic corticosteroids, and 2 of them received dilti-
zem tablets to decrease the dosage of cyclosporine.

The preoperative best-corrected visual acuity on the Snellen chart was less than 20/200 in 14 (93%) of 15 eyes

<table>
<thead>
<tr>
<th>Complications</th>
<th>Duration of CLT to PKP, mo</th>
<th>Post-PKP Follow-up, mo</th>
<th>Post-PKP Follow-up, mo</th>
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<th>Post-CLT Follow-up, mo</th>
<th>Preoperative BCVA</th>
<th>Type of CLT</th>
<th>Origin</th>
<th>Age, y / Sex</th>
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<th>Origin</th>
<th>Preoperative BCVA</th>
<th>Post-CLT Follow-up, mo</th>
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<td>11</td>
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<td>3</td>
<td>15</td>
<td>30/F</td>
<td>Alkali burns</td>
<td>Contralateral-autologous*</td>
<td>PL + PR</td>
<td>HM</td>
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<td>20/50/partial</td>
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<td>CF</td>
<td>CF</td>
<td>6</td>
<td>20/80</td>
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</table>

Abbreviations: BCVA, best-corrected visual acuity; CF, counting fingers; CLT, cultivated limbal epithelium transplantation; HM, hand motions; PKP, penetrating keratoplasty; PL, perception of light; PR, projection of rays.

*Co-cultivated (limbal and conjunctival) limbal epithelium.
†Combined with contralateral conjunctival limbal autograft.
and 20/200 or better in 1 (7%) of 15 eyes. Final best-
corrected visual acuity was less than 20/200 in 2 eyes
(1 of which had primary graft failure), 20/200 to 20/60
in 5 eyes, and better than 20/60 in 8 eyes (Figure 1
and Figure 2). Thirteen corneal allografts (87%) were clear
at the last follow-up. Of the other 2, 1 was a primary fail-
ure and 1 had resolving corneal allograft rejection.

Three of 15 eyes had acute corneal allograft rejec-
tion, of which 2 had living related cultivated limbal epi-
thelium transplantation and 1 had autologous culti-
vated limbal epithelium transplantation. Two eyes had
only endothelial rejection, whereas 1 eye had combined
endothelial and epithelial rejection. One eye with graft
rejection also had glaucoma, which was controlled with
the treatment. All of the rejected corneal allografts re-
sponded favorably to the treatment.

HISTOPATHOLOGIC RESULTS OF RECIPIENT
CORNEAL BUTTONS

All corneal buttons showed a multiple-layered normal cor-
neal epithelium of 3 to 5 layers (Figure 3D). Only 2 cor-
neal buttons showed the presence of residual amniotic
membrane. One of the corneal buttons showed a focal
presence of goblet cells. Immunohistochemical analysis
with monoclonal antibodies (AE5) against cornea-
specific cytokeratin K3 was performed on 12 recipient
corneal buttons, of which 11 showed reactions positive
for the cornea-specific phenotype of the epithelium
(Figure 4F).

COMMENT

The limbal stem cell can be damaged by a variety of in-
sults, of which one of the most common and important
is chemical burns. Most of these cases of chemical burns
have significant stromal scarring, necessitating PKP for
visual rehabilitation. Before the role of limbal stem cells
as a source of corneal epithelium was recognized, PKP in
these eyes invariably failed. This was because the tran-
sient amplifying cells that were transferred onto the cen-
tral corneal surface during PKP had a limited life span
and limited proliferative potential and thus were un-
able to restore the ocular surface epithelium on a long-
term basis. Limbal transplantation is performed in these
cases to maintain the reservoir of corneal epithelial cells
required for a stable and healthy corneal epithelium. Si-
multaneous PKP and limbal transplantation and their ad-
vantages have been addressed previously, but a greater
risk of rejection of corneal grafts exists with an in-
flamed and vascularized recipient corneal stroma.
Therefore, we prefer the 2-staged approach. The first stage
is ocular surface reconstruction by cultivated limbal epi-
thelium transplantation followed by the second stage of
visual rehabilitation by performing PKP.

Various techniques of limbal transplantation have been
reported in the literature, including keratolimbal allo-
graft, which has produced disappointing long-term out-
comes. The need for indefinite immunosuppression is
also an issue in cases of allogenic limbal transplantation.
The other techniques, such as living related conjunctival
limbal allogenic transplantation and conjunctival limbal au-
tografts, may not be useful in total LSCD to replace the lim-
bus in 360° owing to the risk of LSCD at the donor site.
Hence, we prefer the technique of cultivated limbal epi-
thelium transplantation. However, our technique of culti-
vated limbal epithelium transplantation is different from
that reported by others. As reported previously, we
used deep epithelialized human amniotic membrane to cul-
vate limbal epithelium over it without 3T3 fibroblast co-
culture or air lifting. Our culture duration was also shorter
because we did not wait for multiple layers to form. In our
experience, a monolayer ultimately proliferates in vivo to
produce stratified (multilayered) epithelium following trans-
plantation. Our hypothesis is supported by the fact that fol-
lowing PKP, all of the recipient corneal buttons showed
normal stratified corneal epithelium (Figure 3D) with a cor-
nea-specific phenotype (Figure 4F), which had grown into
multiple layers after monolayer transplantation (Sangwan

Figure 1. Change in best-corrected visual acuity (BCVA) of the studied eye
from prelimbal transplantation to the last follow-up after penetrating
keratoplasty.

Figure 2. Comparison of preoperative and postoperative visual acuity at the
last follow-up. CF indicates counting fingers; PL, perception of light.
Cases of severe ocular surface damage with LSCD are often difficult to manage. Apart from limbal damage, conjunctival deficiency usually occurs as well. We tried to address this problem earlier and reported co-cultivation of conjunctival and limbal epithelial cells. Three of the 15 patients in this series had more severe ocular surface damage with symblephara and hence underwent co-cultivated (limbal and conjunctival) epithelium transplantation.

All of the recipients in our study were younger, ranging in age from 3 to 36 years (mean age, 20.3 years) (Table), sustained chemical burns, and subsequently had stromal vascularization in 4 quadrants. Eleven of the 15 patients in our study had a history of ocular surface surgical procedures, and 8 of the patients had symblephara at initial examination. Hence, considering the criteria suggested by the Collaborative Corneal Transplantation Studies Research Group for high-risk PKPs, all PKPs in our series were high risk. Conversely, our cases showed neither a high rejection rate (overall rejection rate, 20%) despite the age of the recipients and stromal vascularization nor a non-rejection-related failure as expected in cases of chemical burns. This substantial decrease of non-rejection-related failure could be explained by the cultivated limbal epithelium transplantation procedure preceding the PKP, which continued to supply healthy epithelium after PKP. Similarly, the fewer corneal graft rejection episodes, notwithstanding age of recipients and vascularized recipient corneal stroma, could be due to our stepwise approach, which included ocular surface reconstruction by cultivated limbal epithelium transplantation in the first step and PKP in the second. Because 4 of the 15 patients underwent allogenic limbal epithelium transplantation and immunosuppression, the effect of immunosuppression on the graft survival also cannot be overlooked. However, if we consider only the autologous limbal epithelium transplantation cases, all of them met the criteria of high-risk grafts and none were immunosuppressed. The corneal graft rejection rate in these cases was 9.1% (1 of 11), which is less than in any other reported series of high-risk grafts without immunosuppression, as described by Hill (73%), Poon et al (53%), and Rumelt et al (42%). To explain this relatively low rejection rate, we speculate that the cultivated limbal epithelium is devoid of Langerhans cells, which are believed to be the antigen-presenting cells and are in abundance at the limbus, forming one of the important components of the afferent arm of corneal allograft rejection. Thus, the recognition of corneal graft alloantigen is down-regulated, which in turn decreases the rate of rejection. However, further studies are needed in this direction to confirm our hypothesis. Similarly, we can-

Figure 3. Slitlamp photographs of case 5. A, Preoperative condition showing total limbal stem cell deficiency with extensive symblephara obliterating the superior and inferior fornices. B, Stable ocular surface and dense corneal scarring after autologous cultivated limbal epithelium transplantation with contralateral conjunctivolimbal autograft (6 weeks postoperatively). Bulbar conjunctiva, in the area of conjunctivolimbal autograft (inferior quadrant), shows a patch of vascularization and pigmentation. C, Clear and compact graft with a stable ocular surface after penetrating keratoplasty (13 months postoperatively). D, Hematoxylin-eosin-stained histopathologic section of the corneal button with multilayered corneal epithelium after autologous cultivated limbal epithelium transplantation (original magnification × 20).
not rule out the effect of the anti-inflammatory property of amniotic membrane,25 which was used as a carrier in these cases. We also noted that despite the central corneal graft rejection in 2 cases of allogenic limbal epithelium transplantation, none showed any signs of limbal allograft rejection. This finding supports similar findings with PKP after keratolimbal allograft transplantation reported by Shimazaki et al.26

Several studies18,27,28 have indicated that all allogenic limbal transplantation cases, including those with living-related limbal allografts, require immunosuppression. We too believe in immunosuppression for allogenic cultured limbal transplantation. Hence, we started administration of cyclosporine preoperatively and then continued with a maintenance dosage of cyclosporine for indefinite periods in the recipients with allogenic cultured limbal epithelium transplantation. Along with systemic cyclosporine, we used diltiazem, a calcium channel blocker. Diltiazem is known to increase the plasma cyclosporine level by competitive inhibition of hepatic enzyme CYP450, which is required for the metabolism of cyclosporine. Thus, the cyclosporine dose can be reduced by 30% to 50% with a drastic reduction in medication cost.16 In high nontherapeutic doses, however, it may exert an immunosuppressive effect.17 It also provides renal protection from cyclosporine-induced nephrotoxicity.29 It is used in other solid organ transplantations but has not been reported for allogenic limbal transplantation.

Previous studies10-12,22 reported a poor final visual outcome of PKP with limbal transplantation. In our study, 14 (93%) of the 15 patients had a preoperative best-corrected visual acuity of hand movements to finger counting. At the last follow-up, 13 patients (87%) had an ambulatory visual acuity of better than 20/200 in the affected eye, of which 8 (53%) achieved a best-corrected visual acuity better than 20/60 (Figure 3). Although ours was a retrospective study, with a small number of cases and long-term results that are still awaited, we observed a definite trend toward better corneal graft survival and excellent visual outcome in these cases.

Certain issues related to PKP following limbal transplantation must be highlighted. Because such patients have already undergone pannus resection with or without superficial keratectomy, the recipient corneal stromal bed is usually thin and irregular, which could result in postoperative astigmatism. Associated conditions, such as eyelid abnormalities, glaucoma, and dry eye syndrome, may affect the final outcome and hence must be treated before PKP. Patients treated with allogenic limbal epithelium transplantation need to undergo immunosuppression even after the PKP.

In summary, we report the early outcome in 15 cases of PKP after cultivated limbal epithelium transplantation, which showed favorable results in the form of corneal graft survival and final visual acuity. However, further studies are required to understand immunologic rejection in cases of allogenic cultivated limbal epithelium transplantation.

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REFERENCES


Correction

Notice of Duplicate Publication of Figure. In the Clinical Sciences article by Sangwan et al titled “Early Results of Penetrating Keratoplasty After Cultivated Limbal Epithelium Transplantation,” published in the March 2005 issue of the ARCHIVES (2005;123:334-340), Figure 3 is the same figure as one previously published in an article by Sangwan et al (Figure 2) that appeared in Bioscience Reports (2003;23:169-174). The authors alerted us to the duplicate publication of the figure when they realized the error. This was an unintentional oversight. The ARCHIVES has since obtained permission from Springer Science and Business Media, the publisher of Bioscience Reports, to reprint the figure.

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