Amniotic Membrane Transplantation With or Without Limbal Allografts for Corneal Surface Reconstruction in Patients With Limbal Stem Cell Deficiency

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Objective: To examine whether amniotic membrane transplantation (AMT), in preparing the perilimbal stroma, enhances the success of allograft limbal transplantation (ALT).

Methods: Thirty-one eyes of 26 consecutive patients had cytologically proven limbal deficiency resulting from chemical burns (14 eyes); Stevens-Johnson syndrome, toxic epidermal necrolysis, or pseudopemphigoid (5 eyes); contact lens–induced keratopathy (3 eyes); aniridia (3 eyes); multiple surgical procedures (2 eyes); atopy (2 eyes); or an unknown cause (2 eyes). Based on the severity of limbal deficiency, group A (mild), comprising 10 eyes, received AMT alone; group B (moderate), comprising 7 eyes, received AMT and ALT; and group C (severe), comprising 14 eyes, received AMT, ALT, and penetrating keratoplasty. All patients except those in group A received continuous oral cyclosporine.

Results: Except for the 2 eyes with atopy, all amniotic membrane–covered surfaces showed rapid epithelialization (in 2 to 4 weeks) and reduced inflammation, vascularization, and scarring, and the surfaces became smooth and wettable. For the mean follow-up period of 15.4 months, 25 (83%) of 30 eyes showed visual improvement, consisting of 6 or more lines (13 eyes), 4 to 5 lines (6 eyes), or 1 to 3 lines (6 eyes). Visual improvement decreased with the severity of limbal deficiency from 8 (100%) of 8 eyes in group A to 5 (71%) of 7 eyes in group B and 11 (79%) of 14 eyes in group C. In group C, corneal graft rejection occurred in 9 (64%) of 14 eyes, and reversible early limbal allograft rejection was noted in 3 (14%) of 21 eyes of groups B and C.

Conclusions: For partial limbal deficiency with superficial involvement, AMT alone is sufficient and hence superior to ALT because there is no need to administer cyclosporine. For total limbal deficiency, additional ALT is needed, and AMT helps reconstruct the perilimbal stroma, with reduced inflammation and vascularization, which collectively may enhance the success of ALT.


When pathologic insults destroy the stem cell–containing limbal epithelium, the corneal surface invariably heals with conjunctival epithelial ingrowth (conjunctivalization), neovascularization, chronic inflammation, and recurrent or persistent corneal epithelial defects.1 These pathologic signs constitute the newly established disease called limbal (stem cell) deficiency, which can be diagnosed by impression cytologic examination in a number of corneal diseases.2 These diseases can be subdivided into 2 major categories. The first category is characterized by total destruction of the limbal stem cell population by chemical or thermal injuries, the Stevens-Johnson syndrome, multiple surgical or cryotherapy procedures at the limbal region, contact lens wear, or severe microbial infection. The finding that such diseases cause limbal deficiency justifies the use of limbal allografts or allografts for corneal surface reconstruction.3-5 The second category includes such diverse diseases as aniridia, multiple endocrine deficiency, limbitis and peripheral ulcerative and inflammatory keratitis, neurotrophic and ischemic keratitis, and pterygium or pseudopterygium. These diseases do not destroy limbal stem cells directly but instead damage the limbal stroma so that it cannot support these stem cells.2

Allograft rejection of transplanted limbal tissues has been combated by using HLA-matched donors,6 by administering oral cyclosporine, or both,7 with success rates of 70% to 80% for a mean follow-up period of 1 to 2 years. When the follow-up period was extended to 5 years, the success rate dropped to about 50% (K.B. and S.C.G.T., unpublished observations, 1996; some of these patients will be described in the “Results” section). This gradual attrition in the success...
PATIENTS AND METHODS

PATIENTS

The study was approved by the Medical Science Subcommittee for the Protection of Human Subjects in Research of the University of Miami School of Medicine, Miami, Fla. At the Bascom Palmer Eye Institute, Miami, from 1993 to 1998, AMT has been performed in many patients with various ocular surface disorders after obtaining their informed consent. Results have been reported of the treatment of persistent corneal ulcer,14 pterygium,15 or conjunctival lesions.16 The present study included 26 consecutively treated patients (31 eyes) with cytologically proven limbal deficiency. The underlying causes of limbal deficiency included chemical burns (14 eyes); Stevens-Johnson syndrome, toxic epidermal necrolysis, or ocular pseudopemphigoid (3 eyes); contact lens–induced keratopathy (3 eyes); aniridia (3 eyes); multiple surgical procedures (2 eyes); atopy (2 eyes); and an idiopathic cause (2 eyes). The patients’ demographic and pertinent clinical data are summarized in the Table. These 31 eyes were further divided according to the severity of limbal deficiency into group A (mild), comprising 10 eyes, which received AMT alone; group B (moderate), comprising 7 eyes, which received AMT and ALT; and group C (severe), comprising 15 eyes, which received AMT, ALT, and penetrating keratoplasty (PKP).

PREPARATION OF PRESERVED HUMAN AMNIOTIC MEMBRANE

Human amniotic membrane was prepared and preserved using a method described elsewhere.14,15 In brief, the human placenta was obtained shortly after an elective cesarean section when the human immunodeficiency virus, human hepatitis type B and C, and syphilis had been excluded by serologic tests. Under a laminar flow hood, the placenta was cleaned of blood clots with a sterile phosphate-buffered saline solution containing penicillin, 50 µg/mL; streptomycin, 50 µg/mL; neomycin, 100 µg/mL; and amphotericin B, 2.5 µg/mL (Life Technologies Inc, Gaithersburg, Md). The amnion was separated from the rest of the chorion by blunt dissection and flattened onto a nitrocellulose paper (Bio-Rad Inc, Gainesville, Fla), with the epithelium–basement membrane surface facing away from the paper. The paper with the adherent amniotic membrane was then cut into 3×4-cm sheets and stored before transplantation at −80°C in a sterile vial containing Dulbecco-modified Eagle medium (Life Technologies Inc) and glycerol (Baxter Healthcare Corp, Stone Mountain, Ga) at a ratio of 1:1 (vol/vol).

AMNIOTIC MEMBRANE TRANSPLANTATION

All surgical procedures were performed by the same surgeon (S.C.G.T.). Following peritomy at the limbus, the perilimbal subconjunctival scarred and inflamed tissue was removed to the bare sclera up to 5 to 7 mm from the limbus. The corneal pannus was then removed mostly by blunt dissection in a manner similar to that described for autograft limbal transplantation.17 For group A, the abnormal vascularized epithelium and pannus were removed, but the normal limbal and corneal adherent epithelia were left intact. For groups B and C, the entire limbal and corneal surfaces were cleaned. For all 3 groups, the amniotic membrane was then removed from the storage medium, peeled off the nitrocellulose filter paper, transferred to the

RESULTS

There were 15 men and 11 women, with a mean (±SD) age of 38.5±13.3 years. All eyes (n=31) were diagnosed as having limbal deficiency by impression cytologic examination that showed goblet cells on the involved corneal surface. Fourteen (45%) eyes had had persistent or recurrent corneal epithelial defects (Table). Four eyes, patient 14 from group B and patients 16, 18, and 20 from group C, had recurrent limbal deficiency after prior ALT for chemical burns. For the mean (±SD) follow-up period of 15.5±9.9 months, 25 (83%) of 30 eyes showed visual improvement (excluding patient 9, who had preexisting tractional reti-
recipient eye, and fitted to cover the entire created defect. Minor modifications were made, depending on the extent of limbal deficiency found in each patient (Table). This membrane was then secured to the corneal edge of the defect by interrupted 10-0 nylon sutures if the covered limbal circumference was less than 2 clock hours, or by a purse-string running suture at the limbal area if it was more than 2 clock hours, and to the surrounding conjunctival edge with episcleral anchorage using interrupted 9-0 or 10-0 Vicryl sutures (Ethicon Inc, Johnson & Johnson Co, Somerville, NJ).

ALLOGRAFT LIMBAL TRANSPLANTATION

The techniques of ALT can be found in a recent review.3 Except for a few exceptions in which a partial limbus was used (Table), a 360° cadaveric donor corneolimbal ring graft, obtained from the donor corneoscleral button stored in the conventional medium without HLA matching, was routinely used in all patients. There was no specific requirement for donor corneas with respect to age, medium, or length of storage. After the central corneal button was removed with an 8.0-mm trephine, the remaining corneoscleral ring was trimmed of excessive sclera and flattened onto a Petri dish with the epithelial surface facing down on a protective layer of Healon (Kabi Pharmacia, Piscataway, NJ). The posterior half of the stroma, including the endothelium, was removed by a razor blade or a sharp scissors. Additional tapering of the corneal and limbusceral edges was done by scissors so that their contacts with the recipient surface would be smooth. For group B, the amniotic membrane would have covered only the denuded perilimbal scleral surface if ALT was performed at the same time as AMT. If ALT was performed after AMT, however, the corneal surface would have been cleaned in the same manner as described above by peritomy made at the site where the purse-string suture had been placed previously. The donor corneolimbal ring was secured to the surrounding conjunctival edge with 9-0 or 10-0 interrupted Vicryl sutures, each with episcleral anchorage, and to the denuded corneal surface with a running 10-0 nylon suture. For group C, ALT was routinely performed together with PKP. In those patients, PKP would be performed first in a conventional manner in the recipient eye, from which the central cornea was removed with a 7.5-mm trephine, followed by other concomitant intraocular surgical procedures if needed. The 8.0-mm donor corneal button would be secured to the recipient eye with 8 cardinal interrupted 10-0 nylon sutures, and the donor corneolimbal rim would then be transferred to the recipient eye to fit exactly with the outside border of the secured central corneal button. It would be secured to the surrounding conjunctival edge in the same manner as described above, but to the donor corneal button using the second set of 8 cardinal interrupted 10-0 nylon sutures, which were passed to include portions of donor corneal button, recipient peripheral cornea, and donor ring graft to replace the first set of cardinal sutures. The donor cornea was finally secured with another running 10-0 nylon suture. A bandage contact lens was then inserted or a partial tarsorrhaphy was done for protection in some patients (Table). This was followed by the topical application of Maxitrol (neomycin sulfate, polymyxin B sulfate, and dexamethasone ointment) (Alcon Laboratories Inc, Fort Worth, Tex). After the operation, all patients received nonpreserved 1% methylprednisolone sodium succinate eye-drops every 2 hours while awake and 0.3% ofloxacin (Allergan Optical Inc, Irvine, Calif) 3 times a day, the dosages of which were tapered to a maintenance dose in 1 to 2 months. Sutures were removed at 3 to 6 weeks. For patients receiving ALT, oral cyclosporine was administered at 6 mg/kg of body weight per day in divided doses starting 1 week before or at the time of ALT. The cyclosporine dosage was then tapered to 2 to 4 mg/kg in 2 to 4 weeks so that the trough blood level was maintained at 100 to 150 µg/L and continued indefinitely for groups B and C. The results of periodic renal and liver function tests were also monitored.

GROUP A (MILD LIMBAL DEFICIENCY): RECEIVING AMT ALONE

The diagnoses for group A, comprising 9 patients and 10 eyes, included aniridia (3 eyes), chemical burns (4 eyes), contact lens–induced keratopathy (2 eyes), and herpetic
<table>
<thead>
<tr>
<th>Patient No./ Age, y/Sex</th>
<th>Eye</th>
<th>Underlying Cause</th>
<th>Surgeries Before AMT</th>
<th>AMT (Extent), °†</th>
<th>Surgeries With AMT</th>
<th>Surgeries After AMT</th>
<th>Vision Before</th>
<th>Vision After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/37/M LE Aniridia</td>
<td></td>
<td>PPV, CE</td>
<td>360 + K</td>
<td>...</td>
<td>...</td>
<td>20/400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/22/F RE Aniridia</td>
<td></td>
<td>...</td>
<td>360 + K</td>
<td>...</td>
<td>...</td>
<td>5/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/24/F LE Aniridia with PED</td>
<td></td>
<td>CE, filter‡</td>
<td>360 + K</td>
<td>...</td>
<td>...</td>
<td>5/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/35/M RE Chemical burn</td>
<td></td>
<td>...</td>
<td>360 + K</td>
<td>...</td>
<td>20/60</td>
<td>20/40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/39/M LE Chemical burn with band keratopathy, PED, and edema</td>
<td></td>
<td>K(c) EDTA</td>
<td></td>
<td>...</td>
<td>20/400</td>
<td>20/30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/26/M RE Chemical burn with symblepharon</td>
<td></td>
<td>... 230 + K(p)</td>
<td></td>
<td>...</td>
<td>...</td>
<td>HM</td>
<td>20/70</td>
<td></td>
</tr>
<tr>
<td>7/40/F LE Chemical burn</td>
<td></td>
<td>... 360 + K(p)</td>
<td>...</td>
<td>20/300</td>
<td>20/70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/44/F LE CL-induced keratopathy</td>
<td></td>
<td>... 300 + K(p)</td>
<td></td>
<td>...</td>
<td>20/200</td>
<td>20/25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/65/F RE CL-induced keratopathy</td>
<td></td>
<td>... 300 + K(p)</td>
<td></td>
<td>...</td>
<td>20/200</td>
<td>20/25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/26/M RE TEN with PED</td>
<td></td>
<td>PO, lash</td>
<td>360 + K</td>
<td>ALT</td>
<td></td>
<td>HM</td>
<td>20/40</td>
<td></td>
</tr>
<tr>
<td>11/65/F RE Pseudopemphigoid</td>
<td></td>
<td>PO, lash 360 ALT</td>
<td>...</td>
<td></td>
<td>1/200</td>
<td>20/50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/36/F RE Idiopathic LD with PED</td>
<td></td>
<td>... 360 + K pannus removal ALT</td>
<td></td>
<td>20/300</td>
<td>CF</td>
<td>20/40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13/73/M RE Chemical burn</td>
<td></td>
<td>... 360 + K</td>
<td>...</td>
<td>ALT</td>
<td></td>
<td>LP</td>
<td>20/60</td>
<td></td>
</tr>
<tr>
<td>14/43/M RE Chemical burn</td>
<td>PKP × 2, PPV, CE, ALT + PKP</td>
<td>360 + K</td>
<td></td>
<td>...</td>
<td>20/200</td>
<td>20/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15/25/M RE Chemical burn</td>
<td>PKP + ALT, CE, IOL, filter</td>
<td>360</td>
<td></td>
<td>ALT + PKP</td>
<td>HM</td>
<td>20/50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16/33/M RE Chemical burn with PED</td>
<td></td>
<td>360</td>
<td></td>
<td>ALT + PKP, tarsorrhaphy</td>
<td>HM</td>
<td>CF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17/46/M RE Chemical burn</td>
<td></td>
<td>360</td>
<td></td>
<td>ALT + PKP</td>
<td>20/80</td>
<td>20/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18/40/M RE Chemical burn</td>
<td>Conjunctival flap, ALT + PKP, CE, IOL, filter</td>
<td>360 + K</td>
<td></td>
<td>ALT + PKP</td>
<td>HM</td>
<td>20/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19/37/M RE Chemical burn with PED</td>
<td>PKP + CE, IOL</td>
<td>360 + K</td>
<td></td>
<td>ALT + PKP, IOL removal, tarsorrhaphy</td>
<td>HM</td>
<td>20/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20/45/M RE Chemical burn</td>
<td>Scleral patch, PKP, ALT + PKP</td>
<td>360</td>
<td></td>
<td>ALT + PKP</td>
<td>2/200</td>
<td>20/60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/39/M RE Chemical burn with band keratopathy and PED</td>
<td>Tarsorrhaphy</td>
<td>K(c) EDTA</td>
<td></td>
<td>ALT + PKP</td>
<td>8/200</td>
<td>20/60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21/21/M RE Chemical burn with PED</td>
<td>Filter</td>
<td>360 + K</td>
<td></td>
<td>ALT + PKP</td>
<td>LP</td>
<td>20/200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22/40/F RE Chemical burn with PED</td>
<td>PKP × 2</td>
<td>360</td>
<td></td>
<td>ALT + PKP</td>
<td>HM</td>
<td>20/50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23/33/F RE CL-induced keratopathy</td>
<td>PKP</td>
<td>360</td>
<td></td>
<td>ALT + PKP</td>
<td>20/400</td>
<td>20/400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24/23/F RE SJS, descemetocele</td>
<td>PKP</td>
<td>360 + K</td>
<td></td>
<td>ALT + PKP, tarsorrhaphy</td>
<td>HM</td>
<td>20/40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25/44/F RE SJS and PED</td>
<td>PKP, CE, filter</td>
<td>360 + K</td>
<td></td>
<td>ALT + PKP, tarsorrhaphy</td>
<td>20/400</td>
<td>20/80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26/38/M RE Atopic KC, descemetocele, and PED</td>
<td>... 360 + K</td>
<td></td>
<td></td>
<td>ALT + PKP, tarsorrhaphy</td>
<td>HM</td>
<td>20/300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LE Atopic KC and PED</td>
<td>... 360 + K</td>
<td></td>
<td></td>
<td>ALT + PKP, tarsorrhaphy</td>
<td>HM</td>
<td>20/300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Group A received AMT only; group B, AMT and ALT; and group C, AMT, ALT, and PKP. LE indicates left eye; PPV, pars plana vitrectomy; CE, cataract extraction; RE, right eye; PED, persistent corneal epithelial defect; HM, hand motion; CL, contact lens; HSV, herpes simplex virus; LP, light perception (vision); RD, retinal detachment; TEN, toxic epidermal necrolysis; PO, punctal occlusion; IOL, intraocular lens; CF, counting fingers (vision); LD, limbic deficiency; IOP, intraocular pressure; ED, epithelial defect; KCS, keratoconjunctivitis sicca; SJS, Stevens-Johnson syndrome; KC, keratoconjunctivitis; and ellipses, not applicable or none.

†Extent of AMT is expressed as follows: number + K indicates degree of perilimbal coverage without cornea; K(c), central cornea only; K(p), partial cornea only; and numbers alone, degree of perilimbal coverage without cornea.

‡Filter indicates filtering procedure using Seton or Molteno tubes.

§For groups B and C, the follow-up period is given as the duration between AMT and ALT vs the duration after ALT for group B or after ALT + PKP for group C. ||The first number indicates the duration after AMT before ALT, and the second number, duration after ALT.
keratitis with failed graft (1 eye). The visual potentials of the 3 patients with aniridia were limited by nystagmus and other posterior segment abnormalities. Following AMT alone, their corneal surfaces became smooth, stable, and transparent enough to regain their preoperative best visual acuity. The use of AMT also reduced chronic inflammation in patient 9, who had had multiple surgical procedures and underlying herpetic keratitis, and its use resulted in a comfortable eye. By excluding this patient with preexisting tractional retinal detachment, the remaining 9 eyes (100%) showed improved vision for a mean (±SD) follow-up period of 12.3±9.3 months. None of the patients took cyclosporine, and no complication has been noted with the transplanted amniotic membrane. Excluding the 3 patients with aniridia and patient 9 with total limbal deficiency, the remaining 6 eyes had partial limbal deficiency and received AMT to cover only the diseased corneal and perilimbal areas (Table). All 6 eyes recovered smooth, stable, and noninflamed corneal surfaces, with improved vision and reduced photophobia (Figure 1). Following AMT, superficial vascularization was still noted in patients 4, 6, and 7 at the peripheral cornea adjacent to the more severely damaged limbus, suggesting the persistence of focal limbal deficiency, which was confirmed by repeating the impression cytologic examination. There has been no recurrence of band keratopathy, and preexisting corneal edema disappeared in patient 5 following EDTA chelation and AMT.

### GROUP B (MODERATE LIMBAL DEFICIENCY): RECEIVING AMT AND ALT

The diagnoses for group B, comprising 5 patients and 7 eyes, included toxic epidermal necrolysis (2 eyes), pseudodendropliod (1 eye), an idiopathic cause (2 eyes), and chemical burn (2 eyes). All patients had diffuse total limbal deficiency with superficial corneal involvement. Following the removal of the pannus, the remaining cornea was clear, and thus no PKP was needed. Three eyes received ALT at the same time as AMT, and 4 eyes received ALT from 1.5 to 3.8 months after AMT. The mean (±SD) follow-up period was 11.3±4.6 months. The visual outcome was comparable for these 2 methods. For example, in patient 10, the corneal pannus was not removed in the right eye during AMT, and the amniotic membrane covered the entire cornea and perilimbal sclera (Figure 2, A and B). During the ensuing 3.2 months, the intensity of preexisting corneal neovascularization was reduced, surface erosion was healed, the perilimbal sclera became noninflamed, and the patient became asymptomatic with visual acuity improved to 20/80 (Figure 2, C and D). The corneal surface was further improved following ALT (Figure 2, E and F). For a comparison, the patient’s left eye received total corneal pannus removal at the time of AMT. During the ensuing 3 months before ALT, we noted that the corneal surface healed slowly and was eventually covered by another layer of superficial pannus (not shown). This new pannus could still be removed during ALT, and the corneal surface was restored with improved vision. We also noted a similar regrowth of superficial corneal pannus in the right eye of patient 12. A regrowth of corneal pannus was not noted in her left eye, which received AMT at the same time as ALT (not shown). For patient 11 with pseudodendropliod, ALT and AMT performed at the same time resulted in improved visual acuity of 20/60 for 12 months. Similar limbal deficiency developed gradually in the next 5 months, however, with a reduced visual acuity of counting fingers. Except for patient 11, no complication has developed in the group B patients. Patient 14 with a severe chemical burn received successful ALT and...
PKP and recovered a visual acuity of 20/70 from hand motion for 31 months until the nasal limbus started to show recurrent limbal deficiency with visual acuity reduced to 20/200. After a sectorial AMT and ALT performed at this region, he regained a visual acuity of 20/70 for 1 year, and the prior corneal transplant developed irreversible rejection with visual acuity reduced to 20/200.

GROUP C (SEVERE LIMBAL DEFICIENCY): RECEIVING AMT, ALT, AND PKP

The diagnoses for group C, comprising 13 patients and 14 eyes, included chemical burns (8 eyes), herpetic keratitis with multiple surgical procedures (1 eye), contact lens–induced keratopathy (1 eye), Stevens-Johnson syndrome (2 eyes), and atopic keratoconjunctivitis (2 eyes). All patients had diffuse limbal deficiency and deep corneal stromal involvement. Their damage was more severe, as shown by an increased number of previous corneal, glaucoma, cataract, and lid operations (12 eyes) and preexisting corneal stromal ulceration and descemetocele (2 eyes). Of the 6 patients with glaucoma or elevated intraocular pressure, 5 had chemical burns involving intraocular structures. Three eyes (patients 16, 18, and 21) required Molteno or Seton filtering procedures before corneal surface reconstruction; 3 eyes (patients 19, 20, and 22) received a Baervelt filtering procedure after reconstruction. The mean (±SD) follow-up period was 19.8±11.1 months. For AMT, 8 eyes were covered by a large piece of amniotic membrane over the entire cornea, including 5 to 7 mm of the perilimbal sclera (labeled as “360+K”; Table). Taking patient 18 as an example, this 40-year-old man had a severe chemical burn of his right eye and required a total conjunctival flap to manage a persistent corneal epithelial defect. All patients had severe photophobia and ocular irritation.

Corneal diseases with limbal (stem cell) deficiency remain some of the most challenging clinical problems. To alleviate patients’ complaints of severe photophobia and reduced vision, the therapeutic role of conventional PKP is limited, partly because the stem cell population is not restored. For patients with unilateral or focal limbal deficiency, autograft limbal transplantation is the treatment of choice. For those with bilateral limbal deficiency, however, the treatment of choice is ALT, which invariably poses the challenge of allograft rejection. In this report, we introduce the use of AMT before ALT. Our results have shown that AMT is useful for restoring a noninflamed perilimbal stromal environment to support the transplanted limbal tissue and to sustain the success of subsequent corneal surface reconstruction. The application of AMT differs among the 3 groups of patients with increasing severity of limbal deficiency and is discussed separately.

When limbal deficiency is partial or focal, AMT alone is sufficient to improve the corneal surface and hence the vision. This effect is demonstrated in group A and has been noted in a rabbit model described earlier. As shown in the Table and Figure 1, the transplanted perilimbal areas became noninflamed, resulting in less photophobia and ocular irritation. Even for patient 9, who had preexisting tractional retinal detachment, AMT relieved the patient’s symptoms. For all others, the use of AMT alone further improved the vision. Because ALT or PKP is not needed, there is no fear of allograft rejection, and patients do not require the administration of cyclosporine. Furthermore, no complication has been noted. For aniridia, a hereditary disease characterized in part by conjunctivalization and abnormal limbal epithelial cells, the use of AMT improved the vision by restoring corneal surface smoothness. For the remainder, excluding patient 9, there was at least 1 clock hour of intact limbus, and AMT further reduced the circumference of limbal deficiency. The diagnosis and extent of limbal deficiency is best made by impression cytologic examination to show conjunctivalization (conjunctival goblet cells) on the involved corneal surface. Other helpful signs include superficial corneal vascularization and a fluorescein-stained and conjunctivalized corneal surface, which is known to
Figure 1. Preoperative (left panel) and postoperative (right panel) appearances of 4 patients in group A. A, Patient 4 had a chemical burn that resulted in nearly total limbal deficiency except at the 6-o’clock position. B, After amniotic membrane transplantation (AMT), the perilimbal conjunctiva became less inflamed, the corneal surface became smooth and stable, and vascularization was noted at the superior cornea (covered by the lid) 7 months later. C, Patient 5 had a chemical burn that resulted in limbal deficiency involving the inferior 4-o’clock position, a persistent corneal epithelial defect, band keratopathy, and edema. D, After EDTA chelation and AMT, covering the area outlined by a purse-string suture, the corneal surface healed without edema and was stable for 3.2 months. E, Patient 6 had a chemical burn that resulted in superior symblepharon and partial limbal deficiency involving the superior half of the cornea. Three weeks after AMT, the symblepharon was released, the perilimbal tissue was not inflamed, and the corneal surface was stable (F; sutures indicate where the amniotic membrane was applied). G, Patient 8’s left eye had nearly total limbal deficiency, except at the 6-o’clock position, due to contact lens–induced keratopathy. H, The use of AMT resulted in a stable and corneal surface with noninflamed perilimbal tissue 2.5 months later.
be more permeable. The reason the extent of limbal deficiency was reduced after AMT might be because it contains a basement membrane, which is known to possess an antiapoptotic effect on epithelial cells.23 This notion is supported by our recent finding on impression cytologic examination that the basal epithelial cell density doubles on the amniotic membrane–reconstructed surface.23 Rapid epithelialization and reduced inflammation, vascularization, and scarring have previously been noted when AMT was used for conjunctival surface reconstruction.15,16 Such an action may be attributed in part to the restoration of a normal conjunctival epithelial phenotype with a higher-than-
normal goblet cell density\textsuperscript{10} and to the effect of the amniotic stromal matrix in suppressing fibroblast apoptosis\textsuperscript{24} and the expression of transforming growth factor $\beta$.\textsuperscript{25}

When limbal deficiency is total and diffuse and corneal involvement remains superficial, ALT needs to be added, as shown in group B. Such a rationale is derived from the impression cytologic finding confirming the presence of conjunctival goblet cells on the peripheral cornea, especially those areas with superficial vascularization in patients in group A receiving AMT alone.\textsuperscript{19} Thus, AMT alone

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**Figure 3.** Patient 18 with a chemical burn (group C). The resulting persistent corneal ulcer required a total conjunctival flap and was complicated by granuloma formation and superior symblepharon (A). Following several operations, he had a noninflamed ocular surface for 14 months (B). The limbal allograft was rejected, resulting in recurrent total limbal deficiency (C) and an elevated intraocular pressure. Amniotic membrane transplantation was performed 25 months after the first allograft limbal transplantation (ALT) and penetrating keratoplasty (PKP), resulting in a quiescent perilimbal conjunctiva (D). Focal engorged and tortuous limbal vessels, a sign suggesting early limbal allograft rejection,\textsuperscript{7} were noted in the 3- to 4-o’clock position of the limbus 8 months after a second ALT and PKP were done (E), but these were decreased 15 months later (F).
cannot restore the limbal cell population when limbal deficiency is diffuse. As shown in the Table and Figure 2, additional ALT performed with or following AMT reconstructed the corneal surface and improved the vision in 5 of 7 eyes. The exact reason for the failure of treatment in patient 11 was not clear; we attribute it to the recurrence of her underlying pseudopemphigoid activity. As occurred in patients in group A, the perilimbal scleral surface covered by the amniotic membrane became noninflamed. As a result, the success of transplanted limbal function can be ensured. While receiving cyclosporine enterally, none of the patients except patient 11 showed recurrent limbal deficiency; no patient showed the early sign of limbal allograft rejection. Because ALT performed after AMT yielded a similar result to ALT performed at the same time as AMT (group B), and because AMT alone might

Figure 4. Patient 15 with a chemical burn (group C). The preoperative appearance showed total limbal deficiency (A). Six months after amniotic membrane transplantation (AMT) was performed in the perilimbal area, the surface is noninflamed (B). A year later, allograft limbal transplantation (ALT) and penetrating keratoplasty (PKP) were performed, resulting in a clear cornea and quiescent perilimbal conjunctiva 1.2 months (C) and 3 months (D) thereafter. The preoperative appearance of patient 22 with herpetic keratouveitis and multiple surgeries led to total limbal deficiency and a failed graft (E). The inflamed perilimbal tissue was first suppressed by AMT, and subsequent ALT and PKP further improved her ocular surface (F).
be sufficient (group A), it is advised that AMT be considered as a first choice, especially for patients with partial limbal deficiency.

When limbal deficiency is diffuse and the damage extends deep into the corneal stroma, corneal surface reconstruction will have to include PKP. This is illustrated in group C. As shown in the Table and Figures 3 and 4, all 14 eyes in group C had more severe damage to their ocular surfaces, external adnexa, and even intraocular structures than those in groups A and B. Before corneal surface reconstruction, it is important to correct dry eyes by punctal occlusion, misdirected lashes and exposure by plastic surgery, and glaucoma by filtering procedures. All patients in group C received AMT as a first-stage procedure. For a reason unknown to us, the transplanted membrane rapidly dissolved in both eyes of patient 27 with atopic keratoconjunctivitis, and subsequent ALT and PKP failed. For the remaining 12 eyes, the transplanted amniotic membrane suppressed the perilimbal stromal inflammation. For those who might eventually require PKP, it is advised that AMT be performed perliminally, when the corneal pannus is quiescent, as shown in Figure 4, but used to cover the entire cornea without removing the pannus if active inflammation or corneal erosion is noted, as shown in Figure 2. The ALT and PKP procedures were performed using the same donor corneoscleral button. There were increased complications such as corneal graft rejection despite oral cyclosporine administration (9/14 eyes, 64%), recurrent surface breakdowns (3/14 eyes, 36%), and uncontrolled intraocular pressure (3/14 eyes, 21%) successfully managed with a Baervelt filtering procedure. Early signs of limbal allograft rejection, ie, tortuous and engorged limbal blood vessels, were noted in 3 eyes in this group, amounting to 14% of a total of 21 eyes that included group B. This number was significantly less than 12 (75%) of 16 eyes receiving ALT without AMT in our previous report7 (P < .001, Fisher exact test). A longer follow-up period is needed to determine whether allograft rejection can indeed be reduced as speculated. All patients in groups B and C require oral cyclosporine treatment indefinitely. We are also intrigued by the finding that limbal allograft rejection was relatively uncommon compared with corneal graft rejection. If AMT indeed protects against limbal allograft rejection mediated by the limbal vascular route, the difference between limbal and corneal rejection would suggest that the transcamenal route might play a more important role in eliciting corneal rejection. If this were the case, we wonder if the use of lamellar keratoplasty, when applicable, might reduce this complication. Alternatively, such a discrepancy might simply be related to the severity of the disease in group C.

CONCLUSION

Our study provides strong evidence that the use of AMT alone or in conjunction with ALT can further enhance the success of corneal surface reconstruction. Research is needed to explore the regulation of limbal stem cells and to study the action mechanism of the inherently avascular amniotic membrane matrix. It will unravel other useful information for enhancing the success of ocular surface reconstruction.

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Dr Tseng has filed an application for a US patent on the method of preparation and clinical uses of preserved human amniotic membrane.


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