Amniotic Membrane Transplantation for Symptomatic Bullous Keratopathy

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**Objective:** To determine whether amniotic membrane transplantation can be used to treat symptomatic bullous keratopathy displaying poor visual potential.

**Methods:** Amniotic membrane transplantation was performed at 5 centers on 50 consecutive eyes (50 patients) with symptomatic bullous keratopathy and poor visual potential. The underlying causes of bullous keratopathy included aphakia (9 eyes), pseudophakia (19 eyes), failed grafts (9 eyes), and others (13 eyes).

**Results:** During the follow-up period of 33.8 weeks (3-96 weeks) after amniotic membrane transplantation, 43 (90%) of 48 eyes with intolerable pain preoperatively became pain free postoperatively. Among the 5 eyes with residual pain, 3 received repeated amniotic membrane transplantation, 1 required a conjunctival flap for pain relief, and 1 had reduced pain. Epithelial defects in 45 (90%) of 50 eyes created and covered by amniotic membrane healed rapidly within 3 weeks. Only 4 eyes (8%) showed recurrent surface breakdown. Epithelial edema or bullae recurred in a smaller area in 5 eyes (10%) and pseudopterygium developed in 1 eye.

**Conclusion:** Amniotic membrane transplantation can be considered as an alternative to conjunctival flaps in alleviating pain, promoting epithelial healing, and preserving cosmetic appearance in patients with symptomatic bullous keratopathy and poor visual potential.

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**Bullous Keratopathy** is a disorder caused by corneal endothelial decompensation and is characterized by corneal stromal edema with or without epithelial blisters or bullae. Insults leading to corneal endothelial decompensation can come from various intraocular surgical procedures including cataract extraction leading to pseudophakia or aphakia. They can also come from nonsurgical trauma, uncontrolled glaucoma, and Fuchs endothelial dystrophy.

Regardless of the source of insult, patients with bullous keratopathy may suffer from reduced vision and ocular pain. These problems can be explained by histopathological changes in the corneal stroma and the epithelium. Without proper endothelial function to maintain corneal stromal hydration, stromal hydration increases with keratocyte loss,1 and the Bowman layer and the epithelial basement membrane attenuate or rupture,2,4 with eventual loss of glycosaminoglycans in the stroma.3 Collectively, these changes lead to increased hydration and intraepithelial edema. The latter surface changes then lead to poor epithelial adhesion and recurrent or persistent erosion, which explains why some conditions may be complicated by infectious keratitis and ulcers.

When there is good visual potential, corneal transplantation is the treatment of choice to alleviate pain and to restore vision and ocular surface defense. However, bullous keratopathy can also develop in transplanted corneas as a result of graft rejection or ensuing intraocular surgical procedures leading to further endothelial atrophy. When there is limited visual potential and corneal transplantation is no longer a feasible choice, ocular pain and surface breakdown can be treated with bandage contact lens,6,7 anterior stromal punctures,8 annular keratotomy,9 epikeratophakia,10 excimer laser phototherapeutic keratectomy,11 or a conjunctival flap.12,13

As an alternative to conjunctival flaps, human amniotic membrane, when appropriately processed and preserved, has recently been shown to be effective in treating persistent corneal epithelial defects with ulceration.14 When used for corneal15-17 and conjunctival18-22 surface reconstruction, amniotic membrane has also been shown to facilitate epithelialization and to reduce inflammation, vascularization, and scar-
PATIENTS AND METHODS

PATIENTS

The study included 50 eyes in 50 consecutive patients with symptomatic bullous keratopathy and poor visual potential enrolled at 5 different locations from November 1996 to November 1998. Forty-eight patients (48 eyes) complained of intolerable ocular pain, and the remaining 2 were pain free but had persistent corneal epithelial defects and stromal ulceration. For those 48 patients with pain, 13 also had persistent or recurrent corneal epithelial defects. Six eyes with epithelial defects were also ulcerated and developed descemetocele. Based on the underlying causes of their disorders, they were further divided into 4 groups: 9 patients had aphakic bullous keratopathy, 19 patients had pseudophakic bullous keratopathy, 9 patients had failed penetrating keratoplasty, and 13 patients had phakic bullous keratopathy. Their demographic data and clinical characteristics are summarized in the Table.

HUMAN AMNIOTIC MEMBRANE PREPARATION AND PRESERVATION

The method of amniotic membrane preparation and preservation has been described previously. In this study, amniotic membrane for one center (S.L.M.) was obtained solely from Bio-Tissue (South Miami, Fla), where amniotic membrane was similarly prepared. All other amniotic membranes were processed at Bascom Palmer Eye Institute, Miami, Fla, and were analyzed by the Department of Biostatistics.

RESULTS

All preoperative and postoperative data were sent to the Bascom Palmer Eye Institute, Miami, Fla, and were analyzed by the Department of Biostatistics.

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Aphakic Bullous Keratopathy</th>
<th>Pseudophakic Bullous Keratopathy</th>
<th>Failed Penetrating Keratopathy</th>
<th>Phakic Bullous Keratopathy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>9</td>
<td>19</td>
<td>9</td>
<td>13</td>
<td>50</td>
</tr>
<tr>
<td>Male/female</td>
<td>5/4</td>
<td>8/11</td>
<td>3/6</td>
<td>6/7</td>
<td>22/28</td>
</tr>
<tr>
<td>Ages ± SD, y</td>
<td>68.4 ± 7.3</td>
<td>67.1 ± 10.4</td>
<td>66.8 ± 15.1</td>
<td>60 ± 18.7</td>
<td>65.4 ± 13.5</td>
</tr>
<tr>
<td>SD of duration of keratopathy, y</td>
<td>4.3 ± 6.3</td>
<td>1.7 ± 1.4</td>
<td>0.2 ± 0.16</td>
<td>1.7 ± 3.0</td>
<td>1.9 ± 3.3</td>
</tr>
<tr>
<td>Suturing, running/interrupted/both</td>
<td>5/1/3</td>
<td>12/3/4</td>
<td>1/2/6</td>
<td>6/4/4</td>
<td>23/10/17</td>
</tr>
<tr>
<td>Contact lens use, yes/no</td>
<td>7/2</td>
<td>9/10</td>
<td>8/1</td>
<td>7/6</td>
<td>31/19</td>
</tr>
<tr>
<td>Mean ± SD of epithelial healing, wk</td>
<td>2.4 ± 2.2</td>
<td>2.2 ± 1.4</td>
<td>2.4 ± 1.5</td>
<td>2.4 ± 1.0</td>
<td>2.3 ± 1.4</td>
</tr>
<tr>
<td>Vision change, better/same/worse</td>
<td>0/8/1</td>
<td>3/15/1</td>
<td>1/7/1</td>
<td>5/8/0</td>
<td>9/38/3</td>
</tr>
<tr>
<td>Membrane, completely dissolved/partially dissolved/intact/intentionally peeled off</td>
<td>1/5/3/0</td>
<td>3/4/10/2</td>
<td>3/15/0</td>
<td>1/4/5/3</td>
<td>10/13/22/5</td>
</tr>
<tr>
<td>Mean ± SD follow-up, wk</td>
<td>35.4 ± 21.2</td>
<td>36.6 ± 24.9</td>
<td>31.1 ± 7.5</td>
<td>30.3 ± 21.3</td>
<td>33.8 ± 20.8</td>
</tr>
</tbody>
</table>

* Data are presented as number of patients unless otherwise indicated.

ring. Based on these actions, we examined whether amniotic membrane can also be used as an alternative treatment for alleviating pain and recurrent epithelial defects in patients with symptomatic bullous keratopathy and poor visual potential.

SURGICAL TECHNIQUE

Informed consent was obtained from each patient. The surgery was performed by 1 surgeon from each of 5 different centers using a similar surgical technique. One such example is illustrated in Figure 1. After a routine retrobulbar injection, the loose epithelium was first removed with a sponge (Microsporge; Alcon Surgical Inc, Fort Worth, Tex), a 0.12 forceps, or a blade (Beaver blade; Alcon Surgical Inc) to where the epithelium became quite adherent. In general, this would create a large corneal epithelial defect up to 1 or 2 mm from the limbus. The preserved amniotic membrane was removed from the storage medium and cut to the same size as the debrided corneal surface with the basement membrane surface up in all cases. The side of the basement membrane could be distinguished from the stromal side by touch with a sponge (Weckcel; Edward Weck & Co, Research Triangle Park, NC). The former was not sticky, while the latter was and could be caught by the sponge. This fashioned membrane was then secured to the edge of the defect by 10-0 nylon sutures, which were either continuously running, interrupted, or a combination of both (Table). Attention was given to make the membrane flattened tightly on to the corneal surface and approximate to the epithelial edge. In some cases a bandage contact lens was applied (Table). Postoperative medication included topical steroid and antibiotic drops or ointments, of which the frequency and duration were no different from those used for cataract surgery.

DATA ANALYSIS

All preoperative and postoperative data were sent to the Bascom Palmer Eye Institute, Miami, Fla, and were analyzed by the Department of Biostatistics.
Figure 1. Preoperative, intraoperative, and postoperative photographs of a patient with painful pseudophakic bullous keratopathy. A, Before surgery, the cornea had several episodes of corneal erosion and 1 episode of bacterial superinfection and the corneal surface was irregular with perilimbal injection. B, The loose epithelium was denuded by Weckcel and blunt sweeping with a surgical blade up to 1 mm within the limbus. C, The amniotic membrane was removed from the storage medium and peeled from the cellulose paper. D and E, This membrane was fastened onto the defect by a running 10-0 nylon suture. F, The membrane surface and the corneal surface were epithelialized 3 weeks later and the conjunctiva was not inflamed. G and H, The healed surface remained noninflamed and stable for up to 5 months, when a central part of the membrane dissolved (indicated by arrowheads) where fluorescein staining showed pooling.
cataract extraction (13 eyes), and phacoemulsification (5 eyes). Additional intraocular surgical procedures included trabeculectomy (4 eyes), retinal detachment repair (3 eyes), and Baerveldt tube implantation (1 eye); other risk factors included glaucoma (3 eyes) and uveitis (1 eye). In failed penetrating keratoplasty, other intraocular surgical procedures included cataract extraction (6 eyes), trabeculectomy (5 eyes), seton tube implantation (2 eyes), cyclocryotherapy (1 eye), and trauma repair (1 eye). In phakic bullous keratopathy, the contributory factors included end-stage or uncontrollable glaucoma (8 eyes), penetrating trauma (2 eyes), and iridoconveal endothelial syndrome (1 eye).

Before amniotic membrane transplantation, bullous keratopathy in these 50 patients had lasted for 1.9 ± 3.3 years (range, 0.03–20 years). All except 2 eyes suffered from ocular surface pain. Thirteen eyes had concomitant epithelial defects. Six eyes with epithelial defects were also ulcerated and 1 developed decemetocoele. Their preoperative visual acuities were all worse than or equal to finger counting: finger counting (22 eyes), hand movement (18 eyes), light perception (5 eyes), and no light perception (5 eyes). All eyes had been treated with topical artificial tears, lubricants, or 5% or 3% saline solution. For those with epithelial defects or ulcers, prophylactic antibiotics were included. Bandage contact lens was used in 2 eyes and excimer phototherapeutic keratectomy in another eye.

With respect to the technique of suturing amniotic membrane, 23 eyes received running sutures, 10 eyes received interrupted sutures, and 17 eyes received both (Table). After the procedure, a bandage contact lens had been used in 31 eyes (62%). The most dramatic finding was that 43 (90%) of 48 patients suffering from ocular pain became pain free the day after transplantation and remained so for the rest of the follow-up period (33.8 ± 20.8 weeks [range, 3–96 weeks]). In the 5 patients who could not be treated successfully, pain was reduced in 4, with 2 receiving repeated amniotic membrane transplantation 1 and 7 months later, respectively. Only in 1 patient was the recurrent pain not relieved until a Gunderson conjunctival flap procedure was performed. The defect created and covered by amniotic membrane healed rapidly within 3 weeks in all except 5 eyes (90%). In these 5 eyes, 2 eyes healed in 4 weeks, 1 healed in 6 weeks owing to the loosening of the running suture, and 1 healed in 8 weeks because of unrecognized aqueous tear deficiency and bacterial keratitis. The healed epithelium developed a defect in 4 (8%) of 50 eyes during the entire follow-up period. Visual acuity remained unchanged in 38 eyes (76%) and improved in 9 eyes (18%). In the latter 9 eyes, 1 eye had visual acuity improvement from hand movement to 20/30 after subsequent extracapsular cataract extraction and corneal transplantation (phakic bullous keratopathy group). 1 eye from finger counting to 20/200, 3 eyes from finger counting to 20/400, 3 eyes from hand movement to finger counting, and 1 eye from light perception to finger counting. The visual acuity of 3 eyes (6%) decreased from hand movement to light perception; all of them had preexisting glaucoma. The fate of amniotic membrane was also recorded. The membrane remained intact in 23 eyes (46%) and became partially dissolved in 14 eyes (28%) or completely dissolved in 8 eyes (16%). In the remaining 5 eyes (10%), the membrane was purposely peeled off as the epithelium migrated underneath and had healed. Preexisting corneal epithelial edema or bullae was resolved in all but 5 eyes (10%), all of which were in a smaller area. One eye developed pseudopterygium after surgery. Figures 1, 2, and 3 illustrate how epithelial healing was facilitated by amniotic membrane transplantation and the fate of the membrane after healing.

This report demonstrates the overwhelming success of amniotic membrane transplantation for ocular pain relief in 43 (90%) of 48 eyes with painful bullous keratopathy. In the 5 eyes that could not be treated successfully, pain was reduced in 4, and in 1 eye the recurrent pain was relieved by a Gunderson conjunctival flap. These results support our clinical impression that amniotic membrane transplantation can be considered an alternative surgical choice for treating this disorder. Because it is technically easier to perform, avoids such potential complications as ptosis, and provides a better cosmetic appearance, amniotic membrane transplantation is superior to conjunctival flap. Furthermore, amniotic membrane transplantation provides an additional advantage in that the resultant cornea does not manifest surgically induced limbal stem cell deficiency, which is invariably created by conjunctival flap. As a result, such corneas are amenable for corneal transplantation if necessary. In 4 eyes with reduced pain, 2 received repeated amniotic membrane transplantation 1 and 7 months later, indicating that temporary relief was achieved by amniotic membrane transplantation.

Pain relief by amniotic membrane transplantation is associated with restoration of corneal epithelial integrity. Before amniotic membrane transplantation, 15 (30%) of 50 eyes had epithelial defects, 13 of which were associated with pain. 8 eyes showed stromal ulceration, and 1 eye developed decemetocoele. All except 4 eyes (92%) maintained a healed and intact epithelium during the entire follow-up period. Preexisting corneal epithelial edema or bullae was resolved in all but 5 eyes (90%), and in these 5 eyes the recurrent bullae were in a smaller area. Even with recurrent epithelial edema in later days, pain did not recur. We thus wonder if amniotic membrane matrix and basement membrane might have decreased the sensory nerve stimulation. It should also be noted that the defect created by surgery healed rapidly (within 3 weeks) in all but 5 eyes (90%). Such rapid epithelialization has been reported previously in corneal and conjunctival surface reconstruction using amniotic membrane.

We attribute the aforementioned therapeutic effects to the new substrate, ie, the basement membrane and the avascular stromal matrix provided by the amniotic membrane. It has been recognized that the basement membrane in general facilitates migration of epithelial cells, which reinforces adhesion of basal epithelial cells, and promotes epithelial differentiation. Recently, the basement membrane has also been found to be important in preventing epithelial apoptosis.
Figure 2. Preoperative and postoperative photographs of a patient with painful aphakic bullous keratopathy. A and B, Before surgery, the cornea suffered from recurrent corneal erosion with microcystic edema and bullae (indicated by arrows). C and D, The membrane was fastened by interrupted 10-0 nylon sutures, and the membrane and the corneal surfaces were nearly completely epithelialized in 2 weeks. E and F, Three and a half months after surgery, the membrane-covered surface was smooth and a small part was dissolved (indicated by arrowheads) where fluorescein staining showed pooling. G and H, The corneal surface continued to improve and was smooth and noninflamed without microcystic edema or bullae 14 months after surgery.
recent laboratory data further suggest that the amniotic basement membrane can prolong the life span of corneal and conjunctival progenitor cells and maintain the slow-cycling label-retaining cells. This action may explain why amniotic membrane transplantation can be used to expand the remaining limbal stem cells and corneal transient amplifying cells during the treatment of partial limbal deficiency and to facilitate epithelialization for persistent corneal epithelial defects with stromal ulceration. Future studies are needed to discern if this epithelial-promoting effect is mediated by the matrix, soluble growth factors, or both.

The stromal side of the membrane contains a unique matrix component that suppresses transforming growth factor β signaling, and proliferation, and myofibroblast differentiation of normal human corneal and limbal fibroblasts. This action explains why amniotic membrane transplantation reduces scars during conjunctival surface reconstruction, prevents recurrent scarring after pterygium removal, and reduces corneal haze after phototherapeutic keratectomy and photorefractive keratectomy. The stromal matrix of the membrane also contains various forms of protease inhibitors, important for promoting epithelial healing and reducing stromal inflammation and ulceration. Future studies are needed to discern the anti-inflammatory action from the antiscarring effect of the amniotic membrane.

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REFERENCES