Amniotic Membrane Inlay and Overlay Grafting for Corneal Epithelial Defects and Stromal Ulcers

Erik Letko, MD; Stephen U. Stechschulte, MD; Kenneth R. Kenyon, MD; Nadia Sadeq, MD; Tatiana R. Romero, MD; C. Michael Samson, MD; Quan D. Nguyen, MD; Stephanie L. Harper, MD; Jonathan D. Primack, MD; Dimitri T. Azar, MD; Martin Gruterich, MD; Claes H. Dohlman, MD; Stefanos Baltatzis, MD; C. Stephen Foster, MD

Objectives: To determine the effect of amniotic membrane transplantation (AMT) on persistent corneal epithelial defects (PEDs) and to compare the efficacy between inlay and overlay techniques.

Methods: Thirty patients (30 eyes) underwent AMT for PED. The use of AMT was restricted to patients in whom all previous measures, including bandage contact lens and tarsorrhaphy, had failed. The amniotic membrane was placed on the surface of the cornea in overlay (group A) or inlay (group B) fashion.

Results: The PED healed after the first AMT in 21 eyes (70%) within an average of 25.5 days after surgery and recurred in 6 eyes (29%). Among the 22 eyes treated with an overlay AMT (group A), the PED healed after the first AMT in 14 eyes (64%) within an average of 24.5 days and recurred in 4 eyes (29%). Among the 8 eyes treated with an inlay AMT (group B), the PED healed within an average of 27.4 days after AMT, which did not statistically significantly differ from group A (P = .72). The PED healed after the first AMT in 7 eyes (88%) and recurred in 2 (29%) of 7 eyes.

Conclusions: The AMT can be helpful in the treatment of PED in which all other conventional management has failed. However, the success rate in our study was not as high as that previously reported, and our results showed a high incidence of recurrences of epithelial defects. We did not find any difference between overlay and inlay techniques in terms of healing time and recurrence rate.

Arch Ophthalmol. 2001;119:659-663

Persistent corneal epithelial defects (PEDs) induced by primary ocular surface disorders, such as chemical injury, neurotrophic keratitis, and keratoconjunctivitis sicca, or associated with systemic diseases, such as ocular cicatricial pemphigoid, Stevens-Johnson syndrome, or toxic epidermal necrolysis, are extremely difficult to treat. Such disorders cause prolonged inflammation of the ocular surface, damaging corneal stem cells and epithelial basement membrane (BM) and resulting in corneal scarring, neovascularization, and decreased vision. In addition, matrix metalloproteinases produced by keratocytes, epithelial cells, and neutrophils can cause progressive stromal ulceration with risk of corneal perforation.

Previous studies have shown that the epithelial BM facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells, promotes epithelial differentiation, and militates against epithelial apoptosis. Even when an extensive PED develops, in the presence of an intact BM, the corneal surface can be replaced by conjunctivally derived epithelium that can acquire a corneal-type phenotype, ie, express 55-kd keratin.

Human placental amnion is composed of a single epithelial cell layer, a BM, and an avascular stroma. Both collagens IV and VII, components of corneal epithelial BM, are present in the BM of amniotic membrane. In addition, collagens I through III and V are also present in human placental amnion. Amniotic epithelium produces basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor β. Amnion prevents inflammatory cell infiltration and reduces apoptosis in keratocytes after transplantation onto the corneal surface. It is noteworthy that matrix metalloproteinases have also been identified in human amniotic membrane.

Davis first reported on the use of amniotic membrane for skin transplantation in 1910. Since then, living rather than preserved amniotic membrane has been used for various purposes, and in 1940, De Roth first reported its use in the eye, using fresh amnion for the reconstruction of conjunctival defects. In the early 1990s, Batlle and Perdomo reintroduced the use...
PATIENTS AND METHODS

Thirty patients (30 eyes) who underwent AMT for PED with or without stromal ulceration between 1997 and 1999 were studied (Table 1). The duration of corneal epithelial defects varied from 1 to 6 weeks. We used a progressively staged approach in the management of PED. Thus, all patients were initially treated with removal of toxic topical antibiotics, lubrication, punctum occlusion if appropriate, and then with a bandage contact lens. If these techniques were unsuccessful, tarsorrhaphy was performed. Amniotic membrane transplantation was reserved for patients in whom all other measures were unsuccessful. In patients who refused to have tarsorrhaphy, mostly for cosmetic reasons, AMT was performed after treatment with bandage contact lenses failed.

Human amniotic membrane grafts were prepared and preserved as previously described. An informed consent was obtained from each patient before the surgery. After peribulbar or topical anesthesia, the base of the epithelial defect or stromal ulcer was debrided with a microsponge, and the poorly adherent epithelium surrounding the defect or ulcer was removed. The amniotic membrane was placed on the surface of the cornea in overlay (group A) or inlay (group B) fashion (Figure). After each AMT, a bandage contact lens was applied and 0.35% ciprofloxacin hydrochloride eye drops 4 times a day and 1% rimexolone eye drops 4 times a day were administered in the postoperative period. The amniotic membrane dissolved under the bandage contact lens during a period of 4 weeks after surgery.

of amniotic membrane transplantation (AMT) for ocular disorders in North America. Kim and Tseng then showed in 1995 that preserved amniotic membrane facilitated corneal surface reconstruction in rabbits after epithelial removal and limbal lamellar keratectomy. Amniotic membrane transplantation has recently been used for ocular surface reconstruction in patients after chemical burn, in patients with advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome, and for pterygium excision, conjunctival surface reconstruction, sterile corneal ulceration, and symptomatic bullous keratopathy.

The purpose of this study was to determine the effect of human amniotic membrane grafts on PEDs of the cornea with or without stromal ulcers and to compare the efficacy between inlay and overlay techniques.

RESULTS

OVERALL OUTCOMES

The overall results for all 30 cases are summarized in Table 2. The mean age of patients was 55.3 years (range, 9-78 years). The male-female ratio was 2:1. The mean follow-up time after AMT was 8.2 months (range, 1-32 months). The mean visual acuity before and after the surgery was 0.04 (range, 0.01-0.13) and 0.05 (range, 0.01-0.20), respectively (P = .45). The PEDs healed within an average of 25.5 days (range, 5-68 days) after surgery. The epithelial defect healed after the first AMT in 21 eyes (70%). In 9 eyes (30%), the defect persisted after the first AMT. The bandage contact lens was removed when the epithelial defect had healed.

GROUP A: OVERLAY AMT

Patients with large epithelial defects with or without stromal ulcer with clinically deficient limbal stem cell function were treated with overlay AMT. Overlay grafts approximately 12 to 14 mm in diameter covered the entire corneal, limbal, and perilimbal surfaces. The overlay AMT was placed with the epithelial BM side up and secured by interrupted 10-0 polyglactin sutures to the surrounding conjunctiva. In some cases, a running 10-0 polyglactin suture was placed in the midperipheral cornea.

GROUP B: INLAY AMT

Patients with localized PED (<20% of corneal surface) with or without stromal ulceration received inlay AMT. Inlay grafts were cut to fit the size of the epithelial defect or stromal ulceration as described by Lee and Tseng. These grafts were placed with the epithelial BM side up and secured to the edge of the defect by interrupted 10-0 polyglactin sutures.

After each AMT, a bandage contact lens was applied and 0.35% ciprofloxacin hydrochloride eye drops 4 times a day and 1% rimexolone eye drops 4 times a day were administered in the postoperative period. The amniotic membrane dissolved under the bandage contact lens during a period of 4 weeks after surgery. The bandage contact lens was removed when the epithelial defect had healed.

Among the 22 eyes treated with an overlay AMT, the mean age of patients was 53.1 years (range, 27-75 years). The male-female ratio was 7:4. The mean follow-up time was 6.8 months (range, 1-22 months) after AMT. The mean visual acuity before and after the surgery was 0.05 (range, 0.01-0.33) and 0.06 (range, 0.01-0.33), respectively (P = .54). The corneal epithelial defect healed within an average of 24.5 days (range, 5-68 days) after surgery. The epithelial defect healed after the first AMT in 14 eyes (64%). The epithelial defect recurred after the first AMT in 4 (29%) of 14 eyes. The average time to the recurrence of the defect was
4.5 weeks (range, 2-9 weeks). The PED did not heal or recurred after the first AMT in 4 patients (45%).

**GROUP B**

Among the 8 eyes treated with an inlay AMT, the mean age of patients was 61.1 years (range, 9-78 years). The male-female ratio was 3:1. The mean follow-up time was 12.2 months (range, 2-32 months) after AMT. The mean visual acuity before and after the surgery was 0.02 (range, 0.01-0.05) and 0.03 (range, 0.01-0.1), respectively ($P = .35$).

The corneal epithelial defect healed within an average of 27.4 days (range, 7-41 days) after AMT, which did not statistically significantly differ from group A ($P = .72$). The epithelial defect healed after the first AMT in 7 eyes (88%). The epithelial defect persisted after the first AMT in 1 eye (12%). The epithelial defect recurred after the first AMT in 2 (29%) of 7 eyes. The PED did not heal or recurred after the first AMT in 3 (38%) of 8 eyes. There was not a statistically significant difference ($P = .53$) in the average time to the recurrence of epithelial defect between group B (6.5 weeks) and group A.

**COMMENT**

The epithelial defects healed after the first AMT in 70% (21/30) of all eyes in our study. By comparison, Lee and Tseng reported an initial success rate of 90% (10/11). In addi-
tion, we found a higher recurrence rate (29%) after the first AMT than that previously reported. Given the intrinsic diversity among PED cases, it might be hypothesized that differences in surgical techniques and/or causes of the epithelial defects might explain these outcome differences. Lee and Tseng did not observe any recurrence of epithelial defects when the amniotic membrane graft was placed into the stromal ulcer and secured by interrupted sutures to the edge of the defect. The corneal epithelium grew over the amniotic membrane graft. We used an identical technique in group B, yet the incidence of recurrences was 29%. Again, the difference in cause of the epithelial defects and extent of damage to the ocular surface might explain this higher rate of recurrences. Interestingly, our results show that there was no difference in recurrence rate between overlay and inlay techniques, although there was a slight difference in success rate after the first AMT when the inlay technique was used. In addition to using amnion in the inlay fashion, we used the amniotic membrane graft to cover the entire corneal surface and perilimbal conjunctiva in some cases, similar to a bandage contact lens. This technique has been used successfully by others in the treatment of patients with symptomatic bullous keratopathy, limbal stem cell deficiency of various causes, and stromal defects after photorefractive keratectomy.

Among our patients, autoimmune diseases affecting the ocular surface were involved in the pathogenesis of epithelial defects and stromal ulcers in 3 of 6 patients with recurrence of epithelial defect. One patient had ocular cicatricial pemphigoid, 1 had toxic epidermal necrolysis, and the other had Sjögren syndrome. Tsubota et al treated a group of patients with ocular cicatricial pemphigoid or Stevens-Johnson syndrome with AMT and limbal stem cell transplantation. They demonstrated that the entire ocular surface with or without PED in this group of patients can be restored by AMT along with limbal allograft and tarsorrhaphy, followed by systemic immunosuppression and topical administration of artificial tears derived from autologous serum. The authors reported a stable ocular surface during the follow-up in 9 of 11 patients. However, final visual acuity remained less than 0.1 in all but 3 patients.

The growth of new epithelium, particularly in patients with multiple causative factors involved in the pathogenesis of epithelial or stromal defects, may be altered by the absence of direct contact with BM. Tseng et al recently showed that AMT alone is superior to AMT with limbal allotransplantation in patients with partial limbal stem cell deficiency, whereas limbal allotransplantation with AMT is needed for patients with total limbal stem cell deficiency.

### Table 2. Results in Groups A and B and the 2 Groups Combined

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 22)</th>
<th>Group B (n = 8)</th>
<th>Combined (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>53.1</td>
<td>61.1</td>
<td>55.3</td>
</tr>
<tr>
<td>Mean visual acuity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before AMT</td>
<td>0.05</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Final</td>
<td>0.06</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Healed PEDs after</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>first AMT, No. (%)</td>
<td>14 (64)</td>
<td>7 (88)</td>
<td>21 (70)</td>
</tr>
<tr>
<td>Mean healing time, d</td>
<td>24.5</td>
<td>27.4</td>
<td>25.5</td>
</tr>
<tr>
<td>Recurrence after</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>first AMT, No. (%)</td>
<td>4/14 (29)</td>
<td>2/7 (29)</td>
<td>6/21 (29)</td>
</tr>
<tr>
<td>Mean interval to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recurrence, wk</td>
<td>4.5</td>
<td>6.5</td>
<td>5.2</td>
</tr>
<tr>
<td>Not healed and recurrent epithelial defects, No. (%)</td>
<td>10 (45)</td>
<td>3 (38)</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Mean follow-up, mo</td>
<td>6.8</td>
<td>12.2</td>
<td>8.2</td>
</tr>
</tbody>
</table>

*Group A underwent overlay amniotic membrane transplantation (AMT); group B, inlay AMT. PEDs indicates persistent epithelial defects.
Active conjunctival inflammation can complicate the postoperative period after ocular surface reconstruction, with resultant failure of the ocular surface. Keratoprosthesis may then be the procedure of last resort in such cases.35

Shimazaki et al36 claimed that the epithelium of the amniotic membrane may survive up to 70 days after cryopreservation. Their study showed that living epithelium of amnion produces basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor β.19 Whether cryopreserved transplanted amniotic membrane confers such benefit is uncertain. Because the epithelium of amnion expresses neither HLA class I nor II antigens,36–38 the use of systemic immunosuppressive therapy in AMT is not required.

Amniotic membrane transplantation can improve visual acuity by both corneal surface restoration and improvement of corneal transparency.39 In our study, the average visual acuity improved after AMT, but the difference between the preoperative and final visual acuities was not statistically significant (P = .45). A statistically significant difference between visual acuity before AMT and final visual acuity was not observed in either group A (P = .54) or group B (P = .35). These results suggest that the effect of AMT on vision rehabilitation is very limited and is unrelated to the technique used.

In summary, our study confirms the results of previous reports that AMT can be helpful in the treatment of epithelial defects and stromal ulcers in which all other conventional management has failed. However, the success rate in our study was not as high as that previously reported, and our results showed a high incidence of recurrences of epithelial defects.

We therefore believe that AMT for PED, with or without stromal ulceration, should not be used as first- or even second-line therapy. The use of AMT for PED should be reserved for cases in which removal of medications toxic to the epithelium, aggressive lubrication and ocular surface protection (eg, bandage contact lens and tarsorrhaphy), and control of inflammation have failed to promote closure of the defect. We did not find any difference between overlay and inlay techniques in terms of healing time, recurrence rate, and effect on vision rehabilitation. Further studies are required to confirm our results, to compare AMT with other strategies in treatment of PEDs and stromal ulcers, to compare the results after different techniques of transplantation, and to estimate the outcomes in various groups of patients.

Accepted for publication December 15, 2000.

Corresponding author and reprints: C. Stephen Foster, MD, Immunology Service, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114 (e-mail: fosters@uvc.edu).

REFERENCES