Postoperative Changes in Amniotic Membrane as a Carrier for Allogeneic Cultured Limbal Epithelial Transplantation

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• PURPOSE: To investigate the morphologic changes and outcomes of the amniotic membrane as a carrier for allogeneic cultivated limbal epithelial transplantation.
• DESIGN: Prospective, noncomparative, interventional study.
• METHODS: A total of 16 eyes receiving allogeneic cultivated limbal epithelial transplantation with amniotic membrane as a carrier were enrolled. Morphologic changes in the amniotic membrane were observed by confocal microscopy and RTVue optical coherence tomography. The paired *t* test was employed to compare the mean best corrected visual acuity (BCVA) and corneal stromal thickness.
• RESULTS: Of the 16 eyes, 12 had stable ocular surfaces (group A), while the other 4 eyes had failed surgeries due to immune rejection (group B). Confocal microscopy showed residual amniotic membrane tissues in 8 eyes in group A at 1 year. However, the amniotic membrane was not detected in group B at 8–10 months. RTVue optical coherence tomography showed discontinuous amniotic membrane tissues in all eyes in group A at 1 year, while highly reflective opacity was seen in the corneal stroma in group B. There were no statistically significant differences in mean BCVA and corneal stromal thickness in group A at 1 month and 1 year after transplantation ($P > 0.05$), but the mean BCVA showed a statistically significant difference at 1 month and after the disappearance of the amniotic membrane in group B ($P < 0.05$).
• CONCLUSIONS: For eyes with stable ocular surfaces after cultivated limbal epithelial transplantation, the amniotic membrane can be present in the cornea for at least 1 year, with no impact on visual acuity or corneal stromal thickness. Chronic inflammation and neovascularization on the ocular surface may accelerate the disappearance of the amniotic membrane. (Am J Ophthalmol 2014;158:1192–1198. © 2014 by Elsevier Inc. All rights reserved.)

ALLOGENEIC CULTIVATED LIMBAL EPITHELIAL transplantation has been found to be effective in the treatment of ocular chemical and thermal burns.[1–3] The amniotic membrane seems to be an ideal vehicle for the in vitro cultivation and transplantation of limbal epithelial stem cells[4,5] because of its unique biologic properties.[6,7] However, neither the morphologic changes and final outcomes of the amniotic membrane on the ocular surface nor their impact on visual acuity, corneal stromal thickness and transparency after transplantation have been reported.

In this prospective study, we enrolled patients who had received cultivated limbal epithelial transplantation for ocular chemical or thermal injuries and observed the changes in the amniotic membrane on the ocular surface postoperatively by various means, including confocal microscopy and corneal optical coherence tomography (OCT).

METHODS

• PATIENTS: This prospective, noncomparative, interventional study was approved by the Institutional Review Board of Shandong Eye Institute and adhered to the tenets of the Declaration of Helsinki. Patients undergoing allogeneic cultivated limbal epithelial transplantation with human amniotic membrane as a carrier at Shandong Eye Institute were recruited on a consecutive basis from January-April 2012. The eligible eyes met the following inclusion criteria: (1) total limbal stem cell deficiency arising from ocular chemical or thermal burns was diagnosed if (a) clinical slit-lamp examination revealed 360-degree loss of limbal palisades of Vogt, chronic ocular surface inflammation, poor epithelial integrity, corneal conjunctivalization, and neovascularization; and (b) corneal impression cytology showed the presence of goblet cells[8,9]; (2) allogeneic cultivated limbal epithelial transplantation was performed at least 6 months after ocular burns; (3) no keratoplasty, limbal tissue transplantation or cultivated limbal epithelial transplantation had been performed previously; (4) a follow-up of at least 12 months was completed at Shandong Eye Institute. Patients with severe dry eye (Schirmer test <5 mm) and eyelid disorders (eg, trichiasis, lagophthalmos and eyelid margin malposition) were excluded. Each patient gave written informed consent to participate in this research after the risks and possible adverse consequences had been explained.

We included 16 eyes (15 patients) in the study. Of the patients, 12 were male, and 3 were female. The mean age

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was 30.6 ± 13.0 years (range, 17–56 years). The original injuries included chemical burns in 11 eyes (10 patients) and thermal burns in 5 eyes (5 patients). The severity was graded as 2 in 9 eyes and 3 in 7 eyes, according to the Roper Hall classifications.[8,9]

• **CULTIVATION OF LIMBAL EPITHELIUM:** Amniotic membrane was prepared as previously reported[10] before it was cut into pieces of 2 × 2 cm for use. Limbal tissue specimens were removed from fresh donor eyes, cut into pieces, and inoculated on denuded amniotic membrane on the transwell insert as an explant culture. The transwell inserts were then transferred into a cell culture plate preseeded with mitomycin C-inactivated NIH 3T3 feeder cells, followed by incubation until confluence and stratification for up to 5 days, with medium changes every 2 days. Dulbecco modified Eagle medium/F-12 (3:1) medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, New York, USA); insulin-transferrin-sodium selenite (100×; Gibco); penicillin-streptomycin (Hyclone, Logan, Utah, USA), 10 ng/mL recombinant human epidermal growth factor (R&D Systems, Minneapolis, Minnesota, USA); 1% nonessential amino acids (Invitrogen, Carlsbad, California, USA); 0.1 nM cholera toxin (Sigma, St. Louis, Missouri, USA); 2 nM 3,3' 5-Triiodo-L-thyronine sodium salt (Sigma); 0.4 ng/mL hydrocortisone succinate (Wako, Osaka, Japan); and 2 nM L-glutamine (Invitrogen) was used for the culture of limbal epithelial cells. The cell sheets with 3 to 5 layers of stratification on the amniotic membrane showing basal column-shaped cells and superficial flattened scale-like cells were applied for clinical transplantation.[11–13]

• **SURGICAL TECHNIQUES:** All surgeries were performed under peribulbar anesthesia. A 360-degree conjunctival peritomy was made before the conjunctiva was retracted posteriorly from the limbus. Fibrovascular pannus presenting over the cornea was removed, and superficial keratectomy was performed to ensure a relatively smooth corneal surface substrate. The human amniotic membrane with cultivated limbal epithelial cells was placed on the bare sclera and corneal stroma with the epithelial side upward and sutured with interrupted 10-0 nylon sutures. The conjunctiva was then sutured onto the limbus to form a conjunctival sac. After a therapeutic soft contact lens was placed, tobramycin and dexamethasone ophthalmic ointments were administered at the end of the surgery.[14]

• **POSTOPERATIVE THERAPY:** Intravenous methylprednisolone (2 mg/kg) was given daily for 3–5 days. Oral prednisolone (1 mg/kg) was then started daily and tapered over a period of 2–3 months. Autologous serum eyedrops, containing 0.02 mg of dexamethasone and 0.1 mg of tobramycin per milliliter of serum, were used every 2 hours for the first week, before 0.02% fluorometholone eyedrops were administered 4 times daily throughout the next 2 weeks. After epithelialization was completed, 1% cyclosporine A eyedrops were given 4 times per day. Tobramycin ophthalmic ointment was administered every night. The above topical therapy was adjusted according to clinical status after 3 weeks. The patients were observed daily during the first week after surgery, weekly during the next 2 months, and monthly thereafter.[14]

OUTCOME MEASURES

COMPLETE OCULAR EXAMINATIONS WERE PERFORMED, including best-corrected visual acuity (BCVA), slit-lamp examination and fluorescein staining. Ocular surface stability was evaluated at 1 year after allogeneic cultivated limbal epithelial transplantation. A stable ocular surface was defined as a clear cornea and maintenance of a normal corneal epithelial phenotype, which was determined by slit-lamp examination without late fluorescein staining. Persistent epithelial defects, recurrent corneal opacity and neovascularization indicated failure of ocular surface reconstruction.[15]

All patients were imaged under an in vivo confocal microscope (HRT3; Heidelberg Engineering, Dossenheim, Germany). A 670-nm diode laser was used as a light source. An automatic z-scan determination of the depth of focus within the cornea was allowed, enabling the collection and storage of high-contrast digital images 400 × 400 mm in size, of all corneal layers. The lateral and transverse resolutions were both 1 mm.

After the eye was anesthetized with 1 drop of 0.4% benoxinate hydrochloride (oxybuprocaine hydrochloride; Santen, Osaka, Japan), 3 consecutive scans from the central cornea and 4 points (superior, inferior, temporal, and nasal) at an average peripheral 6.0 mm arcuate zone were obtained to observe the morphologic change in the amniotic membrane. If the amniotic membrane was not observed in 4 of 5 points, it was considered to have disappeared.[16] All eyes were examined by an experienced operator.

RTVue OCT (Optovue, Fremont, California, USA) was performed to measure the thickness of amniotic membrane and corneal stroma. The cornea anterior module pachymetry protocol enabled anterior segment imaging for pachymetry at the central 6 × 6 mm area. For analysis, 3 pachymetry scans acquired through the center of the cornea for each eye were used. All eyes were examined by an experienced operator.

Statistical analyses were performed using SPSS v 16.0 (SPSS, Chicago, Illinois, USA). All data were described as mean values ± standard deviation. The thickness of the amniotic membrane and corneal stroma, as well as BCVA, were compared using paired samples *t* test. A *P* value of ≤0.05 was considered statistically significant.