Effectiveness of Cultured Human Keratinocyte Onlays on Epithelial Healing and Clinical Outcome After Photorefractive Keratectomy

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ABSTRACT

PURPOSE: To evaluate epithelial healing time, postoperative pain, corneal haze, and visual and refractive outcomes following the application of cultured sheets of human allogeneic epidermal keratinocyte (CEAK) onlays on the photorefractive keratectomy (PRK)-ablated corneal surface as dressing material.

METHODS: In total, 204 eyes from 103 patients with myopia or myopic astigmatism were prospectively evaluated for 6 months after PRK. The ablated cornea was dressed in three different ways. Specifically, CEAK onlays were placed in 80 eyes (CEAK group), CEAK with amniotic membrane (AM) strips in 63 eyes (CEAK with AM group), and therapeutic contact lenses only in 61 eyes (control group). All eyes were covered with bandage contact lens after the operation. Contact lens removal time, intensity of postoperative pain score on postoperative day 2, corneal haze, Snellen visual acuity, and remaining refractive errors were measured.

RESULTS: The mean contact lens removal time was shorter in the CEAK with AM group (1.84 ± 0.72 days) compared to the control (2.77 ± 1.59 days) and CEAK only (2.24 ± 0.79 days) groups (P<.001). However, no significant differences were evident among the groups in terms of immediate postoperative pain, Snellen visual acuity, remaining refractive errors, and corneal haze at 6 months postoperative.

CONCLUSIONS: The CEAK onlay with AM facilitates epithelial healing, and is thus a good candidate dressing material to decrease the epithelial healing time after PRK. However, this onlay method did not affect the intensity of postoperative pain or final visual outcomes after surgery. [J Refract Surg. 2008;24:826-832.]

Excimer laser photorefractive keratectomy (PRK) has re-emerged as a common surgical procedure to correct refractive disorders, as it is less destabilizing to the cornea than LASIK. However, the disadvantages of PRK include slow visual recovery, discomfort in the early postoperative period, and corneal haze, which undermine the efficacy of this surface ablative procedure.1,2

Cultured sheets of human allogenic epidermal keratinocytes (CEAK) promote faster re-epithelialization of skin partial-thickness wounds, skin donor sites, dermabrasion, and thermal burn wounds.3-5 Preserved frozen CEAK are particularly useful in decreasing healing time and curing venous leg and diabetic ulcers.6 Recent studies show that frozen CEAK decrease fibroblast proliferation and inflammatory responses beneath the ablated zone, and generate improved organization of the newly formed epithelium by eliminating significant hyperplasia or discontinuities in rabbit corneas.7 Moreover, CEAK lead to accelerated re-epithelialization. These results support the theory that application of frozen CEAK to the human cornea after PRK affects the epithelial healing pattern and stromal haze.

In this study, we prospectively evaluate the effects of cultured human keratinocyte onlay placement after PRK on epithelial healing time, subjective pain score, postoperative visual outcomes, including refractive status and visual acuity, and corneal opacity.
**PATIENTS AND METHODS**

This study is a prospective, non-randomized and comparative clinical trial performed at the Department of Ophthalmology, Yonsei University, and the Balgeunsesang Ophthalmology Clinic, Seoul, Korea. In total, 103 patients with myopia were enrolled between December 2003 and February 2004. All procedures conformed to the tenets of the Declaration of Helsinki. Informed consent was obtained from all patients after approval from the institutional review board. Preoperative ophthalmic examinations of all patients included slit-lamp microscopy, intraocular pressure, fundus examination, pupil diameter measurements, Schirmer test, manifest refraction, corneal keratometry, corneal topography, and corneal pachymetry.

Prior to surgery, a study author (H.K.L.) explained to patients the merits, disadvantages, and complications associated with the three possible dressing procedures after PRK, specifically, therapeutic contact lens only (Focus; Ciba Vision, Duluth, Ga), CEAK (Kaloeye; TEGO Science, Seoul, Korea) treatment, and application of CEAK with an amniotic membrane (AM) strip on the inferior limbus. Patients were allowed to select their preferred method. If the patient was unable to choose, the dressing material was arbitrarily assigned using a random number table. Consequently, the experimental groups included: 1) control (contact lens only) (n=61 eyes), 2) eye covering with CEAK (n=80 eyes) (CEAK group), and 3) eye covering with CEAK including the AM strip on the inferior limbus (n=63 eyes) (CEAK with AM group).

Exclusion criteria were anterior segment pathology, evidence of significant blepharitis or meibomian gland disease, progressive or unstable myopia and keratoconus, a history of herpetic keratitis, and previous intraocular or corneal surgery. Soft contact lens use was discontinued for at least 3 weeks before examination and treatment. Therapeutic contact lenses were applied to all ablated corneas, ie, those in the control, CEAK, and CEAK with AM groups.

**CONVENTIONAL PRK**

Conventional PRK was performed with the VISX STAR S4 excimer laser (VISX, Santa Clara, Calif) using a standardized protocol. Preoperatively, patients were administered one drop of 0.5% proparacaine (Alcaine; S.A. Alcon-Couvreur, Puurs, Belgium), 0.1% diclofenac (Optanac, Samil, Korea), and 0.3% ciprofloxacin (Ciloxan, Alcon-Couvreur). Lashes and lids were treated with a povidone-iodine swab. A closed-loop lid speculum was placed between the lids of the eye to be treated, and the other eye was occluded. A 7.0-mm optical zone marker was applied to the cornea, centering it over the image of the pupil. A crescent knife was used to remove the central 7.0 mm of the corneal epithelium. Loose epithelium was removed using a blunt spatula, followed by excimer laser stromal ablation using the VISX STAR S4 laser platform. After excimer laser ablation, the CEAK onlay or CEAK with AM strip was applied, according to patient choice, prior to surgery. The CEAK onlay was punched out with 8.0-mm skin trephine, and applied on the ablated cornea. The therapeutic contact lens was placed over the CEAK onlay. For the CEAK with AM group, the AM strip (2×10 mm) was attached with two 10-0 nylon sutures on the inferior limbus, as described previously. Finally, therapeutic contact lenses were applied to all ablated corneas, ie, those in the control, CEAK, and CEAK with AM groups.

**POSTOPERATIVE MEDICATION**

All patients were monitored daily until the epithelial defect had healed completely and instructed to apply one drop of both diclofenac and ciprofloxacin every 6 hours. Artificial tears (Hyalein 0.1% hyaluronic acid; Santen, Osaka, Japan) were applied every hour until complete epithelial healing. Complete epithelialization was determined using daily slit-lamp observation. Once the epithelium had healed completely, all dressing materials (therapeutic contact lenses, CEAK, AM) were removed from the cornea, and 0.3% cipro-
Flouxacin and 0.1% fluorometholone (Fluormetholone, Santen) were administered four times daily for 1 week and twice daily for 1 month, respectively.

**POSTOPERATIVE EXAMINATION**

For subjective pain scores, patients were provided a Faces Pain Scale and asked to rate their individual pain levels on a scale from 0 (no pain) to 5 (worst pain) on the second day after surgery. The Faces Pain Scale is a self-measure used to assess the intensity of a patient’s pain. The method uses a piece of paper on which six faces with expressions ranging from happy (Face 0 = no pain) to very sad (Face 5 = the maximum pain you can imagine) are printed. Patients selected the face that most accurately described how they were feeling.

 Uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), manifest refraction, tonometry, and slit-lamp microscopic analyses were performed by the same investigator (I.H.R.) at 1 week and 1, 3, and 6 months after surgery. At 1, 3, and 6 months postoperatively, two authors (I.H.R., H.K.L.) separately graded subepithelial haze in a masked fashion. Haze levels were determined using a slit lamp, according to the method of Helena et al.10

**EPITHELIAL HEALING**

No case in this study displayed epithelial defects 7 days after the operation. The mean period of re-epithelialization was 2.77 ± 1.59 days (range: 1 to 6 days) in the control group, 2.24 ± 0.79 days (range: 1 to 5 days) in the CEAK group, and 1.84 ± 0.72 days (range: 1 to 5 days) in the CEAK with AM group (P=.008, one-way ANOVA). Successful epithelial healing was defined as complete healing in the operated eye within 48 hours. Successful epithelialization of the eye occurred in 18 (29.5%) eyes in the control group, 26 (32.5%) eyes in the CEAK group, and 35 (59.0%) eyes in the CEAK with AM group (P<.001, Chi-square test) (Fig 1).

**STATISTICAL ANALYSIS**

The pre- and postoperative characteristics of patients were analyzed using a repeated measures analysis of variance (ANOVA) with Tukey’s post-hoc comparisons. Uncorrected visual acuity and BSCVA were compared using a Chi-square or Fisher exact test for tables. The correlations between pre- and postoperative independent variables were analyzed using the Pearson’s correlation coefficient. Data were statistically evaluated using the Statistical Analysis System (Version 6.12; SAS Institute, Cary, NC). A P value <.05 was considered statistically significant.

**RESULTS**

Table 1 shows the preoperative patient characteristics. No significant differences were noted in the preoperative variables among the groups. Postoperative follow-up was possible for all patients up to 1 month. At 3 months after surgery, follow-up studies were performed on 52 eyes from the control (contact lens only) group, 64 eyes from the CEAK group, and 50 eyes from the CEAK with AM group. At 6 months, follow-up studies were performed on 44, 58, and 48 eyes from the control, CEAK, and CEAK with AM groups, respectively.

**TABLE 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n=61 eyes)</th>
<th>CEAK (n=80 eyes)</th>
<th>CEAK With AM (n=63 eyes)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>28.9 ± 6.7 (20 to 44)</td>
<td>27.0 ± 5.5 (19 to 44)</td>
<td>28.9 ± 6.4 (19 to 46)</td>
<td>.40</td>
</tr>
<tr>
<td>Gender (M/F)*</td>
<td>4/27</td>
<td>5/35</td>
<td>4/28</td>
<td>.15</td>
</tr>
<tr>
<td>Spherical equivalent refraction (D)</td>
<td>-4.94 ± 1.82 (1.68 to -9.12)</td>
<td>-5.30 ± 2.35 (-2.25 to -9.12)</td>
<td>-5.28 ± 2.015 (-2.12 to -9.63)</td>
<td>.86</td>
</tr>
<tr>
<td>Cylinder magnitude (D)</td>
<td>1.82 ± 0.95 (0.00 to 3.50)</td>
<td>1.99 ± 0.84 (0.00 to 4.00)</td>
<td>1.87 ± 0.92 (0.00 to 3.50)</td>
<td>.44</td>
</tr>
<tr>
<td>Corneal thickness (µm)</td>
<td>503.8 ± 39.9 (466 to 589)</td>
<td>503.5 ± 31.6 (467 to 582)</td>
<td>494.8 ± 24.2 (461 to 583)</td>
<td>.60</td>
</tr>
<tr>
<td>Pupil size (mm)</td>
<td>5.5 ± 1.3 (3.8 to 7.0)</td>
<td>5.3 ± 1.1 (3.9 to 6.9)</td>
<td>5.5 ± 1.1 (4.0 to 6.8)</td>
<td>.38</td>
</tr>
</tbody>
</table>

CEAK = cultured sheets of human allogeneic epidermal keratinocytes, AM = amniotic membrane

*One-way analysis of variance.
were not significantly different ($P=0.18$, Chi-square test) (Table 2).

**Visual Acuity and Refractive Errors**

At 6 months after surgery, no differences were noted in UCVA and BSCVA among the three groups ($P=0.16$ for UCVA, $P=0.229$ for BSCVA) (Fig 2). Uncorrected visual acuity of all eyes included in the present study was 20/40 or better at 3 months after the operation. The 20/20 rate ranged from 84% in the control group, 88.2% in the CEAK group, and 86.3% in the CEAK with AM group. Mean UCVA until 6 months did not differ among the groups. However, due to earlier epithelial healing, the 20/20 rate in the CEAK with AM group was higher than that of other groups at 1 week after surgery ($P=0.019$). Best spectacle-corrected visual acuity was 20/20 or better in all cases.

The remaining spherical equivalent refraction was similar among the three groups. The mean spherical equivalent refraction of the remaining refractive error was $-0.50 \pm 0.71$ diopters (D) in the control group, $-0.72 \pm 0.33$ D in the CEAK group, and $-0.81 \pm 0.64$ D in the CEAK with AM group ($P=0.26$). A manifest refractive spherical equivalent of $\pm 0.50$ D was achieved in 75% of patients in the control contact lens group (95% confidence interval [CI], 68.3%-79.2%), 73.1% of patients in the CEAK group (95% CI, 69.4%-77.4%), and 74.3% of patients in the CEAK with AM group (95% CI, 69.9%-77.3%; $P=0.139$). The linear regression of attempted versus achieved spherical equivalent refraction was analogous in all three groups ($P=0.33$, Fisher R to z-transformation) (Fig 3). Moreover, the magnitude of cylinder was not different among the groups ($P=0.33$).

**Subepithelial Opacity**

Up to 6 months postoperatively, subepithelial haze was graded separately by two masked practitioners. No differences were evident in subepithelial opacity among the three groups ($P=0.63$). Almost all patients displayed subepithelial opacity below grade 2. However, one eye from the CEAK group was classified as opacity grade 3 (Table 3).

**Discussion**

Pain, corneal haze, and myopic regression remain common problems associated with the conventional PRK procedure. Photorefractive keratectomy techniques have been modified in several ways to overcome these postoperative problems. For example, dressing materials used after excimer laser surface ab-
lation include the occlusive pressure patch, silicone hydrogel lotrafilcon A or etafilcon A, AM onlays or strips, epithelial onlay created by alcohol (LASEK), and epi-LASIK. Although a number of reports have compared postoperative outcomes following epithelial removal methods, few studies have compared epithelial healing, pain, visual acuity, and subepithelial opacity following the use of different dressing methods after PRK. The aim of this study was to prospectively evaluate the clinical outcomes and effects of the novel dressing material, CEAK, on corneal healing. In our patients, subjective pain during the immediate postoperative period, epithelial healing time, remaining refractive error, and visual outcomes were acceptable for each of the methods up to 6 months after surgery.

Despite the existing controversy regarding the degree to which epithelial healing and visual outcomes after refractive surgery are related, severely delayed epithelial healing is possibly an important cause of corneal opacity influencing visual and refractive outcomes. Therefore, improving regulated epithelial regeneration after refractive surgery may improve surgical outcomes and decrease the duration of postoperative pain after PRK. In the present study, epithelial healing generally occurred within 2 days in the CEAK with AM group. However, the control and CEAK groups required more time for epithelial regeneration. The study does not examine why patients in the CEAK with AM group displayed faster epithelial healing. We propose that various cytokines and epithelial growth-associated factors within the AM facilitate epithelial regeneration and wound healing after PRK. The AM expresses epidermal, hepatocyte, and keratinocyte growth factors, and suppresses proinflammatory cytokines during wound healing, similar to interleukin. Moreover, laminin-5, an important extracellular matrix protein for epithelial cell migration that activates the IGF-1 pathway, is expressed in the supernatant from AM cultures. Additionally, when keratinocyte sheets are applied to mouse skin wounds, improved wound healing phenomena, such as early deposition of extracellular matrix, granulation tissue formation, and reorganization, are identified. Although we could not confirm whether these factors are released in the CEAK and AM during epithelial healing, specific roles in corneal epithelial healing are postulated.

We observed no relationship between epithelial healing time, subjective pain scores, and refractive and visual outcomes among the three groups. Previous reports show that the epithelial healing rate influences the refractive and visual outcomes. However, in our experiments, the epithelial healing rate did not significantly affect clinical outcome. The observed difference between previous results and ours may be explained by variations in the severity of delayed epithelial wound healing. Earlier studies addressed the impact of severely delayed epithelial healing on postoperative outcome after surgery. However, we did not encounter severe delays in epithelial wound healing in the patient groups examined. Moreover, these results are supported by our previous report comparing epithelial healing between PRK, LASEK, and transepithelial PRK. Because we used the same treatment nomogram while operating on all eyes, it is not surprising that all three groups displayed similar mean UCVA, BSCVA, and remaining refractive error if epithelial healing was complete within an acceptable period.

Postoperative pain is the primary drawback of
PRK, and its control is an important factor in making preoperative decisions and postoperative compliance of treatment. Despite faster epithelial healing in the CEAK with AM group, the severity of pain was not reduced in patients treated with CEAK or CEAK with AM in comparison to the control group. However, the limitation of this study was not measuring the duration of pain and not quantifying the intensity of pain serially after surgery. Pain from corneal injury is a consequence of the excitation of pain nerve fiber terminals by a noxious stimulus or locally released inflammatory compounds. The corneal nerves are ablated, and inflammatory factors inducing pain are released (eg, prostaglandin E2), which are strong noxious stimuli. In addition, pain from the initial noxious stimulus lasts for 12 to 24 hours, followed by irritation and tearing that continues until epithelial coverage. In the present study, initial insults, epithelial removal, and excimer laser stromal ablation were equivalent among all patients examined. Therefore, it is reasonable to assume that the intensity of pain is similar across all three groups. However, separate studies of pain duration and severity may reveal variations in the duration of pain among the three treatment groups.

A limitation of the present study was that patients were not randomized among the three groups. This may have introduced selection bias, and therefore, randomization is recommended in future studies. Moreover, different techniques were used in patients, which are considered a bias to determine pain scoring. In fact, when both eyes are operated on simultaneously, the pain intensity may differ between the eyes. Consequently, we measured the pain scale of each eye separately. However, it is possible that the pain score analysis of both eyes in the same patient had an impact on statistical independence. In addition, we did not compare the speed of corneal epithelial healing among groups. As expected, it was difficult to measure the epithelial defect size by detaching CEAK before concluding re-epithelialization, and thus the epithelial healing rate could not be determined.

Although CEAK with AM facilitates epithelial healing, there are no significant differences in terms of clinical outcome, postoperative pain, and subepithelial opacity among patients provided a choice of the three dressing techniques (contact lens, CEAK, and CEAK with the AM strip). In terms of cost effectiveness, CEAK usually is more expensive than therapeutic contact lenses and may be more vulnerable to complications (eg, infectious keratitis). Also, CEAK procedures are more time consuming. Therefore, we conclude that the CEAK onlay does not significantly aid in decreasing the intensity of postoperative pain or stromal wound healing in surface-ablated excimer laser surgery, except stimulation of epithelial healing.

**REFERENCES**

17. Solomon A, Rosenblatt M, Monroy D, Ji Z, Pflugfelder SC.


