• Clinical Research •

# Comparison of corneal biological parameters between transepithelial and epithelium-off corneal cross-linking in keratoconus

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## Abstract

● **AIM:** To evaluate the differences in corneal biological parameters between transepithelial and epithelium-off corneal cross-linking in keratoconus.

● **METHODS:** In our prospective clinical trial, 40 patients (60 eyes) with progressive keratoconus were randomized to undergo corneal cross-linking with transepithelial (TE group, *n*=30) or epithelium-off (EO group, *n*=30) keratoconus. Examinations comprised topography, corneal biomechanical analysis and specular microscopy at 6mo postoperatively.

● **RESULTS:** The keratometer values were not significantly different between the TE and EO corneal cross-linked groups in different periods (each *P*>0.05). The corneal thickness of the EO group was greater than that of the TE group at 1wk after the operation (each *P*<0.05). Regarding corneal biomechanical responses, the EO group showed a longer second applanation length than TE group (*P*=0.003). Regarding the corneal endothelial function, standard deviation of the endothelial cell size, and coefficient of variation in the cell area, the values of EO group were larger than those of TE group at 1wk (*P*=0.011, 0.026), and the percentage of hexagonal cells in EO group was lower than that in TE group at 1 and 6mo (*P*=0.018, 0.019).

● **CONCLUSION:** Epithelium-off corneal cross-linking may strengthen corneal biomechanics better than TE-procedure can. However, the TE procedure with a lower ultraviolet-A irradiation intensity would be safer for corneal endothelial function.

● **KEYWORDS:** keratoconus; corneal cross-linking; transepithelial; epithelium-off; corneal biomechanics; corneal topography

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## INTRODUCTION

Keratoconus is a degenerative disorder of the eye in which structural changes in the cornea cause it to thin and develop a more conical shape than the normal gradual curve. Since the first clinical report of corneal cross-linking (CXL) by the Dresden team in 2003, evidence has shown the success of the procedure in halting progressive keratoconus and possible flattening of the cornea[1-3].

The standard protocol of CXL involves debridement of the central epithelium to facilitate penetration of large-molecular-weight riboflavin into the stroma, where it absorbs ultraviolet-A (UVA) light and produces the actual cross-linking between collagen fibrils in the corneal stroma[4]. This treatment increases corneal rigidity and stiffens the anterior corneal stroma[5-6]. The downside of epithelial removal is that it causes significant pain and discomfort during the first postoperative days (delaying visual recovery) and poses potential risks associated with epithelial removal problems[7-8]. To avoid these downsides of epithelium removal, the transepithelial (TE) CXL technique was developed. For TE CXL to work, modification of the standard protocol is required to allow adequate stromal permeation of riboflavin through the epithelial barrier. Using topical benzalkonium chloride (BAC) and tetracaine causes a significant increase in epithelial permeability with loss of epithelial tight junctions[9]. The clinical effects of TE and

epithelium-off (EO) CXL have been reported in some case series and comparative trials. However, the effectiveness and safety of the two techniques remain controversial.

Dynamic Scheimpflug imaging technology is a relatively new method that enables the assessment of corneal biomechanics *in vivo*. It provides more than 10 corneal biomechanical parameters *via* a device equipped with an ultrahigh-speed Scheimpflug camera, which can record the entire deformation process of the corneal response after an air puff[10-13]. In this investigation, a corneal biomechanical analyzer was used to evaluate the difference between TE and EO CXL.

In this study, we compared the safety and efficacy of TE and EO CXL for progressive keratoconus using riboflavin and UVA in the early postoperative period. Both techniques use the same dose of UVA irradiation but differ in the time of irradiation, the riboflavin solution used, and the soaking time.

## SUBJECTS AND METHODS

**Ethical Approval** The study was prospectively approved by the Ethics Review Committee of Hainan Ophthalmological Hospital (No.2017-005) and registered at the Chinese Clinical Trial Registry (No.ChiCTR1900021768). All procedures complied with the Declaration of Helsinki and local laws regarding research on human subjects. Written informed consent was obtained from all patients prior to their participation.

**Study Group and Protocol** The study was a prospective randomized parallel-group trial that included patients diagnosed with progressive keratoconus who were eligible for a CXL procedure at Hainan Eye Hospital from December 12, 2017, through October 11, 2019.

Inclusion criteria were a clear central cornea and documented progression as defined by an increase in the maximum K value or manifest astigmatism of ≥1 D within the previous year based on repeated corneal topography. Exclusion criteria were a minimal pachymetry of less than 400 μm prior to UVA irradiation, previous ocular surgery, ocular surface pathology or infection, collagen vascular disease, and pregnancy.

Each eye was allocated using a computer-generated randomization sequence to either TE CXL groups or EO CXL groups. Patients undergoing surgery for both eyes were treated with TE in one eye and de-epithelialization in the opposite.

**Surgical Technique** All operations were performed under sterile conditions on an outpatient basis. In the TE CXL group, local anesthetic eye drops (0.5% proparacaine hydrochloride) were applied 3 times for 5min. Then, ParaCel Part 1 [riboflavin 0.25% with BAC in hydroxypropyl methylcellulose (HPMC), Avedro Inc.®, Waltham, MA, USA] was applied at an interval of 1 drop every 90s for a total of 4min. Excess ParaCel Part 1 was flushed from the eye with ParaCel Part 2 (riboflavin 0.25%, Avedro Inc.®, Waltham, MA, USA), and additional drops of ParaCel Part 2 were applied at a rate of 1 drop every 90s for a total of 6min. After 10min, the cornea was rinsed with balanced salt solution. After confirming the calibration of the UVA irradiation system (the KXL system, Avedro, Waltham, America), the eye was irradiated for 5min and 20s with a pulsed irradiance (1s on, 1s off) of 45 mW/cm$^2$, which corresponded to a total radiant exposure of 7.2 J/cm$^2$.

In the EO CXL group, local anesthetic eye drops (0.5% proparacaine hydrochloride) were applied 3 times for 5min. Next, the central 9.0-mm-diameter corneal epithelium was removed using a blunt knife. Then, VibeX Rapid (riboflavin 0.1% with HPMC, Avedro, Waltham, America) was instilled every 90s for a total of 10min. After 10min, the cornea was rinsed with balanced salt solution. Then, the eye was irradiated for 4min with a continuous irradiance of 30 mW/cm$^2$ by the KXL system, corresponding to a total radiant exposure of 7.2 J/cm$^2$ (for full CXL details following the standard convention, Table 1).

In both groups, a soft contact lens bandage (Purevision, Bausch & Lomb Incorporated, Florida, USA) was placed on the eye at the end of the procedure. The post-CXL medication consisted of antibiotic eye drops (Levofloxacin Eye Drops, 24.4 mg: 5 mL, Santen-China, Beijing, China) and preservative-free artificial tears (sodium hyaluronate eye drops, URSAPHARM Arzneimittel GmbH, Saarbrücken, Germany), which were used for 4wk. Topical fluorometholone (0.1% fluorometholone eye drops, Santen-China, Beijing, China) and nonsteroidal anti-inflammatory drops (pranoprofen eye drops, Santen-China, Beijing, China) were administered at tapering dosages for 2wk. After 1mo, no medication was used. The bandage lens was removed after 3d in the TE group. In the EO group, the bandage lens was removed after 1wk if epithelial healing was complete.

**Measurements and Devices** Patients were examined at baseline and at 1wk, 1, and 6mo post CXL. Slit-lamp examination, corneal biomechanical analysis (OCULUS Corvis® ST, OCULUS, Wetzlar, Germany), Scheimpflug topography (Pentacam® HR, OCULUS, Wetzlar, Germany), and specular microscopy (SP-3000P, TOPCON CORPORATION, Tokyo, Japan) measurements were performed at each follow-up.

The Scheimpflug topographer recorded the keratometer values, including the flat (K1), steep (K2), and middle (Km) refractive power and astigmatism in the anterior and posterior corneal surface in the 3 mm center area, as well as the steepest curvature value (Kmax) in the anterior corneal surface. The system also recorded the corneal thickness at the pupil center, pachy apex, and thinnest location.

The corneal biomechanical analyzer recorded the entire process of the corneal deformation response to an air jet *via* an ultrahigh-speed Scheimpflug camera. The cornea

**Table 1 CXL methods**

| Parameters | Transepithelial group | Epithelium-off group |
|---|---|---|
| Treatment target | Keratoconus | Keratoconus |
| Fluence (total; J/cm$^2$) | 7.2 | 7.2 |
| Soak time and interval (min) | 10 (q1.5) | 10 (q1.5) |
| Intensity (mW) | 45 | 30 |
| Treatment time (min) | 5min 20s | 4min |
| Epithelium status | On | Off |
| Chromophore | Riboflavin (Avedro Inc.®, Waltham, MA, USA) | Riboflavin (Avedro Inc.®, Waltham, MA, USA) |
| Chromophore carrier | HPMC, BAC | HPMC |
| Chromophore osmolarity | Hypo-osmolar | Iso-osmolar |
| Chromophore concentration | 0.25% | 0.1% |
| Light source | The KXL system (Avedro, Waltham, America) | The KXL system (Avedro, Waltham, America) |
| Irradiation mode (interval) | Pulsed (1s) | Continuous |
| Protocol modifications | Contact lens-assisted | Contact lens-assisted |
| Protocol abbreviation in manuscript | A-CXL (45×5) (accelerated) | A-CXL (30×4) (accelerated) |

HPMC: Hydroxypropyl methylcellulose; BAC: Benzalkonium chloride.

experiences four distinct statuses: first applanation, highest concavity, second applanation, and natural status. The series parameters during each status were derived by analyzing the above processes, including the first applanation length, first applanation velocity, second applanation length, second applanation velocity, maximal deformation amplitude, peak distance (PD), and intraocular pressure (IOP). The definitions of each parameter are summarized in Table 2.

The specular microscope provided the parameters, which included the average size of endothelial cells, the standard deviation of the endothelial cell size, the coefficient of variation in the cell area, the percentage of hexagonal cells, and the endothelial cell density.

**Statistical Analysis** Statistical analysis was performed using SPSS for Windows, version 18.0 (IBM-SPSS, Chicago, Illinois, USA). All changes were calculated as postoperative minus preoperative values and fixed with a delta symbol ( △ ). The normality of the data was tested with the Shapiro-Wilk test. A paired *t*-test was performed to analyze the preoperative and postoperative data within the same group. If the data were not normally distributed, the Wilcoxon rank-sum test was performed. Differences between the two groups were tested with an independent-samples *t*-test; if the distribution of the data was not normal, the Mann-Whitney *U* test was performed. In all tests, statistical significance was defined at a level of $P<0.05$.

## RESULTS

This study enrolled 60 eyes of 40 patients with progressive keratoconus, aged 12-33y (average, 21.14y), who were randomly assigned to either TE ($n=30$) or EO CXL ($n=30$). Both groups were comparable at baseline. Mean keratoconus progression before treatment was not significantly different between the groups. Baseline characteristics are listed in Table 3. In both groups, the time of baseline was 3.72±3.63d preoperative. The follow-up time points were 6.09±3.11d (1wk), 36.96±14.77d (1mo), and 187.34±23.15d (6mo). No delayed re-epithelialization or endothelial damage was detected during follow-up. No signs of inflammation were observed after corneal CXL or throughout the follow-up period in either group.

**Table 2 Parameters measured by Corvis ST**

| Parameters | Explanation |
|---|---|
| IOP (mm Hg) | IOP based on A1 |
| The first applanation (A1) | |
| A1 length (mm) | Length at A1 |
| A1 velocity (m/s) | Velocity of the corneal apex at A1 |
| HC | |
| Deformation maximum (mm) | Maximum deformation amplitude of apex |
| Peak distance (mm) | Distance between nondeformed peaks |
| The second applanation (A2) | |
| A2 length (mm) | Length at A2 |
| A2 velocity (m/s) | Velocity of the corneal apex at A2 |

HC: Highest concavity.

**Corneal Topography** Table 3 summarize the keratometer values, including the flat (K1), steep (K2), and mid (Km) refractive power of the anterior and posterior corneal surface in the 3 mm center area, the steepest curvature (Kmax) value of the anterior corneal surface, and the corneal thickness of the pupil center, pachy apex, and thinnest location in different pre- and postoperative periods.

The keratometer values were not significantly different between the TE and EO corneal cross-linked groups in different periods (each $P>0.05$), except that the corneal thickness of the EO group was significantly increased compared with that of the TE group one week after the operation ($P<0.05$).

**Table 3 The keratometer values comparison between the TE and EO corneal cross-linked groups**

| Parameters | Baseline | | | 1wk | | | 1mo | | | 6mo | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trans | Epi-off | *P* | Trans | Epi-off | *P* | Trans | Epi-off | *P* | Trans | Epi-off | *P* |
| The anterior surface (D) | | | | | | | | | | | | |
| K1 | 46.8±4.34 | 47.51±6.04 | 0.941 | 46.96±4.33 | 48.5±6.59[a] | 0.595 | 47.14±4.82[a] | 48.18±5.47 | 0.583 | 46.15±4.18[a] | 46.76±5.52[a] | 0.928 |
| K2 | 51.62±5.71 | 51.86±7.26 | 0.589 | 52.01±5.74 | 53.03±7.19[a] | 0.965 | 51.91±5.91[a] | 52.47±6.73[a] | 0.819 | 50.58±5.05[a] | 50.61±5.16[a] | 0.516 |
| Km | 49.06±4.82 | 49.57±6.5 | 0.690 | 49.34±4.87 | 50.64±6.79[a] | 0.813 | 49.39±5.17[a] | 50.2±5.94[a] | 0.819 | 48.22±4.42[a] | 48.58±5.2[a] | 0.660 |
| Kmax | 57.87±8.44 | 58.43±11.04 | 0.663 | 58.16±8.33 | 60.33±11.10[a] | 0.842 | 59.05±10.24 | 58.76±9.06 | 0.862 | 55.87±7.85 | 57.08±10.97[a] | 0.818 |
| Astigmatism | 4.81±2.77 | 4.34±2.55 | 0.605 | 5.03±2.59 | 4.55±2.23 | 0.443 | 4.76±2.77 | 4.28±2.65[a] | 0.589 | 4.44±2.56 | 3.84±2.68 | 0.373 |
| The posterior surface (D) | | | | | | | | | | | | |
| K1 | -6.93±0.73 | -7.02±1.12 | 0.871 | -6.9±0.69 | -6.67±0.85[a] | 0.171 | -7.02±0.84[a] | -7.17±1.03 | 0.735 | -6.8±0.7 | -7.05±0.93[a] | 0.273 |
| K2 | -7.87±1.04 | -7.82±1.26 | 0.363 | -7.77±0.94 | -7.48±1.05[a] | 0.169 | -7.94±1.06 | -7.93±1.27 | 0.583 | -7.65±0.86 | -7.84±1.05[a] | 0.756 |
| Km | -7.37±0.82 | -7.39±1.16 | 0.558 | -7.29±0.78 | -7.04±0.92[a] | 0.141 | -7.43±0.92[a] | -7.51±1.14 | 0.883 | -7.19±0.75 | -7.42±0.96[a] | 0.464 |
| Astigmatism | 0.95±0.57 | 0.79±0.49 | 0.306 | 0.88±0.5 | 0.81±0.47 | 0.619 | 0.91±0.45 | 0.76±0.45 | 0.308 | 0.83±0.44 | 0.8±0.47 | 0.733 |
| The corneal thickness (μm) | | | | | | | | | | | | |
| Pupil center | 475.4±48.14 | 487.73±45.74 | 0.487 | 488.13±61.02[a] | 546.4±74.04[a] | 0.002[b] | 475.96±38.9 | 461.3±42.66[a] | 0.234 | 477.25±52.94 | 471±42.67[a] | 0.441 |
| Pachy apex | 471.43±47.76 | 481.7±45.74 | 0.636 | 484.8±56.31[a] | 540.36±79.03[a] | 0.007[b] | 472.15±40.96 | 455.84±45.46[a] | 0.180 | 473.45±54.4 | 465.4±43.33[a] | 0.568 |
| Thinnest location | 462.53±48.59 | 471±51.4 | 0.539 | 478.1±53.08[a] | 522.8±64.42[a] | 0.017[b] | 449.89±54.84 | 456.35±42.51 | 0.698 | 435.73±124.9 | 472.15±55.07 | 0.341 |

Trans: TE group; Epi-off: EO group; D: Diopter. [a]Compared with the baseline $P<0.05$; [b]Statistically significant.

In the TE group, the K1, K2 and Km values of the anterior corneal surface were steeper than the baseline values at 1 and 6mo after the operation (△K1=-0.4±0.57, -0.37±0.57, $P$=0.003, 0.002; △K2=-0.37±0.93, -0.39±0.87, $P$=0.004, 0.021; △Km=-0.4±0.54, -0.37±0.63, $P$=0.002, 0.001). The corneal thickness of the pupil center (CCT), pachy apex (ACT), and thinnest location (TCT) was thicker than the baseline at 1wk after the operation (△CCT=-12.73±47.94, $P$=0.010; △CAT=-13.37±34.35, $P$=0.008; △CTT=-15.57±37.85, $P$=0.005), but the CCT was thinner than the baseline at 1mo after the operation (△CTT= 4.69±10.97, $P$=0.039).

In the EO group, the keratometer value of the anterior and posterior corneal surface was steeper than the baseline at 1wk and 1mo after the operation (each $P<0.05$) but flatter at 6mo after the operation (anterior: △K1=0.48±0.99, $P$=0.003; △K2=0.9±2.81, $P$=0.028; △Km=0.68±1.81, $P$=0.01; △Kmax=0.78±1.72, $P$=0.03, posterior: △K1=0.1±0.35, $P$=0.007; △K2=0.11±0.39, $P$=0.002; △Km=0.12±0.36, $P$=0.003). The corneal thickness of the pupil center (CCT), pachy apex (ACT), and thinnest location (TCT) was thicker than the baseline at 1wk after the operation (each $P<0.05$); however, these values decreased at 1 and 6mo after surgery (△CCT=22.19±32.19, 16.84±41.57, $P\leq0.001$, 0.003; △CAT=21.35±32.58, 17.64±34.97, $P\leq0.001$, 0.004; △CTT=15.73±29.88, 15±31.22, $P$=0.005, 0.006).

**Corneal Biomechanical Analysis** Table 4 summarize the first applanation length (A1L), first applanation velocity (A1V), second applanation length (A2L), second applanation velocity (A2V), maximal deformation amplitude (DA), PD, and IOP values of the two groups in different pre- and postoperative periods.

In the comparison between groups, the A1L of the EO group was higher than that of the TE group at postoperative week 1 ($Z$= -2.026, $P$=0.043), but the A1V was lower ($Z$=-2.095, $P$=0.036). In addition, the A2L of the EO group was significantly higher than that of the TE group at postoperative 6mo ($Z$= -2.095, $P$=0.036). The other biomechanical values were not significantly different in different periods (each $P>0.05$).

In the TE group, A2V was decreased at 1 and 6mo compared to baseline (△A2V=-0.04±0.1, -0.08±0.22, $P$=0.039, 0.028), and DA was lower than baseline at 1wk (△DA=0.09±0.13, $P$=0.001). The IOP was increased at 1wk and 1mo (△IOP= -2.06±4.72, -1.08±2.71, $P$=0.014, 0.048) and then recovered at 6mo. Other parameters that were not significantly changed were observed in the intragroup comparison (each $P>0.05$).

In the EO group, A1V was slower than baseline at 1wk (△A1V=0.01±0.02, $P$=0.004), A2L was longer at 6mo (△A2L=-0.37±0.45, $P$=<0.001), DA was smaller at 1wk and 1mo (△DA=0.11±0.16, 0.06±0.16, $P$=0.001, 0.007), and A2V was slower at all postoperative periods (△A2V=-0.09±0.12,

**Table 4 The corneal biomechanical parameters and IOP comparison between the TE and EO corneal cross-linked groups**

| Parameters | Baseline | | | 1wk | | | 1mo | | | 6mo | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trans | Epi-off | *P* | Trans | Epi-off | *P* | Trans | Epi-off | *P* | Trans | Epi-off | *P* |
| The first applanation | | | | | | | | | | | | |
| A1L (mm) | 1.77±0.34 | 1.88±0.34 | 0.193 | 1.76±0.32 | 1.91±0.32 | 0.043[b] | 1.91±0.37 | 1.77±0.27 | 0.213 | 1.84±0.42 | 1.99±0.4 | 0.139 |
| A1V (m/s) | 0.16±0.02 | 0.15±0.02 | 0.137 | 0.15±0.02 | 0.14±0.02[a] | 0.036[b] | 0.15±0.03 | 0.15±0.04 | 0.761 | 0.16±0.02 | 0.15±0.02 | 0.107 |
| The second applanation | | | | | | | | | | | | |
| A2L (mm) | 1.51±0.49 | 1.37±0.38 | 0.301 | 1.35±0.37 | 1.54±0.55 | 0.248 | 1.28±0.5 | 1.48±0.39 | 0.148 | 1.33±0.46 | 1.71±0.32[a] | 0.003[b] |
| A2V (m/s) | -0.4±0.13 | -0.4±0.13 | 0.137 | -0.37±0.1[a] | -0.32±0.12[a] | 0.058 | -0.34±0.17 | -0.34±0.09[a] | 1.000 | -0.31±0.19[a] | -0.33±0.08[a] | 0.817 |
| The highest concavity | | | | | | | | | | | | |
| DA (mm) | 1.24±0.16 | 1.19±0.18 | 0.515 | 1.17±0.19[a] | 1.09±0.16[a] | 0.161 | 1.21±0.16 | 1.11±0.14[a] | 0.068 | 1.2±0.15 | 1.17±0.18 | 0.236 |
| PD (mm) | 4.39±1.3 | 4.13±1.18 | 0.222 | 4.33±1.24 | 4.25±1.09 | 0.646 | 4.18±1.27 | 4.38±1.12 | 0.721 | 4.62±1.01 | 4.76±0.76 | 0.863 |
| IOP (mm Hg) | 13.12±2.99 | 14.33±3.68 | 0.399 | 15.08±4.53[a] | 14.72±4.53 | 0.909 | 14.24±2.95[a] | 16.11±4.19 | 0.128 | 14.35±4.57 | 14.65±3.39 | 0.242 |

Trans: TE group; Epi-off: EO group. [a]Compared with the baseline $P<0.05$; [b]Statistically significant.

-0.05±0.09, -0.07±0.09, $P$=0.001, 0.016, 0.003). Other parameters that were observed in the intragroup comparison were not significantly changed (each $P>0.05$).

**Specular Microscopy** Table 5 summarize the parameters, including the average size of endothelial cells, the standard deviation of the endothelial cell size, the coefficient of variation in the cell area, the percentage of hexagonal cells, and the endothelial cell density of the two groups in different pre- and postoperative periods.

The standard deviation of the endothelial cell size and the coefficient of variation in the cell area in the EO group were larger than those in the TE group at 1wk ($Z$=-2.536, $P$=0.011; $Z$=-2.233, $P$=0.026), and the percentage of hexagonal cells was lower than that in the TE group at 1 and 6mo ($t$=0.018, $Z$=-2.343; $P$=0.018, 0.019). The rest of the parameters were not significantly different between the two groups in different periods (each $P>0.05$).

In the TE group, the average endothelial cell size and the standard deviation of the endothelial cell size were increased compared with the baseline at 1 and 6mo (△Average=-17.02±33.54, -19.59±34.61, $P$=0.021, 0.003; △SD=-14.34±26.6, -15.73±37.22, $P$=0.015, 0.033), and the endothelial cell density was decreased at 1 and 6mo (△Cell density=121.91±269.48, 145.78±246.36, $P$=0.037, 0.003).

In the EO group, the standard deviation of the endothelial cell size and the coefficient of variation in the cell area were increased compared with the baseline at 1wk, 1, and 6mo (△SD of size=-20.51±35.57, -16.85±35.56, -21.53±33.73, $P$=0.003, 0.018, 0.005; △CD of size=-3.3±7.06, -4.34±7.16, $P$=0.002, 0.02, 0.007), and the percentage of hexagonal cells was decreased at 1wk, 1, and 6mo (△Hexagon=6.3±14.48, 7.71±16.49, 11.5±16.56, $P$=0.024, 0.02, 0.004).

## DISCUSSION

Riboflavin UVA CXL is widely used to halt the progression of keratoconus and to reduce the need for donor keratoplasty[14]. In our study, the corneal topography data recorded by Pentacam showed that cross-linking caused transient corneal edema in the early postoperative period, and this effect was more pronounced in the EO group than in the TE group. The significant difference in the anterior and posterior corneal surface keratometer values can be explained by corneal edema at 1wk and 1mo postoperatively in both groups, and these differences were stabilized at 6mo postoperatively. In addition, the keratometer values of the EO group were decreased at 6mo, which means that the EO group can effectively control the progress of keratoconus. Similar phenomena have been found in previous investigations, which confirms the findings of this study[15-17]. Furthermore, the corneal thickness showed a decreasing trend in the EO group at 1 and 6mo postoperatively, which is in agreement with the published literature[18]. The

**Table 5 The corneal endothelial cell parameters comparison between the TE and EO corneal cross-linked groups**

| Parameters | Baseline | | | 1wk | | | 1mo | | | 6mo | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trans | Epi-off | *P* | Trans | Epi-off | *P* | Trans | Epi-off | *P* | Trans | Epi-off | *P* |
| Average size ($\mu m^2$) | 345.56±38.57 | 368.25±54.91 | 0.058 | 353.98±31.13 | 379.99±60.19 | 0.098 | 371.4±42.64[a] | 381.46±58.72 | 0.490 | 365.14±49.09[a] | 367.88±49.05 | 0.638 |
| SD of size ($\mu m^2$) | 115.03±22.76 | 124.69±29.28 | 0.209 | 122.3±22.1 | 145.2±37.98[a] | 0.011[b] | 129.52±28.44[a] | 143.98±31.94[a] | 0.093 | 130.75±36.79[a] | 139.57±39.98[a] | 0.338 |
| CD of size (%) | 33.48±5.74 | 33.81±4.81 | 0.807 | 34.74±5.54 | 38.11±6.03[a] | 0.026[b] | 34.88±6.38 | 37.74±5.55[a] | 0.090 | 35.68±8.12 | 37.58±6.72[a] | 0.207 |
| Hexagonality (%) | 55.83±12.19 | 57.13±12.29 | 0.574 | 55.26±12.39 | 50.83±12.25[a] | 0.169 | 56.76±13.63 | 48.35±11.45[a] | 0.018[b] | 56.5±12.35 | 47.2±14.92[a] | 0.019[b] |
| Endothelial cell density($/mm^2$) | 2927.85±318.33 | 2765.86±359.65 | 0.058 | 2845.42±241.89 | 2688.31±379.59 | 0.061 | 2727.12±319.06[a] | 2681.41±414.14 | 0.662 | 2782.07±337.81[a] | 2758.08±318.09 | 0.638 |

Trans: TE group; Epi-off: EO group; Average size: The average size of endothelial cell; SD of size: The standard deviation size of endothelial cell; CD of size: The coefficient of variation of the cell; Hexagonality: The percentage of hexagonal cells. [a]Compared with the baseline $P<0.05$; [b]Statistically significant.

decreased corneal thickness in the EO group may be related to epithelial remodeling, compactness of collagen fibrils, corneal dehydration, and keratocyte apoptosis and may be an expression of cross-linking–induced flattening and improved corneal symmetry[17,19-21].

In the second part of the current study, Corvis ST was employed to investigate the corneal biomechanical function pre- and postoperatively. Theoretically, a more deformable cornea in response to an air puff is related to the following features: 1) faster in the first applanation: shorter first applanation time and length, faster first applanation velocity, and larger first applanation deformation amplitude; 2) greater deformation amplitude: greater maximum deformation amplitude, shorter PD, and highest concavity radius; 3) later in the second applanation: longer second applanation time, shorter second applanation length, slower second applanation velocity, and smaller second applanation deformation amplitude[22].

Our study showed that the EO group had a higher A1L and lower A1V than the TE group at 1wk postoperatively and a higher A2L at 6mo postoperatively. This result indicated that the corneal biomechanical strength of the EO group was better than that of the TE group at 1wk and 6mo. The TE group had a smaller DA at 1wk and a slower A2V at 1wk and 6mo, in comparison with the preoperative values. The EO group had a smaller A1V and DA at 1wk, a slower A2V in all postoperative periods, and a longer A2L at 6mo. The change in these parameters suggested that all the biomechanics of the cornea, except for A2V, were enhanced after cross-linking. In an *in vitro* study, Dorronsoro *et al*[23] reported that the DA and the deformation speed decreased with riboflavin and ultraviolet cross-linking, indicating that the viscoelasticity of the cornea decreased and the stiffness increased. This theory may explain the reduction in A2V after cross-linking in this study, but more *in vivo* studies are needed to confirm this finding. Several previous studies have proven that both TE and EO cross-linking can effectively increase the biomechanical strength of the cornea[21,24-25]. From the results of this study, in our opinion, the EO procedure can provide better biomechanical strength than the TE procedure.

During the follow-up period, there was short-term IOP fluctuation in the TE group, which recovered at 6mo. Kymionis *et al*[25] reported that the early increase in IOP after cross-linking may be related to changes in corneal hardness and biomechanics, and the regular use of anti-inflammatory eye drops also causes fluctuations in IOP in the early postoperative period. In addition, the early edema in the EO group may mask the IOP change.

In the corneal endothelial function part, our results showed that the standard deviation of the endothelial cell size and

the coefficient of variation in the cell area in the EO group were higher than those in the TE group at 1wk, and the percentage of hexagonal cells was lower at 1 and 6mo. These differences reflected the finding that the effects of corneal edema and ultraviolet irradiation were more obvious in the EO group. It is noteworthy that the endothelial cell density was not significantly different between the two groups, which is consistent with a previous report by Caporossi *et al*[17]. According to previous studies, an intact epithelium soaked with riboflavin may also in itself be a barrier to UVA irradiation, limiting the depth of keratocyte apoptosis and corneal collagen crosslink formation[26]. An intact epithelial barrier helps to prevent postoperative infection and pain, and cytotoxic damage is restricted to a 200 μm stromal depth[27]. However, the endothelial cell density of the TE group in the present study was decreased at 1 and 6mo. Several studies have reported a similar decreasing trend in endothelial cell density, which was recovered at 3mo or later. In this study, the reduction in endothelial cell density may be partly explained by the higher UVA irradiation intensity (45 mW, pulse mode, 1s interval). In a previous study on the safety of cross-linking, Shetty *et al*[28] reported that cellular apoptosis is inversely correlated with increasing radiation intensity and decreasing exposure time. Hence, based on our results, it can be concluded that a TE procedure with a lower UVA irradiation intensity would be safer for corneal endothelial cells.

In addition, some limitations of the current study should be noted. First, a longer follow-up period is needed to assess long-term results. Second, the difference in the irradiation time, the riboflavin solution used, and the soaking time may affect the results.

In summary, our results show that both procedures can effectively inhibit the progression of keratoconus. In terms of corneal biomechanics, the EO procedure has more advantages than the TE procedure. Nevertheless, even with a smaller effect, the TE procedure may be recommended preferably for use in patients with thin corneas and poor corneal endothelial function along with slowly progressing keratoconus.

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