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Evaluating the safety and efficacy of epi-off corneal cross-linking in patients with thin corneas due to keratectasia

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Abstract

Background: Corneal cross-linking (CXL) is a safe and effective procedure for slowing keratectasia progression in eyes with a corneal thickness of at least 400 µm. Limited research has evaluated the safety and efficacy of epi-off CXL in corneas thinner than 400 µm.

Objective: To evaluate the safety and efficacy of CXL to slow keratectasia progression in eyes with <400 µm preoperative corneal thickness.

Design: Retrospective chart review.

Methods: This retrospective chart review included 37 eyes who underwent epi-off, iso-osmolar riboflavin corneal CXL with a preoperative thinnest point of the cornea <400 µm and had at least 6–12 months of follow-up. Preoperative and postoperative uncorrected visual acuity, best-corrected visual acuity (BCVA), thinnest point of the cornea, flat keratometry, steep keratometry, maximum keratometry (K_{max}), need for penetrating keratoplasty, and cases of endothelial failure were recorded. Data were collected at baseline and months 3, 6, 9, and 12 post-CXL.

Results: Following cross-linking, 18 eyes (47%) had improved BCVA, 13 (35%) had an unchanged BCVA, and 6 eyes (16%) had a worse BCVA ($p = 0.05$). The mean postoperative BCVA was 20/81 (0.61 LogMAR) compared to 20/121 (0.78 LogMAR) preoperatively ($p = 0.06$). K_{max} decreased an average of 1.1 D at 3-month ($p = 0.53$) and 3.4 D at the furthest follow-up ($p = 0.10$). At the farthest follow-up, 22.7% of eyes had >1 D of K_{max} steepening. No patients required keratoplasty and there were no cases of endothelial failure in the follow-up period.

Conclusion: This research supports the safety and efficacy of epi-off, iso-osmolar CXL in eyes with <400 µm baseline corneal thickness with no patients requiring penetrating or endothelial keratoplasty, a trend toward improvement in BCVA, and K_{max} flattening. In the future, prospective studies would be helpful to confirm these findings.

Keywords: corneal biomechanics, corneal cross-linking, keratoconus, safety

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Introduction

Corneal cross-linking (CXL) is an effective treatment for a variety of corneal diseases, notably progressive keratoconus and ectasia as a result of refractive surgery.[1] Overall, with rarely associated risks, the safety of corneal cross-linking is well established when performed within certain parameters.[2] Previously outlined contraindications for CXL include active infectious keratitis and pregnancy.[3] In addition to these conditions, a 400 µm minimum corneal thickness is a suggested criterion for determining procedure eligibility. This requirement is based on studies by Wollensack and Spoerl, which concluded that corneas thinner than 400 µm could experience endothelial damage.[4,5]

Multiple recent studies have reported favorable outcomes following CXL in corneas with corneal thickness measurements <400 µm.[6–8] These

studies suggest that the minimum corneal thickness recommendation may be too conservative. Recent work has led to groups proposing new minimal requirements including a paper by Hafenzi and colleagues suggesting a minimal corneal thickness of 330 μm, based on the lack of adverse outcomes in their subjects with thin corneas.[9] In addition, Kymonis and colleagues had only one patient with substantially decreased endothelial cell count after they irradiated 14 eyes with the corneal thickness of nearly 300 μm.[10] Additional studies have failed to demonstrate a significant decrease in endothelial cell count following corneal cross-linking.[11–13]

A number of surgical techniques have been described to evaluate the safety of CXL in patients with thin corneas. Among the range of described techniques, a common method for <400 μm CXL involves the instillation of a hypo-osmolar riboflavin solution to swell the cornea to at least 400 μm immediately prior to irradiation,[14,15] with an overall accelerated protocol to hasten UVA delivery.[16] Multiple additional techniques have been described including the use of an epithelial pocket[17] or epithelial flap[18] to deliver riboflavin during the procedure. Furthermore, other studies have evaluated the use of riboflavin-soaked contact lenses to artificially increase corneal thickness with favorable outcomes.[19]

Overall, despite the aforementioned studies, the literature remains limited regarding outcomes of 'Epi-off' CXL in patients with corneas <400 μm. The most common adverse event includes corneal edema, but case reports reporting single instances of endotheliitis exist.[9,20–22] More research is needed to determine the central corneal thickness in which cross-linking procedures are safe to confidently increase the number of eligible patients. This corneal thickness cutoff is clinically significant as up to 25% of patients presenting for keratectasia evaluation have corneal thickness values <400 μm.[23]

Given the overall limited published work, the present study aimed to evaluate the clinical response, safety profile, and effectiveness of epi-off, iso-osmolar riboflavin corneal CXL for patients with corneas <400 μm.

Materials and methods

Charts were reviewed for all patients who had undergone corneal cross-linking at a single, private practice setting (Sioux Falls, SD) from 2018 to 2020, totaling 163 patients (218 eyes) with keratoconus or post-Lasik corneal ectasia. The diagnosis of keratoconus was made by utilizing both tomographic and clinical characteristics. Patients with preoperative minimum corneal thickness of less than 400 μm (including epithelium) were included for analysis, resulting in 37 eyes from 34 patients. Demographics recorded included gender, age, related diagnosis, and which eye underwent surgery. In total, 13 females and 21 males aged 16–69 (mean = 43.6 years) met the screening criteria for the study. Eligibility for CXL was based on the determination of disease progression. Progression of keratoconus was defined as a maximum keratometry (K_{max}) increase of least 1 D, an increase in the manifest refraction spherical equivalent of at least 1 D, a decrease of 5% in the minimum pachymetry, or loss of at least two lines of the corrected distance visual acuity during the past 12 months. One patient (age = 16) qualified as a pediatric keratoconus patient and was cross-linked after they had confirmed disease progression. Patients with corneal opacities in the central visual axis, active corneal infection, current pregnancy, or a history of herpetic eye disease were excluded from the study.

All corneas underwent CXL by utilizing a 30 min irradiation, 'epi-off' technique using iso-osmolar riboflavin similar to the standard Dresden protocol, which has previously been described in detail.[24] This technique entails the removal of the central 8–10 mm of corneal epithelium followed by the application of an applying an iso-osmotic riboflavin solution (0.1% riboflavin-5-phosphate and 20% dextran T-500) to the corneal surface. UVA irradiation (370 nm) was then administered for 30 min with an intensity of 3 mW/cm^2, during which the riboflavin solution was reapplied every 5 min.

Preoperative and postoperative uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA), thinnest point of the cornea, flat keratometry (K1), steep keratometry (K2), and K_{max} were recorded. K_{max} values were analyzed at 3-month and furthest (6–12 months) follow-ups. Pentacam tomography (Oculus; Wetzlar, Germany) data were used to measure corneal thickness and keratometry values. Patients were instructed to discontinue contact lens use (2 weeks for rigid gas permeable lenses, 1 week for soft contact lenses) before corneal measurements. Need for penetrating keratoplasty (PKP) or scleral contacts and cases of endothelial failure were recorded.

Statistical analysis

Data were analyzed using SAS version 9.5 (Statistical Analysis System Institute Inc. Cary, NC, USA). To compare preoperative and postoperative values, analyses of repeated values were utilized using PROC GLIMMIX. All tests were performed with an alpha level of 0.05.

Results

Visual acuity in Logarithm of the Minimum Angle of Resolution (LogMAR) equivalents before and after surgery are reported in Table 1.

In all, 18 eyes (47%) had improved visual acuity and 13 eyes (35%) had stable visual acuity after 3 months following surgery ($p = 0.05$). More specifically, 8.1% ($n = 3$) lost 3 or more Snellen lines of BCVA, 5.4% ($n = 2$) lost 2 lines, and 2.7% ($n = 1$) lost 1 line. In addition, 18.9% ($n = 7$) of eyes gained 1 line, 10.8% ($n = 4$) gained 2 lines, and 16.2% ($n = 6$) gained 3 or more lines of BCVA, as seen in Figure 1.

Changes in mean UCVA (F-value: 1.12, $p = 0.30$) and BCSVA (F-value: 0.41, $p = 0.06$) were not significant when compared with preoperative and postoperative values.

The average preoperative K_{max} was 69.51 D ± 10.45 D (range 49.9–97.3 D). K_{max} decreased an average of 1.1 D at 3-month and 3.4 D at the furthest follow-up ($p = 0.10$) (Figure 2). The average furthest follow-up was 10.5 months after the operation. At the furthest follow-up, 23% of patients had >1 D of steepening while 77% of patients had less than 1D of steepening. Change in K_{max} was not significant at 3-month (F-value: 0.4, p-value: 0.53), 6-month (F-value: 0.03, $p = 0.87$), 9-month (F-value: 0.00, $p = 0.99$), or 12-month (F-value: 0.99, $p = 0.32$) follow-ups.

Since not all patients had 3-month follow-ups, preoperative K_{max} was compared with the minimum postoperative timepoint, an average of 4.6 months. There was a significant flattening in K_{max} between preoperative and nearest postoperative timepoints (F-value: 8.51, $p = 0.005$). The average thinnest point of the cornea was 331.88 µm ± 63.06 µm (range 136–399 µm) preoperatively and 306.10 µm ± 73.59 µm (range 187–422 µm) 3 months postoperatively. Change in the thinnest point of the cornea was not statistically significant (F-value: 2.61, $p = 0.11$).

No patients required PKP. There were no cases of endothelial failure for the duration of the follow-up period. There were no cases of persistent corneal haze (defined as corneal haze >6 months from surgery) at the farthest follow-up.

Discussion

This retrospective case review evaluated the safety and efficacy of 30 min, epi-off, iso-osmolar riboflavin CXL in eyes with corneal thickness <400 µm due to keratectasia. The results of this study corroborate prior work supporting the safety and efficacy of CXL in this group of patients, a population that has traditionally been labeled as poor candidates for CXL owing to safety concerns. In this study, the vast majority (77%) of patients experienced <1D of steepening during follow-up. Furthermore, no patients had persistent corneal haze, required PKP, or experienced endothelial failure within the 12-month follow-up period. These results are notable given past hesitancy to perform

Table 1. Preoperative and postoperative visual acuities in LogMAR equivalents in patients with corneas less than 400 µm who underwent corneal cross-linking. All values are reported as mean ± standard deviation and range.

Visual assessment	Preoperative		Postoperative		*p*-Value
	Mean ± SD	Range	Mean ± SD	Range	
UCVA	1.57 ± 1.53	1–1.9	1.38 ± 1.22	0.54–1.70	0.30
BCVA (contacts)	0.70 ± 0.85	–0.12–1.30	0.51 ± 0.56	0–1.01	0.28
BSCVA (glasses)	0.78 ± 0.77	0.18–1.30	0.61 ± 0.63	0.18–1.01	0.06

BCVA, best-corrected visual acuity; BSCVA, best spectacle corrected visual acuity; SD, standard deviation; UCVA, uncorrected visual acuity.

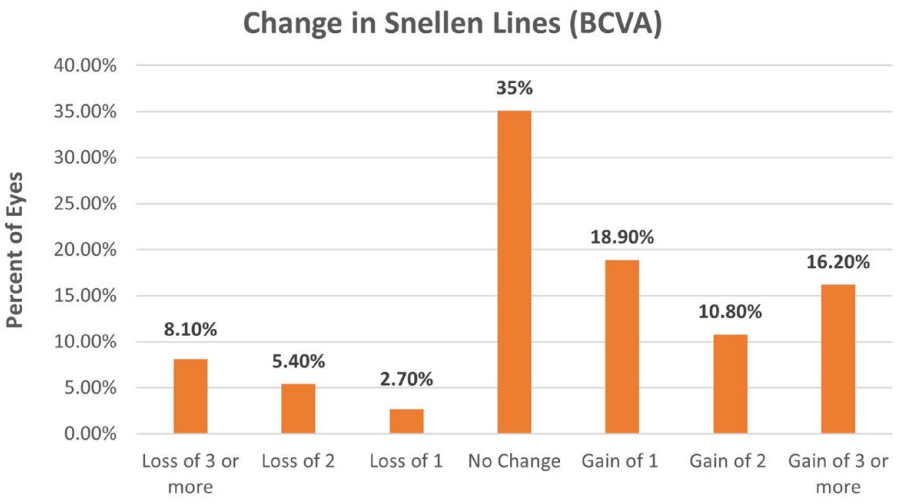

Figure 1. Change in BCVA Snellen lines from preoperative to postoperative values based on the farthest available follow-up.
BCVA, best-corrected visual acuity.

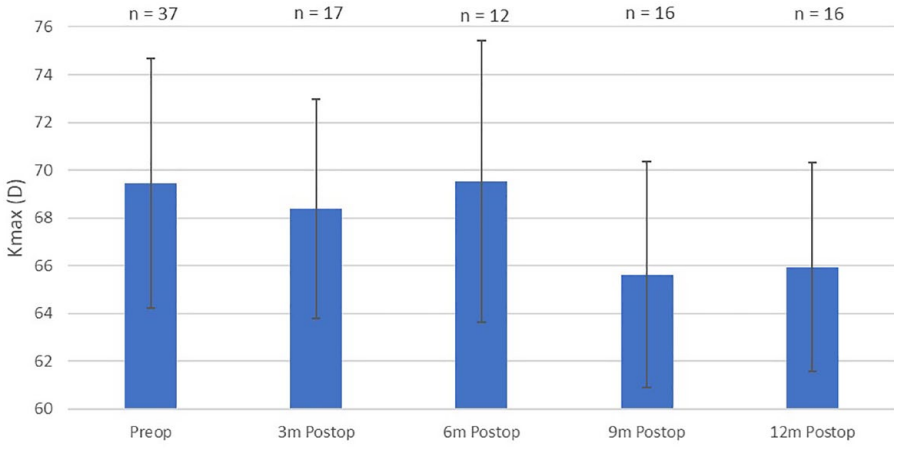

Figure 2. Average K_{max} absolute values, preoperatively and postoperatively. Error bars represent standard deviations for the mean.
K_{max}, maximum keratometry.

CXL on corneas <400 µm. Though scarcely published, the primary safety concerns related to CXL include potential complications such as endotheliitis,[15] corneal edema,[13,20] and corneal scarring.[3] In a multicenter clinical trial by Hersh and colleagues, corneal haze was reported in 68% of patients 3 months after treatment, but only found in 5.5% of patients at 1-year follow-ups.[11] Many studies considering the safety of corneal cross-linking report no long-term or serious complications of the procedure,[10,12,25] as reflected in our study.

Despite the apparent safety of cross-linking, endothelial cytotoxicity remains a particular concern. Wollensak *et al.*[26,27] published that, when 3 mW/cm^2 UVA was used with riboflavin, keratocyte damage occurred down to a depth of 300 µm. These findings led to a recommendation for a 400 µm minimum (after epi removal) threshold for cross-linking procedure eligibility. There are several reasons to question the 400 µm minimum in regard to endothelium safety. One reason includes the endothelial riboflavin concentrations may be less than previously estimated, meaning less reactive oxygen species and endothelial damage would occur. Recently, Seiler and colleagues measured endothelial riboflavin concentrations of approximately 0.015%,[28] almost half the previously

reported values.[4,5] This could mean current cross-linking parameters are causing less endothelial damage than previously thought and are potentially safe for thinner corneas. Seiler's article also points out that the original studies performed on rabbits, though *in vivo*, may not translate to human corneas in clinical practice. They mention that rabbit corneas are thinner at baseline and able to regenerate, meaning they may have a lower damage threshold. They also note that Wollensak's study design may have caused the corneas to shrink before treatment and their estimated UV transmission may be too high.[28] Other studies have attempted to determine the threshold for corneal toxicity. Mooren and colleagues treated human cadaver corneas with 0.025% riboflavin and 18 mW/cm^2 UVA irradiance for 5 min. After 5 days, they had no signs of endothelial cell apoptosis or necrosis.[29] Their study's radiant exposure of 5.4 J/cm^2 is significant when compared with Wollensak's radiant exposure of 0.63 J/cm^2 in his porcine corneas.

There are a number of prior studies evaluating CXL in thinner corneas. Hafezi *et al.*[8] used an algorithm to give personalized irradiation dependent on each patient's minimal corneal thickness after riboflavin soaking but before applying UVA irradiation in patients with corneal thickness less than 400 µm. The researchers found CXL with individualized fluence was able to stop KC progression of corneas as thin as 214 µm with 90% success rates at 1-year post-CXL. In addition, no corneas showed clinical signs of endothelial compromise, although the total fluence in the study never exceeded 5.4 J/cm^2. With their algorithm, the authors aimed to place the demarcation line 70 µm from the endothelium, but the average distance was 93 µm ± 47 µm following CXL. This may suggest the depth of radiant energy does not penetrate the stroma as deep as previously thought, meaning CXL can be done safely in corneas thinner than 400 µm.

Another study by Salman and colleagues reported the use of a 30 min, 'epi-off' CXL technique with hypo-osmolar riboflavin to swell corneas as thin as 310 µm (mean preoperative thickness = 337 µm). The authors reported an average of 2.1 D of K_{max} flattening at 10 years, similar to our reported 3.4 D of flattening at farthest follow-up. They also reported 82% of eyes had stable or improved BCVA following CXL and noted zero cases of persistent stromal haze or endothelial failure, which were both seen in our study. Although their studies' preoperative corneal thickness was slightly higher than ours (370 *versus* 332 µm), we both observed a nonsignificant reduction in postoperative corneal thickness. Their 10 years of data displaying anterior K_{max} flattening and posterior K_{max} steepening are indicative of keratectasia stabilization and likely indicate the safety and efficacy of CXL in thin corneas are consistent long term.[14]

Perhaps most important to our study, past research has found no significant difference in endothelial cell counts before and after cross-linking.[11,12] Specifically, Knyazer *et al.*[13] found no significant change in endothelial cell counts (ECC) at 18-month follow-ups. Even when ECC decrease during the procedure, there is reason to question whether this causes clinical detriment. Kymionis *et al.*[10] encountered significant endothelial cell loss when performing corneal cross-linking on thin corneas (2733 ± 180 cells/mm^2 *versus* 2441 ± 400 cells/mm^2 at last follow-up). However, these findings were not correlated with adverse clinical outcomes. In addition, the cohort of patients selected for our study had severe, progressive keratoconus as evidenced by their high K_{max} and thin central corneal thickness. Some of these patients may have required a PKP in the future due to progressive visual deterioration. Even if cross-linking had caused endothelial cell toxicity in these patients, it would have had minimal clinical significance to their long-term treatment, as a PKP or endothelial keratoplasty would be performed regardless. This research implies it is reasonable to perform cross-linking on thin corneas to prevent disease progression as this procedure is much more likely to prevent the need for PKP than cause it in this population.

Our study aimed to determine the necessity of a minimal corneal thickness for cross-linking procedures. This threshold is clinically significant given that approximately one-quarter of patients presenting for keratoconus treatments have pachymetry values less than 400 µm.[23] In addition, we had no cases of persistent corneal haze or endothelial failure, and no patients required penetrating or endothelial keratoplasty. In fact, CXL may have prevented many of these patients from progressing to transplant.[30] These findings make our study increasingly valuable, as we suggest that CXL can be offered to, and therefore benefit, a wider patient population.

Of interest to our study, it is important to note some research has shown waiting to CXL the sequential eye in bilateral disease, as compared to CXL both eyes on the same day, has undesirable outcomes such as a high chance of disease progression and increased healthcare-related costs.[31] However, in a study population with thin corneas, despite the aforementioned low risks of complications, waiting for a favorable outcome in the first eye before CXL the sequential eye is most reasonable. Yet, it is also important to not wait too long to CXL the sequential eye to prevent even more disease progression in already advanced patients with bilateral disease, but future research should aim to evaluate the outcomes of immediate *versus* delayed bilateral CXL corneas.

This study is not without limitations. There was no control group and the retrospective nature of the study led to some patients not having values for all 3-, 6-, and 9-month follow-up visits. Due to KC being a chronic disease, having more than 12 months of follow-up is preferred to evaluate long-term results and disease progression. In addition, although we report a large sample size of patients with very thin corneas, it is still a relatively small sample size that can yield unpredictable results. It should be noted that endothelial cell count measurements were not obtained in this study, which can be a helpful tool for evaluating the safety of CXL in eyes with thin corneas. Ideally, demarcation line measurements would be collected on all our subjects, but the retrospective nature limited this possibility. We also report no pachymetry measurements following epithelium removal. We also acknowledge there were two patients with a post-LASIK keratectasia diagnosis included in this study, which may differ in disease progression from keratoconus. Despite these limitations, this study adds to the literature supporting the safety and efficacy of epi-off, iso-osmolar CXL in patients with thin corneas and promotes future, large-scale work.

In conclusion, our study supports past research suggesting CXL procedures are safe in patients with a central corneal thickness of less than 400 µm. In contrast to other studies, we collected pachymetry values and found no corneas underwent endothelial failure or required keratoplasty during the follow-up. We hope that our findings will broaden the number of patients able to receive corneal cross-linking for treating progressive keratectasia.

Declarations

Ethics approval and consent to participate

This study was approved by the University of South Dakota Institutional Review Board (approval number IRB-20-84). This retrospective chart review collected data from procedures that had already occurred and were de-identified; therefore, the University of South Dakota Institutional Review Board waived the requirement for informed consent to participate in this study.

Consent for publication

Not applicable.

Author contributions

Abigail Nieuwsma: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft.

Brandon L. Vander Zee: Data curation; Investigation; Methodology; Validation; Writing – review & editing.

John P. Berdahl: Conceptualization; Methodology; Project administration; Supervision; Writing – review & editing.

Mitch Ibach: Formal analysis; Investigation; Project administration; Writing – review & editing.

Tanner J. Ferguson: Methodology; Resources; Supervision; Writing – review & editing.

Daniel Terveen: Conceptualization; Methodology; Project administration; Supervision; Writing – review & editing.

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Competing interests

One of the authors is a lecturer and consultant for Glaukos. Another author is a lecturer and consultant for Glaukos. The other authors have no financial or proprietary interest in any material or method mentioned.

Availability of data and materials

Due to institutional review board stipulations, a limited amount of supplementary data materials may be available upon request of the corresponding author.

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