Clinical Ophthalmology

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ORIGINAL RESEARCH

Visual Recovery with Cryopreserved Amniotic Membrane After Epi-Off Corneal Cross-Linking in Keratoconus Patients: A Non-Randomized, Controlled, Retrospective Study

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Video abstract

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Background: This study assessed whether application of cryopreserved amniotic membrane (AM) improves recovery after collagen cross-linking (CXL) using the Dresden protocol in patients with progressive keratoconus.

Methods: This single-centre, retrospective, non-randomized, comparative study included consecutive patients with progressive keratoconus who underwent epi-off CXL followed by bandage contact lens (BCL) or cryopreserved AM (Prokera Slim). Patients were assessed preoperatively and at 1 week, 1 month, 3 months, and 6 months postoperatively. Outcomes included best-corrected visual acuity (BCVA), maximum keratometry (K_{max}), complete re-epithelialization, and complications.

Results: A total of 62 eyes (46 patients) were treated with AM after CXL, and 46 eyes (39 patients) received BCL. At 6 months post-op, a significantly greater proportion of eyes in the AM group returned to baseline BCVA versus the BCL group (85.7% vs 64.9%, $p < 0.05$). BCVA logMAR was significantly better in the AM treatment group compared to the BCL group at 6-months (0.19 ± 0.21 vs 0.31 ± 0.28, respectively; $p=0.046$). The change in K_{max} values was comparable between the groups at all time points. The proportion of eyes with a healed epithelial defect at 1 week postoperatively was comparable between the groups (69.6% BCL vs 66.1% AM, $p = 0.71$). There were three complications (one each of infiltrative keratitis, infectious keratitis, and persistent epithelial defect) in the BCL group and none in the treatment group ($p = 0.07$).

Conclusion: The use of AM after epi-off CXL resulted in no post-operative complications and improved BCVA six months after surgery compared to a BCL. Prospective, randomized controlled studies are warranted to verify these findings.

Keywords: amniotic membrane, corneal crosslinking, epi-off, Prokera, keratoconus

Introduction

Keratoconus is a progressive, bilateral ocular disease that results in non-inflammatory thinning and steepening of the central or paracentral cornea.[1,2] The incidence of keratoconus varies globally across different ethnic groups, which is attributed to genetic, environmental, biomechanical, and biochemical factors.[3,4] Older epidemiology studies estimated that keratoconus affects 1 in 2000 people in the general population, although more recent studies have found a higher prevalence of 1 in every 375 people or 1 in every 84 people.[1,5,6] If left untreated, surgical intervention, such as keratoplasty, may be indicated.

Corneal cross-linking (CXL) is a minimally invasive treatment for keratoconus that uses ultraviolet (UV) light and riboflavin to markedly stiffen the cornea, increase its biomechanical strength, and halt the progression of keratoconus.[7,8] There are multiple surgical protocols for CXL, however, performing CXL with the corneal epithelium removed (ie "epi-off") is the gold standard technique.[9,10] While this technique improves the CXL process and halts the progression of keratoconus,[11] there are potential disadvantages to the procedure, including postoperative pain and risk of complications

https://doi.org/10.2147/OPTH.S569518
Received: 11 October 2025
Accepted: 16 February 2026
Published: 23 February 2026

such as persistent epithelial defects, infectious keratitis, and persistent stromal haze.[12–14] Furthermore, 25.5% of patients do not recover pre-operative visual acuity at one year post-operatively.[15] Hence, adjunctive measures to minimize these risks and improve postoperative outcomes, including visual acuity and overall patient satisfaction, are needed.

The amniotic membrane (AM) is the innermost layer of the placenta and is known to possess anti-inflammatory and anti-scarring properties.[16,17] Because of these properties, AM has been used in patients to promote wound healing and has been successfully used to treat many ocular surface conditions, including persistent epithelial defects and infectious keratitis.[18] Notably, AM has been shown to suppress transforming growth factor-β (TGF-β) signalling and subsequent myofibroblast differentiation as well as promote apoptosis of pro-inflammatory cells, which are proposed to reduce keratocyte apoptosis and stromal haze following photorefractive/phototherapeutic keratectomy.[16,17] While these properties of AM could be beneficial for epi-off CXL to promote faster corneal re-epithelialization and reduce the risk of complications such as infection and haze, its use in epi-off CXL has not yet been reported. Herein, a comparative retrospective study was performed to assess the clinical outcomes of epi-off CXL with and without the in-office application of AM on post-operative day one.

Materials and Methods

A retrospective chart review was performed in accordance with the tenets of the Declaration of Helsinki on consecutive patients with progressive keratoconus who underwent epi-off CXL with or without AM at a single centre. The Institutional Review Board (Sterling IRB, Atlanta, GA; #12869-MRosetta) approved the waiver of consent, and appropriate measures were taken to protect the confidentiality of the study participants. Patients were included in the study if they were 13 years of age or older, had been diagnosed with keratoconus based on corneal topography (inferior steepening relative to superior ≥ 1.5 dioptres (D) and elevated posterior float), underwent epi-off CXL between May 2022 and May 2024, had best-corrected visual acuity (BCVA) worse than 20/20 at baseline, and had demonstrated progression of astigmatism (≥ 1.00 D), myopia (≥ 0.50 D), minimum corneal thickness (thinning of 10–20μm), and/or BCVA (loss of 1 line). Exclusion criteria were central corneal thickness (CCT) <350 μm, history of any corneal surgery, including refractive surgery and intracorneal ring segments, and any history of corneal disease that could interfere with postoperative healing, such as limbal stem cell deficiency or previous herpetic infection.

All patients underwent CXL following the Dresden protocol by debriding the central 7–9 mm of corneal epithelium, applying Photrexa (Glaukos, Aliso Viejo, CA) photosensitizing riboflavin drops containing 0.1% riboflavin 5-phosphate and 20% dextran solution every 2 minutes to saturate the stroma, and then exposing the stromal bed to 370 nm UVA light at an irradiance of 3 mW/cm^2 at a 1 cm distance for 30 minutes using the KXL System (Glaukos, Aliso Viejo, CA). Surgery was performed by one surgeon, and all patients were treated with moxifloxacin 0.5% four times a day for 7 days postoperatively or until the epithelial defect healed, prednisolone acetate 1% four times a day for 1 week and then tapered over the following 3 weeks, ketorolac 0.5% four times a day for up to 3 days as needed for pain, non-preserved artificial tears every hour, and over-the-counter pain relief if needed (acetaminophen 1000 mg alternating with ibuprofen 600 mg every four-six hours). A bandage contact lens (Air Optix Night & Day Aqua; Novartis, Inc.) was placed on the eye immediately after surgery in all cases. The bandage contact lens (BCL) is a silicone hydrogel soft lens (lotrafilcon A) approved for extended wear for up to 30 nights. In each case, a lens with 13.8 mm diameter, 8.6 mm base curve and plano power was used. The BCL was replaced with cryopreserved AM (Prokera Slim; BioTissue, Miami, FL) on post-operative Day 1 for eyes in the treatment group. The cryopreserved AM as the brand Prokera (BioTissue, Inc). is a self-retaining biologic corneal bandage that treats superficial corneal disease. In brief, the cryopreserved AM was thawed at room temperature for several minutes, rinsed with sterile saline, and placed into the superior fornix while the patient looked down and then slid under the lower eyelid. All eyes had the BCL or AM removed at the 1-week post-operative visit.

The patients were assessed pre-operatively and at 1 week, 1 month, 3 months, and 6 months postoperatively. The primary outcome of this study was BCVA, which was measured using Snellen acuity and converted to logMAR units for analysis. Maximum keratometry (K_{max}) was compared between groups as a secondary outcome measure. Change in K_{max} is a useful measure to assess recovery following CXL, as spectacle refraction and contact lens fit can be performed to maximize acuity once topography has stabilized. Topography was performed at baseline as well as 1 month, 3 months,

and 6 months post-operatively using a Pentacam (Oculus, Germany) and analysed using the Belin/Ambrósio Ectasia Report. The proportion of patients with complete re-epithelialization was assessed at 1 week, and any complications (ie, infection, infiltrates, or persistent epithelial defects) were recorded and compared.

All statistical analyses were performed using SPSS Software (IBM; Armonk, NY, USA). Continuous data are reported as mean ± standard deviation or median and range, and categorical data are described using frequency and percentage. The normality of data distribution was confirmed using the Shapiro–Wilk test. Any missing data points were left as missing without data imputation. For continuous variables, a linear mixed model was performed to assess differences between groups, with treatment group set as a fixed effect and eye (OD or OS) set as a random effect. A repeated measures linear mixed model was used to assess differences across timepoints within groups to account for the inclusion of both eyes of one patient. The Chi-Square test was used to assess the distribution of binomial data between groups, and Fisher's Exact Test was used when at least one cell had an expected count of <5. Post-hoc analysis was done to compute the statistical power of the complication rates between groups (power=0.32). A p-value less than 0.05 was considered statistically significant.

Results

A total of 108 eyes from 71 patients underwent CXL performed by a single surgeon and were included in the study analysis. Of these, 62 eyes of 46 patients (31 males, 15 females) received AM after CXL, and 46 eyes of 39 patients (24 males, 15 females) received only BCL after CXL. Fourteen patients received treatment with AM in one eye and without AM in the other eye. A complete list of patient demographics and baseline characteristics is presented in Table 1. The only significant difference noted in the baseline characteristics between the groups was best sphere, which was −5.2 D ± 4.2 in the treatment group and −7.6 D ± 5.8 in the control (p = 0.02). Surgery was performed uneventfully in all cases.

Table 1 Patient Demographics and Baseline Characteristics for the AM Treatment Group and Control Group

Patient Characteristics	AM Treatment Group	Control Group	P-value
Number of Patients (Eyes)	46 (62)	39 (46)	
Age (Years)	28.5 ± 9.2 29 (13–55)	29.2 ± 9.4 29 (14–56)	0.69
Gender, n (%)			
Male	31 (67.4%)	24 (61.5%)	0.57
Female	15 (32.6%)	15 (38.5%)	
Eyes, n (%)			
OD	30 (48.4%)	21 (45.7%)	0.78
OS	32 (51.6%)	25 (54.3%)	
K_{Max} (D)	58.6 ± 9.6 57.3 (44.4–83.5)	61.3 ± 9.5 60.45 (45.2–85.3)	0.14
Flat K (D)	46.7 ± 4.9 45.5 (39.9–62.2)	48.3 ± 5.3 47.4 (40.1–62.6)	0.17
Steep K (D)	50.9 ± 5.8 49.4 (42.7–69.5)	52.5 ± 6.7 52.3 (43.2–76.1)	0.30
Cylinder (D)	4.1 ± 2.1 3.7 (0.6–9.3)	4.3 ± 2.5 3.9 (0–13.4)	0.78

(*Continued*)

https://doi.org/10.2147/OPTH.S569518

Table 1 (Continued).

Patient Characteristics	AM Treatment Group	Control Group	P-value
Best Sphere (D)	−5.2 ± 4.2 –4.1 (−17.1–1)	−7.6 ± 5.8 –6.2 (−24-0.4)	0.02
Central Cornea Thickness (μm)	462.9 ± 40.5 457 (383–564)	454.5 ± 45.3 443 (371–549)	0.34

Notes: Values reported as mean ± standard deviation, median (range).
Abbreviations: AM, amniotic membrane; BCL, bandage contact lens; K, keratometry; OD, oculus dexter; OS, ocular sinister.

Table 2 Proportion of Eyes with a Healed Epithelial Defect at 1 Week Postoperatively and Proportion of Eyes with Corneal Infection, Infiltrates or Persistent Epithelial Defects After CXL

Outcome	AM Treatment Group (n=62)	Control Group (n=46)	P-value
Eyes with a Healed Epithelial Defect by 1 Week, n (%)	41 (66.1%)	32 (69.6%)	0.71
Eyes with Corneal Infection, Infiltrate, or Persistent Epithelial Defect, n (%)	0 (0.0%)	3 (6.5%)	0.07

The proportion of eyes with complete re-epithelialization, corneal infection, infiltrates, and/or persistent epithelial defects was assessed and compared between the groups (Table 2). In the control group, 69.6% of eyes had complete re-epithelization by 1 week postoperatively compared to 66.1% in the AM treatment group, which was not statistically significant ($p = 0.71$). However, there were three complications in the control group (one infiltrative keratitis, one infectious keratitis and one persistent epithelial defect) and none in the AM treatment group ($p = 0.07$).

The proportion of eyes in each group that returned to baseline BCVA (or better) at each postoperative timepoint is shown in Figure 1. Although the proportion was higher in the AM treatment group at all follow-up visits, statistical significance between groups was observed only at 6-months postoperatively (Figure 1). At 6 months, 85.7% of eyes in

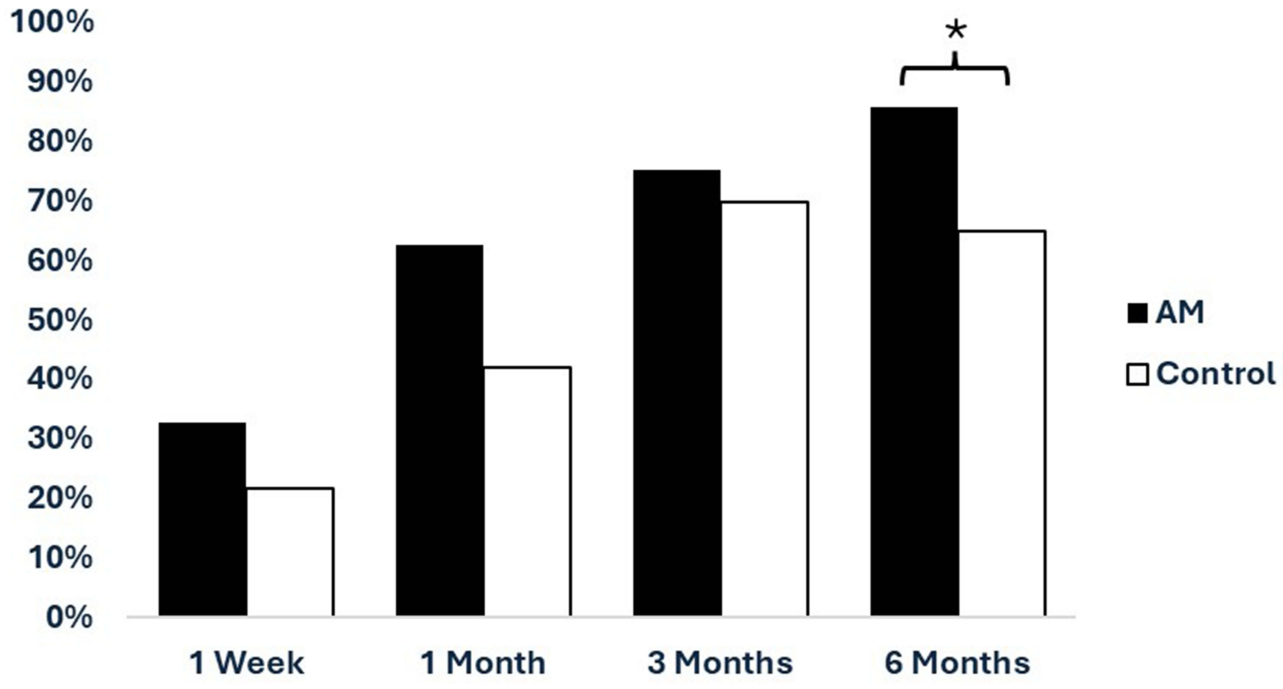

Figure 1 Proportion of Eyes with Return to Preoperative BCVA or Better in the AM Treatment Group and the Control Group at Each Postoperative Timepoint. (*$p < 0.05$).

https://doi.org/10.2147/OPTH.S569518

Table 3 BCVA logMAR Before and After CXL

Group	Baseline	Week 1	Month 1	Month 3	Month 6
AM Treatment	0.32 ± 0.24	0.52 ± 0.34	0.30 ± 0.19	0.22 ± 0.17*	0.19 ± 0.21*
Control	0.38 ± 0.33	0.53 ± 0.37	0.41 ± 0.34	0.32 ± 0.23	0.31 ± 0.28
p-value	0.51	0.99	0.09	0.052	0.046

Notes: *p<0.05 within group compared to baseline BCVA.

the AM treatment group returned to baseline BCVA (or better) compared to 64.9% of eyes in the control group (p=0.03). BCVA at each follow-up visit is demonstrated in Table 3. At 6 months, BCVA was significantly better in the AM treatment group compared to the control group (0.19 ± 0.21 vs 0.31 ± 0.28, respectively; p=0.046). No other significant differences in BCVA were observed between groups at 1 week, 1 month, and 3 months (Table 3). When assessing the AM treatment group, BCVA significantly improved from baseline at 3- and 6-months (Table 3). BCVA initially worsened by −0.20 ± 0.36 logMAR as expected at 1 week ($p < 0.001$), improved by 0.02 ± 0.17 logMAR at 1 month ($p = 0.35$), significantly improved by 0.09 ± 0.16 logMAR at 3 months ($p < 0.001$), and significantly improved by 0.13 ± 0.19 logMAR at 6 months ($p < 0.001$). In the control group, BCVA significantly worsened by −0.15 ± 0.37 logMAR at 1 week (p=0.007), worsened by −0.03 ± 0.21 logMAR at 1 month ($p = 0.32$), improved by 0.03 ± 0.19 logMAR at 3 months ($p = 0.31$), and improved by 0.05 ± 0.23 logMAR at 6 months ($p = 0.20$), however, these improvements were not statistically significant compared to baseline.

The average K_{max}, the proportion of eyes with a reduction in baseline K_{max}, and the proportion of eyes with progression > 1D were not significantly different between groups at any time point. In the AM treatment group, the average K_{max} was 58.7 ± 9.6 D (range: 44.4−83.5 D) before surgery, 60.2 ± 9.7 D (44.6–89.3 D) at postoperative 1 month ($p = 0.002$), 59.1 ± 9.3 D (47.7−85.5 D) at 3 months ($p = 0.08$), and 56.8 ± 7.9 D (48.0−83.7 D) at 6 months ($p = 0.26$). In the control group, the K_{max} was 61.2 ± 9.5 D (range: 45.2−85.3 D) before surgery, 63.2 ± 9.0 D (48.2−81.9 D) at postoperative 1 month (p=0.004), 62.6 ± 9.3 D (45.0−81.4 D) at 3 months ($p = 0.36$), and 60.3 ± 9.3 D (45.2−85.6 D) at 6 months (p=0.12). A total of 12 eyes (30.8%) in the AM treatment group and 14 eyes (46.6%) in the control group exhibited > 1D progression from baseline K_{max} values at 6 months (p=0.18). Furthermore, 64.1% of the eyes in the AM treatment group demonstrated a reduction (or improvement) in baseline K_{max} values (D) at 6 months compared to 53.3% of eyes in the control group (p=0.37).

For the 3 patients in the control group that had complications, all cases resolved successfully. The patient with infectious keratitis healed after 30 days; BCVA improved from 20/30+1 to 20/25-1 at 6 months post-CXL but K_{max} regressed 7D. The patient with infiltrative keratitis resolved in 14 days; BCVA changed from 20/40 to 20/40+2 at 6 months post-CXL and K_{max} remained constant. The patient with a persistent epithelial defect resolved in 28 days; BCVA changed from 20/200 to 20/60 and K_{max} improved 1D.

Discussion

Epi-off CXL is a widely adopted treatment for stiffening the cornea in patients with keratoconus, halting disease progression, and reducing the potential need for corneal transplantation.[7,8] While epi-off CXL is generally considered a safe and successful procedure, potential complications, such as pain, haze, and fluctuations in vision during the healing process, can affect patient satisfaction. These complications may be related to excessive inflammation generated by CXL or secondary to limbal stem cell injury due to prolonged UV light exposure.

Commonly used adjunctive treatments applied after CXL to reduce pain include a BCL to cover the underlying epithelial defect and topical steroids to reduce inflammation. However, these treatments are also associated with an increased risk of microbial infiltration.[12] A large prospective trial in the United Kingdom reported microbial keratitis in 0.71% of eyes treated with a BCL, topical steroid, and topical antibiotic immediately postoperatively compared to zero cases in eyes that did not have a BCL placed after surgery.[14] This is comparable with the rates reported in a systematic review by Abbouda et al,[19] which range from 0.17% to 1.3%. Moreover, sterile infiltrates have been reported in 7.6% of

cases and may occur because of staphylococcal antigen deposition in areas of static tear pooling beneath the BCL.[20,21] In this study, it was similarly found that 6.4% (3/47) of the eyes developed complications after epi-off CXL with postoperative topical steroids and BCL. In contrast, no eyes treated with AM developed corneal infiltrates, infection, or a persistent epithelial defect postoperatively. While this difference was not statistically significant, post-hoc analysis demonstrated that it was underpowered (power = 0.32) and required a larger sample size. Nevertheless, the reduced complication rate with AM holds clinical relevance, as it provides a safer alternative treatment method to aid in post-operative healing. Cryopreserved AM provides therapeutic effect due to the innate wound healing properties of the tissue, and Prokera Slim (BioTissue Inc.) is designated by the Food and Drug Administration as promoting healing through anti-inflammatory, anti-scarring and antiangiogenic mechanisms. The three patients who experienced a postoperative complication all underwent CXL with AM in their fellow eye without complication. These results are consistent with those of other studies that have shown the benefits of AM in promoting healing in cases with infectious keratitis.[18] AM may help prevent infection as it provides a physical mechanical barrier and can help absorb and slowly release topical antibiotics that are exogenously applied.[22] In addition, some studies have shown a direct anti-microbial property of AM, although further confirmatory studies are warranted.[23] Nonetheless, AM has consistently shown a favourable safety profile in the literature and may help reduce complications after CXL.

Another relatively common side effect of CXL is stromal haze, which causes a reduction in contrast sensitivity and visual acuity. The exact physiology of post-CXL haze is not known, but it is suggested to be a response to keratocyte apoptosis after exposure to UV-A light and corresponds to the reorganization of stromal fibrils.[24] CXL is thought to be dependent on the creation of free radicals/oxygen species generated by riboflavin and UV light, which trigger the formation of covalent bonds between collagen chains, ultimately stiffening and strengthening the cornea.[25] Hence, some inflammatory insult is necessary for halting the progression of ectasia, and the presence of haze in the anterior stroma is typically viewed as an indicator of successful CXL.[11] In this cohort, haze was qualitatively minimal, and no eyes had persistent visually significant haze at the final timepoint. Quantitative measurement of haze was beyond the scope of this study. Nevertheless, BCVA logMAR was significantly better in the AM group compared to the BCL control group at 6-months. At 6-months, BCVA changed by 0.13 ± 0.19 logMAR in the AM group and 0.05 ± 0.23 logMAR in the BCL group, respectively. Comparatively, Hersh et al[26] reported a mean BCVA change of 0.114 logMAR (5.7 letters) at 12-months. AM may reduce stromal haze and could explain the faster return of BCVA after CXL due to its anti-inflammatory and anti-scarring properties.[27,28] AM reduces TGF-β signalling in keratocytes and prevents pro-scarring myofibroblast differentiation; this mechanism may also reduce stromal haze. Corneal haze has also been shown to be reduced in rabbit corneas after the application of human AM following excimer laser photoablation.[17] This reduction of haze has not yet been demonstrated in humans; one study compared epithelial healing and stromal haze after photo-refractive keratectomy (PRK) between two groups: one treated with a BCL and the other with AM (Prokera). There was no difference in visual outcomes, with both groups having excellent distance visual acuity at 1 year postoperatively and similar incidence of clinically significant haze between the groups.[29] AM could have a different effect on keratocyte healing after CXL compared to PRK as the mechanism differs between the two procedures; CXL causes photochemical and radiation damage via UV-A light and activated riboflavin, whereas PRK causes traumatic ablation from the excimer laser.[9,10,29] To date there has been no controlled study comparing haze post-CXL with and without AM which may be a future avenue of research.

In this study, the rate of re-epithelialization was similar between groups at 1 week post-operatively (66.1% in the AM group vs 69.6% in the control group). Nevertheless, patients were not assessed daily, thus, it is possible that the rates of epithelialization may have differed on post-operative Days two through six. For example, Vlasov et al[29] found that patients treated with AM (Prokera) had expedited corneal reepithelialization 1 day after PRK but was not better than the BCL group in hastening complete reepithelialization of the cornea. Relative to other degenerative conditions, the subjects in these subjects are also young and otherwise healthy so likely to re-epithelialize quickly for both groups.

Both groups followed the typical postoperative recovery course with initial topographical steepening followed by stabilization and in some cases, improvement, compared to baseline. While neither group showed a statistically significant flattening at 6 months postoperatively, the magnitude of change is consistent with other trials. A large observational study by Ferdi et al[15] reported K_{max} flattening of 1.2 D over a 12-month period for 976 eyes (of 794

patients) who underwent epi-off CXL. Similarly, a prospective controlled trial by Hersh et al[26] showed an improvement of 1.6 D over the same 1-year time frame. This cohort was smaller with a shorter follow-up, and while the improvement in K_{max} was not statistically significant at 6 months, both groups trended towards an improvement in K_{max} compared to baseline. Overall, this cohort responded well to surgery with either AM or BCL when comparing K_{max} changes postoperatively.

The current study had some limitations; the design was retrospective in nature, and the eyes were not randomized to a given treatment. Furthermore, both groups contain results from two eyes for patients who underwent surgery bilaterally. While this could be managed via the inclusion of only one eye or propensity scores matching to create groups with similar pre-treatment characteristics, this approach rejects valid data and would have resulted in the loss of statistical power. As a result, we used a linear mixed model analysis to account for any potential inter-eye correlations and prevent incorrect variance estimates to ensure valid statistical inferences. Best sphere was significantly different between groups at baseline which may suggest milder keratoconus in the AM treatment group. Severe keratoconus is associated with higher myopia as the cornea steepens and is also associated with higher K_{max}, higher astigmatism and lower corneal thickness, all of which were comparable between groups in this cohort. BCVA was similar between groups at baseline, which was the primary outcome measure assessed. This study is also limited by its shorter follow-up duration of 6 months; visual function continued to improve between 6 and 12 months in the Hersch et al[26] trial with most improvement between 1 and 3 months. Future trials employing controlled randomization schema with assessments up to one year post-operatively would be necessary to expand upon and verify the current findings.

Conclusions

In conclusion, AM improved BCVA at 6 months postoperatively compared to BCL alone and was well-tolerated without any postoperative complications, suggesting that it can be a safe, adjunctive treatment for epi-off CXL to improve visual function. These results provide real-world experience with a relatively large sample size and are applicable to physicians performing CXL in their practice and to optometrists co-managing surgical care for these patients. Randomized, controlled trials with longer post-operative follow-up are necessary to expand upon and verify these findings.

Acknowledgments

This paper/abstract was presented at the American Academy of Ophthalmology and the American Academy of Optometry 2025 Annual Meeting as a poster/podium presentation with interim findings.

Funding

There is no funding to report.

Disclosure

Dr Rosetta is a paid speaker for BioTissue. Dr Gupta has no affiliation with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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