

# Efficacy of iontophoresis-assisted epithelium-on corneal cross-linking for keratoconus

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## Abstract

• **Corneal cross-linking (CXL) is a noninvasive therapeutic procedure for keratoconus that is aimed at improving corneal biomechanical properties by induction of covalent cross-links between stromal proteins. It is accomplished by ultraviolet A (UVA) radiation of the cornea, which is first saturated with photosensitizing riboflavin. It has been shown that standard epithelium-off CXL (S-CXL) is efficacious, and it has been recommended as the standard of care procedure for keratoconus. However, epithelial removal leads to pain, transient vision loss, and a higher risk of corneal infection. To avoid these disadvantages, transepithelial CXL was developed. Recently, iontophoresis has been adopted to increase riboflavin penetration through the epithelium. Several clinical observations have demonstrated the safety and efficacy of iontophoresis-assisted epithelium-on CXL (I-CXL) for keratoconus. This review aimed to provide a comprehensive summary of the published studies regarding I-CXL and a comparison between I-CXL and S-CXL. All articles used in this review were mainly retrieved from the PubMed database. Original articles and reviews were selected if they were related to the I-CXL technique or related to the comparison between I-CXL and S-CXL.**

• **KEYWORDS:** corneal cross-linking; keratoconus; iontophoresis; riboflavin; ultraviolet A

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## INTRODUCTION

**K**eratoconus is a bilateral and progressive corneal disorder that affects one in 2000 individuals in the younger population<sup>[1]</sup>. It usually results in progressive corneal thinning, corneal deformation, and impaired vision.

At the University of Dresden, Spoerl and Seiler<sup>[2]</sup> developed photochemical corneal cross-linking (CXL) using ultraviolet A (UVA) (370 nm) light and riboflavin (vitamin B2). The basic mechanism of CXL is the formation of covalent bonds predominantly at the surface of collagen fibrils and in the protein network surrounding the collagen, which then results in a long-term increase in corneal biomechanical rigidity and improvement in its strength and stability<sup>[3-4]</sup>. Since its advent in the late 1990s, CXL has remained the primary treatment for corneal ectatic diseases worldwide. Standard epithelium-off CXL (S-CXL) has been recommended as the gold standard of care for progressive keratoconus<sup>[5]</sup>. However, it is disadvantageous because it cannot be used in thin corneas, results in pain, temporarily reduces visual acuity, causes infections and a haze that is likely to persist for 1 to 12mo, it is also difficult to perform in children<sup>[6-10]</sup>. All of these defects have been shown to correlate with corneal epithelium removal. Therefore, the concept of epithelial-on cross-linking is more appealing.

Iontophoresis has been used for ophthalmological drug administration since 1908 and applied in various medical fields such as transdermal delivery of anti-inflammatory agents, local anesthetics or analgesics, and transmucosal antiviral administration<sup>[11-17]</sup>. More recently, it has been found to be efficacious for transcorneal drug delivery<sup>[18]</sup>. Studies have demonstrated its safety and efficacy in drug penetration through the cornea and into other ocular tissues<sup>[19]</sup>. Recently, iontophoresis and CXL have been combined to form iontophoresis-assisted epithelium-on CXL (I-CXL). But can I-CXL achieve the same therapeutic effects as S-CXL? In this article, we have reviewed the available literature concerning this topic. All articles used in this review were mainly retrieved from the PubMed database. Original articles and reviews were selected if they were related to the technique of I-CXL or related to a comparison between I-CXL and S-CXL. Letters and case reports were excluded.

## FACTORS THAT INFLUENCE CORNEAL CROSS-LINKING EFFICACY

Seiler and Hafezi<sup>[20]</sup> demonstrated that the depth of effective CXL treatment depends on UVA intensity and the concentration of corneal intrastromal riboflavin. Lombardo *et al*<sup>[21]</sup> showed that intact corneal epithelium filters out an average of 20% of the UVA radiation passing through the

cornea in epithelium-on CXL. Bottos *et al*<sup>[22]</sup> reported that the reduced effects of conventional epithelium-on CXL (in comparison to S-CXL) was mainly the result of limited riboflavin penetration through the corneal epithelium and also demonstrated that it was not a barrier to UVA transmittance. The total UVA dose was consistent throughout different CXL protocols. Thus, corneal intrastromal riboflavin concentration appears to be the key factor limiting epithelium-on approaches. More recently, Richoz *et al*<sup>[23]</sup> reported that oxygen is also an essential element for photopolymerization in CXL in order for CXL to occur. Kamaev *et al*<sup>[24]</sup> hypothesized that oxygen plays a role in CXL *via* oxygen transformation into reactive oxygen species. Intact corneal epithelium may act as a barrier in order to reduce rapid oxygen diffusion into the stroma. However, whether this reduction leads to a decrease in photopolymerization in I-CXL has not yet been experimentally confirmed.

### **CORNEAL INTRASTROMAL RIBOFLAVIN CONCENTRATION**

Several studies<sup>[25-28]</sup> have reported the differences in corneal intrastromal riboflavin concentrations after using three CXL imbibition techniques (S-CXL, conventional epithelium-on CXL, and I-CXL delivery) with a 0.1% riboflavin solution. These results demonstrated that I-CXL imbibition produced greater and deeper riboflavin saturation than the conventional epithelium-on technique but did not reach the level acquired with the S-CXL technique. All of the above studies used a 0.1% riboflavin solution plus enhancers. Novruzlu *et al*<sup>[29]</sup> obtained the same results after administration of a simple 0.2% riboflavin solution by iontophoresis without using penetration enhancers. In a rabbit model, Cassagne *et al*<sup>[30]</sup> compared the iontophoresis-riboflavin delivery technique using a charged riboflavin solution that contained 0.1% riboflavin, 0.1% ethylenediaminetetraacetic acid, and 0.05% trometamol for I-CXL with S-CXL. Using a high-performance liquid chromatography analysis, they discovered that iontophoresis allowed riboflavin imbibition with two-fold less concentrations than the S-CXL technique (936.2±312.5 vs 1708±908.3 ng/mL,  $P<0.05$ ).

Hayes *et al*<sup>[31]</sup> used spectrophotometry to indirectly measure and compare intrastromal riboflavin penetration in *ex vivo* porcine corneas following the standard protocol, clinically routine iontophoretic protocols, or two modified iontophoretic protocols. The St. Thomas'/Cardiff iontophoresis protocol B, which included two 5min iontophoresis-assisted deliveries of Ricrolin+ with a 15min soak time in between the two iontophoresis periods, still showed significantly less intrastromal riboflavin concentrations than just S-CXL. However, corneas treated with this protocol have a higher optical density than corneas treated with St. Thomas'/Cardiff iontophoresis protocol A (iontophoresis-assisted delivery for

5min with a 20min riboflavin soak) and routine iontophoretic protocols (iontophoresis-assisted delivery for 5min).

In addition, intrastromal riboflavin concentrations in different corneal segments after different delivery techniques exhibited obvious changes. It was reported that the intrastromal riboflavin concentration decreased in conjunction with depth enhancement, and the differences in the extent of decrease for the three delivery techniques were statistically significant. In a human cadaver corneal study<sup>[26]</sup> done with high-performance liquid chromatography, the mean riboflavin content in the superficial slice (0-150  $\mu\text{m}$ ) in the epithelium-off group was about 2-fold greater than that of the iontophoresis group (50.5±5.3 vs 23.6±2.5 mg/g) and 4-fold greater than that of the epithelium-on group (11.7±3.3 mg/g). Similar differences among three groups were observed for the intermediate and posterior stromal slices (150-300  $\mu\text{m}$  and >300  $\mu\text{m}$ , respectively), presenting an evident riboflavin concentration reduction with increasing depth in all groups. Using two-photon fluorescence microscopy, Gore *et al*<sup>[32]</sup> confirmed the depth-dependent riboflavin concentration differences and differences between conventional administration and iontophoresis with Ricrolin+ in fresh postmortem rabbit eyes. The epithelium-off and iontophoresis groups obtained peak riboflavin at concentrations of 0.09%±0.01% and 0.031%±0.003% within the most superficial stroma (0-10  $\mu\text{m}$ ), respectively. At a depth of 300  $\mu\text{m}$ , the stromal riboflavin concentration was 0.075%±0.006% and 0.016%±0.002% in epithelium-off groups and iontophoresis groups, respectively.

Using two-photon fluorescence microscopy, Gore *et al*<sup>[33]</sup> measured corneal intrastromal riboflavin concentrations in rabbits using different transepithelial iontophoresis protocols. The authors found that epithelium-on iontophoresis administration with higher-concentrations of riboflavin solutions, greater iontophoresis dosage, and longer solution contact times achieved greater intrastromal riboflavin penetration. A protocol utilizing 0.25% (wt/vol) riboflavin with benzalkonium chloride (BAC) 0.01% and two cycles of applied current and subsequent soaking (1 mA for 5min, soak for 5min; 0.5 mA for 5min, soak for 5min) attained similar intrastromal riboflavin penetration to conventional epithelium-off protocol. The best-performing non-BAC containing protocol produced intrastromal riboflavin concentrations approximately 60% that of conventional epithelium-off protocol. Riboflavin solutions containing saline can lead to minimal stromal penetration.

Using Scheimpflug photography, Lombardo *et al*<sup>[34]</sup> analyzed corneal light backscattering before and after transepithelial I-CXL in donor eyes. Light backscattering significantly increased after iontophoresis and decreased significantly after I-CXL, approaching the baseline values in specimens with and without intact epithelium. After standard corneal soaking with riboflavin, a significant increase in corneal light backscattering

was detected and remained unchanged up to 30min after S-CXL. The light backscattering increase after iontophoresis in corneas with epithelium was lower than that after standard soaking, whereas in corneas without epithelium it was similar to that after standard stromal soaking.

Hypoosmotic riboflavin saline buffer including enhancers were adopted in most of the above reports. Li *et al*<sup>[35]</sup> investigated the imbibition of 0.1% riboflavin-distilled water solution into corneal stromata by iontophoresis-assisted delivery at a current of 1 mA and duration of 10min; identical stromal yellow changes were observed when compared with the standard protocol. This suggested that iontophoresis using 0.1% riboflavin-distilled water solution yielded the same corneal intrastromal riboflavin concentrations as the standard protocol for two reasons: 1) fewer parasitic ions in the distilled water, thus resulting in less interference with riboflavin permeability; 2) a hypoosmotic pressure in the riboflavin-distilled water solution that damaged the barrier function of the epithelium. However, these are qualitative observations. Whether this protocol can attain the same intrastromal riboflavin concentration as the standard technique needs further quantitative assessment.

Although the intrastromal riboflavin concentration obtained by iontophoresis did not reach the levels seen with the S-CXL imbibition technique, it was superior to the conventional epithelium-on protocol. It is not yet exactly clear which riboflavin concentration is necessary in the corneal stroma to achieve a sufficient cross-linking effect for corneal stabilization. Therefore, the I-CXL technique needs further clinical studies.

#### **Corneal Biomechanics, Biomolecules and Morphology**

Mencucci *et al*<sup>[36]</sup> researched the I-CXL-induced early modifications in *ex vivo* human corneas. They found that biomolecular and morphological alterations of corneas treated with I-CXL at 10 mW/cm<sup>2</sup> for 9min were similar (but more superficial) than corneas treated with S-CXL. These alterations included maldistribution, decreaseD in quantity, and increased apoptosis of anterior stromal keratocytes in addition to reduced subepithelial interweaving of corneal collagen I fibers. Similarly, other reports showed that the apoptotic keratocyte effect following I-CXL was seen only at a depth of 210-230  $\mu$ m, while it was 270-300  $\mu$ m following the S-CXL procedure<sup>[37-39]</sup>. Bikbova and Bikbov<sup>[40]</sup> reported a similar outcome with 150-210  $\mu$ m and 240-309  $\mu$ m for the same set of parameters. These results implied that the efficacy of I-CXL may be inferior to that of S-CXL.

Cassagne *et al*<sup>[30]</sup> compared I-CXL with S-CXL in a rabbit model and found that I-CXL and S-CXL induced a similar increase in anterior and intermediate stromal collagen packing. The stress at 10% strain displayed comparable stiffness in I-CXL- and S-CXL-treated corneas. Furthermore, they

observed an equal increase in resistance against corneal collagenase degradation after I-CXL and S-CXL. In addition, Lombardo *et al*<sup>[41]</sup> found Young's modulus (E) of the anterior cornea increased by a mean of 1.8 times (from 1.6 to 2.9 MPa) and 1.9 times (from 1.3 to 2.5 MPa) after I-CXL and S-CXL in human donor eyes, respectively. Vinciguerra *et al*<sup>[27]</sup> also observed that stress-strain in human corneas did not show significant differences between I-CXL and S-CXL.

Mastropasqua *et al*<sup>[42]</sup> measured the deformation amplitude index in human cadaver corneas after different cross-linking protocols. The deformation amplitude index is a biomarker indicating corneal biomechanical rigidity. The results showed that the trend in deformation amplitude index reduction was more evident in corneas treated with I-CXL (10 mW/cm<sup>2</sup>, 9min) than S-CXL and I-CXL (3 mW/cm<sup>2</sup>, 30min) even if this difference was not statistically significant. Lanzini *et al*<sup>[43]</sup> reported that the stress values for 10% strain and the Young's modulus after I-CXL (10 mW/cm<sup>2</sup>, 9min) were higher than those after S-CXL and I-CXL (3 mW/cm<sup>2</sup>, 30min) in human cadaver corneas. This suggested that I-CXL (10 mW/cm<sup>2</sup>, 9min) produced the greatest mechanical resistance.

However, the corneas analyzed in the above studies were healthy, not keratoconic. Moreover, explanted fresh corneas may be slightly different from *in vivo* corneas. In addition, corneas were analyzed immediately after I-CXL in the aforementioned studies. Manetti *et al*<sup>[44]</sup> evaluated type I collagen fiber and keratocyte distribution in the corneal stroma of *in vivo* I-CXL in a patient with advanced keratoconus. The corneal tissue was collected six months after I-CXL at 10 mW/cm<sup>2</sup>, and the authors indicated an attempt to restore parallel distribution of type I collagen fibers even when fiber interweaving appeared less organized than in healthy corneas and S-CXL-treated keratoconic corneas. In the above study, I-CXL improved the distribution of CD34-positive keratocytes in keratoconic corneas although scattered CD34 immunoreactivity was still evident in the subepithelial stroma. It has previously been demonstrated that CD34-positive keratocytes are responsible for corneal matrix synthesis and maintenance<sup>[39]</sup>.

Aldahlawi *et al*<sup>[45]</sup> reported that in porcine eyes, S-CXL-treated keratocytes showed greater enzymatic resistance to pepsin digestion than I-CXL. This result indicated that S-CXL may be more effective than I-CXL in preventing keratoconus progression.

#### **CLINICAL STUDIES OF IONTOPHORESIS-ASSISTED EPITHELIUM-ON CORNEAL CROSS-LINKING**

Several clinical studies about I-CXL for keratoconus have been reported<sup>[1,40,46-60]</sup>. The results of these studies are promising (Table 1).

**Case Series** In the first prospective clinical study, Bikbova and Bikbov<sup>[46]</sup> used I-CXL (iontophoresis: 0.2-1.0 mA,

**Table 1 Results reported in literature for I-CXL procedures**

First author (y)	Iontophoresis		Radiation		Follow-up (mo)	Results		Type	No. of eyes	Indication
	Electric (mA)	Time (min)	Energy (mW/cm <sup>2</sup> )	Time (min)		Kmax	CDVA			
Bikbova <sup>[46]</sup> (2014)	0.2-1.0	10	3	30	12	D	S	PCS	22	KC
Lin <sup>[47]</sup> (2015)	1.0	5	9	10	12	D	S	PCS	23	KC
Bouheraoua <sup>[11]</sup> (2014)	0.2-1.0	5	10	9	6	S	S	PCS	15	KC
Bouheraoua <sup>[48]</sup> (2015)	0.2-1.0	5	10	9	6	S	--	PCS	--	KC
Vinciguerra <sup>[49]</sup> (2014)	1.0	5	10	9	12	S	I	PCS	20	KC
Li <sup>[50]</sup> (2014)	1.0	5	3	30	6	S	S	PCS	15	KC
Buzzonetti <sup>[51]</sup> (2015)	0.5-1.0	5	10	9	15	S	I	PCS	14	Pediatric KC
Magli <sup>[52]</sup> (2016)	1.0	5	10	9	18	S	S	PCS	13	Pediatric KC
Bikbova <sup>[40]</sup> (2016)	0.2-1.0	10	3	30	24	D	I	RCT	76	KC
Lombardo <sup>[53]</sup> (2016)	1.0	5	10	9	6	D	I	RCT	22	KC
Vinciguerra <sup>[54]</sup> (2016)	1.0	5	10	9	12	S	I	PCS	20	KC
Jouve <sup>[55]</sup> (2017)	1.0	5	10	9	24	S	I	PCS	40	KC
Lombardo <sup>[56]</sup> (2017)	1.0	5	10	9	12	S	I	RCT	22	KC
Jia <sup>[57]</sup> (2017)	1.0	5	3	30	24	D	I	PCS	94	KC
Cantemir <sup>[58]</sup> (2017)	1.0	5	10	9	36	D	I	RCS	40	KC
Bilgihan <sup>[59]</sup> (2017)	1.0	10	9	13.3	12	D	I	RCS	46	KC
Rossi <sup>[60]</sup> (2017)	1.0	5	10	10	12	D	I	PCS	10	KC

CDVA: Corrected distance visual acuity; D: Decreased; S: Stabilized; I: Improved; PCS: Prospective clinical study; RCT: Randomized controlled trial; RCS: Retrospective clinical study; KC: Keratoconus; Kmax: Maximum keratometric reading. The 0.2 (0.5)-1.0 mA indicated the electric intensity was initially 0.2 (0.5) mA and gradually increased to 1.0 mA.

10min; radiation: 3 mW/cm<sup>2</sup>, 30min) in 19 patients (22 eyes) with progressive keratoconus. They found decreases in the average keratometry values and stabilization of uncorrected visual acuity (UCVA), corrected distance visual acuity (CDVA), corneal astigmatism, and endothelial cell density with a 1-year follow-up. Thereafter, several other clinical reports followed. Lin *et al*<sup>[47]</sup> displayed similar outcomes as Bikbova and Bikbov<sup>[46]</sup> in a prospective nonrandomized trial in which the maximum keratometric reading (Kmax) values showed a statistically significant decrease and UCVA, CDVA, and endothelial cell density remained stable after I-CXL (iontophoresis: 1.0 mA, 5min; radiation: 9 mW/cm<sup>2</sup>, 10min) in 23 patients (23 eyes) with progressive keratoconus. However, Kmax values showed no statistically significant decreases and remained stable<sup>[1,48-52]</sup>, but CDVA improved significantly in some studies<sup>[49,51]</sup> and was stabilized in other reports<sup>[50,52]</sup>. Jia *et al*<sup>[57]</sup> reported a 24-month follow-up clinical observation of I-CXL (iontophoresis: 1.0 mA, 5min; radiation: 3 mW/cm<sup>2</sup>, 30min) in 75 patients (94 eyes) with 0.1% riboflavin in distilled water for progressive keratoconus. The authors showed that Kmax and average keratometry had significantly decreased, and CDVA had significantly improved.

Vinciguerra *et al*<sup>[49]</sup> enrolled patients with advanced keratoconus with a Kmax value of up to 64 D. Using I-CXL, Buzzonetti *et al*<sup>[51]</sup> and Magli *et al*<sup>[52]</sup> treated pediatric patients who had progressive keratoconus. The progression of pediatric keratoconus is usually faster than adult keratoconus, treatment

compliance is poor, permanent complications appear easily, and the risk of requiring keratoplasty is high<sup>[61-62]</sup>. However, even in these advanced keratoconus or pediatric patients, I-CXL safely and effectively halted keratoconus progression.

**Comparative, Non-randomized Trials** Several prospective, comparative, non-randomized clinical studies have been published. Vinciguerra *et al*<sup>[54]</sup> have shown a stable Kmax in I-CXL (20 eyes; iontophoresis: 1.0 mA, 5min; radiation: 10 mW/cm<sup>2</sup>, 9min), a statistically significant reduction of Kmax in S-CXL (20 eyes), and a statistically significant improvement in CDVA in both groups at the 1-year follow up for progressive keratoconus. However, CDVA showed a quicker improvement in the I-CXL group compared to the S-CXL group. Spherical equivalent refraction reduced significantly at all postoperative follow-up times in the I-CXL group. Conversely, it was stable after S-CXL. Jouve *et al*<sup>[55]</sup> reported identical outcomes in comparative 24-month follow-up studies between I-CXL (40 eyes; iontophoresis: 1.0 mA, 5min; radiation: 10 mW/cm<sup>2</sup>, 9min) and S-CXL (40 eyes) in terms of Kmax and CDVA.

Recently, Rossi *et al*<sup>[60]</sup> evaluated the efficacy differences between S-CXL (10 eyes) and I-CXL (10 eyes; iontophoresis: 1 mA, 5min; radiation: 10 mW/cm<sup>2</sup>, 10min) with the differences between 12mo and baseline data. The differences of UDVA, CDVA, spherical error, aberrometric outcomes, corneal astigmatism, and flat, mean, and apex keratometries were not statistically significant. However, the differences

in spherical equivalent, steep keratometry, and superior-inferior symmetry index were statistically significant. The improvement in these three parameters in the S-CXL group was superior to that in the I-CXL group.

In a retrospective study, Cantemir *et al*<sup>[58]</sup> compared the 3-year I-CXL (40 eyes; iontophoresis: 1.0 mA, 5min; radiation: 10 mW/cm<sup>2</sup>, 9min) outcomes with S-CXL (40 eyes) for early stages of keratoconus. Visual acuity significantly improved at month 36 in both groups. In the I-CXL group, UDVA recovered more rapidly than S-CXL after 3mo. The trend in CDVA improvement in the S-CXL group was more favorable than that in the I-CXL group. Kmax values showed a significant reduction by 0.9 D in the I-CXL group and by 1.2 D in the S-CXL group after 36mo. Spherical equivalent did not experience statistically significant changes in both groups throughout 36mo follow-up.

In another retrospective trial comparing S-CXL (47 eyes) and diluted alcohol and iontophoresis assisted CXL (DAI-CXL) (46 eyes; iontophoresis: 1.0 mA, 10min; radiation: 9 mW/cm<sup>2</sup>, 13.3min) for keratoconus, Bilgihan *et al*<sup>[59]</sup> used a 10% alcohol solution as an enhancer, enhancer-free 0.2% riboflavin solution as a photosensitizer, and a total dose of 7.2 J/cm<sup>2</sup> for UVA radiation. In this study, CDVA improved at months 3 and 6 after DAI-CXL and S-CXL, respectively. Higher-order aberrations, coma, and spherical aberrations improved significantly at month 12 in both groups. Kmax values decreased in both groups at month 6.

**Randomized Controlled Trials** Bikbova and Bikbov<sup>[40]</sup> published the first prospective randomized controlled trial (RCT) comparing I-CXL (iontophoresis: 0.2-1.0 mA, 10min; radiation: 3 mW/cm<sup>2</sup>, 30min) with S-CXL. They enrolled 119 patients (149 eyes) with Amsler classification of keratoconus I-II (73 and 76 eyes in the S-CXL and in I-CXL groups, respectively) and found statistically significant differences in CDVA between the two groups with a better result in the I-CXL group after 6mo; however, no significant differences were observed 24mo after the procedures. Keratometric value stabilization and decrease were obtained in both groups, but S-CXL was more effective after 24mo of follow-up.

Another RCT was performed by Lombardo *et al*<sup>[53]</sup>. They enrolled 34 eyes of 25 patients, including 12 eyes with S-CXL and 22 eyes with I-CXL (iontophoresis: 1.0 mA, 5min; radiation: 10 mW/cm<sup>2</sup>, 9min). A significant decrease in Kmax and a significant improvement CDVA were observed in both groups at 6mo. However, the manifest spherical equivalent refraction changed on average by 0.65±1.20 D ( $P=0.02$ ) and 0.24±0.77 D ( $P=0.32$ ) in the I-CXL and S-CXL groups, respectively. Changes in these three parameters between the two groups did not show statistical significance. The contrast sensitivity function recovery was slower in S-CXL than in I-CXL. At 6mo, no significant central corneal thickness

differences were found in I-CXL, whereas it was seen in S-CXL. In this trial, I-CXL showed comparable results with S-CXL with respect to halting keratoconus progression during a 6mo follow-up.

When the follow-up period was extended to 12mo in the above RCT, the decrease of Kmax was not significant and CDVA had significantly improved<sup>[56]</sup>. The change of Kmax from baseline in I-CXL was not statistically significant; however, it was opposite if participants <24 years old were removed from the analysis. The change in Kmax from baseline was statistically significant in S-CXL. CDVA, manifest spherical equivalent refraction, and contrast sensitivity function improved significantly in the I-CXL group; however, they changed slightly in the S-CXL group. The changes in Kmax, CDVA, manifest spherical equivalent refraction, and contrast sensitivity function from baseline between the two groups did not show statistical significance. The central corneal thickness measures did not change significantly in both groups at 12mo. Most of the above clinical studies adopted a hypoosmolar 0.1% riboflavin solution enriched with penetration enhancers (trometamol and ethylenediaminetetraacetic acid) without dextran or sodium chloride, specifically formulated to facilitate quick penetration via corneal iontophoresis into the corneal stroma through an intact epithelium. Nevertheless, Bouheraoua *et al*<sup>[48]</sup> used hypoosmolar 0.1% riboflavin without dextran, Jia *et al*<sup>[57]</sup> and Li *et al*<sup>[50]</sup> both used 0.1% riboflavin-distilled water solution only. In all of the above reports, corneal endothelial cell density was not significantly reduced, and no severe postoperative complications were found. These findings confirmed I-CXL safety.

**Corneal Stromal Demarcation Line** Lin *et al*<sup>[47]</sup> reported that within one postoperative month, I-CXL-treated keratoconus corneas exhibited a demarcation line in the anterior stroma with maximal depth of about 133 μm, whereas Bouheraoua *et al*<sup>[48]</sup> showed that the demarcation line was not clearly measurable at a mean depth of 214 μm in 46.5% of the patients treated with I-CXL. Similarly, in several other studies, the demarcation line after I-CXL appeared to be shallower and less easily distinguishable than after S-CXL; however, it exhibited features more similar to those observed after S-CXL with respect to depth and visualization when compared with enhancer-assisted transepithelial CXL<sup>[1,37,49,63]</sup>. Even using iontophoresis at 1.0 mA and for 10min (usually it was 5min) in a prospective RCT, Bikbova and Bikbov<sup>[40]</sup> only observed a corneal demarcation line with mean depths of 172 μm and 292 μm in the I-CXL and S-CXL groups, respectively. Until now, the deepest demarcation line after I-CXL was observed by Jia *et al*<sup>[57]</sup> who reported a mean depth of 298.95 μm at one month postoperatively after using a 0.1% riboflavin-distilled water solution.

In Bikbova and Bikbov's<sup>[40]</sup> RCT, the depth of the corneal stromal demarcation line paralleled the clinical efficacy between I-CXL and S-CXL. In other words, the corneal stromal demarcation line in the I-CXL group was shallower than that in the S-CXL group (172  $\mu\text{m}$  vs 292  $\mu\text{m}$ ), and the efficacy of the I-CXL group was also less than that of the S-CXL group. However, until now, whether the depth of corneal stroma demarcation line indicates the effectiveness of CXL treatment has not been confirmed.

**Corneal Anterior Stromal Keratocyte** Bouheraoua *et al*<sup>[11]</sup> found that the mean corneal anterior stromal keratocyte densities were significantly lower at 6mo in the S-CXL group when compared to preoperative values, whereas they returned to preoperative values in the I-CXL group by *in vivo* real-time confocal microscopy scans. In another prospective *in vivo* confocal microscopy study in S-CXL, Jordan *et al*<sup>[64]</sup> revealed a significant decrease in the mean anterior keratocyte density at 1, 3 and 6mo postoperatively with return to the baseline values at 12mo postoperatively. Similarly, Touboul *et al*<sup>[65]</sup> also confirmed the decrease of corneal anterior stromal keratocyte within six months postoperatively in S-CXL. These findings suggested that corneal anterior stromal keratocyte in I-CXL recovered faster than in S-CXL.

### CONCLUSION

I-CXL has the major advantage of retaining the corneal epithelium and avoiding de-epithelization-related complications such as early postoperative pain, vision impairment, and infection risks<sup>[66]</sup>. Moreover, it has the advantage of shortening riboflavin delivery time from 30 to 5min. Clinical studies have confirmed the safety and efficacy of I-CXL for halting the progression of keratoconus. There are several advantages of clinical results in I-CXL compared to S-CXL: 1) I-CXL had significantly faster recovery of visual acuity and contrast sensitivity function than S-CXL; and 2) spherical equivalent refraction was significantly less myopic 6 and 12mo after I-CXL than that after S-CXL. However, I-CXL is inferior to S-CXL with respect to many factors that affect efficacy such as corneal intrastromal riboflavin concentration and transmissivity of UVA. Long-term I-CXL effectiveness needs to be further observed.

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