Enhanced-Fluence Pulsed-Light Iontophoresis Corneal Cross-linking: 1-Year Morphological and Clinical Results

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ABSTRACT

PURPOSE: To assess the safety and efficacy of a novel pulsed-light enhanced-fluence iontophoresis corneal cross-linking (EF I-CXL) procedure in patients with progressive keratoconus.

METHODS: This prospective interventional pilot study included 12 eyes of 10 patients. Iontophoresis with Ricrolin+ solution (Sooft, Montegiorgio, Italy) was used for stromal imbibition. The treatment energy dose (fluence) was optimized at 30% (from 5.4 J to 7 J/cm^2) and ultraviolet-A (UV-A) power set at 18 mW/cm^2 × 6.28 minutes of exposure time, pulsing the light 1 second on/1 second off with a total irradiation time of 12.56 minutes. Uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), Scheimpflug corneal tomography data, and corneal optical coherence tomography (OCT) at baseline and 1, 3, 6, and 12 months postoperatively were evaluated.

RESULTS: Twelve-month statistically significant average data ($P < .05$) showed UDVA decreased from 0.50 ± 0.10 to 0.36 ± 0.08 logMAR, maximum keratometry decreased from 52.86 ± 1.50 to 51.49 ± 0.90 diopters (D), surface asymmetry index decreased from 2.34 ± 0.36 to 2.13 ± 1.12 D, symmetry index decreased from 4.22 ± 1.01 to 3.56 ± 0.90 D, and coma decreased from 0.25 ± 0.05 to 0.14 ± 0.06 μm. Corneal OCT showed greater than 80% demarcation line detection at 295.8 ± 20.2 μm depth on average in the first postoperative month.

CONCLUSIONS: The preliminary results of the EF I-CXL protocol demonstrate its capability to increase I-CXL efficacy closer to standard CXL.

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Corneal cross-linking with epithelium removal (standard CXL) is currently considered the gold standard treatment for progressive keratoconus and secondary corneal ectasia. Long-term functional data in young and pediatric patients showed that standard CXL is able to halt progressive ectasia for up to 10 years of follow-up.[1-3]

The Dresden protocol of standard CXL[1] and Siena modifications[2] involves the removal of corneal epithelium to allow the homogeneous imbibition of the corneal stroma via passive diffusion by riboflavin (a large hydrophilic molecule that cannot penetrate an intact epithelium) before continuous light ultraviolet-A (UV-A) irradiation of the cornea with a UV-A power of 3 mW/cm^2 and total energy dose (fluence) of 5.4 J/cm^2.[4,5]

Transepithelial CXL penetration has been largely unsatisfactory.[6-11] To overcome the limitations of transepithelial techniques based on chemical disruption and uneven alteration of the epithelial barrier,[9-11] an electric-assisted methodology of riboflavin transport with epithelium in situ called iontophoresis-assisted CXL (I-CXL) was used with interesting results that were better compared to standard CXL.[12,13] Early clinical data on I-CXL have shown an increased permeation of the riboflavin into the stroma compared to transepithelial techniques and clinical data reported in the literature showed the efficacy of I-CXL in stabilizing keratoconus in the short-term follow-up (less than 24 months).[13] The 24-month follow-up evaluation concluded that I-CXL halted progression of keratoconus less efficiently than did standard CXL.[14] Nonetheless, the demarcation line assessed by optical coherence tomography

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(OCT) and in vivo confocal microscopy was found in fewer than 50% of cases and was more superficial than with the standard procedure.[14,15]

In the reported studies on I-CXL,[13-15] despite the presence of epithelium in situ, the total energy dose (fluence) delivered to corneal stroma was identical to that used in the standard CXL with epithelium removal[2] (5.4 J/cm^2) with continuous UV light irradiation. A laboratory study[16] demonstrated that a 25% increase in UV-A radiance (from 5.4 to 6.5 J/cm^2) significantly increased the corneal resistance against enzymatic degradation. This study[16] concluded that, although epithelium-on CXL appeared to be inferior to epithelium-off CXL in terms of enzymatic resistance to pepsin digestion, the outcome of epithelium-on CXL may be significantly improved by increasing the UV-A radiance and exposure time of 25%. Interestingly, another study[17] evaluating the stromal demarcation line depth following a modified accelerated CXL protocol by using 18 mW/cm^2 UV-A power with 7 minutes of exposure time, corresponding to a total fluence of 7.5 J/cm^2, instead of the standard dose of 5.4 J/cm^2 for 5 minutes of UV-A exposure, demonstrated that increasing the fluence and consequently the UV-A exposure time of 30% to 40%, the depth of demarcation line was similar to standard CXL.[17]

Another important issue that can be considered an additional limitation of the transepithelial treatments is the higher oxygen consumption by the corneal epithelium.[18-20] On the basis of volumes of oxygen per unit volume corneal tissue, epithelial oxygen use was demonstrated to be approximately 10 times that of the whole stroma.[20,21] It is known that oxygen is the "driver" of CXL reaction and the biomechanical effect of CXL is oxygen dependent.[22] To increase oxygen diffusion,[23] we added the pulsed-light UV-A emission[24,25] in this new enhanced-fluence I-CXL (EF I-CXL) protocol to partially compensate for the major oxygen consumption, also calibrating the UV-A power and the exposure time to maintain an overall treatment duration of 18 to 20 minutes on balance. The aim of this study was to investigate the 1-year clinical outcomes of the EF I-CXL pulsed-light protocol together with the OCT evaluation of the demarcation line.

PATIENTS AND METHODS

This pilot interventional study included 12 eyes of 10 patients (8 unilateral, 2 bilateral) affected by progressive stage II keratoconus according to Krumeich's classification. Patients were treated by the EF I-CXL protocol at the Siena Crosslinking Center. Eight patients were men and 2 were women, with a mean age of 24.8 years (range: 15 to 36 years). All patients included in the study completed the entire 12-month follow-up.

TABLE 1
EF I-CXL Methods

Parameter	Variable
Treatment target	Keratoconus stabilization
Fluence (total)	7 J/cm^2
Soak time and interval	Iontophoresis (5 minutes)
Intensity	18 mW/cm^2
Epithelium status	On
Chromophore	Riboflavin
Chromophore carriers	Trometamol, Na-EDTA, no dextran
Chromophore osmolarity	Hypotonic
Chromophore concentration	0.1%
Light source	KXL I (Avedro, Waltham, MA)
Irradiation mode (interval)	Pulsed (1 second on/1 second off)
Protocol modifications	I-CXL
Protocol abbreviation	EF I-CXL

EF I-CXL = enhanced-fluence iontophoresis corneal cross-linking

Iontophoresis was used for stromal imbibition and performed with the Ricrolin+ riboflavin solution (Ricrolin TE; Sooft, Montegiorgio, Italy) delivered by the electric generator I-ON CXL (Sooft), at 1 mAmpere × 5 minutes, 0.8 mm of corneal surface via suction ring and inox electrode placement, and 0.35 mL riboflavin 0.1% volume. The fluence was optimized at 30% (7 J/cm^2) and UV-A power set at 18 mW/cm^2 × 6.28 minutes of exposure time, pulsing the light 1 second on/1 second off with a total prolonged UV-A irradiation time of 12.56 minutes (30%). The EF I-CXL protocol is displayed in **Figure A** (available in the online version of this article) and summarized in **Table 1**.

The treatment goals were explained to the patients: stabilization of corneal ectasia, reduction of postoperative pain compared to standard CXL, reduction of infectious risk, and prevention of complications related to wound healing. After informed consent was obtained, the treatments were conducted at the Siena Crosslinking Center by the same operator (CM).

Patients underwent a preoperative full ophthalmological examination including: uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), Scheimpflug corneal tomography (Sirius; C.S.O. Florence, Italy), corneal OCT examination (AS-OCT; OptoVue, Freemont, Irvine, CA), and endothelial cell count (I-Conan, Noncon Robo, NSP-9900; Konan Medical, Tokyo, Japan). Follow-up examinations were performed at 3 days and 1, 3, 6, and 12 months. All patients completed the 12-month follow-up visit.

TABLE 2
Demographic Baseline Data

Parameter	Value
Patients	10
Eyes	12
Age (y)	24.8 (range: 15 to 36)
Male/female ratio	8/2
UDVA (logMAR)	0.50 ± 0.10
CDVA (logMAR)	0.23 ± 0.13
Maximum keratometry (D)	52.86 ± 1.50
Surface asymmetry index (D)	2.34 ± 0.36
Symmetry index (D)	4.22 ± 1.01
Coma (μm)	0.25 ± 0.05
Minimum corneal thickness (μm)	470.3 ± 39.7

UDVA = uncorrected distance visual acuity; CDVA = corrected distance visual acuity

Surgical Technique

After topical anesthesia (oxybuprocaine hydrochloride drops) instilled 10 minutes before treatment and application of a Barraquer's closed valve eyelid speculum, the ocular surface was dried with a micro-sponge, an iontophoresis suction ring was placed and centered on the corneal surface, and Ricrolin+ solution was administered and transferred by the I-ON CXL electric delivering system. After 5 minutes of imbibition, the remaining solution was aspirated by a syringe and suction was interrupted. The ocular surface was rinsed abundantly with sterile saline sodium chloride solution, eliminating all residual riboflavin on the corneal surface, and pulsed-light UV-A exposure was started. The UV-A source used was the KXL I system (Avedro, Waltham, MA) setting a fluence of 7 J/cm^2 delivered by a UV-A power of 18 mW/cm^2 with pulsed-light emission (1 second on/1 second off) in a total UV-A exposure time of 12.56 minutes to maintain the overall treatment time of less than 20 minutes. During the pulsed UV-A light emission, the epithelial surface was rinsed with the sodium chloride solution every 2 minutes. At the end of the UV-A irradiation, the corneal surface was treated with ofloxacin drops (Monoflox; Sooft) and hyaluronic acid and amino acids (Trium Free; Sooft) and dressed with a therapeutic soft contact lens (Schalcon, Rome, Italy) for 48 hours. The EF I-CXL method is outlined in **Table 1**.

Statistical Analysis

The paired Student's *t* test for statistical analysis was performed using GraphPad Prism software (version 6.0; GraphPad Software, La Jolla, CA) Differences with a *P* value of less than .05 were considered statistically significant.

RESULTS

Morphology

Baseline demographic data are shown in **Table 2**. Corneal biomicroscopic examination performed immediately after iontophoresis imbibition and at the end of the EF pulsed-light UV-A irradiation showed a superficial punctate epithelial defects also shown by riboflavin dye test instilled via a riboflavin preservative-free drop (Drop test IROS, Naples, Italy) (**Figure BA**, white arrow, available in the online version of this article). Epithelial dye test performed 72 hours after treatment at the time of therapeutic soft contact lens removal was negative, documenting a normal epithelial status (**Figure BB**). The slit-lamp examination of the cornea revealed a clearly distinguishable demarcation line (**Figure BC**, yellow arrow).

Corneal OCT examination 72 hours after treatment, before therapeutic contact lens removal, showed a clear and homogeneous demarcation line with a marked tissue hyperreflectivity (**Figure 1A**). Demarcation line depth, 72 hours after treatment, was evident in 83.3% of cases and averaged at 295.8 ± 20.2 μm depth. At the first postoperative month, the demarcation line was detectable with less intensity of stromal reflectivity, reasonably due to less stromal edema (**Figure 1B**). At the third month, the demarcation line was still visible on OCT examination, disappearing between 3 and 6 months after treatment (**Figure 1C**). Preoperative mean endothelial cell density was 2,352 cells/mm^2 (range: 2,052 to 2,996 cells/mm^2). Postoperative endothelial cell count at month 12 was 2,316 cells/mm^2 on average (range: 2,112 to 2,979 cells/mm^2).

Functional Outcomes

The overall functional outcomes are summarized in **Figure 2**.

According to the Visual Analogue Scale pain scale, the average value reported in our series after EF I-CXL was 1.5 (range: 0 to 5) on a pain scale from 0 (no pain) to 10 (unbearable pain).

DISCUSSION

According to the preliminary results and observations of the current study, despite the small sample size, the EF I-CXL pulsed-light Ricrolin+ protocol showed its capability in stabilizing progressive keratoconus. No adverse events were recorded during the entire 12-month follow-up period. This new epithelium-on method achieved all study aims. The preliminary results suggest that this EF I-CXL protocol with pulsed-light UV-A irradiation might be able to overcome some of the limits of the previous iontophoresis protocol: demarcation line evidence less than 50% and shallower stromal treatment penetration (less than 250 μm epithelium included).[14,15]

First, 30% of the UV-A photoattenuation provided by the corneal epithelium and Bowman's lamina antioxidant systems[18] was compensated for by increasing the UV-A energy dose from 5.4 to 7 J/cm^2. According to Kamaev et al.,[23] the pulsed light irradiation was introduced to potentially increase intraoperative oxygen diffusion, thus enhancing treatment efficiency. Moreover, another advantage of pulsed-light irradiation could explain the increase in treatment depth penetration according to corneal OCT and in vivo confocal microscopic evidences comparing continuous versus pulsed-light accelerated CXL treatments.[24,25] These two variations from the original I-CXL protocol allowed a superior and repeatable visualization of the demarcation line that was considered a potential cause of less efficacy of epithelium-on I-CXL compared to epithelium-off standard CXL.[26-28] The demarcation line was clearly visible in more than 80% of the examined patients at an average depth of 295.8 ± 20.2 µm at contact lens removal (72 hours after treatment) and in more than 80% of cases in the first postoperative month. These data are comparable with standard CXL evidence in the absence of complications related to wound healing (haze) and endothelial damage.

The demarcation line is morphological data that can be measured by means of in vivo confocal microscopy and corneal OCT.[26-28] It is thought to represent the estimated depth of photo-oxidative cellular damage (eg, keratocyte apoptosis and associated corneal edema) induced by CXL that modifies the reflectivity of the stroma. Basically, the demarcation line is morphological data nearly documenting the penetration of CXL treatment.[28] According to literature review data,[27,29] we know that the best functional outcomes were recorded in the epithelium-off treatments where the demarcation line was deeper and detected at least at two-thirds of the whole stromal thickness, whereas the poorer functional results were recorded after transepithelial CXL treatments, where the demarcation line was found to be superficial, poorly visible, or invisible.

The variability and unpredictability of collagen and extracellular matrix proteoglycan redistribution and rearrangement after CXL[28] may explain the nonlinear correlation between demarcation line depth and functional results. This means that there is no direct correlation between the depth of demarcation line and the functional results after CXL, but there is incontrovertible evidence that the best postoperative clinical response was statistically and practically achieved after epithelium-off CXL, where the demarcation line was deeper. Moreover, it is important to remember that CXL is not a refractive procedure but has a long-acting, unpredictable refractive impact on the cornea. The treatment goal is stiffening of the corneal structure to achieve biomechanical stabilization of the progressive ectatic diseases, and the basic ex vivo and vivo CXL studies clearly demonstrated that the best biomechanical stability can be achieved if the photo-oxidative process includes a sufficient volume of corneal stroma of at least two-thirds of the stromal thickness.[29]

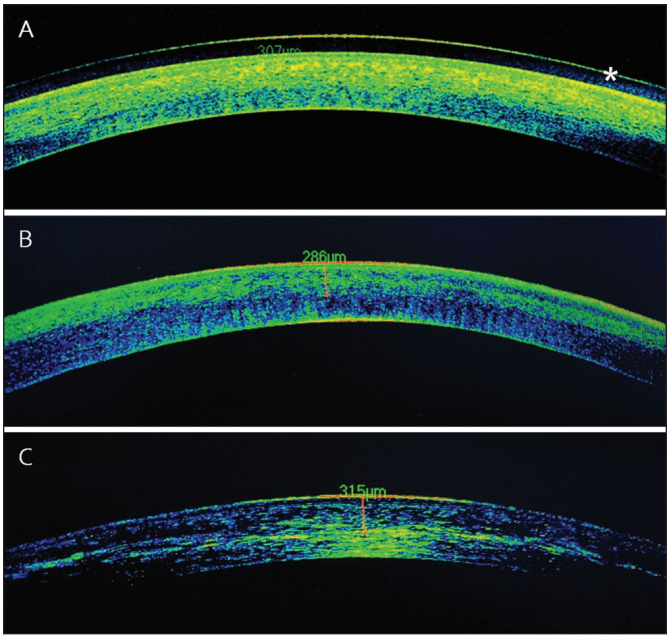
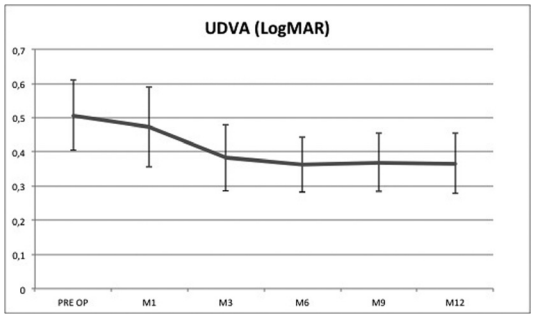


Figure 1. (A) Corneal optical coherence tomography (OCT) examination performed 72 hours after enhanced-fluence iontophoresis cross-linking (EF I-CXL) Ricrolin+ solution (Sooft, Montegiorgio, Italy) protocol showing a clear and homogeneous demarcation line with a marked tissue hyperreflectivity (asterisk). (B) Demarcation line depth, 72 hours after treatment, was evident in 83.3% of cases and averaged at 295.8 ± 20.2 µm depth. At 1 month postoperatively, the demarcation line was detectable with less intensity of stromal reflectivity due to less amount of stromal edema in more than 80% of cases. (C) At 3 months postoperatively, the demarcation line was still visible on OCT examination.

Vinciguerra et al.[29] demonstrated that when removing the epithelium after I-CXL imbibition, the efficacy of the technique improved in both functional and demarcation line detection, but the overall advantages of the epithelium-on CXL were lost.

A previous study comparing I-CXL and standard CXL reported that the demarcation line after I-CXL was inconsistent and superficially visible in only 35% of treated corneas versus 95% after standard epithelium-off CXL.[14] In another study, the same authors reported a visible demarcation line in 47.7% at a mean depth of 212 ± 36.5 µm, which was shallower compared with 97% of the standard CXL at 345 ± 24.5 µm.[15] It is hypothesized that to achieve ectasia stabilization after CXL it is advisable to achieve a treatment depth of at least 250 µm epithelium.[29] Functional results were encouraging through



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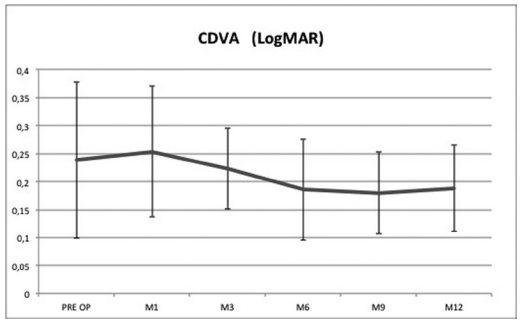

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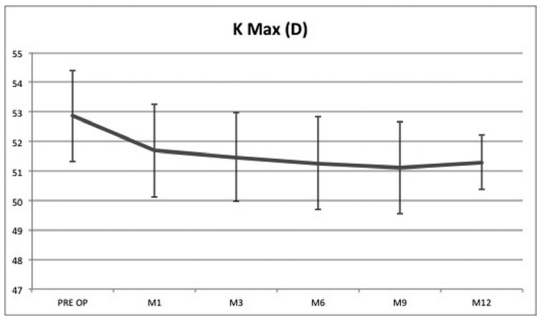

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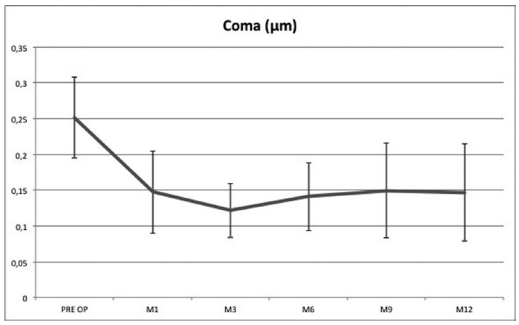

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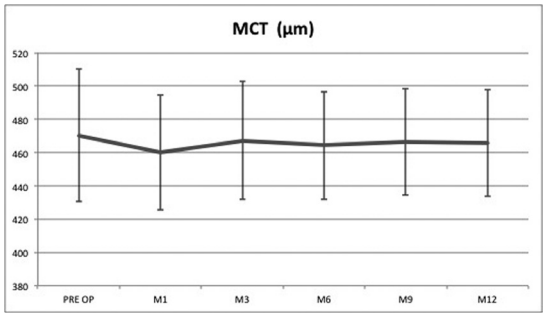

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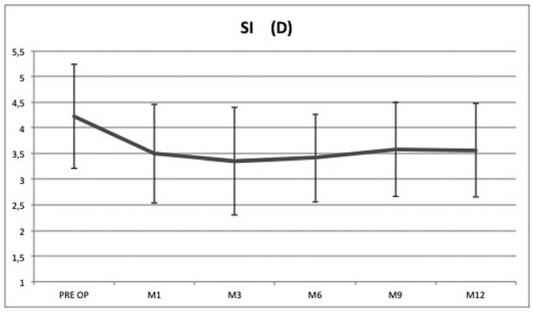

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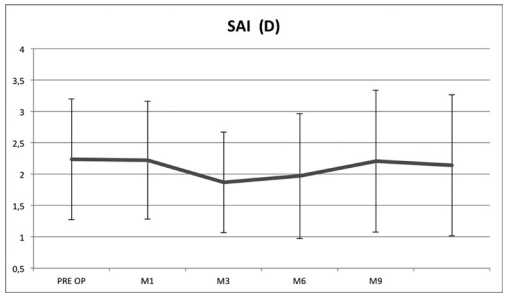

G

Figure 2. (A) Average uncorrected distance visual acuity (UDVA) decreased from a baseline value of 0.50 ± 0.10 to 0.36 ± 0.08 logMAR at 12 months postoperatively, becoming statistically significant from 3 months postoperatively to the end of follow-up ($P = .001$). (B) Average corrected distance visual acuity (CDVA) decreased from a baseline value of 0.23 ± 0.13 to 0.18 ± 0.07 logMAR at 12 months, which was not statistically significant. (C) Average maximum keratometry (Kmax) value, measured with tangential algorithm, showed a reduction from 52.86 ± 1.50 diopters (D) at baseline to 51.49 ± 0.90 D at 1-year follow-up (average delta: -1.40 ± 0.80 D), becoming statistically significant at 1 month postoperatively ($P = .0091$). (D) Average coma value showed a statistically significant improvement in the overall follow-up, starting at 1 month postoperatively, passing from an average baseline value of 0.25 ± 0.05 to 0.14 ± 0.06 µm ($P = .001$). (E) Average minimum corneal thickness (MCT) decreased from a baseline value of 470.3 ± 39.7 to 465.78 ± 32 µm at 12 months postoperatively, which was not statistically significant. (F) The average topographic symmetry index (SI) decreased from a baseline value of 4.22 ± 1.01 to 3.56 ± 0.90 D at 12 months postoperatively, becoming statistically significant at 1 month postoperatively ($P = .046$). (G) Average topographic surface asymmetry index (SAI) values decreased from a baseline value of 2.34 ± 0.36 to 2.13 ± 1.12 D at 12 months postoperatively, which was statistically significant at 1, 3, and 6 months postoperatively ($P < .05$).

a statistically significant improvement of UDVA and CDVA associated with a significant reduction of coma aberration. Together with the increase in visual acuity, morphological results showed a significant reduction of average maximum keratometry from 52.80 to 51.40 D (average delta: -1.40 ± 0.80 D) at 12-month follow-up that was closer to the standard epithelium-off procedure 1-year average results.[1-6] Indeed, Vinciguerra et al.[13] reported that morphological parameters after I-CXL showed a significant reduction of maximum keratometry in the epithelium-off standard CXL group (by -1.05 ± 1.51 D after 12 months), whereas the I-CXL group curvature was only stable (-0.31 ± 1.87 D).[13]

The 24-month study of Jouve et al.[14] comparing I-CXL with standard CXL indicated that the I-CXL halted the progression of keratoconus less efficiently than did standard CXL after 2 years of follow-up, requiring longer prospective studies to confirm long-term efficacy.[14]

A possible explanation of the inferior flattening effect of the original I-CXL procedure might be the partially absorbed energy dose[18] and less oxygen diffusion through epithelium in situ.[20]

The riboflavin intrastromal concentration after I-CXL is halved compared with the concentration obtained after epithelium removal passive diffusion.[30] This could be another limitation that requires further investigation. However, no one knows the "best" riboflavin intrastromal concentration to achieve a good CXL effect. Even if the above mentioned study[30] showed the intrastromal concentration of riboflavin with iontophoresis is 50% with respect to the standard CXL technique, the percentage of riboflavin in the anterior two-thirds of the corneal stroma would be efficacious to provide good results.

Based on our knowledge of the basic laboratory studies and science of CXL, we modified the original I-CXL protocol first of all by increasing the fluence at 7 J/cm^2 (30% over the original I-CXL) and adding the pulsed-light emission (1 second on/1 second off) according to our experience in the accelerated CXL protocols with pulsed light. The preliminary outcomes of our study, even in a small pivotal case series of 12 eyes, open the way to discovering a more efficacious transepithelial CXL procedure. Indeed, the preliminary demarcation line evidence and the 1-year functional results were encouraging and closer to standard CXL compared to the initial I-CXL protocol. Larger prospective studies with longer follow-up are needed to confirm these promising results.

AUTHOR CONTRIBUTIONS

Study concept and design (CM); data collection (CM, SAB, MF); analysis and interpretation of data (CM, RV, PV); writing the manuscript (CM, SAB, MF); critical revision of the manuscript (CM, RV, PV); statistical expertise (SAB)

REFERENCES

1. Raiskup F, Theuring A, Pillunat LE, Spoerl E. Corneal collagen crosslinking with riboflavin and ultraviolet-A light in progressive keratoconus: ten-year results. *J Cataract Refract Surg.* 2015;41:41-46.
2. Mazzotta C, Traversi C, Baiocchi S, et al. Corneal collagen cross-linking with riboflavin and ultraviolet A light for pediatric keratoconus: ten-year results. *Cornea.* 2018;37:560-566.
3. Vinciguerra R, Romano MR, Camesasca FI, et al. Corneal cross-linking as a treatment for keratoconus: four-year morphologic and clinical outcomes with respect to patient age. *Ophthalmology.* 2013;120:908-916.
4. Wollensak G, Iomdina E. Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. *J Cataract Refract Surg.* 2009;35:540-546.
5. Baiocchi S, Mazzotta C, Cerretani D, Caporossi T, Caporossi A. Corneal crosslinking: riboflavin concentration in corneal stroma exposed with and without epithelium. *J Cataract Refract Surg.* 2009;35:893-899.
6. Ghanem VC, Ghanem RC, de Oliveira R. Postoperative pain after corneal collagen cross-linking. *Cornea.* 2013;32:20-24.
7. Dhawan S, Rao K, Natrajan S. Complications of corneal collagen cross-linking. *J Ophthalmol.* 2011;2011:869015.
8. Greenstein SA, Fry KL, Bhatt J, Hersh PS. Natural history of corneal haze after collagen crosslinking for keratoconus and corneal ectasia: Scheimpflug and biomicroscopic analysis. *J Cataract Refract Surg.* 2010;36:2105-2114.
9. Leccisotti A, Islam T. Transepithelial corneal collagen cross-linking in keratoconus. *J Refract Surg.* 2010;26:942-948.
10. Caporossi A, Mazzotta C, Paradiso AL, Baiocchi S, Marigliani D, Caporossi T. Transepithelial corneal collagen crosslinking for progressive keratoconus: 24-month clinical results. *J Cataract Refract Surg.* 2013;39:1157-1163.
11. Gatzioufas Z, Raiskup F, O'Brart D, Spoerl E, Panos GD, Hafezi F. Transepithelial corneal cross-linking using an enhanced riboflavin solution. *J Refract Surg.* 2016;32:372-377.
12. Bikbova G, Bikbov M. Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin. *Acta Ophthalmol.* 2014;92:e30-e34.
13. Vinciguerra P, Romano V, Rosetta P, et al. Transepithelial iontophoresis versus standard corneal collagen cross-linking: 1-year results of a prospective clinical study. *J Refract Surg.* 2016;32:672-678.
14. Jouve L, Borderie V, Sandali O, et al. Conventional and iontophoresis corneal cross-linking for keratoconus: efficacy and assessment by optical coherence tomography and confocal microscopy. *Cornea.* 2017;36:153-162.
15. Bouheraoua N, Jouve L, El Sanharawi M, et al. Optical coherence tomography and confocal microscopy following three different protocols of corneal collagen-crosslinking in keratoconus. *Invest Ophthalmol Vis Sci.* 2014;55:7601-7609.
16. Aldahlawi NH, Hayes S, O'Brart DP, O'Brart ND, Meek KM. An investigation into corneal enzymatic resistance following epithelium-off and epithelium-on corneal cross-linking protocols. *Exp Eye Res.* 2016;153:141-151.
17. Kymionis GD, Tsoulnaras KI, Liakopoulos DA, Skatharoudi CA, Grentzelos MA, Tsakalis NG. Corneal stromal demarcation line depth following standard and a modified high intensity corneal cross-linking protocol. *J Refract Surg.* 2016;32:218-222.

18. Kolozsvári L, Nógrádi A, Hopp B, Bor Z. UV absorbance of the human cornea in the 240- to 400-nm range. *Invest Ophthalmol Vis Sci.* 2002;43:2165-2168.
19. Spoerl E, Seiler T. Techniques for stiffening the cornea. *J Refract Surg.* 1999;15:711-713.
20. Freeman RD. Oxygen consumption by the component layers of the cornea. *J Physiol.* 1972;225:15-32.
21. Harvitt DM, Bonanno JA. Oxygen consumption of the rabbit cornea. *Invest Ophthalmol Vis Sci.* 1998;39:444-448.
22. Richoz O, Hammer A, Tabibian D, Gatzioufas Z, Hafezi F. The biomechanical effect of corneal collagen cross-linking (CXL) with riboflavin and UV-A is oxygen dependent. *Trans Vis Sci Tech.* 2013;2:6.
23. Kamaev P, Friedman MD, Sherr E, Muller D. Photochemical kinetics of corneal cross-linking with riboflavin. *Invest Ophthalmol Vis Sci.* 2012;53:2360-2367.
24. Mazzotta C, Traversi C, Caragiuli S, Rechichi M. Pulsed vs continuous light accelerated corneal collagen crosslinking: in vivo qualitative investigation by confocal microscopy and corneal OCT. *Eye (Lond).* 2014;28:1179-1183.
25. Peyman A, Nouralishahi A, Hafezi F, Kling S, Peyman M. Stromal demarcation line in pulsed versus continuous light accelerated corneal cross-linking for keratoconus. *J Refract Surg.* 2016;32:206-208.
26. Bonnel S, Berguiga M, De Rivoyre B, et al. Demarcation line evaluation of iontophoresis-assisted transepithelial corneal collagen cross-linking for keratoconus. *J Refract Surg.* 2015;31:36-40.
27. Raiskup F, Spoerl E. Corneal crosslinking with riboflavin and ultraviolet A: Part II. Clinical indications and results. *Ocul Surf.* 2013;11:93-108.
28. Mazzotta C, Hafezi F, Kymionis G, et al. In vivo confocal microscopy after corneal collagen crosslinking. *Ocul Surf.* 2015;13:298-314.
29. Vinciguerra P, Romano V, Rosetta P, et al. Iontophoresis-assisted corneal collagen cross-linking with epithelial debridement: preliminary results. *Biomed Res Int.* 2016;2016:3720517.
30. Mastropasqua L, Nubile M, Calienno R, et al. Corneal cross-linking intrastromal riboflavin concentration in iontophoresis-assisted imbibition versus traditional and transepithelial techniques. *Am J Ophthalmol.* 2014;157:623-630.

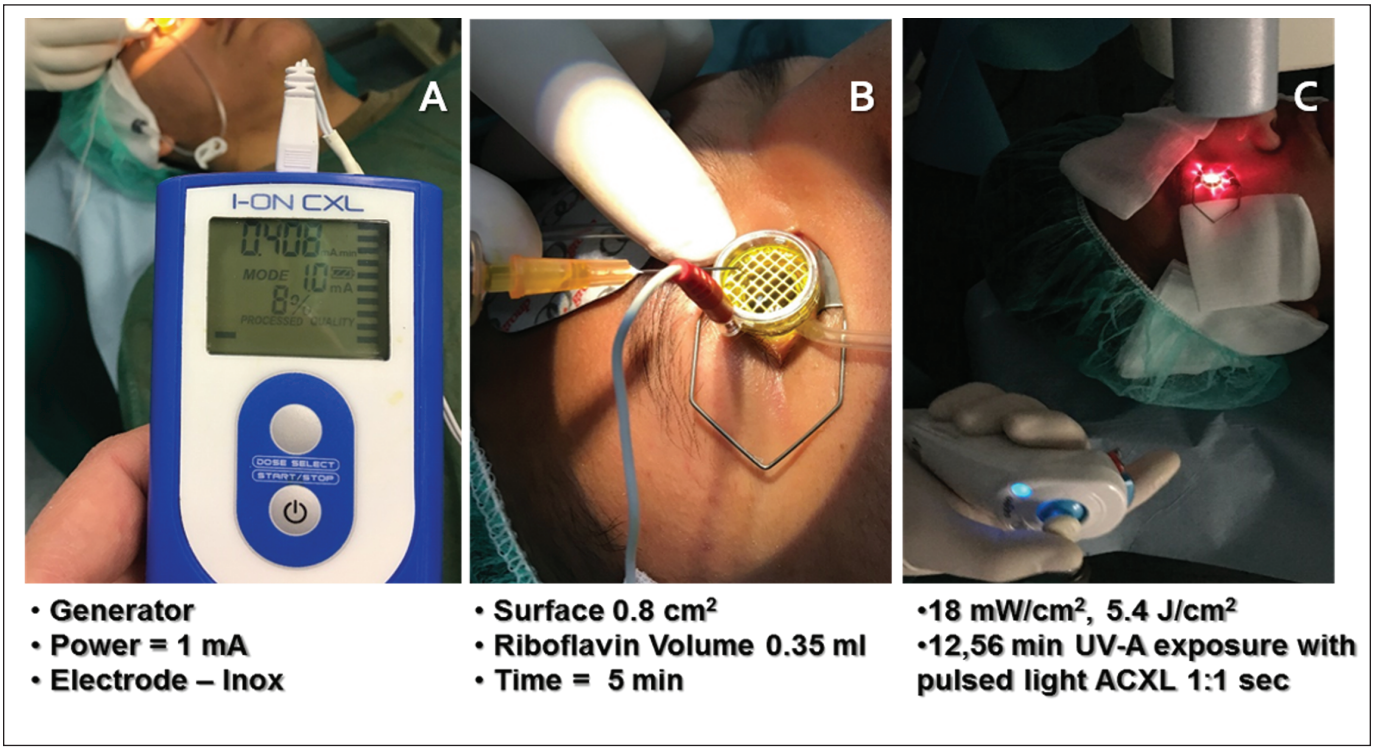

Figure A. Enhanced-fluence iontophoresis cross-linking (EF I-CXL) Ricrolin+ solution (Sooft, Montegiorgio, Italy) protocol. (A) Electric generator I-ON CXL (Sooft) set at 1 mAmpere × 5 minutes of corneal imbibition. (B) Iontophoresis-assisted corneal imbibition using a 0.8-mm diameter suction ring and 0.35 mL riboflavin 0.1% volume of Ricrolin+ solution. (C) Pulsed-light (1 second on/1 second off) ultraviolet-A (UV-A) irradiation with optimized 30% (7 J/cm^2) fluence and UV-A power set at 18 mW/cm^2 × 6.28 minutes of exposure time, with a total prolonged UV-A irradiation time of 12.56 minutes.

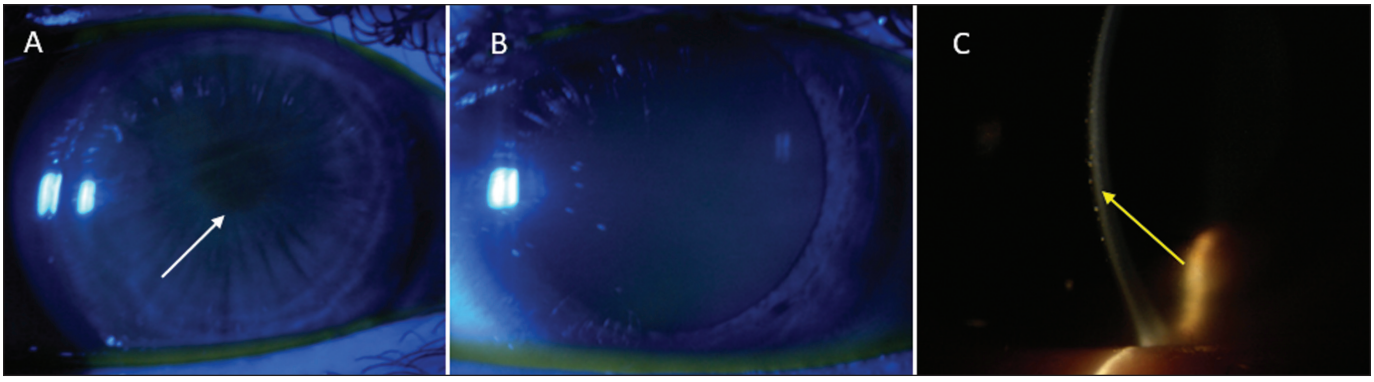

Figure B. (A) Epithelial status after enhanced-fluence iontophoresis cross-linking (EF I-CXL) protocol showed superficial punctate epithelial defects as documented by riboflavin dye test (Drop test IROS, Naples, Italy) (white arrow). (B) The epithelial dye test performed at the time of therapeutic soft contact lens removal (72 hours after treatment) was negative, showing a normal epithelial status. (C) The biomicroscopic examination of the cornea revealed a distinguishable demarcation line (yellow arrow).