Depósito de investigación de la Universidad de Sevilla

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"This is an Accepted Manuscript of an article published in Journal of Refractive Surgery on 2021 available at: https://doi.org/10.3928/1081597X-20210802-01"

Epithelial Flap Corneal Cross-linking

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Corneal cross-linking (CXL) is the current treat- ment for reducing the progression of keratoconus.[1,2] CXL predominantly affects the anterior 300 µm of the corneal stroma, causing a free radical reaction resulting from the uncorrected distance vi- sual acuity and riboflavin interaction, which in turn creates chemical bonds within the stroma.[3] Epithelial removal is the first step in CXL, aiding penetration of riboflavin into the stroma by bypassing the epithelial tight junctions.[4] Removal of epithelium exposes the subepithelial corneal nerve plexus and is associated with significant pain until the surface reepithelializes. The pain can be reduced by the use of a bandage con- tact lens or transepithelial CXL approaches, but these modifications can increase the risk of infection[5] or duce efficacy.[6-8] The use of an epithelial flap after CXL and excimer refractive surgery has been shown to re- duce postoperative pain. CXL is often associated with an anterior stromal reaction and may negatively affect vision.[9-11] This may be seen as haze at the slit lamp or using Scheimpflug imaging densitometry.[12] It has been speculated from excimer laser treatment studies that the removal of epithelium may induce addition- al haze,[13] although a study comparing conventional Epi-Off CXL to only partial removal of the epithelium did not demonstrate any difference in haze.[14]

In this study, we compared standard Epi-Off CXL to a modified Epi-Off CXL technique also known as epithelial-flap CXL (Epi-Flap CXL), which was sug- gested as an adaptation CXL by Li et al.[15] The Epi-Flap technique involves creating a hinged epithelial flap, which can be displaced to aid stromal penetration of riboflavin and can be repositioned at the end of sur- gery to cover the previously denuded stroma. The aim of this study was to investigate whether there was a difference in postoperative pain and corneal haze fol- lowing Epi-Flap CXL and Epi-Off CXL.

PATIENTS AND METHODS

Patients with progressive keratoconus referred to B-Medical Center, Sesto Calende, Italy, undergoing bi- lateral CXL were included. The tenets of the Declara- tion of Helsinki were followed with informed consent for surgery obtained from all participants at the time of intervention. One eye was treated with Epi-Off CXL and the fellow eye with Epi-Flap CXL. The first eye was treated with Epi-Flap CXL and the second with Epi-Off CXL and vice versa in a random manner. If the flap was not successful or was amputated, standard Epi-Off CXL was performed and Epi-Flap CXL was performed in the second eye. Keratometry, pachymetry, and corneal haze were measured with a Scheimpflug camera (Pentacam; Oculus Optikgeräte GmbH). The Epi-Off CXL was de- livered as described previously.[5,16] Postoperative pain was recorded using the validated Verbal Rating Scale[17] for each eye. The different levels of pain were described with adjectives and scored from 0 to 4, with “no pain” marked as 0, “mild pain” as 1, “moderate pain” as 2, “severe pain” as 3, and “unbearable pain” as 4.

CXL Technique

One minim of proxymetacaine hydrochloride 0.5% w/v eye drops was instilled (Bausch & Lomb House). An 18% alcohol solution was instilled for 30 seconds inside a MST 9-mm LASEK Epithelial Trephine (Micro-Surgical Technology) centered on the pupil (**Figure AA**, available in the online version of this article) A cellulose sponge was used to remove the alcohol solution and the trephine was removed. Balanced salt solution was used to rinse the ocular surface. For the Epi-Off technique, the epithelium was removed as previously described. For the Epi-Flap technique, the epithelium was detached and peeled back, creating a flap with a superior hinge using a 27-gauge cannula, flat with a 0.4-mm superior hole, especially de- signed for the procedure by e.Janach srl **(Figures AB-AD**) (**Video 1**, available in the online version of this article). If the flap was less than 9 mm, the peripheral corneal epithe- lum was manually removed. The flap was kept moist with riboflavin during the CXL procedure.

VibeX Rapid (Avedro) riboflavin solution was then instilled every 2 minutes for 15 minutes (**Figure 1E**). The cornea was then irradiated with ultraviolet-A 365-nm light for 15 minutes using the KXL machine (Avedro) at an irradiance of 6 mW/cm^2 delivering a total energy dose of 5.4 J/cm^2 (**Figure AF**). For the Epi-Flap CXL group, the epithelial flap was then repositioned using balanced salt solution (**Figures AG-AH**) (**Video 2**, avail- able in the online version of this article).

Postoperative Treatment

No bandage contact lenses were used in any patient. Chloramphenicol 1% ointment and cyclopentolate 1% drops were instilled. Patients were then prescribed chloramphenicol 1% ointment hourly for 3 days. Af- ter the third day, preservative-free dexamethasone eye drops were applied four times a day for 4 weeks.

Statistical Analyses

The statistical analyses were performed using STATA 14.0 software (StataCorp). All measurements were ex- pressed as mean ± standard deviation. The normality of all data was estimated using the Shapiro-Wilk normal- ity test. Pain score, as an ordinal non-continuous vari- able, was expressed with median and interquartile range (IQR). Comparisons between the two different groups at baseline and at 12 months of follow-up were performed with the two-sample *t* test in case of normally distributed variables and with the Wilcoxon rank-sum test in case of non-normally distributed variables. Comparisons for the same patient at 12 months of follow-up versus baseline were performed with a paired *t* test in case of normally distributed variables and with matched-pairs Wilcoxon signed-rank test in case of non-normally distributed vari- ables. A *P* value of less than .05 was considered statisti- cally significant and was adjusted with the Bonferroni correction according to the number of tests performed.

RESULTS

Twenty-four eyes of 12 patients (mean age: 27.15 ± 5.15 years, 8 women and 4 men) were included. In the 12 eyes in which the epithelial flap was completed, 2 had tears and small lacerations. Other than tearing of the epi- thelial flap, no intraoperative or postoperative complica- tions occurred. All flaps were in place the next day. There were no significant differences between eyes in the two groups before treatment (**Table 1**). For both groups, there were significant changes in corneal thickness, maximum keratometry, and densitometry following CXL, but not in corrected distance visual acuity (**Table 2**). There was no difference in the change in these parameters compared to baseline between the Epi-Off CXL and Epi-Flap CXL groups, except for anterior densitometry, which was higher in the Epi-Off

CXL group (**Table 3** and **Figure B**, available in the online version of this article).

The Verbal Rating Scale pain scores (0 to 4) were significantly lower for the Epi-Flap CXL group com-

TABLE 1
Baseline Epi-Off CXL and Epi-Flap CXL Parameters[a]

Parameter	Epi-Off CXL	Epi-Flap CXL	*p*b
CDVA (logMAR)	0.27 ± 0.17	0.31 ± 0.21	.69
Corneal thickness (µm)	471.0 ± 22.14	454.1 ± 32.55	.12
Kmax (D)	52.55 ± 6.58	56.91 ± 7.32	.072
Anterior densitometry	20.21 ± 2.11	20.56 ± 2.10	.39
Center densitometry	13.09 ± 1.21	13.56 ± 1.22	.62
Posterior densitometry	10.51 ± 1.22	10.60 ± 0.91	.70

Epi-Off CXL = standard epithelium-off corneal cross-linking; Epi-Flap CXL = corneal cross-linking with an epithelial flap; CDVA = corrected distance visual acuity; Kmax = maximum keratometry; D = diopters
[a]*Values are reported as mean ± standard deviation.*
[b]*According to the Bonferroni correction, the level of significance is* P < *.008.* P *value highlights that there is no difference between the two populations at baseline.*

TABLE 2
Epi-Off CXL and Epi-Flap CXL Baseline and 12-Month Follow-up Parameters[a]

Parameter	Baseline	12 Months Postoperative	*p*b
Epi-off CXL			
CDVA (logMAR)	0.27 ± 0.17	0.25 ± 0.36	.58
Corneal thickness (µm)	471.0 ± 22.14	449.73 ± 33.58	.0011
Kmax (D)	52.55 ± 6.58	51.41 ± 6.03	.0007
Anterior densitometry	20.21 ± 2.11	25.04 ± 2.69	.0007
Central densitometry	13.09 ± 1.21	15.23 ± 1.72	.0007
Posterior densitometry	10.51 ± 1.22	11.58 ± 1.39	.0016
Epi-Flap CXL			
CDVA (logMAR)	0.31 ± 0.21	0.28 ± 0.22	.503
Corneal thickness (µm)	454.1 ± 32.55	439.27 ± 35.16	.0006
Kmax (D)	56.91 ± 7.32	55.55 ± 7.23	.0007
Anterior densitometry	20.56 ± 2.10	22.74 ± 2.30	.0007
Central densitometry	13.56 ± 1.22	14.87 ± 1.57	.0006
Posterior densitometry	10.60 ± 0.91	11.01 ± 1.05	.0248

Epi-Off CXL = standard epithelium-off corneal cross-linking; Epi-Flap CXL = corneal cross-linking with an epithelial flap; CDVA = corrected distance visual acuity; Kmax = maximum keratometry; D = diopters

[a]*Values are reported as mean ± standard deviation.*

[b]P *value expresses whether there is a statistical difference between the baseline and the 12-month follow-up in each group. Bonferroni correction has been applied, so the accepted level of significance is* P < *.008.*

pared to the Epi-Off CXL group on both the first (1.00 [IQR: 0.00 to 1.00] vs 3.00 [IQR: 3.00 to 3.75], *P* = .01)
and third (0.00 [IQR: 0.00 to 1.00] vs 1.00 [IQR: 0.00
to 1.00], *P* = .01) postoperative days. No pain was ob- served in both groups after the third day.

DISCUSSION

Our results demonstrate that there was no differ- ence in outcome between eyes that underwent Epi-Off or Epi-Flap CXL in terms of corrected distance visual acuity, maximum keratometry, and corneal thickness at 1 year postoperatively. Of note, however, patients reported significantly less postoperative pain from the eye that underwent Epi-Flap CXL compared to the eye that had Epi-Off CXL. Postoperative pain after CXL is a significant concern for patients and can lead to tempo- rary disability for patients lasting up to 5 days follow- ing treatment.[18] The pain has been attributed to exposed and injured nerve fibers aggravated by pain-inducing factors such as eyelid movement.[19] For eyes that un- derwent Epi-Flap CXL, the retention of the epithelium, although unstable, may help reduce these factors. With the Epi-Flap technique, there was a comparative signifi- cant reduction in pain on days 1 and 3 after CXL. This is consistent with the findings of Liu et al,[15] who dem- onstrated a reduction in pain following Epi-Flap CXL in a study of 27 eyes receiving standard Epi-Off or Epi- Flap CXL. Similarly, pain reported after excimer refrac-

TABLE 3
Difference (D) Between Epi-Off CXL and Epi-Flap CXL Parameters at Baseline and at 12-Month Follow-up in Each Group[a]

Parameter	D **Epi-Off CXL**	D **CXL Epi-Flap**	***p*b**
Corneal thickness (μm)	-23.3 ± 21.0	-16.8 ± 12.3	.42
Kmax (D)	-1.44 ± 0.96	-1.61 ± 1.43	.96
Anterior densitometry	3.6 ± 1.1	2.0 ± 1.0	.0003
Center densitometry	1.0 ± 0.7	0.9 ± 0.7	.25
Posterior densitometry	0.6 ± 0.8	0.3 ± 0.7	.14

Epi-Off CXL = standard epithelium-off corneal cross-linking; Epi-Flap CXL = corneal cross-linking with an epithelial flap; Kmax = maximum keratometry; D = diopters

[a]*Values dre reported as mean ± standard deviation.*

[b]P *value expresses whether there is a statistical difference between the* D *of the two groups. Bonferroni correction has been applied, so the accepted level of signifi- cance is* P < *.01*.

tive surgery was reduced by the use of epithelial flaps compared to transepithelial treatments.[20]

Corneal haze after CXL has been well document- ed.[10,11,21,22] Transparency of the cornea is dependent on uniformly sized and regularly organized collagen fibrils and stationary keratocytes.[23,24] Corneal haze following CXL is believed to develop from transient changes in collagen fibril arrangements and the cellular compo- nents of the stroma.[22,24] CXL is understood to demon- strate the maximum reaction in the anterior stroma. In a study of 31 eyes, Pircher et al[25] reported an increase in corneal haze during the first 3 months following Epi-Off CXL with a slow subsequent reduction. In our cohort, although corneal haze was present in both groups, we found significantly less haze in the anterior stroma of the Epi-Flap CXL group. Bouheraoua et al[26] compared standard Epi-Off CXL to transepithelial CXL and re- ported that the keratocyte density in the anterior stroma was significantly greater following transepithelial CXL at 6 months, suggesting a more rapid recovery in the latter. The exact mechanisms for this are not fully elu- cidated but suggest a supportive effect from the remain- ing corneal epithelium in regenerating the stroma after the CXL. Similarly, a protective role of an epithelial flap in aiding recovery and preventing haze has been dem- onstrated after laser refractive surgery.[13]

Stromal penetration of the riboflavin is a crucial step in the CXL process.[27] It is well known that the epitheli- um is a barrier for the penetration of riboflavin. Studies in vitro have demonstrated that the stromal concentration is significantly lower with transepithelial application of riboflavin.[28] This may partially explain why results from transepithelial CXL are inferior to standard Epi-Off CXL at halting keratometric progression.[6-8] Modalities such as iontophoresis to aid riboflavin penetration have been explored. Lombardo et al[29] and Pagano et al[30] compared 22 eyes treated with CXL with iontophoresis and 12 eyes with conventional Epi-Off CXL. Although progression was halted in both, the Epi-Off CXL group showed flat-

tening and improvement in corneal topography readings not observed in the iontophoresis group.[29-30] Recurrent corneal erosions are a possible complication after laser epithelial keratomileusis. We did not observe any late re- current erosion in our patients, possibly indicating that the preservation of the Bowman membrane during CXL procedures could play a role in corneal epithelial healing. An important limitation to this study is the patient being able to distinguish pain separately for each eye. Pain is variable between patients but less so within pa- tients, and we suggest that the within-eye differences in pain are relevant. Unfortunately, we did not collect data on the stability of the epithelium in the Epi-Flap CXL group and to the extent to which it became disrupted following treatment.

Another limitation of the study could be that we did not use a bandage contact lens. This could possibly ex- plain a higher pain level and corneal haze postopera- tively in the Epi-Off CXL group. However, we did not apply a bandage contact lens in this group because it has been reported that it may entail an increased risk of microbial keratitis.[5]

Additionally, the use of chloramphenicol ointment could act in favor of the Epi-Flap CXL group, influenc- ing the difference of pain feeling in the third postop- erative day.

Despite these limitations, our study would suggest that Epi-Flap CXL is associated with less pain and an- terior stromal haze than Epi-Off CXL and this would support its use in patients undergoing CXL for kerato- conus. Further studies with more patients, perhaps a confocal analysis of the corneal stroma, and the evalu- ation of the demarcation line would be helpful in the understanding of this new surgical technique for CXL.

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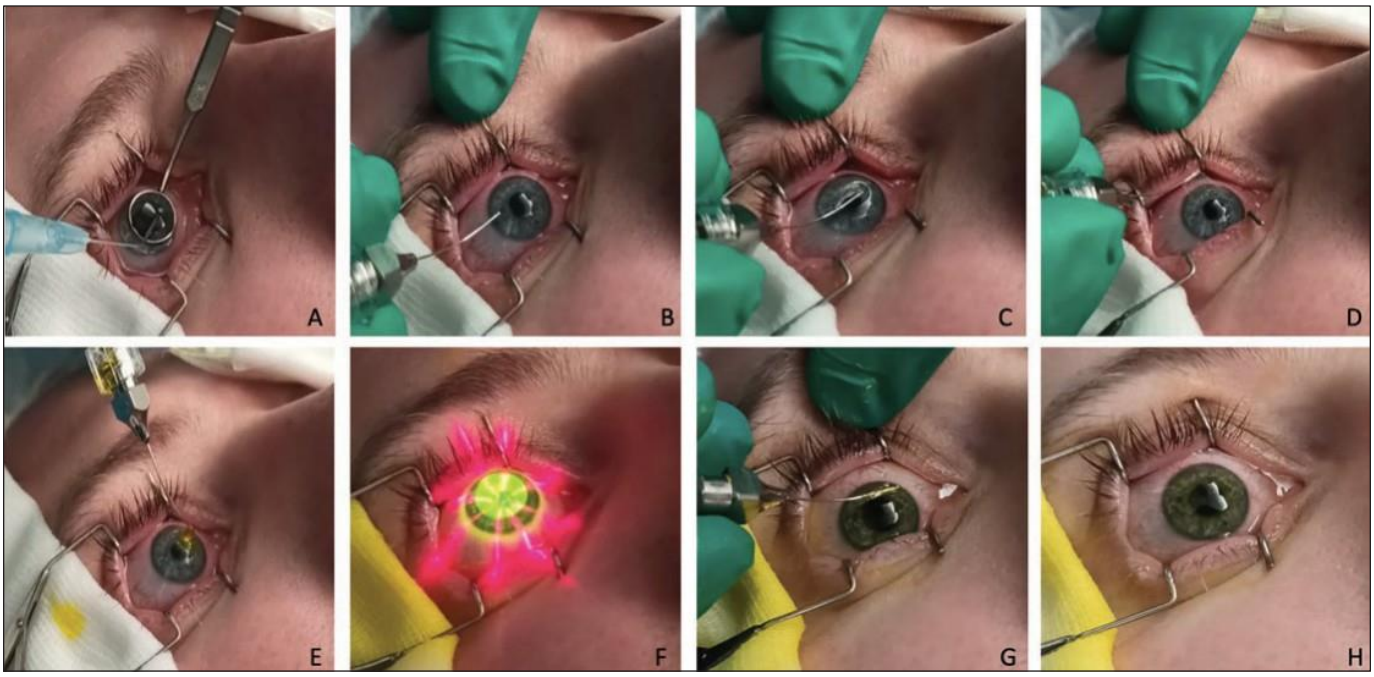
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