Video Article

Three Different Protocols of Corneal Collagen Crosslinking in Keratoconus: Conventional, Accelerated and Iontophoresis

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Abstract

Keratoconus is a bilateral and progressive corneal ectasia. In order to slow down its progression, corneal collagen cross-linking (CXL) has recently been introduced as an efficient treatment option. In biological and chemical sciences, crosslinking refers to new chemical bonds formed between reactive molecules. Hence, the aim of corneal collagen CXL is to synthetically increase the formation of crosslinks between collagen fibrils in the corneal stroma. Despite the fact that the efficiency of the conventional CXL (C-CXL) protocol has already been shown in several clinical studies, it might benefit from improvements in duration of the procedure and removal of corneal epithelium. Hence, in order to provide a coherent evaluation of two new and optimized CXL protocols, we studied keratoconus patients who had undergone one of the three CXL treatments: iontophoresis (I-CXL), accelerated CXL (A-CXL), and conventional CXL (C-CXL). A-CXL is a 6 time faster CXL procedure using a ten time higher UVA irradiance but still including an epithelium removal. Iontophoresis is a transepithelial non-invasive technique in which a small electric current is applied to improve riboflavin penetration throughout the cornea. Using anterior segment optical coherence tomography (AS OCT) and *in vivo* confocal microscopy (IVCM), we conclude that regarding the depth of treatment penetration, conventional CXL protocol remains the standard for treating progressive keratoconus. Accelerated CXL seems to be a quick, effective and safe alternative to treat thin corneas. The use of iontophoresis is still being investigated and should be considered with greater caution.

Video Link

The video component of this article can be found at https://www.jove.com/video/53119/

Introduction

Keratoconus is a bilateral and progressive corneal ectasia usually reported in 1 in 2,000 in the general population[1] resulting in modification of the corneal shape and thus decreased vision[2]. Keratoconus is usually present in early puberty and progresses until the third to fourth decade of life when the disease typically tends to stabilize, although progression can be variable throughout a patient's life. By halting keratoconus progression, cross linking aims at postpone or avoid keratoplasty.

To date, the only efficient and safe treatment of progressive keratoconus proven in clinical studies is the conventional corneal collagen cross-linking (C-CXL) protocol, which aims to increase stiffness and hence halt keratoconus progression[3-8]. In order to reduce operation time and other possible risk factors of C-CXL, such as infectious keratitis or stromal haze[9], several improved protocols have been described. First, in accelerated CXL (A-CXL), a higher irradiance of UVA is delivered to the cornea over a reduced time[10]. Secondly, to avoid the necessity for epithelial debridement, transepithelial approaches have been employed. Unfortunately, they have limited success when compared to the conventional protocol[11]. The most recent transepithelial method for corneal riboflavin delivery during CXL is iontophoresis (I-CXL), but rigorous evaluation of this treatment has not yet been performed[12]. Iontophoresis is a non-invasive technique in which a small electric current is applied to improve an ionized drug's penetration through a tissue. In CXL by iontophoresis, the riboflavin is ionized to penetrate the cornea through the epithelium.

In vivo confocal microscopy (IVCM) is a method of imaging the cornea that can highlight the cellular changes of abnormal corneas in diseases such as keratoconus[13]. Indeed, IVCM has demonstrated alterations to all layers of the cornea in keratoconus with a particular reduction in density of the sub-basal nerve plexus and stromal keratocytes[13-15]. Plus, IVCM has proven to be highly convenient for microstructural analysis of the cornea after C-CXL[16].

The corneal demarcation line is described as a hyperreflective line seen in anterior segment optical coherence tomography (AS OCT) 1 month after C-CXL at a depth of 300 µm[17,18]. IVCM following C-CXL provides information about corneal structural alterations, including the absence of

corneal keratocytes to a depth of 300 µm. The depth of this acellular zone, as well as the depth of the demarcation line within the corneal stroma revealed on AS OCT, seems to be associated with the effective depth of CXL treatment[19], and measurement of the corneal demarcation line depth in AS OCT 1 month after CXL has been proposed as an efficient clinical method for evaluation of CXL effectiveness[18].

In the present study we investigate the efficiency of three different protocols of corneal collagen crosslinking (conventional, accelerated, and iontophoresis) using measurement of the corneal stromal demarcation line by AS OCT and confocal microscopy. We furthermore used IVCM to quantitatively analyze corneal microstructure changes after the three treatments.

Protocol

These protocols follow the guidelines of our institution's human research ethics committee.

1. Conventional Corneal Collagen CXL (C-CXL)

1. Preparation of the Patient

1. 5 days before the surgery, put 1% pilocarpine drops twice a day in the treated eye.
2. In the operating room, in aseptic conditions, lie the patient on his/her back.
3. Administer topical anesthesia such as oxybuprocaine 0.4%.
4. Clean the eye and the skin around the eye with iodine antiseptic twice.
5. Use a lid speculum to keep the eye open.

2. Epithelial Removal

1. Mark the central 9.0 mm of the cornea with a circle corneal marker.
2. Remove the central 7.0 to 9.0 mm of corneal epithelium by mechanical debridement using a blunt spatula.

3. Riboflavin Application

1. Apply 0.1% riboflavin with 20% Dextran on the cornea every min for 20 min.

4. UVA Irradiation

1. Irradiate the cornea with a 370 nm wavelength UVA light at an irradiance of 3 mW/cm^2 (5.4 J/cm^2 surface dose) and at a 5 cm working distance for 30 min.

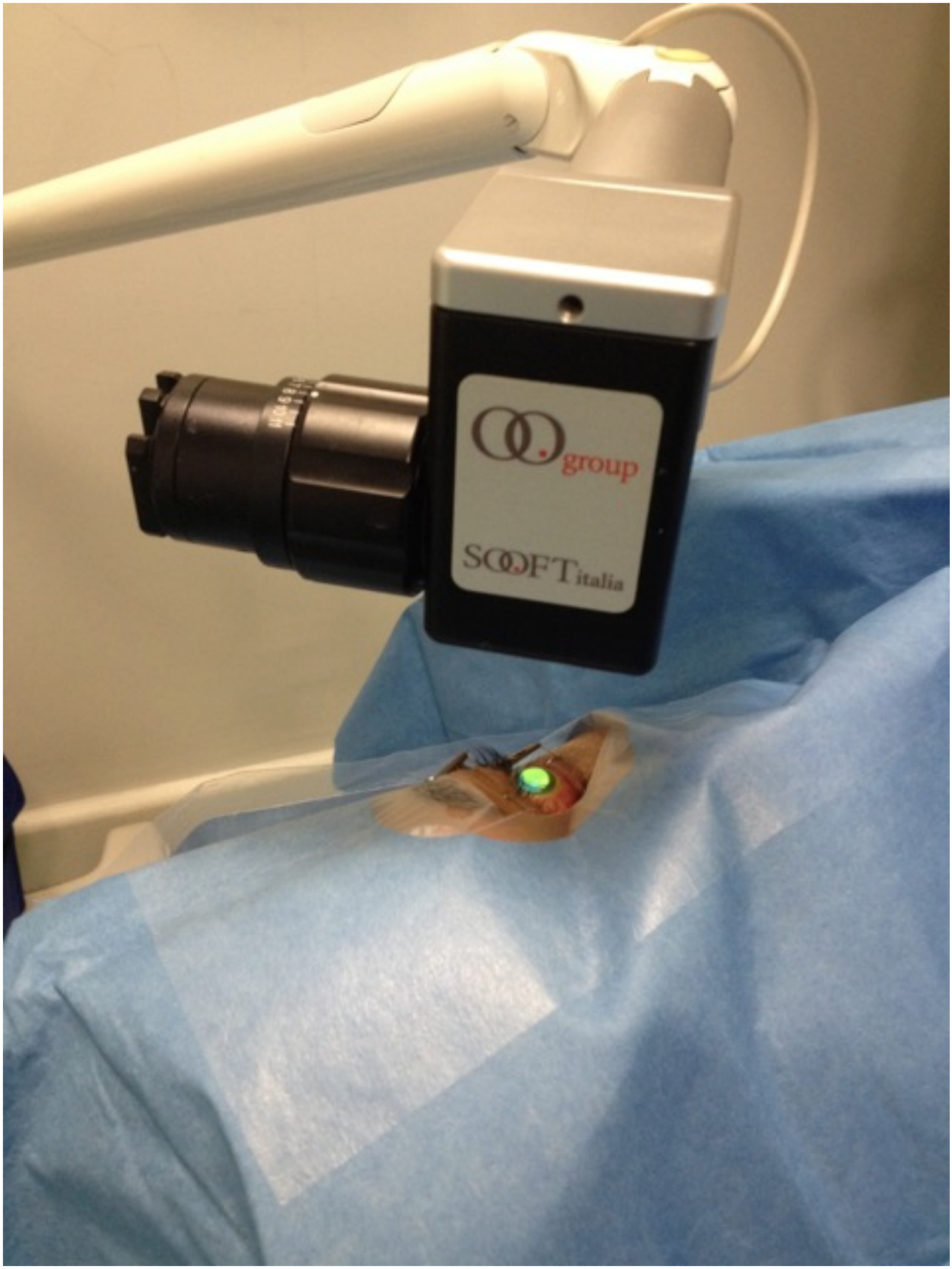
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Figure 1: UVA irradiation in C-CXL. The cornea is irradiated with a 370 nm wavelength UVA light at an irradiance of 3 mW/cm^2 (5.4 J/cm^2 surface dose) and at a 5 cm working distance for 30 minutes. Please click here to view a larger version of this figure.

2. During irradiation, apply drops of riboflavin to the cornea every 5 min.
3. During irradiation, add topical anesthesia (oxybuprocaine 0.4%) if necessary.

5. End of the Surgery

1. Put antibiotic drops (tobramycin 0.3%) and artificial tears (hyaluronate drops 0.18%) into the operated eye.
2. Place a soft bandage contact lens at the end of surgery until re-epithelialization is complete. Re-epithelialization usually takes 3 days.
3. Prescribe analgesics such as paracetamol (500 mg) plus codeine (30 mg), 6 pills a day.
4. After corneal re-epithelialization, initiate topical therapy with steroids (topical dexamethasone 1 mg/ml) and continue for 3-4 weeks. Plus, use artificial tears 4 times a day for 1 month.

2. Accelerated Corneal Collagen CXL (A-CXL)

1. Preparation of the Patient

1. 5 days before the surgery, put 1% pilocarpine drops twice a day in the treated eye.
2. In the operating room, in aseptic conditions, lie the patient on his/her back.
3. Administer topical anesthesia such as oxybuprocaine 0.4%.
4. Clean the eye and the skin around the eye with iodine antiseptic twice.
5. Use a lid speculum to keep the eye open.

2. Epithelial Removal

1. Mark the central 9.0 mm of the cornea with a circle corneal marker
2. Remove the central 7.0 to 9.0 mm of corneal epithelium by mechanical debridement using a blunt spatula.

3. Riboflavin Application

1. Apply 0.1% riboflavin without Dextran on the cornea every 2 min for 10 min.

4. UVA Irradiation

1. Irradiate the cornea with a 370 nm wavelength UVA light at an irradiance of 30 mW/cm^2 (5.4 J/cm^2 surface dose) and at a 5 cm working distance for 3 min.
2. During the irradiation, add topical anesthesia (oxybuprocaïne 0.4%) if necessary.

5. End of the Surgery

1. Place antibiotic drops (tobramycin 0.3%) and artificial tears (hyaluronate drops 0.18%) into the operated eye.
2. Place a soft bandage contact lens at the end of surgery until re-epithelialization is complete. Re-epithelialization usually takes 3 days.
3. Prescribe analgesics such as paracetamol (500 mg) plus codeine (30 mg), 6 pills a day.
4. After corneal re-epithelialization, initiate topical therapy with steroids (topical dexamethasone 1 mg/ml) and continue for 3-4 weeks. Plus, use artificial tears 4 times a day for 1 month.

3. Iontophoresis (I-CXL)

1. Preparation of the Patient

1. 5 days before the surgery, put 1% pilocarpine drops twice a day in the treated eye.
2. In the operating room, in aseptic conditions, lie the patient on his/her back.
3. Administer topical anesthesia such as oxybuprocaine 0.4%.
4. Clean the eye and the skin around the eye with iodine antiseptic twice.
5. Use a lid speculum to keep the eye open.

2. Position the Iontophoresis Device.

1. Apply the sticky passive electrode on the forehead under the operative field.
2. Apply the active electrode, a suction ring, to the open eye. Center the suction ring on the periphery of the cornea before releasing the suction.

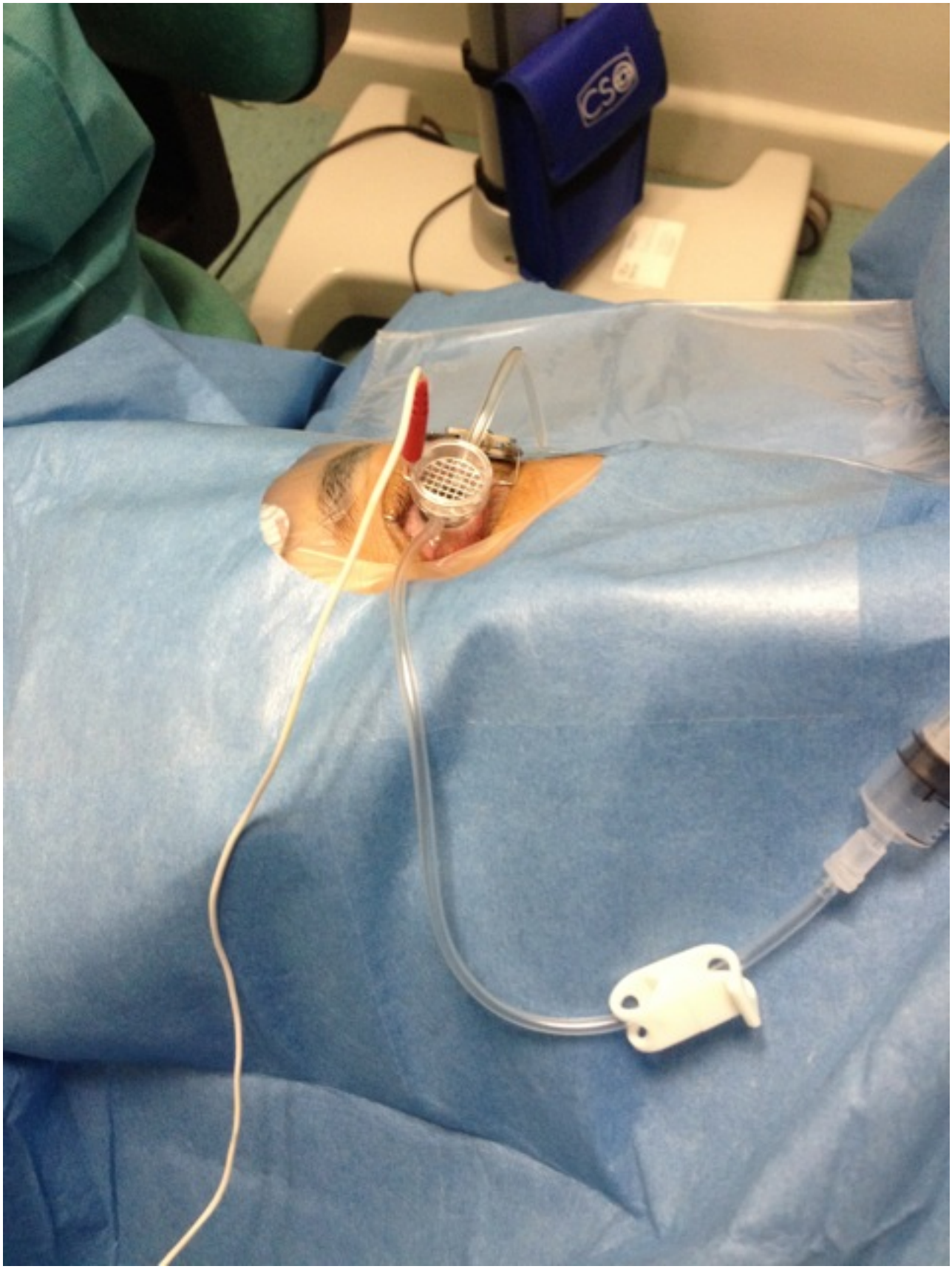

Figure 2. Iontophoresis device. The passive electrode is applied on the forehead under the operative field and the active electrode, a suction ring, is applied to the open eye. Please click here to view a larger version of this figure.

3. Riboflavin Application

1. Fill the suction ring with hypoosmolar 0.1% riboflavin without Dextran.

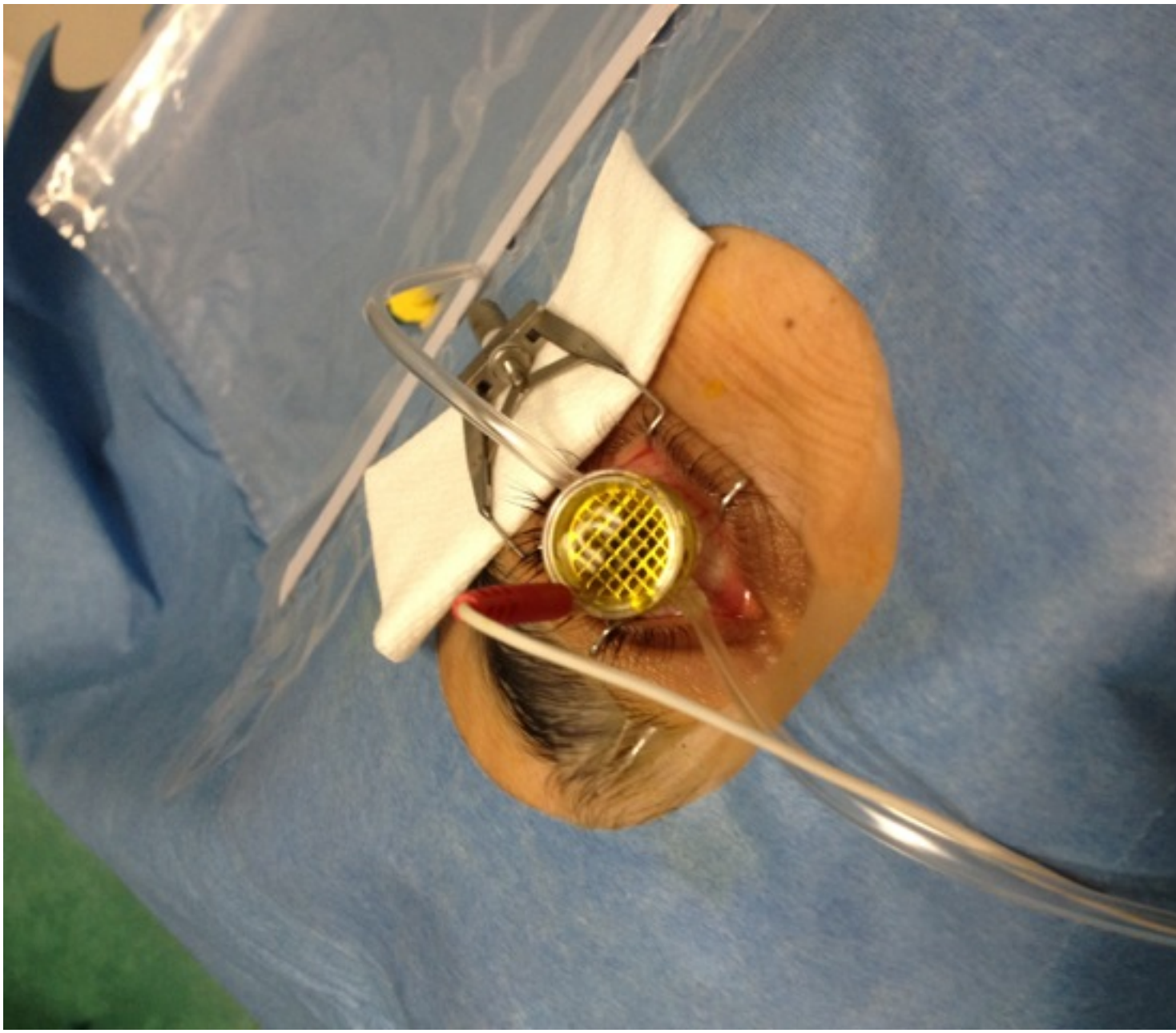

Figure 3. Riboflavin application in I-CXL. The suction ring is filled with hypoosmolar 0.1% riboflavine without Dextran. Please click here to view a larger version of this figure.

2. Start the electrical current at 0.2 mA and gradually increase to 1.0 mA for a total iontophoresis time of 5 min (**Figure 4**).

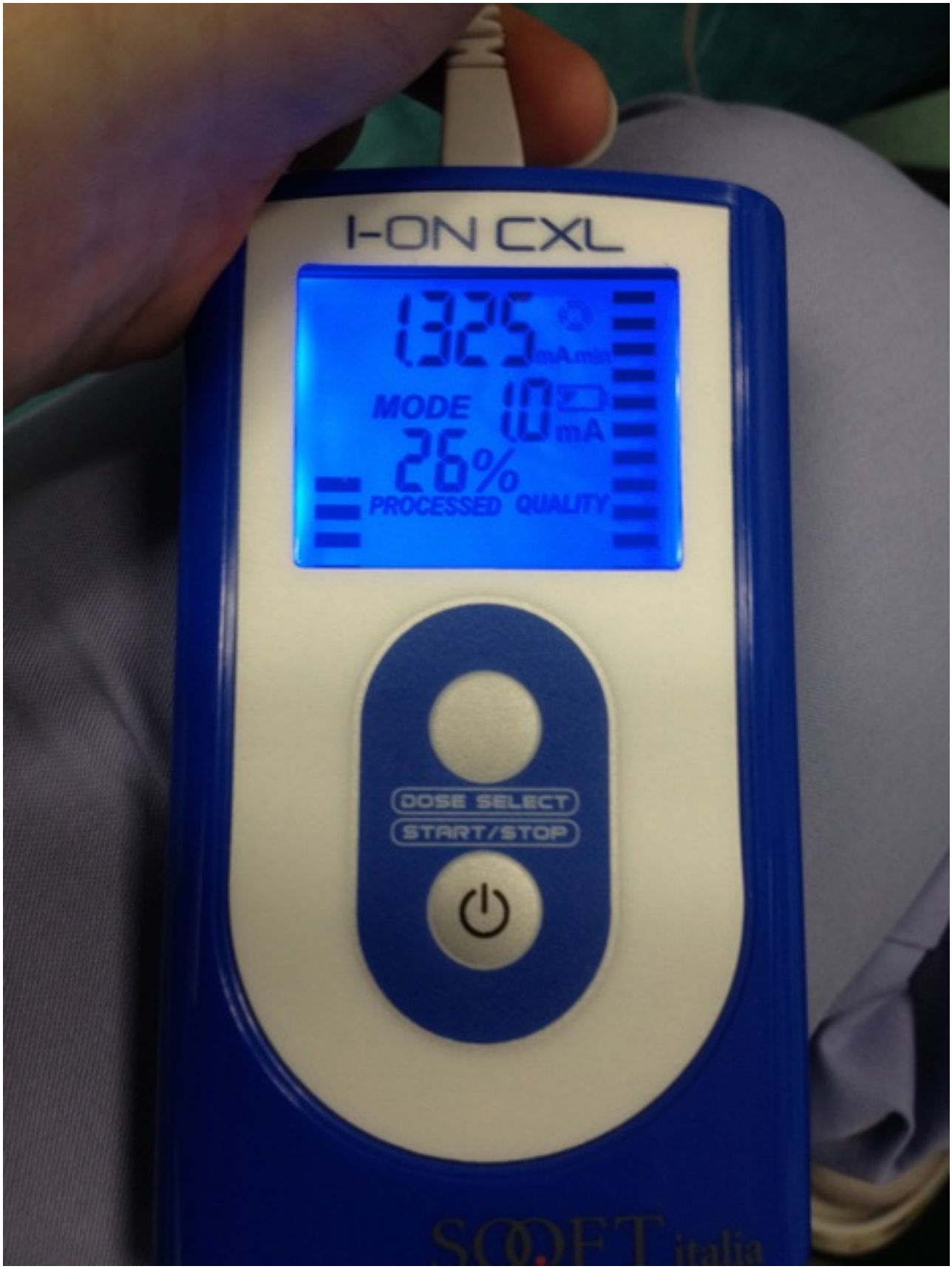
I-ON CXL
1325
mA min
MODE
10
mA
26%
PROCESSED QUALITY
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Figure 4. Iontophoresis device for riboflavin penetration. The electrical current is initially 0.2 mA and gradually increased to 1.0 mA. Total iontophoresis time is 5 minutes. Please click here to view a larger version of this figure.

4. UVA Irradiation

1. Irradiate the cornea with a 370 nm wavelength UVA light at an irradiance of 10 mW/cm^2 (5.4 J/cm^2 surface dose) and at a 5 cm working distance for 9 min.
2. During the irradiation, add topical anesthesia (oxybuprocaïne 0.4%) if necessary.

5. End of the Surgery

1. Place antibiotic drops (tobramycin 0.3%) and artificial tears (hyaluronate drops 0.18%) into the operated eye.
2. Prescribe analgesics such as paracetamol (500 mg) plus codeine (30 mg), 6 pills a day.
3. After surgery, initiate topical therapy with steroids (topical dexamethasone 1mg/ml) and continue for 3-4 weeks. Plus, use artificial tears 4 times a day for 1 month.

Representative Results

The corneal demarcation line was visible in AS OCT in 92% of cases at a mean depth of 301.6 µm (SD, 73.6)

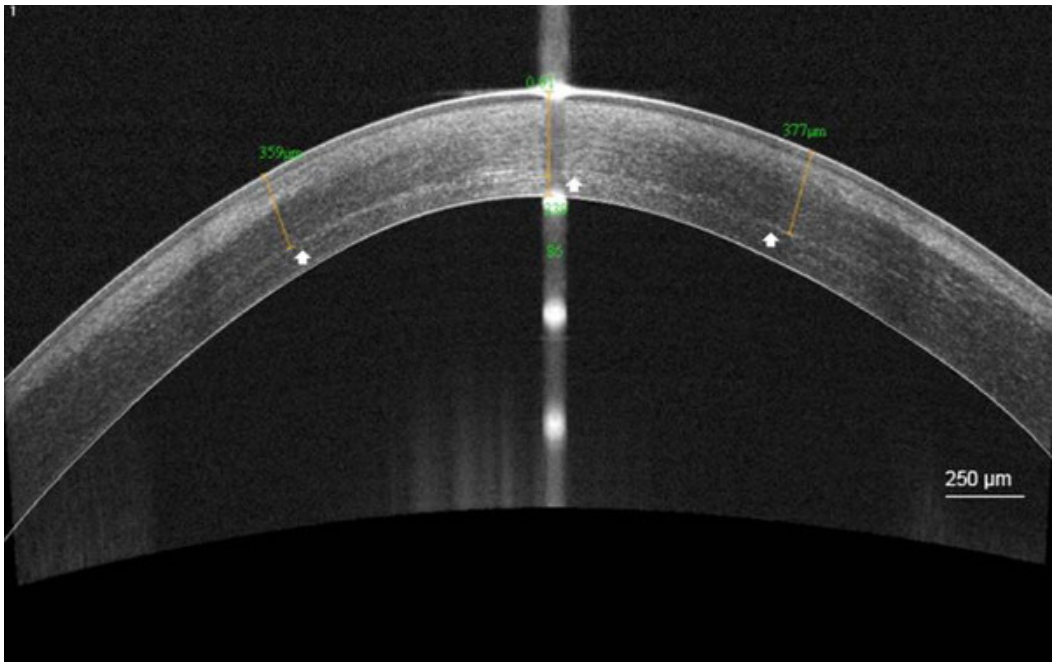

Figure 5. Demarcation line after C-CXL. High-resolution corneal anterior segment optical coherence tomography scan (AS OCT) visualizing the corneal stromal demarcation line at a mean depth of 358 µm (white arrow), 1 month after conventional corneal collagen cross-linking (C-CXL). Scale bar 250 µm. Please click here to view a larger version of this figure.

1 month after C-CXL, whereas after A-CXL it was seen in 85.5% of cases at a mean depth of 183.1 µm (SD, 39.6).

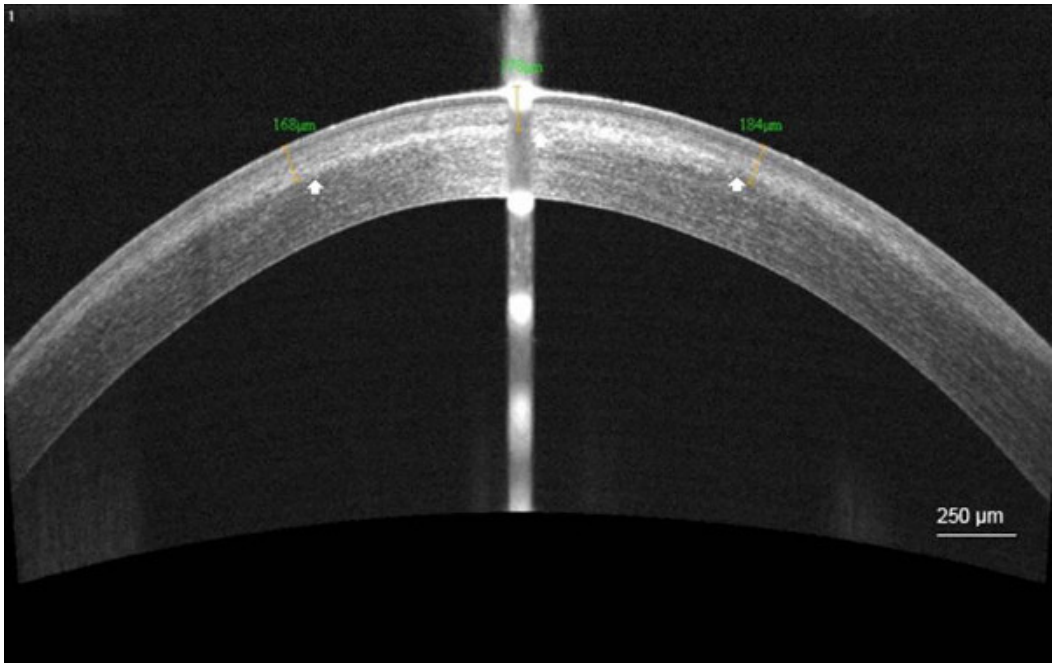

Figure 6. Demarcation line after A-CXL. High-resolution corneal anterior segment optical coherence tomography scan (AS OCT) visualizing the corneal stromal demarcation line at a mean depth of 176 µm (white arrow), 1 month after accelerated corneal collagen cross-linking (A-CXL). Scale bar: 250 µm. Please click here to view a larger version of this figure.

Finally, after I-CXL, the corneal demarcation line was only seen in 46.5% of cases at a mean depth of 214 µm (SD, 37.5). The differences in corneal demarcation line depth following either C-CXL, A-CXL or I-CXL were statistically significant ($p < 0.001$ and $p = 0.01$). The demarcation line was present significantly more often after C-CXL and A-CXL than after I-CXL ($p = 0.005$).

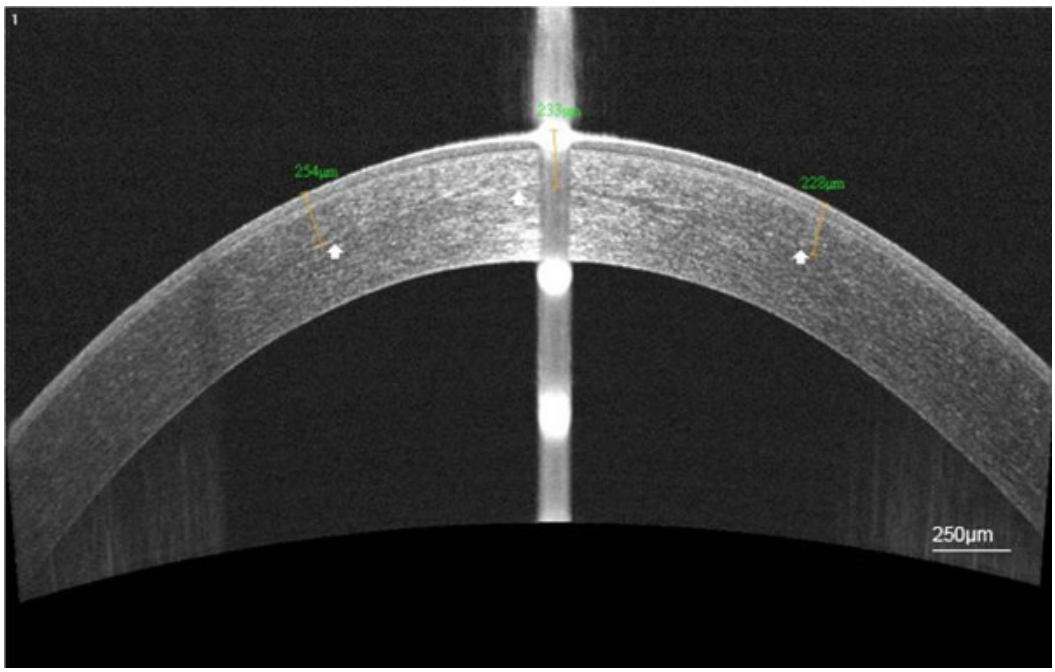

Figure 7. Demarcation line after I-CXL. High-resolution corneal anterior segment optical coherence tomography scan (AS OCT) visualizing the corneal stromal demarcation line at a mean depth of 238.5 µm (white arrow), 1 month after iontophoresis (I-CXL). Scale bar: 250 µm. Please click here to view a larger version of this figure.

No intra or postoperative complications were detected in patient follow-ups within 6 months after application of any of the three protocols, including no significant differences in endothelial cell counting. Moreover, the maximum K-value (Kmax) remained stable for each of the protocols after a 6 month follow-up.

	C-CXL	A-CXL	I-CXL
Evolution of the higest K-value (D) :			
Preoperative, mean (SD)	52.7 (5.9)	48.9 (6.6)	49.8 (5.6)
6 months, mean (SD)	50.9 (5.5)	49.4 (6.8)	50.2 (5.0)
p wilcoxon signed rank test	0.09	0.06	0.28
Evolution of specular microscopy :			
Preoperative, mean (SD)	2764 (251)	2592 (433)	2520 (336)
6 months, mean (SD)	2760 (336)	2612 (353)	2433 (274)
p wilcoxon signed rank test	0.46	0.85	0.15

Table 1. Efficacy and safety of each protocol of CXL. Evolution of the maximum K-value (dioptry, D) and the endothelial density following conventional (C-CXL), accelerated (A-CXL), and iontophoresis (I-CXL) cross-linking.

For each of the protocols, in the 1-3 month postoperative period, anterior stromal edema with extracellular lacunae and fragmented keratocyte nuclei was observed with IVCM. At 6 months, repopulation of the anterior stroma with keratocyte nuclei was seen and was greater after I-CXL than after the two other protocols. The demarcation between cross-linked and non-cross-linked corneal stroma was seen as a region where keratocytes became elongated and surrounded by large hyper-reflective stromal bands.

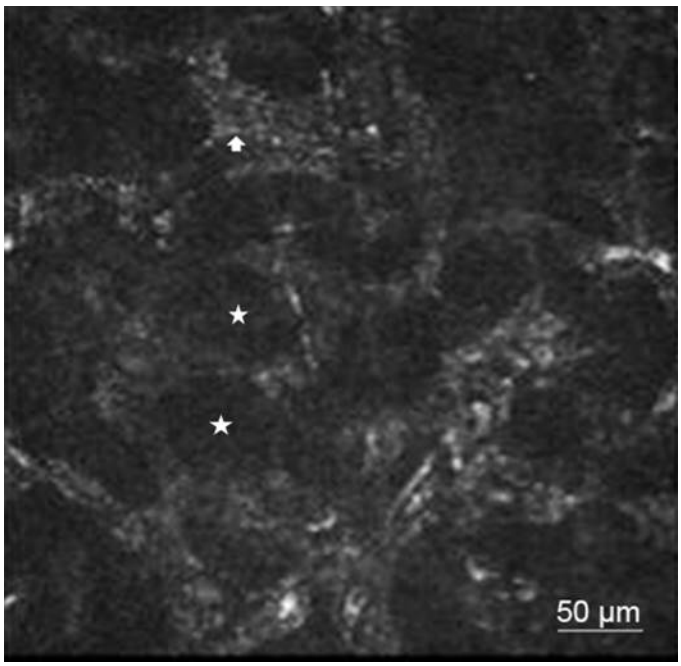

Figure 8.: Microstructural corneal changes after C-CXL. *In vivo* confocal microscopy scans (IVCM) of the corneal stroma obtained 1 month after conventional collagen cross-linking (C-CXL): anterior stromal edema with hyper-reflective cytoplasm (white arrows) and extracellular lacunae (asterisks) are observed. Scale bar: 50 µm.

Discussion

CXL using UVA irradiation and riboflavin is the standard treatment for arresting the progression of keratoconus. Riboflavin is a photosensitizer which induces chemical covalent bonds (cross-links) when irradiated with UVA[3]. In the cornea, this phenomenon creates cross-links between collagen fibrils that increase corneal stiffness. Although this phenomenon is well described, until now there has been no direct evidence of intracorneal cross-links. Nonetheless, several studies have reported a stabilization of the Kmax after the procedure thus demonstrating the effectiveness of C-CXL[3-8]. The question of whether this efficacy is due to cross-links or to other microstructural changes in the corneal stroma remains unanswered.

One of the indirect clinical outcomes of CXL efficacy is the corneal demarcation line detected with AS OCT and IVCM 1 month after CXL. Recently, Kymionis et al. showed that the hyperreflectance evaluated using AS OCT corresponds to the transition area between the acellular and the cellular zone seen on confocal microscopy[20]. Thus, the corneal demarcation line seen on AS OCT must correspond to a zone of activated keratocytes and fibroblastes under an acellular stroma and above a normal one. Nonetheless, Yam and associates[21] failed to demonstrate a correlation between the CXL demarcation line depth with change of visual acuity and of maximum K-value 6 months after C-CXL. The question of whether a greater amount of CXL can result in a greater enhancement in visual acuity and greater reduction of keratometry remains the subject of studies with longer postoperative follow-up. In addition, regarding the mean anterior stromal keratocyte density count on IVCM, a significant reduction was observed during the first 6 months, with normalization at 12 months with C-CXL and A-CXL, and at 6 months with I-CXL[22-24]. Consequently, varying microstructural corneal changes appear dependent on the type of collagen cross-linking protocol used. This

result and the fact that the corneal demarcation line appeared significantly deeper after C-CXL than after A-CXL or I-CXL allows us to discuss the indication and effectiveness of these three protocols.

The conventional protocol has already proved its efficacy and safety with a maximum follow-up of 6 years[3-8]. C-CXL requires corneal pachymetry of at least 400 µm to protect the endothelial cells[25]. Its major drawbacks are related to time duration (1 hr) and the necessity to remove the epithelium. Indeed, this leads to patient discomfort and pain and can cause several complications such as infectious keratitis and stromal haze[9]. Nonetheless, for now, this protocol is still recommended to treat progressive keratoconus, especially when the evolution is aggressive.

Given that one of the major drawbacks of C-CXL was the duration of the procedure, the accelerated protocol initially aimed at reducing operation time by delivering a higher irradiance to the cornea[26]. However, reducing the soaking time to 10 min may limit the intra-stromal penetration of the riboflavin into the cornea, thus leading to the observed superficial corneal demarcation line. Even if it has not been reported and despite the fact that the same number of photons touch the fibrils, it is possible that the 10 times higher irradiance in A-CXL extends the risk of endothelial injuries[27, 28]. In this context, it is important to remark that the absence of Dextran in the riboflavin used for A-CXL may explain the absence of endothelial damage despite the higher irradiance. Indeed, Dextran is known to have an osmotic effect that leads to corneal thinning during the procedure and thus to potential endothelial damage[29]. Consequently, accelerated CXL appears to be a safe CXL modality. Plus, the A-CXL protocol seems to be efficacious; indeed the maximum K-value remained stable at 6 months follow-up. Nonetheless, as for C-CXL, its major limitation is the desepithelialization leading to pain and potential complications such as haze and corneal infection[9]. Touboul *et al.* carried out a qualitative study using confocal microscopy of patients treated with A-CXL[23]. Indeed, when compared to C-CXL, the stiffening effect of the UVA-riboflavin seemed to be most prominent in the anterior 150-200 µm of the cornea with greater keratocyte apoptosis and increased stromal reflectivity. This finding suggests that patients with thin corneas (minimal thickness of 350-400 µm) may benefit from the accelerated CXL. At this time, a hypo-osmolar riboflavin that leads to swelling of thin corneas before C-CXL is used since this protocol still requires corneal pachymetry of at least 400 µm to prevent endothelial damage[25]. Nonetheless, accelerated CXL may be preferentially used in the future as a faster and less penetrating treatment to stabilize keratoconus for thinner corneas. However, long-term studies are needed to conclusively correlate the depth of the demarcation line with the effect on corneal biomechanics.

Iontophoresis CXL is one transepithelial protocol recently developed to avoid epithelial debridement[12, 30]. Application of an electric current forces the hypo-osmolar riboflavin to penetrate the corneal stroma. Vinciguerra and co-workers examined 20 eyes which underwent iontophoresis CXL in a prospective study. They showed that the Kmax was stable 1 year after the procedure. However, the demarcation line was not clearly measurable with AS OCT during the follow-up[31]. Similarly, in our study, the corneal demarcation line assessed with AS OCT was hardly seen at a mean depth of 214 µm in less than half of the patients (46.5%). Plus, confocal microscopy revealed much less keratocyte apoptosis and increased stromal reflectivity after I-CXL than after the two other protocols. Indeed, using confocal microscopy and a modified riboflavin (Ricrolin TE), Caporossi et al. investigated another protocol of transepithelial crosslinking. As for iontophoresis, they find that apoptosis of stromal keratocytes was superficial (mean depth of 140 µm) and unevenly seen in the anterior stroma[11]. Plus, they confirmed that this Epi-ON protocol resulted in keratoconus evolution after 24 months of follow-up, adding a note of caution to its application in pediatric patients that often suffer from more aggressive forms of the disease[32]. Indeed, as for other transepithelial protocols, iontophoresis does not seem to ensure an improvement in topographic indices in pediatric patients[33]. This absence of efficacy can be explained by limited riboflavin and UVA penetration with the epithelium *in situ*[11,34-36]. Indeed, the epithelium is a physical barrier for both riboflavin and UVA penetration, limiting the depth of apoptosis and thus of corneal biomechanical effects[11]. In addition, riboflavin concomitantly serves as a photosensitizer and a UV blocker during UV exposure[28]. Consequently it is conceivable that, as for other transepithelial protocols, insufficient riboflavin penetration during iontophoresis will not only limit the efficacy of the procedure, but also increase the risk of endothelial cell damage. Nonetheless, no endothelial cell loss was noted as yet after iontophoresis. Finally, in our study, similarly to Vinciguerra *et al.*[31], the highest K-value appeared stable 6 months after I-CXL. However, it remains to be seen from longer follow-ups whether this new procedure remains reliable. Thus, as with other Epi-ON protocols, caution is require when using iontophoresis. Nevertheless, the enthusiasm for transepithelial CXL is understandable, considering the decrease of potential CXL complications. With Epi-OFF CXL, complications occur in approximately 1% of cases essentially caused by temporary haze[9]. Unfortunately, this haze occasionally leaves corneal scars. Consequently, we believe that currently, iontophoresis CXL should be used with caution on pediatric patients and we would mostly propose this protocol to patients with thin corneas and slowly progressing keratoconus.

Conclusively, regarding penetration, the conventional CXL protocol remains the standard option for treating progressive keratoconus. Accelerated CXL appears to be a quick, effective and safe alternative to treat particularly thin corneas. Iontophoresis is associated with less damage of anterior keratocytes and a less visible demarcation line and should thus be considered with greater prudence.

Disclosures

The authors have nothing to disclose.

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