Review Article

Current concepts in crosslinking thin corneas

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Corneal cross-linking (CXL), introduced by Wollensak *et al.* in 2003, is a minimally invasive procedure to halt the progression of keratoconus. Conventional CXL is recommended in eyes with corneal thickness of at least 400 microns after de-epithelialization to prevent endothelial toxicity. However, most of the keratoconic corneas requiring CXL may not fulfill this preoperative inclusion criterion. Moderate-to-advanced cases are often found to have a pachymetry less than this threshold. There are various modifications to the conventional method to circumvent this issue of CXL thin corneas while avoiding the possible complications. This review is an update on the modifications of conventional CXL for thin corneas.

Key words: Cross-linking, keratoconus, thin cornea

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Keratoconus is a chronic, progressive corneal disease characterized by progressive stromal thinning and corneal ectasia causing irregular astigmatism and visual impairment. It typically starts in the pubertal age group and progresses up to mid-30s.[1,2] It is estimated that the corneal biomechanical strength is only 60% that of normal, which causes the conical protrusion of the cornea.[3] Prior to the introduction of corneal crosslinking (CXL), the management of keratoconus included spectacles and rigid gas permeable (RGP) contact lenses to provide visual improvement. Once the disease was advanced, patients were subjected to either deep anterior lamellar keratoplasty (DALK) or penetrating keratoplasty (PK). However, spectacles and contact lenses could not halt the progression of the disease, and keratoplasty was accompanied by potential complications. These included possible graft rejection, suture-related problems, irregular astigmatism, and recurrence of the disease in host cornea. Hence, keratoplasties are done as a last resort.[4] Current treatment options include corneal collagen CXL to halt the progression of keratoconus, intrastromal corneal ring segments (ICRS) to improve the visual quality by regularizing the shape of the cornea and anterior lamellar keratoplasty in advanced cases. PK is reserved for patients with hydrops and full-thickness corneal scarring. It has been estimated that around 10–20% of keratoconus patients require surgical intervention in the form of keratoplasty.[5]

Progression of keratoconus is defined as an increase of 1D or more in steepest keratometry value, an increase of 1D or more in manifest cylinder, or an increase of 0.5D or more in spherical equivalent.[6] Corneal CXL introduced by Wollensak *et al.* in 2003, is a minimally invasive procedure to halt the progression of keratoconus.[7] In this procedure, riboflavin (vitamin B2) acts as a photosensitizer and also protects the underlying ocular structures from the effects of ultraviolet A (UVA) radiation. The interaction between riboflavin and UVA radiation causes a photo-oxidation reaction creating reactive oxygen species and forming new cross-links on the surface of the collagen fibrils and within the proteoglycan coating that surrounds them.[8] This has been shown to increase the biomechanical stiffness of the keratoconic cornea and halt its progression.[9-12]

Conventional CXL, also known as the "Dresden protocol," involves epithelial debridement followed by corneal soakage with riboflavin solution. The cornea is then exposed to UVA radiation (370 nm) at 3 mW/cm^2 for 30 min to achieve a surface dose of 5.4 J/cm^2.[13] Conventional CXL is recommended

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in eyes with corneal thickness of at least 400 microns after de-epithelialization to prevent endothelial toxicity.[14,15] Studies have estimated that progression is seen in around 25–30% of cases diagnosed with keratoconus.[16] However, most of the keratoconic corneas requiring CXL may not fulfill this preoperative inclusion criterion. Moderate-to-advanced cases are often found to have a pachymetry less than this threshold. According to a study, around 25% of keratoconus patients have a pachymetry of <400 microns at initial presentation.[17] It has also been reported that corneal thickness significantly reduces intraoperatively during CXL owing to possible corneal desiccation and dehydration during the prolonged period of UVA exposure.[18] There are various modifications to the conventional method to circumvent this issue of CXL thin corneas while avoiding the possible complications. These modifications have been discussed in the current review.

Hypo-osmolar Riboflavin

Conventional CXL by the Dresden protocol uses 0.1% riboflavin in 20% dextran (402.7 mOsmol/L) and 3 mW/cm^2 of UVA for 30 min.[7] It effectively treats anterior 300 microns of the stroma.[19] However, when corneal pachymetry after de-epithelialization is <400 microns, the endothelial cytotoxicity threshold of 0.35 mW/cm^2 is reached.[14] Raiskup *et al.* also reported that in eyes with thinner corneas and steeper keratometry values, like those with advanced keratoconus, a permanent stromal scar developed after CXL using standard iso-osmolar riboflavin.[20]

Corneal stroma has a normal swelling pressure of 50–60 mm Hg.[21] When exposed to a hypo-osmolar solution, the cornea can swell up to double its thickness.[22] This property was used by Hafezi *et al.* to temporarily induce corneal swelling in thin corneas by using hypo-osmolar riboflavin without dextran (310 mOsm/L).[23] After corneal de-epithelialization, isotonic riboflavin was applied to the cornea every 3 min for 30 min. Following this, five repetitive measurements were taken at the thinnest point of the de-epithelialized cornea using ultrasound pachymetry. Hypo-osmolar riboflavin (without dextran) was then instilled every 20 s till the corneal thickness increased to a minimum of 400 microns [Fig. 1a-c]. The eye was then irradiated with UVA radiation at 3 mW/cm^2. This technique was used in 20 eyes and the authors reported stabilization of the keratectasia at 6 months of follow up. There was no endothelial cell loss in any case. They reported that the absolute increase in pachymetry using hypo-osmolar riboflavin ranged from 36 to 110 microns in their study.

Another study by Raiskup and Spoerl showed that their cases were stable at 1 year of follow-up following CXL. In their study, they used hypo-osmolar riboflavin alone every 2 min for 30 min to increase corneal pachymetry. They also reported that the use of hypo-osmolar riboflavin alone resulted in no stromal scar,[24] which was seen with the use of iso-osmolar riboflavin.[20] However, a study of pachymetric changes during CXL by Schmidinger *et al.*, revealed that despite the use of hypo-osmolar riboflavin, the thinnest corneal thickness was <400 microns due to corneal dessication during the irradiation phase.[25] This limitation was overcome when a study showed that the accelerated protocol of CXL using 9 mW/cm^2 for 10 min could effectively halt progression of keratoconus in thin corneas.[26]

Stojanovic *et al.* found that although the procedure using hypo-osmolar riboflavin with standard irradiation of 3 mW/cm^2 for 30 min halted the progression of keratoconus, the effect was lower than that seen with CXL in normal corneas.[27] There are a few possible explanations for this. The increase in the corneal stromal thickness after using hypo-osmolar riboflavin is due to the hydrophilic properties of the stromal proteoglycans causing "collagen-free lakes" to form.[23] This could effectively dilute the number of collagen fibrils available for CXL. Also, the

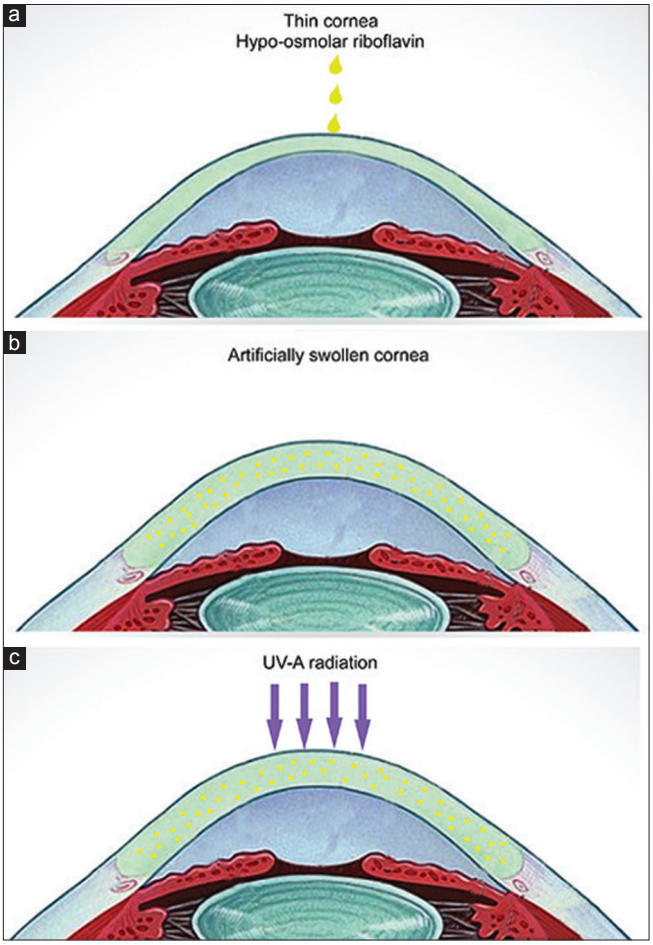

Figure 1: Collagen cross-linking using hypo-osmolar riboflavin. (a) Hypo-osmolar riboflavin instilled on the thin cornea. (b) Increased corneal pachymetry. (c) UVA radiation exposure given to the swollen cornea

Table 1: Collagen cross-linking using hypo-osmolar riboflavin

Author, year	Number (eyes)	Concentration of riboflavin used (%)	Follow-up (months)	Topography changes	Endothelial cell loss	Other complications
Hafezi *et al.*, 2009	20	0.1	6	Stable topography	No	No
Raiskup and Spoerl, 2011	32	0.1	12	Stable topography	–	No
Stojanovic *et al.*, 2014	20	0.5	12	Stable topography	No	No

diffusion factor of oxygen through hydrated corneal stroma is lower than normal corneal stroma.[28] This could lead to a lower oxygen transport, reducing the efficacy of CXL.

Hafezi *et al.* reported failure of CXL in a cornea that was 268 microns thick. This case demonstrated that a certain minimum stromal thickness is required for successful CXL to occur. Assuming that 75% of the corneal stroma gets normally cross-linked, and 250 microns of cross-linked stroma is essential to prevent ectasia, it was proposed that the preoperative thickness should be least 330 microns.[29] [Table 1] Koç *et al.* demonstrated that the anatomical results were better with accelerated CXL in thin corneas as compared to thicker ones. However, there was no difference in the improvements of visual acuity in both the groups.[30]

Transepithelial Crosslinking

Corneal collagen CXL without epithelial debridement is another technique that allows thinner corneas to be treated. Standard CXL with epithelial debridement may cause irreversible endothelial damage in thin cornea.[14] Transepithelial CXL allows corneas with advanced keratoconus to be treated.[31,32] However, an intact epithelium poses a few challenges. Riboflavin being a high molecular weight, hydrophilic molecule, does not penetrate the intact epithelium.[33] Hence, substances like ethylenediaminetetraacetic acid (EDTA), benzalkonium chloride (BAC), gentamicin, and trometamol are combined with riboflavin to enhance its permeability through the epithelium[34,35] [Fig. 2a-c]. Secondly, the riboflavin film and the riboflavin-soaked epithelium might absorb the incident UVA light causing attenuation of the CXL effect.[36] A study by Bottós *et al.* showed that the CXL effect was limited in eyes with intact epithelium due to inadequate stromal concentration of riboflavin, and not by reducing UVA transmittance.[37] An intact epithelium might diminish oxygen diffusion into the stroma further attenuating the CXL effect.[38] Also, the depth of stromal demarcation line in cases of transepithelial CXL is approximately 200 microns indicating that the actual CXL effect might be less as compared to the standard protocol. It is estimated that the biomechanical rigidity increases by approximately 64% after transepithelial CXL as compared to 320% after standard CXL.[31] Sun *et al.* used an irradiance of 45 mW/cm^2 and pulsed illumination (1:1) to improve the oxygen availability during the treatment. They reported results similar to standard CXL using this protocol.[39] Performing CXL with intact epithelium reduces the risk of infective keratitis, improves patient comfort, reduces stromal haze, and intraoperative corneal thinning,[40,41] probably because of less tissue damage and reduced wound healing reaction.

Caporossi *et al.* used riboflavin with dextran, and EDTA and trometamol were used as permeability enhancers. They reported an initial improvement in uncorrected distant visual acuity (UDVA) and best-corrected distant visual acuity (BDVA) in the first 3–6 months following CXL. Thereafter, the UDVA and BDVA returned to preoperative levels gradually. Topographically, keratoconus was stable up to 12 months after transepithelial CXL, but a subsequent worsening in maximum keratometry (K_{max}) was observed at 24 months.[42] Gatzioufas

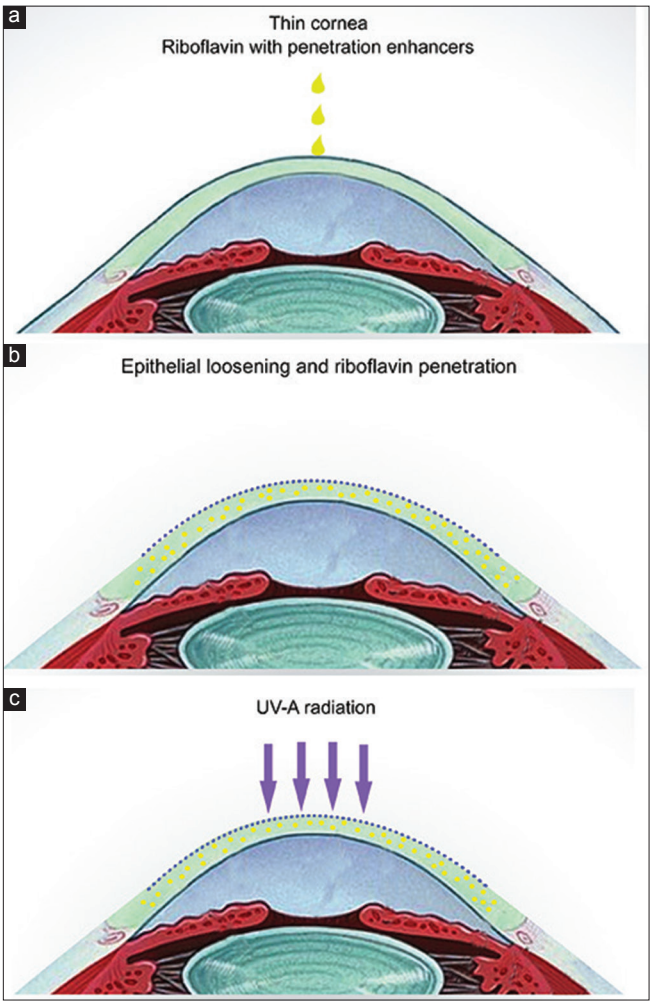

Figure 2: Transepithelial collagen cross-linking. (a) Thin cornea soaked with riboflavin with penetration enhancers. (b) Epithelial loosening (blue-dotted line) and riboflavin penetration into the corneal stroma. (c) UVA radiation exposure given

Table 2: Transepithelial collagen cross-linking

Author, year	Number (eyes)	Follow-up (months)	Topography changes	Endothelial cell loss	Other complications
Caporossi *et al.*, 2013	26	24	Stable up to 12 months, then worsening noted at 24 months follow-up	–	No
Gatzioufas *et al.*, 2016	26	12	Progression in 46% cases	–	Epithelial defects, loose epithelium
Filippello *et al.*, 2012	20	18	Stable topography	–	No
Leccisotti and Islam, 2010	51	12	Stable topography	No	No

et al. used riboflavin with BAC 0.01% as the enhancer in 26 eyes. There was no change in UDVA, BDVA, or corneal pachymetry at 6 and 12 months. They observed progression in 46% eyes at 12 months of follow up.[43]

On the other hand, Filippello *et al.* reported topographic improvement after using riboflavin with dextran, EDTA, and trometamol in 20 eyes. There was a significant improvement in UDVA, BDVA, K_{max}, and higher order aberrations as well.[40] Leccisotti and Islam used riboflavin with dextran, EDTA, BAC, and trometamol and reported improvement in vision and topography. They reported that there was a definite but limited favorable effect of transepithelial CXL. The effect was less than conventional CXL by epithelial removal.[41] Recently, techniques like iontophoresis have been developed to enhance the riboflavin penetration into the stroma and improve the effects of CXL [Table 2].

Iontophoresis-assisted Crosslinking (I-CXL)

Transepithelial CXL by using enhancers may not be the only way to facilitate riboflavin penetration. Iontophoresis is a non-invasive technique wherein a small electric current is used to facilitate penetration of an ionized substance in a tissue.[44] Riboflavin, being a negatively charged molecule with a molecular weight of 376.4 g/mol, is an ideal molecule for iontophoresis.[45] In this method, the passive electrode (anode) is placed on cervical vertebrae or on the patient's forehead. The active electrode is applied to the cornea using a suction ring. The annular suction ring of the iontophoresis device is irrigated with 0.1% riboflavin in distilled water till the grid is submerged. Following this, a small current of 1 mA is given for 5 min. Stromal soakage with riboflavin is confirmed on slit lamp and UVA exposure is given [Fig. 3a and b].[45,46]

A study by Bikbova and Bikbov demonstrated that I-CXL effectively stabilized the progression of keratoconus up to 12 months. The UDVA and BDVA remained stable with no significant corneal haze. The level of keratocyte apoptosis was 210 microns in their study and there was no endothelial damage.[45] Vinciguerra *et al.* reported that although they did not observe a clear stromal demarcation line, I-CXL was able to halt progression in their cases followed up to 1 year. They reported a significant improvement in BDVA and a non-significant improvement in K_{max} and aberrometry.[47] Studies comparing conventional CXL and I-CXL in early stages of keratoconus have shown I-CXL to be effective in halting progression and achieving stabilization.[48,49] Another study compared I-CXL with conventional CXL and found that after 2 years following treatment, I-CXL halted progression, although less efficiently than conventional CXL. The authors reported a demarcation line at a depth of 216 microns in 35% of the patients who had undergone I-CXL. The failure rate after I-CXL was 20% as compared to a 7.5% of failure rate following conventional CXL.[50] As in transepithelial CXL, CXL efficacy could be limited due to riboflavin-soaked epithelium reducing the UVA penetration into deeper parts of the stroma. In addition, oxygen diffusion is reduced because of epithelial presence. In a study by Mastropasqua *et al.*, I-CXL demonstrated deeper saturation of riboflavin with respect to conventional CXL but did not reach the concentrations with conventional CXL.[51]

Iontophoresis does have its own advantages. Studies have shown that there was a significant improvement in contrast sensitivity in patients that underwent I-CXL as compared to conventional CXL.[52,53] This could be explained by the epithelial debridement and wound healing in standard CXL [Table 3].[52]

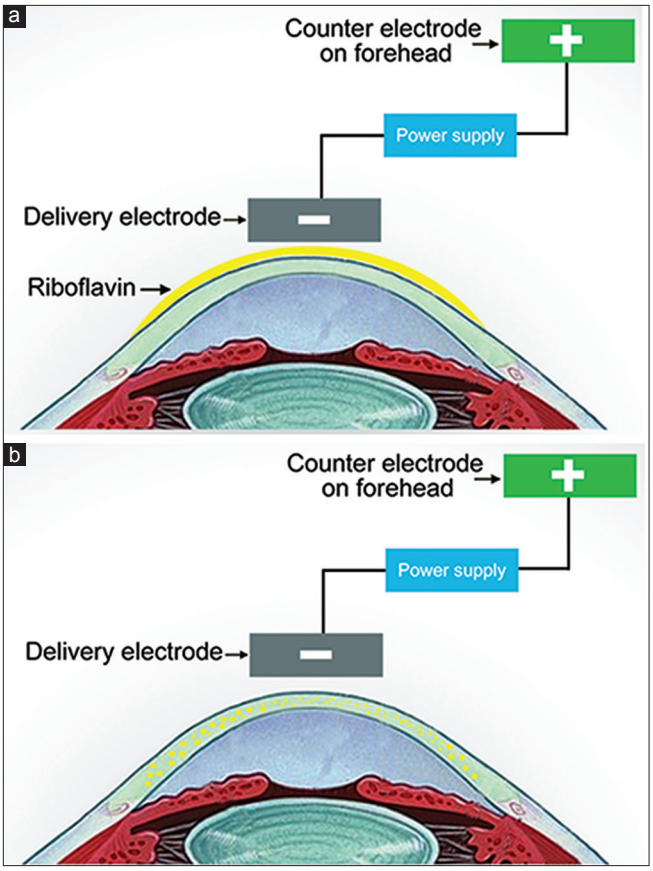

Figure 3: Iontophoresis-assisted transepithelial collagen cross-linking. (a) Process of iontophoresis – delivery electrode placed on riboflavin-soaked cornea and counter electrode placed on forehead/cervical vertebrae of the patient. (b) 1 mA of current causes riboflavin penetration into corneal stroma

Table 3: Iontophoresis-assisted transepithelial collagen cross-linking

Author, year	Number (eyes)	Follow-up (months)	Topography changes	Endothelial cell loss	Other complications
Bikbova *et al.*, 2014	22	12	Stable topography	No	No
Vinciguerra *et al.*, 2014	20	12	Stable topography	No	No
Jouve *et al.*, 2017	80	24	Failure rate of 20% in I-CXL vs 7.5% in conventional CXL	No	No

Iontophoresis significantly reduces the riboflavin soakage time during CXL to 5 min as compared to 30 min in conventional CXL. Also, there is a reduction in post-operative pain and incidence of infective keratitis due to its transepithelial nature. However, longer term follow-ups are needed to establish its efficacy.[54]

Customised Epithelial Debridement Technique

In 2009, Kymionis *et al.* described a technique that involved epithelial debridement of the keratoconic cornea sparing the epithelium over the apex of the cone as determined on topography.[55] The intact island of epithelium soaked with riboflavin causes UVA attenuation and acts as a protective shield over the thinnest corneal point. At the same time, the paracentral cornea, where epithelium is removed, allows better riboflavin penetration resulting in increased biomechanical stiffening effect as compared to transepithelial CXL. The edge of the epithelial island, refracts the UVA radiation and deviates the impact of CXL in the intermediate stromal level which,[56] theoretically, has a better biomechanical impact on the cornea than transepithelial CXL.[56,57] Kymionis *et al.* performed this technique in two patients and reported stabilization of the ectasia up to 9 months of follow-up and no intraoperative or postoperative complications.[55] Following this, in 2011, Kaya *et al.* performed anterior segment optical coherence tomography (AS-OCT) and an *in vivo* confocal microscopy study on corneas that underwent CXL by this technique. They observed that the stromal demarcation line was detectable in the de-epithelialized, peripheral cornea, but not in the area where epithelium was left intact. There was total loss of keratocytes in the de-epithelialized areas, whereas in areas with intact epithelium, the keratocytes were preserved.[58] However, Mazzotta and Ramovecchi reported that the CXL effect is seen at a depth of 150 microns in the epithelium-on area as compared to 250 microns in the epithelium-off area indicating a definite, but lower CXL effect under the intact epithelium.[56] A study by Cagil *et al.* in 19 eyes, demonstrated that there was a halt in the progression of keratoconus at 12 months. They reported a significant endothelial cell loss after this procedure, however, there was no pleomorphism or polymegathism seen.[57] Larger cohorts with longer follow-ups are however needed to establish the efficacy of this technique [Table 4].

Lenticule-assisted Crosslinking

Sachdev *et al.* described a technique of tailored stromal expansion in corneas with pachymetry <400 microns. In this technique, they used the stromal lenticule removed from patients undergoing small incision lenticule extraction (SMILE) for myopic correction. After epithelial debridement, stromal lenticule of appropriate thickness was placed over the patient's cornea making sure that the center of the lenticule is placed over the apex of the cone as seen on topography. Intraoperative ultrasound pachymetry was performed to confirm the augmented stromal thickness. Riboflavin 0.1% was instilled every 5 min for 30 min and UVA radiation was given as in conventional CXL [Fig. 4a-c]. They claimed the technique to be safe in their few cases. The technique does have some advantages in that; the thickness of the lenticule can be customized based on the pachymetry of the keratoconic cornea. The stromal lenticule is also biologically similar to the cornea being treated.

The potential limitations of this technique include reduced oxygen availability for CXL; however, there need to be long-term studies to establish the safety and efficacy of this technique.[59] Currently, there is no other clinical study supporting this method.

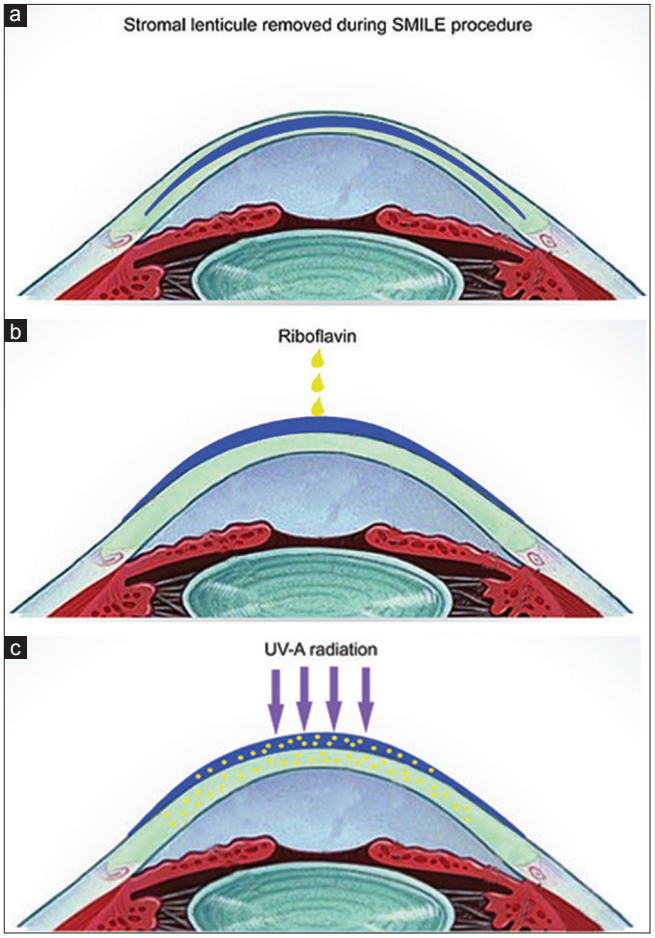

Figure 4: Lenticule-assisted Crosslinking. (a) Stromal lenticule removed during SMILE procedure. (b) Lenticule placed over de-epithelialized cornea and soaked with riboflavin drops. (c) UVA radiation given after confirming riboflavin penetration

Table 4: Collagen cross-linking with customized epithelial debridement

Author, year	Number (eyes)	Follow-up (months)	Topography changes	Endothelial cell loss	Other complications
Kymionis *et al.*, 2009	2	9	Stable topography	No	No
Mazzotta and Ramovecchi, 2014	10	12	Stable topography	No	No
Cagil *et al.*, 2017	19	12	Stable topography	Yes	No

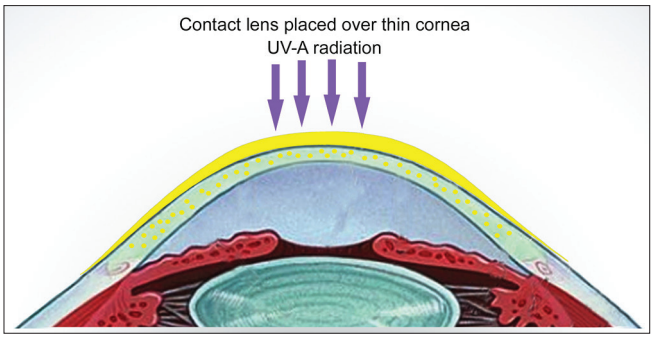

Figure 5: Contact lens-assisted Crosslinking contact lens soaked in riboflavin solution placed over thin cornea and UVA radiation given

Contact lens-assisted Crosslinking (CACXL)

Jacob *et al*. described this technique for corneas having pachymetry of 350–400 microns after epithelial removal. A bandage contact lens having thickness of approximately 0.9 mm was soaked in 0.1% iso-osmolar riboflavin for 30 min. When intraoperative pachymetry was confirmed to be >400 microns, UVA irradiance was given as in conventional CXL [Fig. 5]. They found this technique to be effective in their study of 14 eyes.[60] The advantage of this technique is that it is not dependent on the swelling properties of the cornea. On the other hand, the riboflavin-soaked contact lens reduces oxygen availability and absorbs UVA radiation to reduce the surface irradiance level by 40–50%.[4] Studies with larger sample size and longer follow-ups are needed.

Individualized Corneal Crosslinking

Instead of absorbing excessive UV light in front of the corneal surface, also a lower UV dose can be administered from the beginning, e.g., by shortening the irradiation time while maintaining standard UV irradiance. This approach has the advantage that oxygen diffusion into the stroma is not hindered. Recently, an experimentally validated numerical algorithm[61] has been developed that accounts for oxygen, riboflavin, and UV availability in the stroma during CXL treatment. It allows predicting the biomechanical stiffening effect after CXL, but also required UV irradiation time for a certain penetration depth. In a recent case series of keratoconus patients with ultrathin corneas,[53] individualized CXL treatment was investigated clinically. The authors aimed at a penetration depth of up to 70 µm distance from the endothelium and did show promising results. No endothelial complications were observed within the 1-year follow up, however, a higher failure rate (11% vs 7.6%) was observed, compared to standard CXL.

Conclusion

Corneal CXL by conventional Dresden protocol has been shown to effectively halt progression in eyes with keratoconus having corneal pachymetry of 400 microns or more. Owing to the newer diagnostic techniques, most of the cases with keratoconus are diagnosed at an early stage and it is possible to treat these cases with conventional CXL. However, certain cases that are present in an advanced stage have thinner corneas and cannot be subjected to CXL by the Dresden protocol.

There are various modifications to the conventional protocol that have been used to make CXL possible in thin corneas without causing endothelial damage.

Hypo-osmolar riboflavin has been shown to be effective in cases with pachymetry of 320 microns or more. Transepithelial CXL and I-CXL are also good alternatives in early stages of keratoconus. Customized epithelial debridement has an added theoretical advantage over transepithelial CXL in having a more biomechanical stiffening effect in paracentral cornea where epithelium has been removed. However, studies with larger sample size are needed.

Lenticule-assisted CXL and CACXL have emerged as promising new surgical techniques as well. Studies to analyze the long-term effects of these techniques are awaited.

Most of the protocols have been shown to effectively halt the progression of keratoconus without causing any adverse effects intra- or postoperatively. However, the evidence regarding the safety and efficacy of these modified protocols is still limited. Studies with longer-term follow-ups and larger sample sizes are needed.

Individualized CXL treatment involves a patient-specific adaptation of the UV irradiation time and theoretically can be performed with any corneal thickness. This type of treatment approach may be particularly promising for very advanced stages of keratoconus. Nevertheless, the minimally required UV dosage to prevent keratoconus progression and hence the threshold below which individualized CXL treatment is not effective anymore, is unknown yet.

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Conflicts of interest

There are no conflicts of interest.

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