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REVIEW ARTICLE

Corneal Cross-linking can halt the Progression of Keratoconus, but what is the Best Approach to Treatment?

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ABSTRACT

There are a number of treatment options for keratoconus, but only corneal collagen cross-linking (CXL) appears to halt the progression of the disease. To guarantee effective cross-linking, CXL treatment involves removal of the corneal epithelium prior to riboflavin application and ultraviolet light illumination to ensure that riboflavin reaches the collagen in the stroma—epithelial-on cross-linking ('Epi-off' CXL). Several methods of 'Epi-on' (transepithelial) CXL have been proposed, as keeping the corneal epithelium intact should be less painful and help avoid other CXL-associated adverse events. The evidence to date is that Epi-off CXL remains the most effective method of strengthening the cornea and slowing keratoconus progression—but transepithelial methods are gaining ground.

Keywords: Corneal collagen cross-linking, 'Epi-off' cross-linking, 'Epi-on' cross-linking, Keratoconus, Transepithelial cross-linking.

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INTRODUCTION

Keratoconus is a common corneal disorder, affecting around one in 1500 of the general population, where the central or paracentral cornea undergoes progressive thinning and steepening to generate the classic cone-shaped cornea. As keratoconus progresses, it results in irregular astigmatism, and scarring begins to occur on the exposed high points of the cornea. This leads to a slow but clear decrease in best-corrected visual acuity (BCVA), with low-contrast visual acuity deteriorating more rapidly than high-contrast.[1,2] It is not just visual acuity (VA) that is progressively lost with keratoconus, though it is also quality of life. The disease typically has a young age of onset, and this has two main implications: significantly impaired vision-related quality of life, and the lifetime economic burden of its treatment.[3] For context, in 2011, the average annual cost of routine vision care for someone in the US with myopia was estimated to be US$ 200, whereas the estimated annual price tag for keratoconus was US$ 653; the lifetime cost of the disease for a single patient, including clinic visits, fitting fees, contact lenses, surgical procedures, and potential complications, was estimated to be US$ 25,168.[3] It is a significant, lifelong public health burden. In patients with mild-to-moderate keratoconus, VA is typically corrected with spectacles or contact lenses (and, in some cases, intracorneal ring segment implantation). Advanced disease typically requires corneal surgery, including deep lamellar keratoplasty or penetrating keratoplasty. But if you want an intervention that's able to slow—and even halt—the progression of keratoconus, you need to look to corneal collagen cross-linking (CXL).

Corneal cross-linking involves a photochemical reaction. Ultraviolet (UV) A illumination activates riboflavin, which leads to oxidative cross-linking of collagen in the corneal stroma, strengthening the cornea in the process[4]—which is why keratoconus progressions is one of the principal indications for CXL. The procedure is indicated in several circumstances, particularly when keratoconus progression (measured by changes in K values, astigmatism, pachymetry, corneal hysteresis or VA) is evident. Children with keratoconus are at a particularly high risk of progression, which is often rapid, so the diagnosis of keratoconus in patients, particularly boys aged under 18 years is a prompt indication for CXL.[5,6]

Cross-linking with Removal of Epithelium

In clinical use since 1999, the original CXL procedure is known as the 'Dresden protocol', and is an 'Epi-off' procedure.[7] After anesthetizing the eye, the central 8 mm of the corneal epithelium is removed to expose the collagen-rich stroma, and riboflavin solution (0.1% riboflavin-5-phosphate and 20% dextran T-500) is applied to the surface of the cornea both 30 minutes before

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irradiation and at 5-minute intervals during the course of a 30-minute exposure to 370 nm UVA with a fluence of 3 mW/cm^2. After treatment, antibiotic drops are applied and some patients also receive a bandage contact lens. All patients use antibiotics, steroid drops and lubricants postoperatively until re-epithelialization is complete.

The evidence is that Epi-off CXL works and that its effect lasts for at least 10 years,[8] as recently demonstrated by the results of a retrospective interventional case series that enrolled 24 patients whose eyes (n = 34) were treated with the classic Dresden protocol for progressive keratoconus between the years of 2000 and 2004. Patients' mean age (standard deviation) at the time of the procedure was 28.4 (±7.3) years, and the mean follow-up period was 10.9 (±1.7) years. What was found was that on average, compared with baseline values: K_{max}, K_{min}, K_{apex} values were significantly lower 10 years after CXL was first performed and mean astigmatism was also reduced.

Having said that a recent systematic review and meta-analysis[9]—which was published before the 10-year study paper[8] was available—did register some concerns, not about the technique *per se*, but about the quality of the available evidence. Their analysis incorporated 49 studies that involved patients receiving Epi-off CXL, of which 39 were graded as 'very low quality evidence'. A number of reasons were given, including study design, lack of a comparator arm, high loss to follow-up, and incomplete reporting. The investigators also stated that 'uncertainty remains about duration of benefit.' However, they did recognize that 'delaying or preventing the need for corneal transplant and improving the fitting of contact lenses could be benefits that are highly valued by people with keratoconus.' Importantly, the most common side-effects were pain, corneal edema, and corneal haze, which are usually associated with wound response, but usually resolve within a few days of the procedure. Epi-off CXL also carries a small risk of viral reactivation, haze, melting, infectious ulceration and the development of permanent stromal scars.[10] However, most of these adverse events are avoidable or manageable with topical antibiotics, steroids and appropriate peri- and postoperative analgesia.

Transepithelial Approach

There is another approach: transepithelial (TE) or 'Epi-on' CXL. Leaving the corneal epithelium intact should eliminate wound-related complications and pain associated with Epi-off CXL.

The reason the Dresden protocol involved epithelial cell removal was the fact that riboflavin is a large hydrophilic molecule that cannot penetrate an intact epithelium; it also does not help that the epithelium blocks about 20% of the UV illumination administered. Accordingly, a number of approaches have been taken to try and get the riboflavin to the stroma, including pharmacological cleavage of epithelial tight junctions, intrastromal application of riboflavin through injections or femtosecond laser-created pockets, and iontophoresis.

A preclinical study performed in rabbits has shown that pharmacological disruption of the epithelial tight junctions with the surfactant benzalkonium chloride (BAK) 0.005% (prior to the regular Dresden illumination and riboflavin application protocol) does increase corneal stiffness—but only by approximately one-fifth of what regular, Epi-off CXL achieves.[11] Brillouin microscopy of CXL-treated porcine eyes that had received either an Epi-off or TE (with 0.02% BAK and 0.44% NaCl as the penetration enhancers) protocol showed that TE-CXL was 70% less effective in terms of biomechanically strengthening the cornea than standard CXL.[12]

When evaluated clinically, this protocol managed to improve corrected distance VA and corneal topography parameters on placido disk topography remained stable, although the K_{max} on Scheimpflug imaging and I-S value on placido topography deteriorated.[10] Treatment failure rates were 7% (similar to Epi-off CXL failure rates in the literature), and no haze or other complications were noted in the 18-month follow-up period reported. Another study that used different penetration enhancers both for a 30-minute pretreatment soak and throughout the 30-minute illumination period (riboflavin 0.1%, dextran T500, trometamol and EDTA; Ricrolin TE) appeared to halt keratoconus progression with a statistically significant improvement in VA and topographic parameters—according to placido topography. Another study, using Ricrolin TE for TE-CXL resulted in keratoconus instability, particularly in patients aged 18 years and younger. The study reported that '50% of pediatric patients were retreated with Epi-off CXL due to significant deterioration of all parameters after 12 months of follow-up'.[13] A number of riboflavin solutions have now been developed, and their transepithelial corneal stiffening effect has been compared, relative to standard Epi-off CXL in rabbits, with Epi-off CXL having the greatest effect.[14]

A summary of recent clinical studies of Epi-on CXL is presented in Table 1, and the last one in the list is worthy of the attention:[15] the protocol involved instilling gentamicin, BAK and EDTA for 3 hours, followed by oxybuprocaine for 30 minutes. Riboflavin 0.1% in 20% dextran T500 and oxybuprocaine were then instilled for 30 minutes, finally being followed by 30 minutes of UV-A irradiation to the central 7.5 mm of the cornea while

Table 1: Summary of key transepithelial CXL publications

Reference	*Paper title*	*Conclusion*	*Study type*
Filippello et al J Cataract Refract Surg (2012)	Transepithelial corneal collagen-cross-linking: bilateral study	Appeared to halt keratoconus progression, with a statistically significant improvement in VA and topographic parameters	Cohort study
Koppen et al J Cataract Refract Surg (2012)	Refractive and topographic results of benzalkonium chloride-assisted transepithelial cross-linking	Transepithelial CXL is (…) less effective than standard CXL	Cohort study
Caporossi et al J Cataract Refract Surg (2013)	Transepithelial corneal collagen cross-linking for progressive keratoconus: 24-month clinical results	Functional results after TE-CXL showed keratoconus instability, in particular in pediatric patients	Prospective case series
Kocak et al J Fr Ophthalmol (2014)	Comparison of transepithelial corneal collagen cross-linking with Epi-off in progressive keratoconus	TE-CXL does not effectively halt the progression of keratoconus	Retrospective case review
Touboul et al J Refract Surg (2012)	Corneal confocal microscopy following conventional, transepithelial, and accelerated corneal collagen cross-linking procedures for keratoconus	*In vivo* corneal confocal microscopy (…) TE-CXL did not appear to alter corneal morphology	Prospective case series
Caporossi et al Eur J Ophthalmol (2012)	Transepithelial corneal collagen cross-linking for keratoconus: qualitative investigation by *in vivo* HRT II confocal analysis	TE-CXL showed a limited apoptotic effect (…) about one-third of classic Epi-off	Prospective case series
Mastropasqua et al Cornea (2013)	Morphological modification of the cornea after standard and transepithelial corneal cross-linking as imaged by anterior segment optical coherence tomography and laser scanning *in vivo* confocal microscopy	Marked corneal modification (…) which was poorly evident in the TE-CXL…	Prospective case series
Leccisotti and Islam J Refract Surg (2010)	Transepithelial corneal collagen cross-linking in keratoconus	A limited but favorable effect. The effect appears to be less pronounced than (…) CXL with de-epithelialization	Prospective consecutive study

riboflavin was instilled every 5 minutes. Few epithelial cells would remain after that process and many would question its nomenclature as an 'Epi-on' procedure.

FEMTOSECOND LASER POCKETS

Porcine eye studies have shown that 'the biomechanical effect of CXL using the femtosecond laser pocket technique is about 50% less pronounced than that after standard CXL',[16] and it has been tried in the clinical setting too. John Kanellopoulos performed a small trial, taking 10 eyes with early keratoconus, using the femtosecond laser to make an incision 100 µm in depth, and irradiating the eyes with UV-A illumination with a fluence of 7 mw/cm^2 for 15 minutes.[17] Initial outcomes were good in terms of mean uncorrected and best spectacle-corrected VA, and no ectasia progression was noted during the mean 26 months follow-up period. It shows promise, but the data are not there yet.

Iontophoresis

Another method under active investigation is the iontophoretic delivery of riboflavin into the corneal stroma. Riboflavin is water-soluble, negatively charged at physiological pH, and has a low molecular weight—all of which means that the application of a low-intensity electrical current flowing between a negatively charged delivery electrode placed on the cornea and a counter electrode (placed on the patient's forehead) should be able to drive riboflavin across the intact epithelium and into the corneal stroma.

As it turns out, the composition of the solution in which the riboflavin is prepared matters a great deal. It was established early on (in rat corneas) that the standard Dresden protocol riboflavin preparation (0.1% riboflavin, 20% dextran T500) failed to penetrate the corneal epithelium into the stroma in any significant amounts with iontophoresis, but that the far more soluble 0.1% riboflavin-5'-phosphate did.[18]

A comparison of Epi-off *vs* iontophoretic TE-CXL (I-CXL) was performed in rabbit eyes using a different riboflavin solution, Ricrolin+ (0.1% riboflavin, EDTA and tromethamine), 1 mA for 5 minutes. That study showed that I-CXL was associated with a 45% lower corneal riboflavin concentration than corneas soaked using the traditional Epi-off method. However, when these corneas were subjected to stress-strain assessments and challenged with collagenase digestion, both the Epi-off

Table 2: Summary of recent clinical iontophoretic, transepithelial CXL publications.

Reference	*Paper title*	*Conclusion*	*Study type*
Bouheraoua et al Invest Ophthalmol Vis Sci (2014)	Optical coherence tomography and confocal microscopy following three different protocols of corneal collagen cross-linking in keratoconus	The demarcation line was present in less than 50% of cases and was more superficial than with the traditional procedure	Prospective, consecutive, non randomized study
Bonnel et al J Refract Surg (2015)	Demarcation line evaluation of iontophoresis-assisted transepithelial corneal collagen cross-linking for keratoconus	I-CXL creates a demarcation line (…) which seems less easily distinguishable and shallower than in conventional CXL seems less easily distinguishable and shallower than in conventional CXL. However, its depth and visualization seems to be more similar to conventinal CXL than transepithelial CXL. It is conclusion of the study published in JRS in 2015. Its depth and visualization seems to be more similar to conventional CXL than transepithelial CXL	Prospective, consecutive, non randomized study

and I-CXL-treated corneas exhibited similar median stress at 10% strain and similar collagenase resistance.[19]

Mastropasqua et al compared the concentration of riboflavin in human cadaver corneas as introduced by Epi-off, TE and iontophoresis, finding that the mean riboflavin content in the superficial slice in the Epi-off group was about two-fold greater than that of the iontophoresis group (50.5 ± 5.3 µg/gm and 23.6 ± 2.5 µg/gm, respectively) and four-fold greater than that of the TE group (11.7 ± 3.3 µg/gm).[20] Clinical experience with I-CXL is beginning to be reported (Table 2), too, and it appears to tell a similar story: the cross-linking effect appears to be better than regular TE-CXL, but not as great as Epi-off CXL.

One study that reported 1 year results of I-CXL on 20 eyes from 20 patients with progressive keratoconus has provided some initial, encouraging results: significant improvements in CDVA, with no progression noted over that period and nonsignificant trends toward topographic improvement.[21] The study authors speculated that I-CXL 'has the potential to become a valid alternative for halting the progression of keratoconus while reducing postoperative patient pain, risk of infection, and treatment time in select patients,' but noted that 'the relative efficacy of this technique compared to standard epithelium-off techniques remains to be determined.'

AVAILABILITY OF EVIDENCE

The evidence for Epi-off CXL is a known quantity; it works and its effects persist for at least a decade. Removing the corneal epithelium might be uncomfortable for the patient, it is a lengthier and more painful procedure than TE or I-CXL and it does carry a small increase in risk of corneal infection or other adverse events like haze. But, these risks can easily be mitigated, and the pain and hazing can be managed. Transepithelial CXL—either with or without iontophoresis—would be a great option for patients with keratoconus, if it could work as effectively as Epi-off CXL, but there is just not enough proof at the moment that either Epi-off technique can. Until there is good evidence that a TE-CXL protocol can come close to the efficacy of Epi-off CXL with the Dresden protocol, it is going to remain that way.

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