Major review

Corneal cross-linking

J. Bradley Randleman, MD[a,b,*], Sumitra S. Khandelwal, MD[c], Farhad Hafezi, MD, PhD[d,e,f]

[a] Department of Ophthalmology, Emory University, Atlanta, Georgia, USA
[b] Emory Vision, Emory Eye Center, Atlanta, Georgia, USA
[c] Baylor College of Medicine, Cullen Eye Institute, Houston, Texas, USA
[d] ELZA Institute, Zurich, Switzerland
[e] Laboratory for Ocular Cell Biology, University of Geneva, Geneva, Switzerland
[f] Department of Ophthalmology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

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ABSTRACT

Since its inception in the late 1990s, corneal cross-linking has grown from an interesting concept to a primary treatment for corneal ectatic disease worldwide. Using a combination of ultraviolet-A light and a chromophore (vitamin B2, riboflavin), the cornea can be stiffened, usually with a single application, and progressive thinning diseases such as keratoconus arrested. Despite being in clinical use for many years, some of the underlying processes, such as the role of oxygen and the optimal treatment times, are still being worked out. More than a treatment technique, corneal cross-links represent a physiological principle of connective tissue, which may explain the enormous versatility of the method. We highlight the history of corneal cross-linking, the scientific underpinnings of current techniques, evolving clinical treatment parameters, and the use of cross-linking in combination with refractive surgery and for the treatment of infectious keratitis.

1.   Introduction

Corneal cross-linking represents a physiologic principle of tissue biomechanical alteration that may affect every facet of corneal disease, from ectatic corneas and cornea-based refractive surgical procedures to corneal transplantation, infectious keratitis management, corneal edema management, resistance to collagenase activity, and beyond. Cross-linking represents a testament to translational science, and this rigorous basic science foundation has allowed the cross-linking principle to permeate our treatment regimens and inspire novel approaches.

1.1.   History

The theory that induction of cross-links in cornea tissue could result in stiffening and strengthening of ectatic cornea tissue sparked the development of cornea cross-linking in the late

1990s.[140] Observational studies demonstrating decreased rates of keratoconus in patients with diabetes revealed that natural cross-linking occurs in these patients from the nonenzymatic glycosylation of proteins, which results in the formation of advanced glycosylation end products.[135] This, combined with Theo Seiler's inspiration to use ultraviolet (UV) light to stimulate cross-linking in the cornea, similar to the manner in which dentists use cross-linking to strengthen gums, led to the advent of this revolutionary treatment (Theo Seiler, personal communication, 2014).

Initial treatment in porcine eyes showed up to 70% increase in cornea rigidity compared to controls[140] that was repeated in other studies using porcine,[143,166] rabbit,[144] and human cadaver eyes.[144,166] In these models, the safety of cross-linking was related to cornea thickness to avoid damage to the cornea endothelium and other ocular structures.[167]

Wollensak and colleagues treated 23 eyes with progressive keratoconus, resulting in halting progression in all eyes and corneal flattening in up to 70%.[165] Further clinical studies showed similar promising results in patients with ectasia after refractive surgery.[48]

2. Fundamental concepts in corneal cross-linking

The basic requirements for corneal cross-linking include a photoinducer, a light source with adequate intensity but safe parameters, and a photochemical reaction that induces free radicals while creating a chemical bond between collagen fibrils.[36]

2.1. Riboflavin

Riboflavin (vitamin B2) is the standard photoinducer in cross-linking, as its alkylisoalloxazine structure allows for absorption over a wide range of the light spectrum, including an absorption peak in UV-A range.[30] All flavins are thermostable, yet photosensitive, which allows for molecular changes in a short amount of time.[62] Riboflavin is safe for systemic absorption, readily available in fortified foods and food coloring, but is water insoluble; therefore, the more soluble riboflavin-5 phosphate is commonly used in cross-linking protocols.

Adequate absorption of riboflavin is required for effective cross-linking; however, corneal epithelial tight junctions limit the penetration of its large molecules (molecular weight 376 g/mol). To allow for sufficient riboflavin concentration in the corneal stroma, epithelial debridement is required in standard protocols.[7] Variations in riboflavin soak time[62] and the role of the riboflavin in tear film[164] have the goal of providing adequate penetration to allow for effective stromal cross-linking treatment.

2.2. UV light

UV light is the second necessary component for cross-linking, with important safety parameters that depend on wavelength,[119] irradiance, and time of irradiation.[51] The absorption peak of riboflavin at 370 nm (E. Spoerl, personal communi-
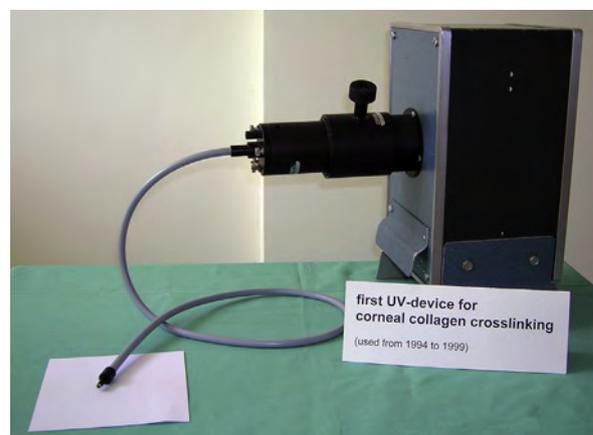
Fig. 1 – The first ultraviolet (UV) light–emitting device used for collagen cross-linking. Image courtesy of Eberhad Spoerl.

cation, 2014) is ideal for the effectiveness of cross-linking and the protection of other ocular structures.[141] Because of the limited availability of light-emitting diodes at that specific wavelength, the first devices used a wavelength of 365 nm (Fig. 1). Variations to the intensity and duration of UV exposure in preclinical studies led to the development of the original standard Dresden protocol, which was found to provide maximum efficacy of tissue stiffening using 3 mW/cm^2 of energy for 30 minutes, which corresponds to a total energy dose (fluence) of 5.4 J/cm^2.

In attempts to accelerate the treatment, variations on these parameters promoted use shorter treatment times at higher intensities. The Bunsen-Roscoe law of reciprocity states that a photochemical effect should be similar as long as total fluence remains constant. Laboratory studies showed that the Bunsen-Roscoe law may apply over a limited range in the cornea. At intensities higher than 45 mW/cm^2, the increase in biomechanical stiffness may drop significantly.[54,160] Alteration of the protocol timing, termed accelerated cross-linking, is discussed in more detail in Section 7.3.

2.3. The cross-linking photochemical reaction

The photosensitizer riboflavin absorbs UV-A energy and excites into a triplet state that can undergo 2 types of reactions: aerobic type 2 and, to a limited extent, anaerobic type 1. Both create reactive oxygen species that induce covalent bonds between collagen molecules and also between proteoglycans and collagen.[171] Clinically, the extent of this effect can be seen as a demarcation line, initially observed at the slit lamp[134] and later confirmed with confocal microscopy[104] and anterior segment ocular coherence tomography.[29,88,168] This line typically presents at 300–350 μm depth after cross-linking with the standard protocol and might be produced by changes in the reflectivity of the cross-linked part of the corneal stroma. Although this has not been definitively established, many clinicians believe that the demarcation line indicates the depth or extent of cross-linking treatment.

2.4. Role of oxygen in cross-linking

Oxygen plays a fundamental role in the cross-linking reaction that needs to be further understood before recommending major changes in treatment protocols. Hammer and colleagues performed cross-linking on *ex vivo* porcine corneas in a low-oxygen environment.[54] Specimens treated under these conditions failed to show an increase in the biomechanical stability, indicating that oxygen is essential for the biomechanical part of the cross-linking process. This may explain why high-intensity and epithelium-on treatment protocols have to date failed to increase the biomechanical stiffness to levels that arrest keratoconus progression. Further studies are needed on the role of oxygen during cross-linking.

2.5. Cross-linking safety parameters

The safety parameters for cross-linking focus on protection of limbal stem cells, corneal endothelium, lens, and retina. Safe protocols require corneal thicknesses of at least 400 μm before application of riboflavin and again before UV-A light exposure. Modifications to protocols include the use of hypoosmolar riboflavin solution to swell the cornea after epithelial debridement,[50] with some concern for decreased endothelial cell count in chemically swollen corneas.[95] For patients who start with adequate corneal thickness, dehydration may cause thinning during the procedure,[59] so careful monitoring is needed. An iatrogenically hydrated cornea may not respond to cross-linking as well. One theory is that increased intracollagen molecule distance limits cross-linking efficacy.[47]

2.6. Cross-linking complications

With proper adherence to the safety limits described previously to prevent UV toxicity to the corneal endothelium, most potential complications related to cross-linking arise from epithelial removal. These include infection, sterile infiltrates, delayed re-epithelialization, transient corneal edema, and corneal haze or scarring.[113] Table 1 lists the most common complications associated with cross-linking.

Keratocyte damage is of concern with regard to scarring; however, repopulation occurs several weeks after the procedure. Corneal healing is slower after cross-linking, and corneal nerve damage, although reversible, may occur.[65]

3. Clinical applications for corneal ectasias

The primary clinical indications for corneal cross-linking include progressive keratoconus in adults and corneal ectasia after laser *in situ* keratomileusis (LASIK). More recently, indications have expanded to include pediatric keratoconus, with treatment offered at the time of diagnosis.

3.1. Stabilization of progressive keratoconus in adults

Quality of life studies suggest there is a significant effect on mental health in patients with progressive keratoconus that affects activities of daily living[84] as well as visual quality.[83] The progressive nature of keratoconus calls for not only visual rehabilitation but also a treatment halting progression.

3.1.1. Clinical results

The first clinical study by Wollensak and colleagues showed both halting of keratoconus progression with induced corneal flattening throughout the first 2 years of follow-up, along with continued improvements in visual acuity (Fig. 2).[165] Several other pilot and retrospective studies followed, all showing improvement in both uncorrected and best corrected acuity with corneal flattening on steepest keratometry.[6,15] Comparative studies using the fellow eye as control had equally impressive results, showing improvement in uncorrected and best corrected visual acuity, mean simulated keratometry, coma, and other high order aberrations in the treated eye,[155] whereas progression continued in the nontreated eye.[25]

The first randomized controlled trial by Wittig-Silva and colleagues found statistically significant flattening of the steepest keratometry and a trend toward better visual acuity,[163] with long-term follow-up showing continued flattening up to 4 years after treatment.[162] Additional long-term data confirm these findings, with reduction of spherical aberration and topographic steepening at 4 years[16] and improvement in keratometry on an average of 4.84D in one study.[112]

Hersh and colleagues published the results from the first US-based prospective clinical trial for cross-linking, demonstrating improvement in visual acuity and maximum keratometry in patients with keratoconus and ectasia after LASIK.[58] In this study, the keratoconus patients had more topographic flattening than ectasia patients, and both groups had transient corneal steepening and reduced corneal thickness during the first 3 months.[45] In addition, improvement in corneal topographic indices did not correlate with visual acuity.[42]

Table 1 — Complications of cross-linking			
Primary processes	Wound healing processes	LASIK flap complications*	Limbal stem cell
Keratocyte apoptosis	Delayed re-epithelialization	Flap striae	Superficial keratitis
Nerve fiber damage	Sterile infiltrates	Diffuse lamellar keratitis	Stromal clouding
Endothelial toxicity	Infectious infiltrates	Epithelial ingrowth	Cell infiltration
Treatment failure	Cornea haze	Flap melt	
	Cornea scarring		

LASIK, laser *in situ* keratomileusis.
* Complications that may arise in patients with a history of LASIK who undergo cross-linking treatment.
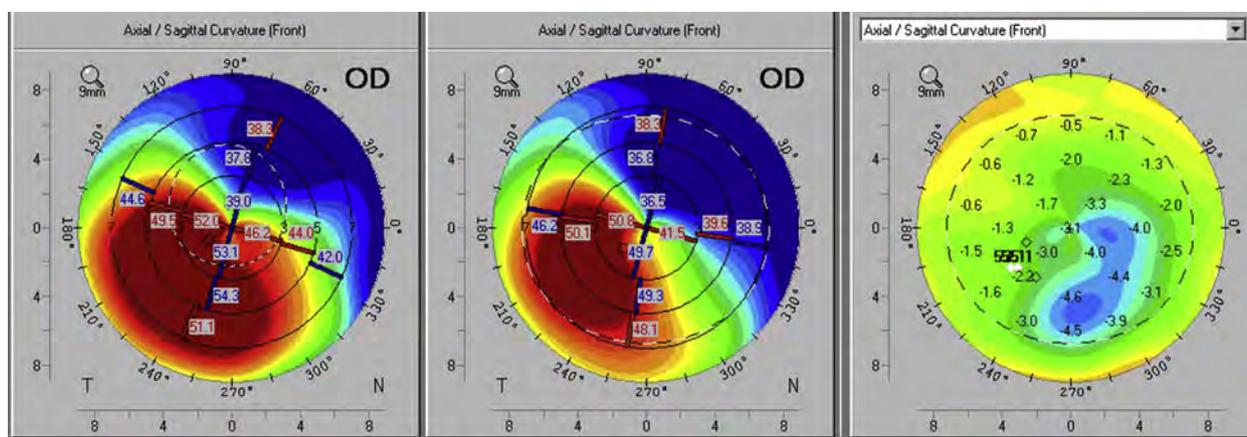
Fig. 2 – Sagittal curvature difference map from Scheimpflug imaging of a patient with keratoconus showing before cross-linking (far left) and after cross-linking (middle), demonstrating improved regularization of the anterior corneal curvature, with up to 4.6 diopters of flattening in the difference map (far right).

3.2.   Postoperative corneal ectasia

Ectasia after corneal refractive surgery occurs from focal interlamellar and interfibrillar biomechanical collagen fiber slippage that is biomechanically similar to keratoconus, with resultant corneal weakening and thinning and progressive irregular astigmatism.[27] Ectasia occurs more frequently after LASIK[116] owing to the depth-dependent reduction in corneal tensile strength in deeper stroma[115,131] but occurs to a lesser extent after photorefractive keratectomy (PRK).[114]

Initial reports showed cross-linking can be used to halt ectasia progression, with improvement in corneal topography and reduced maximum keratometry at 2-year follow-up (Fig. 3).[48] Prospective randomized control data confirmed these results, showing improvement in corrected distance visual acuity[124] and maximum keratometry at 1 year.[58,101,156] Several studies comparing the results between keratoconus and postoperative ectasia revealed a trend to less improvement in the ectasia group; however, the enrolment criteria regarding progression were different between the 2 groups.[42]

3.3.   Pediatric keratoconus

Considering the progressive nature of keratoconus, pediatric patients may benefit the most in treatment. The ectatic disease process likely starts at an age much younger than the age of clinical presentation, and children with keratoconus are more likely to progress to significant vision loss.[100,105] There are special considerations in the pediatric population, including protocol, techniques, time of treatment, duration of effect, and safety.

Initial studies have shown a rapid response to cross-linking in the first year, with continued improvement in uncorrected and best corrected visual acuity, spherical equivalent refraction, and keratometry.[5,17,153] Eyes with more advanced keratoconus show more flattening during the first year.[137] A longer-term study followed patients for 3 years, finding that, despite the initial improvement in parameters during the first year, some pediatric patients may continue to progress long term.[21] A case series of advanced pediatric keratoconus patients showed similar results, with continued progression in most eyes.[138] This suggests that pediatric
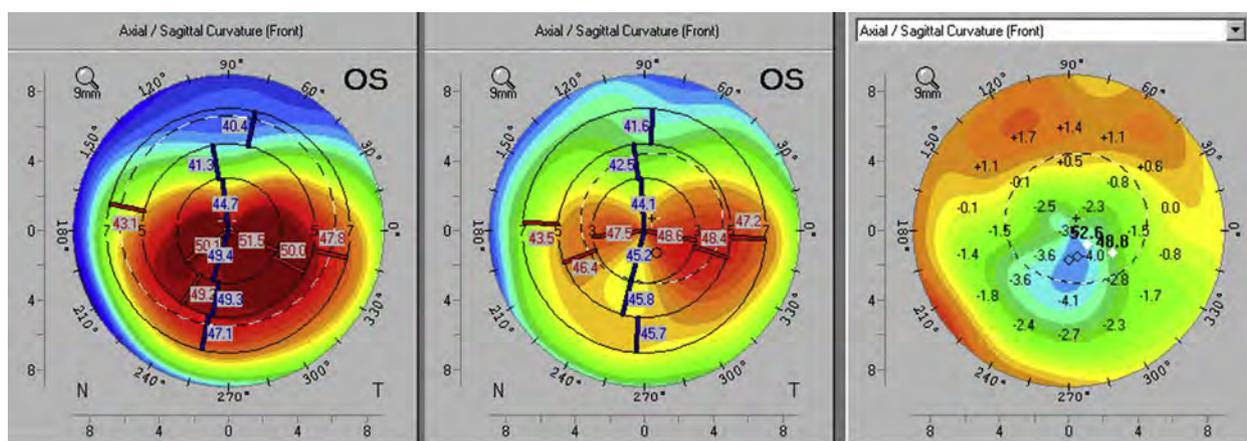
Fig. 3 – Sagittal curvature difference map from Scheimpflug imaging of a patient who developed ectasia after laser in situ keratomileusis showing before cross-linking (far left) and after cross-linking (middle), demonstrating improved regularization of the anterior corneal curvature, with up to 4.1 diopters of flattening in the difference map (far right).

patients may have more aggressive disease, and long-term data with possible technique changes are needed. It is therefore important to counsel the family about the possible shorter duration of benefit after cross-linking and the need for close follow-up of all pediatric keratoconus patients.

Epithelium-off techniques in children carry potentially increased risk of infection, haze,[153] scarring,[138] delayed epithelial healing,[21] and patient intolerance of the procedure because of postoperative pain. Techniques that spare the epithelium (transepithelial) would be of particular usefulness in this population; however, transepithelial studies show worsening keratometry values over 18 months, so epithelial-off techniques are still recommended.[14]

Long-term data in pediatric patients are still needed. As opposed to adult ectatic disease, where one may elect to wait for clear signs of progression before cross-linking, pediatric cases often present with more aggressive keratoconus and need treatment immediately after the diagnosis is confirmed. Although the general recommendation has been to perform cross-linking if progression is documented over a 3-6 month period,[35] one study found that 88% of pediatric keratoconus patients progressed over a relatively short time period.[21] Thus, treatment of pediatric keratoconus is often recommended on initial presentation without waiting for progression.[35] There is no consensus on treatment timing yet, and the risks of treatment need to be weighed against the benefits.

4.   Optimizing cross-linking success

Determining preoperative factors that predict increased cross-linking efficacy is of particular interest, as there are currently a wide range of topographic and visual improvement outcomes reported. To date, results based on age at the time of treatment have been contradictory. Vinciguerra and colleagues found over a 4-year follow-up in patients older than 18 years that although all age groups showed improvement in ectasia, more functional and morphologic improvements occurred in those aged 18–39 years.[159] Another study that included patients aged less than 18 years found that younger patients have more central cornea steepening, and cross-linking caused more improvement in vision and cornea flattening than older patients.[139] Meanwhile, another study found that patients aged more than 30 years did better after cross-linking than other age groups.[150]

Another factor may be maximal corneal steepness, as Koller and colleagues found that steeper corneas have more flattening after cross-linking, without any correlation to age, sex, time of diagnosis, or corneal shape factors.[78] Greenstein and Hersh obtained similar results, with steeper corneas with worse visual acuity having better results.[44] Another study, however, suggested that steep preoperative keratometry greater than 58D was associated with higher complication rates.[77] Certain patterns of steepening may also predict better outcomes, with some studies suggesting that peripheral topographic cones may not have successful outcomes.[43] Eyes with peripheral steepening with pellucid marginal corneal degeneration, however, may also benefit from cross-linking.[89,107] There is likely more than preoperative keratometry or age that determines outcomes.[49]

4.1.   Measuring treatment success with cross-linking

Definitively measuring progression in corneal ectasias has proved challenging. Measuring the success of cross-linking is equally difficult. The most common measurement in large prospective studies has looked at maximum keratometry (KMax) using Scheimpflug imaging (Figs. 2 and 3)[48,58,165] This strategy is limited, however, as these data points are frequently not repeatable and often do not correlate with changes in visual acuity.[58]

The cornea has dynamic and elastic properties, so static topographic or tomographic imaging may only provide some of the data necessary to evaluate patients fully. Diagnosis and measurement of progression of disease may require the measurement of corneal biomechanics.[60] The Ocular Response Analyzer (Reichert Ophthalmic Instruments, Buffalo, NY, USA), measures corneal hysteresis, and early studies suggested a transient increase in this parameter after cross-linking.[39,154] More advanced waveform-derived variables may provide a better picture of the effect of cross-linking but need further validation.[40,53]

Dynamic Scheimpflug technology (Corvis ST; Oculus Optikgeräte GmbH, Wetzlar, Germany) produces highly repeatable measurements of cornea biomechanics in normal[109] and keratoconic eyes.[148] Factors such as deformation amplitude are repeatable and comparable methods to follow keratoconus progression[1] and may provide a way to analyze success of treatment after cross-linking, although early studies have been inconclusive.[8] Other technologies such as the applanation resonance tonometer demonstrate increased ocular hysteresis after cross-linking.[9] Repeatability and comparability studies may provide confirming data. Computer simulation modeling may provide insight into the biomechanical changes after cross-linking and provide better clinical metrics to follow.[127,136]

5.   Corneal cross-linking combined with refractive surgical procedures: CXL plus

Although cross-linking can be used to prevent progression of cornea ectasia and partially reverse maximal steepening, its typical effect on visual function is limited.[155] Adjunctive treatments in conjunction with cross-linking have been introduced to improve visual function while maintaining the biomechanical benefits of cross-linking. The term "CXL plus" encompasses these refractive treatments.[90]

5.1.   Cross-linking combined with intrastromal corneal ring segments

Intrastromal corneal ring segments may improve visual and topographic outcomes in patients with keratoconus[96] and ectasia after LASIK.[97] These rings do not appear to prevent disease progression, so combination treatment with cross-linking may be more beneficial than either treatment alone. Intacs (Addition Technology, Des Plaines, IL, USA) with cross-linking may reduce keratometry and topographic parameters compared to Intacs alone,[20] although some studies find no difference between the groups[120] or that Intacs alone gives

better refractive results.[99] Keraring (Mediphacos, Belo Horizonte, Brazil)[31,32] and Ferrara Ring (Ferrara Ring-AJL, Boecillo, Spain)[57,128] segments have also produced favorable results with cross-linking.[26] Creation of ring segment channels may be performed with modified femtosecond laser settings, with riboflavin introduced into the channels; however, the long-term effects of this combination have not been studied.[3]

Options for timing of surgery include simultaneous same-day cross-linking with ring segments or sequential surgery several days apart to several months apart.[90] Both techniques have shown improvement in visual acuity and topography in keratoconus and ectasia.[170] Simultaneous single and paired ring segments may be efficacious in improvement of visual acuity when combined with CXL.[169]

5.2.   Photorefractive keratectomy and cross-linking

The first specifically refractive treatment to be combined with cross-linking was PRK. Topography-guided PRK allows remodeling of the shape of the cornea and specifically addresses visual function affected by irregular astigmatism[19,76] but does not halt progressive keratoconus. Cross-linking in combination with topography-guided PRK, however, may both halt progression and reduce irregular astigmatism. Long-term safety and efficacy remain unknown.

The timing and sequence of PRK with cross-linking should maximize biomechanical impact and positive changes in corneal curvature while minimizing risk of complications, especially corneal haze. Both simultaneous and sequential treatments have been studied. Kanellopoulos and Binder reported a patient with bilateral progressive keratoconus who underwent standard cross-linking, followed one year later by topography-guided PRK, who achieved 20/20 uncorrected acuity 18 months after PRK.[70] Kymionis and colleagues described similar outcomes in a series of patients who underwent same-day topography-guided PRK with cross-linking for keratoconus[92] or pellucid marginal degeneration.[89]

A retrospective study comparing simultaneous versus sequential PRK with cross-linking suggested that simultaneous (same-day) treatment was superior to sequential treatment with regard to visual acuity, spherical equivalent refraction, and change in keratometry.[67] Simultaneous treatment creates a unique posterior linear stromal haze that improved, but did not resolve, at 1 year.[94] Corneal haze was less in simultaneous PRK and cross-linking treatments as compared to sequential PRK and cross-linking.[67]

Protocols have included variable recommendations concerning maximum ablation depth and the use of mitomycin C in this high-risk group with thinner corneas and greater propensity for corneal scarring. Kymionis and colleagues recommended a maximum ablation depth of 50 μm and no use of antimetabolites, thinking that depopulation of keratocytes during cross-linking may reduce haze.[91,92] Kanellopoulos recommended maintaining a residual corneal thickness of 350 μm and used mitomycin C.[67] Stojanovic and colleagues advised a more conservative approach of 60-μm maximal ablation depth and minimum final corneal thickness of 400 μm.[145] Lin and colleagues recommended a maximum stromal ablation depth of 80 μm and a minimum residual stromal

depth of 300 μm in a study population that included both keratoconus and ectasia after LASIK. Although long-term outcomes need to be assessed, topography-guided PRK in conjunction with cross-linking improves not only visual acuity but also visual function and quality of life indices.[98]

Under certain circumstances, sequential treatment is the only possible approach, for example, in patients who have undergone cross-linking previously and now seek topography-guided PRK. Cross-linking appears to influence the ablation rate of excimer laser pulses. Chen and colleagues[22] and Richoz and colleagues[120] investigated this issue on *ex vivo* porcine corneas using different commercially available excimer laser systems. Both groups found similar reductions of the ablation rate per pulse of 9%[22] and 12%,[120] for the first 200 μm of stromal tissue. These results should allow for nomogram adjustments for cross-linked corneas.[121]

5.3.   Phototherapeutic keratectomy and cross-linking

Options for epithelium debridement for cross-linking include mechanical debridement or excimer laser phototherapeutic keratectomy (PTK). A prospective comparative study using transepithelial PTK (termed the Cretan protocol) resulted in better visual and refractive outcomes than mechanical debridement with a rotating brush.[86] This was corroborated by other comparative studies.[73] The authors hypothesized that PTK decreases corneal irregularities but caution about PTK over the cone as this may create deeper corneal damage.

Using PTK to remove the epithelium in conjunction with topography-guided PRK and cross-linking may also be effective. The Athens protocol used PTK for removal of epithelium, followed by partial topography-guided PRK with mitomycin C, then the cross-linking procedure.[70]

Regional epithelial thickness is highly variable in ectatic corneas,[117,126] and the epithelium undergoes remodeling after cross-linking.[118,125] These anatomic findings may explain the benefits of PTK for cross-linking, and direct epithelial measurements may ultimately prove useful for planning combined treatments.

5.4.   Phakic intraocular lenses and cross-linking

Phakic intraocular lenses (PIOLs) used to correct refractive error without loss of corneal tissue are available in various designs including iris-fixated, angle-supported, and posterior chamber lenses. PIOL use in keratoconus and ectasia after LASIK without cross-linking is generally not recommended because of progressive astigmatism in these eyes. A toric posterior chamber PIOL, in combination with cross-linking, was successfully used in 2011 in high myopic eyes with progressive keratoconus.[85] Further case series with cross-linking and toric posterior chamber PIOLs confirmed excellent outcome at 6 months.[34,82] Similar results have been shown with iris-claw PIOLs combined with cross-linking.[46,64] Proper counseling is needed in these patients regarding the potential for continued refractive change after cross-linking.
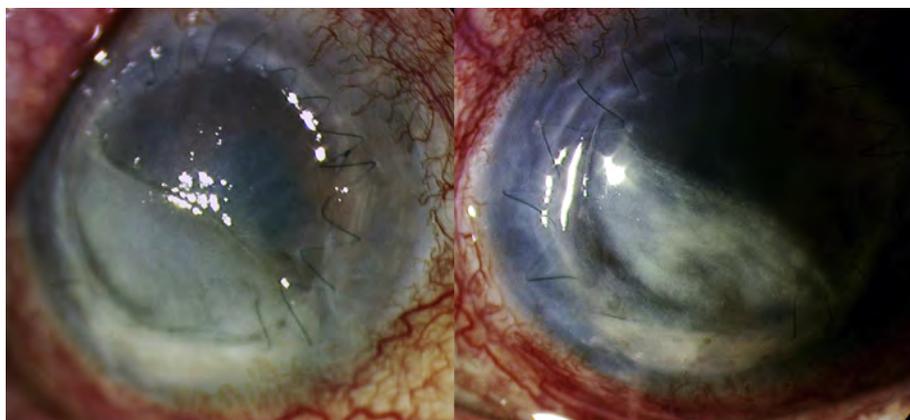
Fig. 4 – **Slit lamp image of a patient who developed infectious keratitis in the right eye after penetrating keratoplasty (left) who underwent PACK-CXL with subsequent resolution of the ulcer resulting in a sterile scar (*right*). Image courtesy of Jes Mortensen.**

5.5. Multiple refractive treatments combined with cross-linking

Treatments that combine cross-linking with corneal ring segments and PRK yield positive results in various protocols.[24,61,79] Another case series described ring-segment implantation followed by cross-linking, followed by toric PIOL implantation for patients with high refractive error not amenable to PRK.[24] Long-term follow-up will be important in these patients to determine the stability of visual outcomes.

6. Cross-linking for infectious keratitis: PACK-CXL

Microbial keratitis is a leading cause of blindness globally. In developed countries, microbial keratitis is frequently related to contact lens wear, whereas minor corneal trauma concomitant with limited access to medical care is the most common etiology in developing countries.[161] In India, there are an estimated 2 million new cases of corneal ulcers per year, leading to the term "silent epidemic."[41] Cross-linking has been used in both recalcitrant and primary cases of microbial keratitis with variable success depending on the causative organism and the depth of the ulcer (Fig. 4).[11]

At the ninth Annual International Cross-Linking Congress held in Dublin, Ireland, in December, 2013, the phrase "photo-activated chromophore for keratitis" was advanced, and the term "PACK-CXL" adopted for cross-linking for infectious keratitis to facilitate future communications on this topic.[52]

6.1. PACK-CXL background

The combination of riboflavin and UV light has been used as a disinfectant for some time. Riboflavin exposed to UV light in the 1960s inactivated tobacco mosaic virus RNA[152] and inactivated pathogens in the blood and plasma.[23] In addition to direct damage to microbes, mechanisms of action include increased corneal resistance to enzymatic degradation,[142] prevention of microbial replication via intercalation of riboflavin with the pathogen's DNA,[108] generation of reactive oxygen species with direct cytotoxic effects,[80,81] and alteration of the ocular surface to be a more hostile environment for microbes.[146] *In vitro* and animal studies demonstrate efficacy of cross-linking as adjunct treatment for challenging keratitis pathogens such as methicillin-resistant *Staphylococcus aureus*,[102,132] *Candida albicans*, *Aspergillus fumigatus*,[130] *Fusarium solani*,[37] and *Acanthamoeba*[10] (Table 2).

6.2. PACK-CXL clinical results

The first clinical case series for PACK-CXL included 5 eyes recalcitrant to medical treatment with atypical and fungal infections after LASIK or contact lens use, 4 of which immediately improved after cross-linking. Pathology on the remaining cornea showed an immune reaction without active fungal disease.[63] The largest series included 40 patients and showed the treatment to be safe and effective, with only 6 cases not resolving with cross-linking treatment.[111] In addition, the success rate was better in this series in bacterial than in fungal infections. Cross-linking may activate the *Herpes simplex* virus[93] and should be avoided in eyes with a history of herpetic keratitis.

Table 2 – CXL antimicrobial spectrum			
Class	Organism	*In vitro* efficacy	Clinical efficacy
Bacterial			
Gram positive	*Staphylococcus aureus*	Good	Moderate to good
	Staphylococcus epidermis		
	Streptococcus viridans		
	Mycobacterium		
Gram negative	*Pseudomonas*	Good	Good
	Serratia		
	Haemophilus influenza		
Fungal	Mold	Good	Low to moderate
	Yeast		
Protozoa	*Acanthamoeba*	Good	Mixed
Viral	Herpes simplex	Poor	Poor

Recently, Said and colleagues published a prospective study analyzing the effect of PACK-CXL with medication against medication only in advanced corneal ulcers. They showed that the time to healing was comparable in both groups, with a tendency in the "PACK-CXL plus medication" group for fewer complications. PACK-CXL, with its current irradiation settings, might be better suited for superficial (early) ulcers.[129]

Alio and colleagues performed a systemic review and meta-analysis of 12 case series with 104 eyes in total treated with PACK-CXL.[2] Protocols varied, with almost all involving epithelial debridement and use of antimicrobial treatment. Most treated pathogens were bacteria (58%), fungus (12%), *Acanthamoeba* (7%), and 25% of eyes had negative cultures. The analysis suggested better time to re-epithelialization in bacterial cases compared to fungal, *Acanthamoeba*, and culture-negative cases, with a higher risk of requiring corneal transplantation in fungal and *Acanthamoeba* cases.[74]

All but 1 clinical studies used PACK-CXL in combination with antimicrobial treatment, and the ethical concern of not treating known infections with antibiotics likely will prevent a large prospective trial of cross-linking alone. An *in vitro* study has shown that fluorescein absorbs UV light at 365 nm at a level similar to riboflavin. Therefore, fluorescein may reduce the antimicrobial effect of the treatment by competing with riboflavin if an ulcer is stained with fluorescein immediately before PACK-CXL.[122]

7. Cross-linking controversies and future directions

Although cross-linking has been used for more than 15 years, many elements remain undetermined. The most important of these include the potential to overcome the epithelial barrier to riboflavin penetration, optimal timing for treatment in adults with keratoconus, the efficacy of accelerated cross-linking techniques, and the prophylactic use of cross-linking in eyes without clear evidence of ectatic disease at the time of LASIK or PRK.

7.1. Overcoming the epithelial barrier for riboflavin penetration

As discussed in Section 2.1, intact epithelium acts as a near-complete barrier to riboflavin absorption. There are numerous disadvantages to epithelium-off techniques, namely postoperative pain, decreased vision, and increased risk of complications including infection.[36] Finding an effective cross-linking technique that allows for epithelial layer retention is the ultimate goal; however, the efficacy of epithelium-on (transepithelial) techniques has been disappointing. Available transepithelial techniques do not allow sufficient riboflavin absorption.[7,56] Alternate techniques to avoid full epithelial removal include disruptors such as tetracaine,[56] superficial epithelial scraping,[56] benzalkonium chloride,[75] ethylenediaminetetraacetic acid,[4,18] mechanical epithelial disrupters, stromal channels, and flaps.[4,28,66] To date, no studies have shown the same efficacy for any of these techniques as epithelium-off techniques.[51] Iontophoresis, the

application of a low electric gradient to enhance molecular transport,[33] allows some penetration of riboflavin through an intact epithelium. Studies have shown that, although improved over other transepithelial techniques, iontophoresis still does not achieve riboflavin concentrations comparable to the standard cross-linking protocol.[103,157] Initial clinical results have shown some efficacy for iontophoresis but likely less than standard epithelium-off techniques.[12] The demarcation line found after iontophoresis is more faint and less deep than that found after traditional epithelium-off techniques (Fig. 5). Recent biomechanical studies in rabbits suggest some potential advantages for transepithelial approaches, but these need to be validated in human eyes.[4,151]

7.2. Treatment timing: Treating at diagnosis or signs of progression

Treatment timing has been debated, with most studies focusing on patients who have progressive keratoconus or ectasia after refractive surgery, which is by definition progressive. Treatment at initial diagnosis could be a good option to halt the progression before actual visual loss; however, there are some challenges. Patients with better visual acuity before cross-linking are those with the greatest chance for visual loss after treatment.[77] It is difficult to know who is going to progress, especially for patients in their 20s or 30s. Treating forme fruste keratoconus requires counseling to the patient that they may or may not progress without treatment and that there are risks associated with epithelium-off cross-linking.

7.3. Varying the treatment time: Accelerated cross-linking

Treatment protocols are still in evolution, especially concerning the relationship between treatment time and UV intensity. Accelerated protocols, with shorter treatment times and higher-intensity UV exposure, yield variable results. Clinical studies suggest that the setting of 7 mW/cm^2 for 15 minutes might induce sufficient cross-links to arrest keratoconus progression.[68] Limited data are available for 18 mW/cm^2 for 5 minutes treatment, which showed good safety[38] but questionable efficacy.[123]

Recent laboratory data also show conflicting results. Wernli and colleagues found equivalent biomechanical responses, measured as a change in Young's modulus compared to control eyes, for standard (3 mW/cm^2, 30 minutes) and rapid (10 mW/cm^2, 9 minutes) treatment protocols.[133] Hammer and colleagues found a decreased stiffening effect with increasing UV-A intensity, also measured as comparative changes in Young's modulus at 10% strain. They found significant differences between 3 mW/cm^2 versus 9 mW/cm^2, 3 mW/cm^2 versus 18 mW/cm^2, and both 3 mW/cm^2 and 9 mW/cm^2 compared to the control group, but no difference between the 18 mW/cm^2 and the control groups.[54]

In contrast to the standard protocol, in some studies, the demarcation line is less dense, less uniform, and demonstrably present in fewer cases after equivalent accelerated treatments (Fig. 5).[13,157,158] In contrast, Kymionis and colleagues found no difference in the demarcation line between
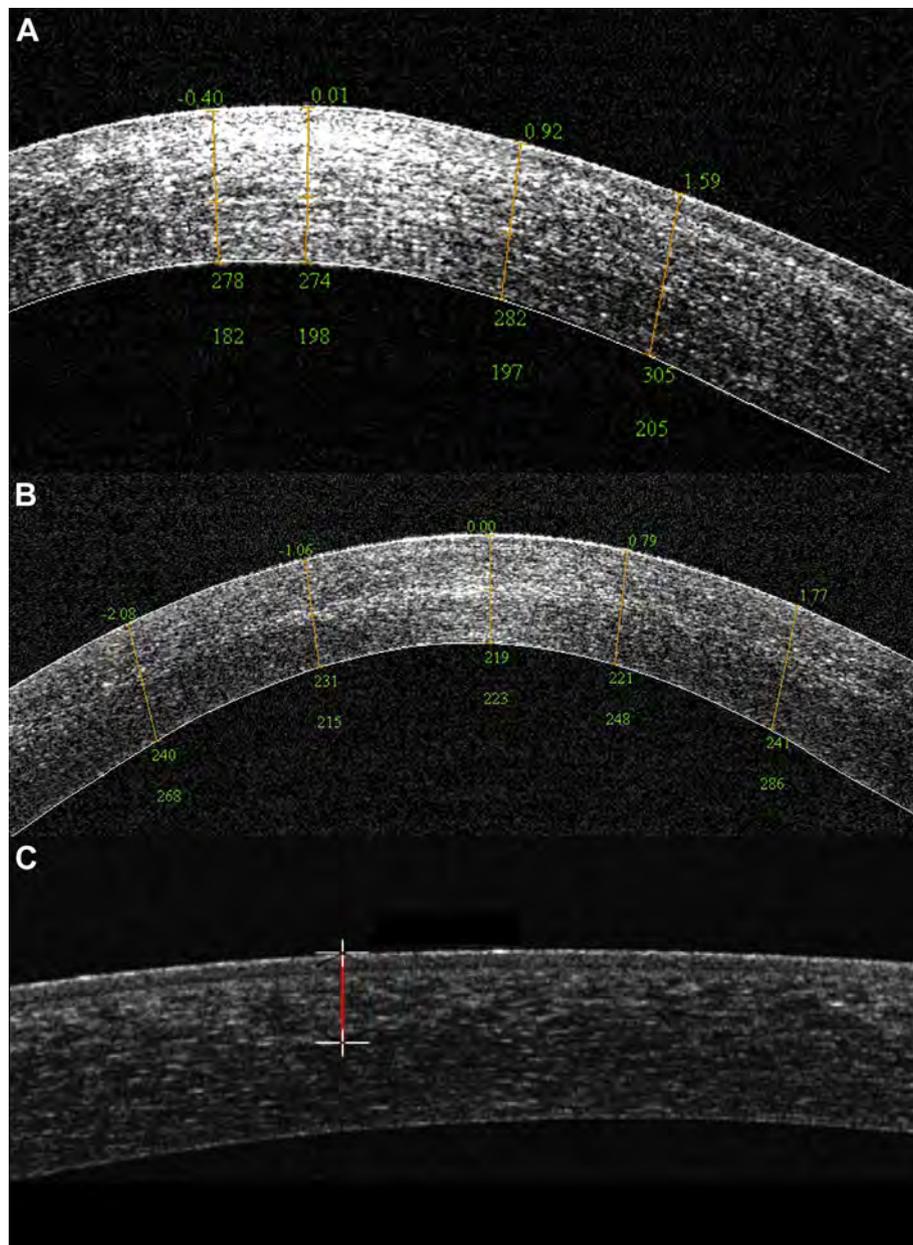
Fig. 5 — Corneal ocular coherence tomography demonstrating the demarcation line after A: standard protocol epithelium-off cross-linking, B: accelerated protocol epithelium-off cross-linking, and C: iontophoresis cross-linking. Note the slightly increased depth and intensity of the demarcation line with the standard protocol (274—305 μm) as compared to the accelerated protocol (219—240 μm) and the reduced depth and intensity of the line with iontophoresis cross-linking (approximately 230 μm).

standard CXL and a modified accelerated protocol (9 mW/cm^2 for 14 minutes).[87,88]

Clinical results have been generally positive to date. Tomita and colleagues reported 2 separate cohorts with similar accelerated protocols (30 mW/cm^2 for 3 minutes)[106,149] but different riboflavin soak times (10 minutes[106] or 15 minutes)[149] and found all measured outcomes were similar to those of the standard protocol. Hashemian and colleagues[55] reported a 15-month follow-up of standard and accelerated (30 mW/cm^2 for 3 minutes) protocols with similar equivalent outcomes. They also found less decrease in anterior stromal keratocyte density with the accelerated protocol and less disruption of the subbasal nerve plexus in the accelerated group. Ozgurhan and colleagues also observed less subbasal nerve disruption with an accelerated protocol.[110] These findings imply that accelerated treatment may have more rapid overall corneal recovery after CXL, which could improve safety profiles.

7.4. Prophylactic and additive CXL

Perhaps most controversial is cross-linking as an adjunct treatment at the time of LASIK as prophylaxis against myopic[147] or hyperopic[72] regression and possibly to reduce the

incidence of postoperative ectasia.[69] Although intriguing, there is limited evidence that prophylactic cross-linking will be efficacious, and as traditional cross-linking can induce progressive, continued flattening over time, it may prove challenging to titrate a cross-linking effect with enough biomechanical effect to protect against corneal shape change but not enough to induce unwanted progressive corneal flattening.

Selective, focal cross-linking may be able to induce specific changes in corneal shape, but this too remains largely theoretical.[71]

8. Conclusion

Corneal cross-linking remains a fascinating physiological phenomenon with a steadily growing list of indications. With more than 400 articles in the peer-reviewed literature, the treatment has developed with solid bench and clinical studies supporting its role in the treatment of cornea ectasias, infectious keratitis, and several more disease categories. New indications and treatment protocols are in development, and we look forward toward carefully performed comparative studies to provide more data to support further refinement and improvements in current cross-linking protocols.

9. Methods of literature search

MEDLINE and PubMed database searching the keywords CXL, corneal cross linking, collagen cross linking, keratoconus, ectasia after LASIK, infectious keratitis and cross linking, infectious keratitis, and cross-linking, from 1980 to 2014. Detailed search was limited to English language manuscripts or at least available abstracts in English.

10. Disclosures

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