Corneal Cross-Linking: The Science Beyond the Myths and Misconceptions

Roy S. Rubinfeld, MD, MA,*† Ciro Caruso, MD,‡ and Carmine Ostacolo, PharmD§

Purpose: There has been a recent explosion in the variety of techniques used to accomplish corneal cross-linking (CXL) for the treatment of ectatic corneal diseases. To understand the success or failure of various techniques, we review the physicochemical basis of corneal CXL and re-evaluate the current principles and long-standing conventional wisdom in the light of recent, compelling, and sometimes contradictory research.

Methods: Two clinicians and a medicinal chemist developed a list of current key topics, controversies, and questions in the field of corneal CXL based on information from current literature, medical conferences, and discussions with international practitioners of CXL.

Results: Standard corneal CXL with removal of the corneal epithelium is a safe and efficacious procedure for the treatment of corneal ectasias. However, the necessity of epithelium removal is painful for patients, involves risk and requires significant recovery time. Attempts to move to transepithelial corneal CXL have been hindered by the lack of a coherent understanding of the physicochemistry of corneal CXL. Misconceptions about the applicability of the Bunsen–Roscoe law of reciprocity and the Lambert–Beer law in CXL hamper the ability to predict the effect of ultraviolet A energy during CXL. Improved understanding of CXL may also expand the treatment group for corneal ectasia to those with thinner corneas. Finally, it is essential to understand the role of oxygen in successful CXL.

Conclusions: Improved understanding of the complex interactions of riboflavin, ultraviolet A energy and oxygen in corneal CXL may provide a successful route to transepithelial corneal CXL.

Key Words: cornea, corneal cross-linking, CXL, epi-on, epi-off, transepithelial, review, riboflavin, irradiation, UVA, Dresden protocol, oxygen

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From the *Department of Ophthalmology, Georgetown University Medical Center/Washington Hospital Center, Washington, DC; †Re:Vision Private Practice, Rockville, MD; ‡Corneal Transplant Centre, Pellegrini Hospital, Naples, Italy; and §Department of Pharmacy, University Federico II of Naples, Naples, Italy.

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Correspondence: Roy S. Rubinfeld, MD, MA, Re:Vision, 11200 Rockville Pike, Suite 150, Rockville, MD 20852 (e-mail: rsrubinfeld@gmail.com).

The term "cross-linking" describes chemical reactions that covalently bond 2 or more molecules (especially proteins and other biomolecules) and modify their physicochemical properties. These reactions involve the use of cross-linker molecules that can be activated in situ by heat, pressure, changes in pH, or radiation.[1] Corneal cross-linking (CXL) uses riboflavin 5′-phosphate and ultraviolet A (UVA) irradiation as activators and is used for the treatment of ectatic corneal diseases, particularly keratoconus (KCN) and post-surgery ectasia, as well as infectious keratitis, corneal edema, myopia, and other corneal diseases. Introduced in the 1990s, corneal CXL has become an effective and safe procedure when specific criteria are met.[2]

Reactive oxygen species (primarily singlet oxygen) are produced when UVA irradiation photoactivates riboflavin. The cross-links that strengthen and improve the biomechanical properties of treated corneas and increase their resistance to enzymatic digestion[3–5] appear to reside on the surface of the collagen fibrils and within the surrounding glycosaminoglycan network.

The 3 critical reagents required in the corneal stroma for effective CXL to occur are riboflavin, UVA radiation, and oxygen. Riboflavin does not easily penetrate the corneal stroma, so a variety of techniques have been developed over the past decade to enhance riboflavin penetration of the corneal stroma. These include 1) riboflavin diffusion through the corneal stroma after epithelium removal (standard or epithelium-off CXL), 2) transepithelial riboflavin stromal diffusion (TE-CXL), 3) direct riboflavin introduction into the stroma (stromal pocket), 4) iontophoresis-assisted CXL (I-CXL) to promote transepithelial penetration of riboflavin, and 5) oral administration to achieve riboflavin distribution throughout the cornea.

Corneal CXL TECHNIQUES

Standard Corneal CXL (Dresden or Epi-off CXL)

The first international research paper describing the preclinical assessment of corneal CXL procedures was published in 1998.[6] This paper served as proof-of-concept for CXL and proposed riboflavin 5′-phosphate as a suitable and safe CXL agent. The procedure was gradually refined over the subsequent years,[4,7–10] leading to the standard CXL procedure, often referred to as the "Dresden protocol" or as "conventional" or "epi-off" CXL. This protocol involves surgical removal of the corneal epithelium followed by the application of ~0.1% riboflavin formulation drops every 1 to

2 minutes for 30 minutes. The patient's eye is then examined under blue light, to confirm riboflavin diffusion into the anterior chamber, which is seen as a "flare." (Using this endpoint for stromal loading before UVA irradiation has substantial limitations, as seen in Fig. 1). The cornea then undergoes continuous UVA irradiation (~370 nm wavelength) with a power output of 3 mW/cm^2 for 30 minutes, constituting 5.4 J/cm^2 of total energy. Riboflavin drops are periodically applied to the cornea to avoid endothelial damage.[8] The efficacy of the standard protocol in slowing or halting the progression of KCN has been widely confirmed by clinical trials with follow-ups ranging from 1 to 10 years. These studies consistently demonstrate a statistically significant improvement in corneal stability as well as in visual acuity, refractive outcomes, and topographic outcomes. They also support the relative safety of the protocol as assessed by macroscopic (corneal haze and scarring) and microscopic (endothelial cell count) parameters[11–14] and cost effectiveness of CXL.[15]

Despite its general safety, several significant risks have been reported with standard CXL, including infections, keratitis, edema and scarring, and even corneal perforation.[16] These adverse events may lead to further loss of vision or even loss of the eye.[17] Moreover, substantial pain and reduced vision for weeks after standard CXL slow postoperative patient recovery.[18–20] These drawbacks are mainly the consequence of surgical removal of the cornea's protective epithelial layer and usually persist until this layer heals. This necessitates that patients miss work or school for a period of prolonged recovery. In unilateral CXL, this would be repeated for the second eye. Thus, less invasive alternatives to standard CXL techniques continue to be pursued (Stulting D. Predicting and Treating Corneal Ectasia. Presented at the ASCRS Symposium on Cataract, IOL and Refractive Surgery as the Binkhorst Lecture, May 2016; New Orleans, LA).[21]

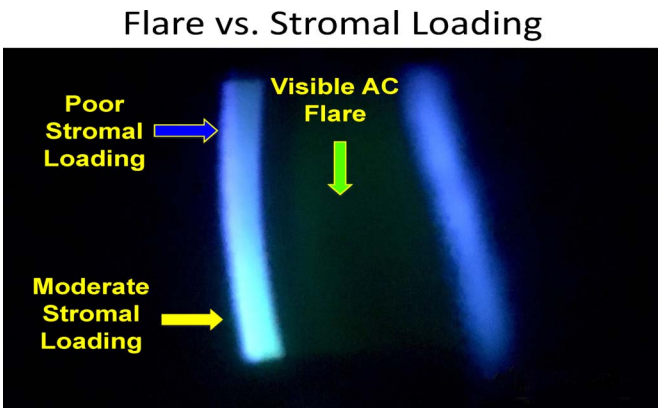

FIGURE 1. Cobalt blue slit-lamp photograph of cornea being checked before UVA application. Riboflavin "flare" in the anterior chamber has often been used as an endpoint for adequate riboflavin stromal loading. Although riboflavin is clearly present in the anterior chamber as a flare (green arrow), the corneal stroma is inadequately and nonuniformly loaded. The yellow arrow shows the inferior cornea that has moderate stromal loading, and the blue arrow shows very limited superior riboflavin stromal loading.

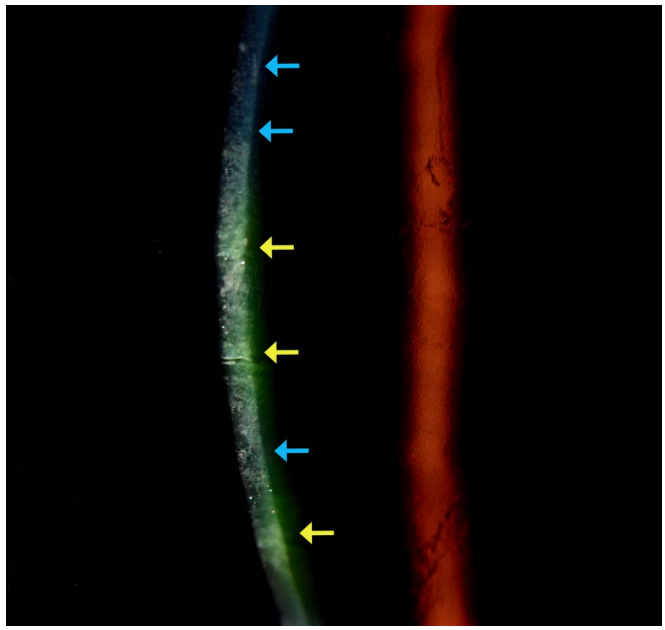

FIGURE 2. Slit-lamp photograph of stromal riboflavin loading after mechanical disruption of the epithelium in a crisscross pattern. The yellow arrows show patchy areas of mild stromal loading [compare with Fig. 7, below], and the blue arrows show areas of little or no stromal riboflavin loading.

Transepithelial CXL (Epi-on)

Retaining the corneal epithelium would reduce many of the risks, patient pain, and inconvenience of standard CXL. However, getting adequate riboflavin through intact epithelium has been considered impractical or impossible based partly on misconceptions about riboflavin 5′-phosphate's corneal pharmacokinetics. The riboflavin molecule is often described as "too large" to cross the epithelial barrier.[22] In reality, the riboflavin phosphate sodium salt, the ingredient most commonly used in CXL riboflavin eye drops, has a molecular weight of only 478.33 g/mol. Riboflavin phosphate is a hydrophilic molecule, and it is its negative charge, not its molecular size, that accounts for its low corneal epithelial permeability[23] such that standard riboflavin formulations do not reach the desired intrastromal concentration through an intact epithelium.[24] One early strategy for performing effective TE-CXL used riboflavin formulations with "permeation enhancers" such as alcohol,[25] combinations of benzalkonium chloride,[26] 1,3-Propanediol, 2-amino-2-(hydroxymetyl)- (Trometamol or TRIS) and ethylenediaminetetraacetic acid (EDTA),[27,28] and/or local anesthetics,[29,30] designed to overcome epithelial permeability barriers. This approach frequently resulted in inadvertent partial or total epi-off procedures as epithelial disruption or postoperative sloughing of the epithelium occurred. These early TE-CXL approaches substantially failed clinical, and even preclinical, assessments[25,28,31–37] and are rarely used in clinics today.

Similar findings have been reported with partial surgical debridement or disruption techniques.[38,39] Although prospective clinical case series with these methods have shown improvement in visual outcomes and corneal shape,[40–42] superior topographic results were found with complete

FIGURE 3. The Daya Disruptor device for mechanical disruption and micropenetration of the epithelium to enhance riboflavin stromal loading without surgical removal of the epithelium. Courtesy of Sheraz Daya, MD. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

epithelial debridement.[39,43] Furthermore, partial debridement (or mechanical disruption) (Figs. 2, 3) has the same safety risks associated with standard CXL. It broaches the integrity of the protective corneal epithelium (Fig. 4), and stromal haze, sterile infiltrates, infectious keratitis, and loss of ≥2 lines of vision have been reported with these techniques.[44,45] In addition, partial epithelial debridement or disruption has not allowed sufficient homogeneous stromal distribution of riboflavin for effective CXL.[25]

Two new (and not yet United States Food and Drug Administration [FDA]-approved) riboflavin formulations for TE-CXL that do not alter the integrity of the epithelium are currently in clinical trials: Ribocross TE (IROS Srl; Napoli, Italy)[46] and Ribostat CXLO (CXL Ophthalmics, Encinitas, CA).[47] Ribocross TE, used in a prospective, nonrandomized clinical trial of 25 eyes with a 2-year follow-up, demonstrated statistically significant improvements in visual acuity, refraction, and corneal topography.[48]

Ribostat CXLO riboflavin demonstrated homogeneous penetration of the corneal stroma of rabbit eyes to a stromal concentration more than adequate for effective CXL.[49] Clinically, Ribostat CXLO–treated eyes (n = 592) demonstrated significant improvements in visual acuity, higher-order aberrations, and topography with no loss of effect 2 years after treatment.[47]

At earlier stages of technical development, nanotechnology has been pressed into service to deliver riboflavin to the corneal stroma. A sustained riboflavin stromal release without epithelium removal has been reported using cyclodextrins,[50,51] amphiphilic nanoemulsion,[52] and biodegradable polymeric nanoparticles plus EDTA and trometamol.[53] The proteoglycan, decorin, has shown some preclinical promise in corneal strengthening without epithelial removal, UVA radiation, or photosensitizer.[54]

Stromal "Pocket" Corneal CXL

In stromal pocket corneal CXL introduced by Kanellopoulos, riboflavin solution is injected into a 100-μm-deep intrastromal pocket incision created by a femtosecond laser followed by UVA irradiation at 7 mW/cm^2 for 15 minutes. The early results were promising in terms of riboflavin loading, efficacy, and safety,[55] but larger clinical trials have not followed. An ex vivo study in porcine eyes reported that the biomechanical effect after loading the corneal stroma using a "pocket" was about half that of the standard epi-off procedure.[37]

Iontophoresis-Assisted Corneal CXL

The physicochemical properties of riboflavin phosphate, while potentially impeding its transepithelial stromal absorption, make it a candidate for iontophoresis-assisted transepithelial delivery. Riboflavin is of relatively small size, negatively charged at physiological pH, and water soluble. In laboratory studies, transepithelial iontophoretic riboflavin loading demonstrated corneal stromal penetration similar to the standard CXL protocol.[56–59] Clinical results of I-CXL have shown some promise. Using the treatment parameters of 1 mA for 5 to 10 minutes, and standard riboflavin 0.1% solution, cessation of keratoconic progression with improvements in

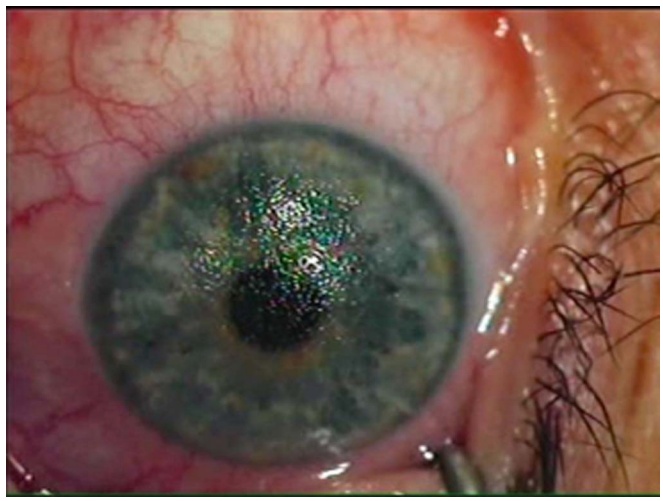

FIGURE 4. Postoperative appearance of a cornea after mechanical disruption of the epithelium with the Daya Disruptor to enhance stromal riboflavin loading. Courtesy of Sheraz Daya, MD. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

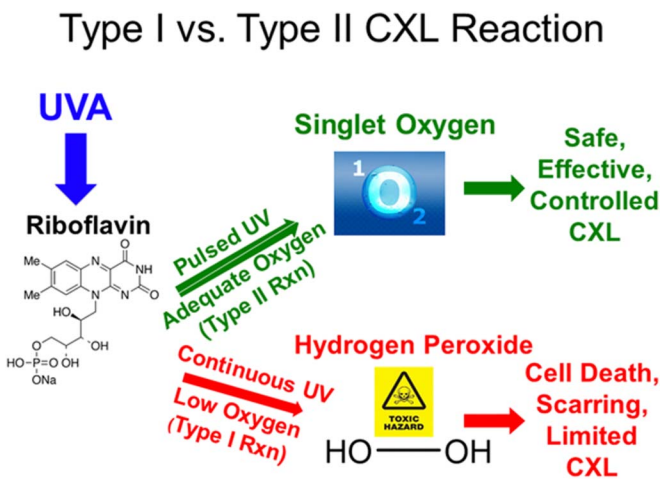

FIGURE 5. Schematic description of the 2 main types of CXL photochemical reactions. Type II is less toxic than type I.

visual acuity and topographic outcomes has been documented for 6 to 15 months, including in pediatric patients.[60–63] However, a 2-year study of I-CXL was less successful, with a 20% failure rate.[64]

Iontophoretic application of riboflavin to the cornea was developed to reduce the time needed to fully load the

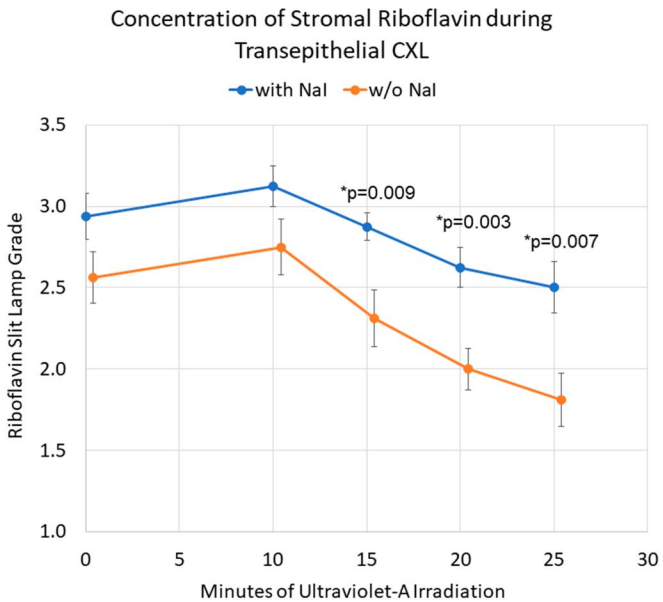

FIGURE 6. Analysis performed by contract laboratory demonstrating iodide's protective effect against UVA-induced photo-degradation. The chart shows riboflavin in the cornea of New Zealand white rabbits (n = 16) observed as green upon masked grading of slit-lamp photographs. The orange line shows grades during UVA exposure after loading with riboflavin solution; the blue line shows the grades in the contralateral eyes where the solution contained sodium iodide. Error bars show standard error of the mean; the final time points are significantly different ($P < 0.05$ by a Wilcoxon Ranked-Sum test). Absorption Systems, LLC, San Diego, CA, data on file 2013 and 2018.

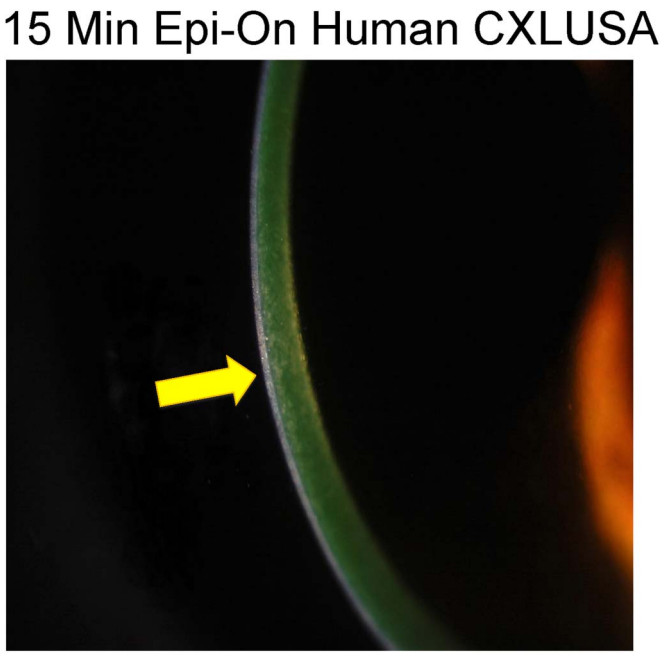

FIGURE 7. Slit-lamp photo of well-loaded cornea ready for UVA application using a novel transepithelial CXL system. Yellow arrow denotes the epithelium, which is devoid of riboflavin, thus permitting high UVA transmission [compare with Fig. 2, above].

corneal stroma with riboflavin, decreasing patient pain and epithelial damage. However, patients report that the procedure is painful, and examination of the cornea shows disruption of the epithelium, resulting in postoperative epithelial defects.[65] Long-term stabilization of the cornea has not been demonstrated definitively, and little is known about possible unexpected side effects. When iontophoresis is used to deliver treatments to the skin, tingling, itching, and discomfort from the electric current have been reported as well as mild erythema and edema.[66] Clinicians performing I-CXL have reported poor stromal loading and patient complaints of marked discomfort during the procedure; as well as postoperative epithelial sloughing and subsequent postoperative pain. Moreover, there is a risk of epithelial abrasion during placement and removal of the iontophoretic corneal applicator.[67]

To improve riboflavin stromal loading, a "mixed" technique combining iontophoretic riboflavin delivery with epithelium removal has been proposed.[68] Reported clinical results were comparable to standard CXL.

Oral Riboflavin for corneal CXL

An unusual approach to corneal CXL without epithelial abrasion uses dietary riboflavin followed by sunlight exposure. A very small (7 patients) prospective study using oral administration of riboflavin and 15 minutes of natural sunlight exposure daily demonstrated no adverse effects. However, the small sample size limits the assessments of efficacy. A larger clinical study is ongoing.[69]

INNOVATIONS IN CXL PROCEDURES: SUCCESSES AND CRITICISMS

The Energy Issue: Accelerated CXL

In 2004, an upper limit for safe UVA irradiation of the cornea during standard CXL was calculated as 3 mW/cm^2 based on levels of endothelial cell damage and keratocyte apoptosis in an ex-vivo rabbit model after riboflavin/UVA treatment.[10,70] A 2016 study by Mooren et al[71] suggests that the safe limit of UVA irradiation may be higher. Human corneas were loaded with riboflavin and irradiated from the endothelial side in vitro. Despite UVA dosages 8 times the established cytotoxic threshold, there was no statistically significant difference in endothelial cell counts between the irradiated and control cornea samples.[71] These findings further encouraged preclinical evaluations of high fluence protocols, known as accelerated CXL (A-CXL),[72–74] using UVA power intensities of up to 90 mW/cm^2.[75] In vitro results [using demarcation lines (DL) as a CXL efficacy metric] suggest a comparable efficacy of A-CXL to standard CXL in terms of corneal stiffening when fluence rates are maintained below 40 to 50 mW/cm^2. Above this threshold, however, the efficacy suddenly decreases.[75]

Here, it should be noted that the DL may not be an appropriate measure of CXL efficacy. It is generally accepted that the DL represents the transition zone between the upper cross-linked stroma and the underlying untreated tissue. However, a study that increased the fluence and decreased exposure time in A-CXL yielded DLs that were shallower than in standard CXL, but not deeper.[76] And in I-CXL-treated patients, there was an almost total absence of a DL.[63] Later findings noted the presence of DL in approximately half of patient eyes 1 month after I-CXL followed by its disappearance at 3 months postoperatively.[77] Finally, a 24-month study of standard CXL, A-CXL, and TE-CXL showed no correlation between DL and topographic changes.[78]

Clinical results with A-CXL have been mixed. Several case series of A-CXL demonstrate a lack of KCN progression and improvements both in topographic and in visual parameters with 6 months,[79,80] 1 year[81] and 2 years of follow-up,[82] as well as in pediatric cases.[83,84] Some authors have found similar refractive and topographic outcomes,[85–88] whereas others have reported more effective topographic flattening with standard protocols.[89,90] This reduced efficacy of A-CXL has been correlated with reports of more superficial corneal stromal DL.[91,92] Moreover, Lombardo et al[93] provided evidence of a low stromal riboflavin consumption, when accelerated UVA irradiation is performed, that may correspond to fewer cross-links and less corneal strengthening.

Unfortunately, A-CXL is based on a scientific misconception of the Bunsen–Roscoe law of reciprocity.[94] This law states that the effect is directly proportional to the total cumulative energy dose irrespective of fluences or how the dose is administered. This suggests that corneal strengthening can be achieved with a higher fluence and reduced exposure duration. However, the Bunsen–Roscoe reciprocity law was developed in the field of darkroom photography. The complex biochemical reactions involved in CXL are fundamentally different than the simpler inorganic reactions on which the reciprocity law was based. Beyond threshold energy levels, the CXL photochemistry reaction changes entirely and the reciprocity law is not applicable in the complex biological milieu of the cornea.[95–98] Instead of being dependent on the total cumulative energy dose, corneal CXL photochemical reactions appear to be dependent on energy intensity.[99,100] One simplified everyday example of how the Bunsen–Roscoe law does not hold true can be described as follows: If a brownie recipe calls for baking at 300 degrees for 30 minutes, applying the Bunsen–Roscoe law to speed the process would indicate baking the brownies at 3000°F for 3 minutes.

Returning to corneal CXL physicochemistry, taking the well-tested standard CXL parameters of 3 mW/cm^2 of UVA energy applied for 30 minutes, the Bunsen–Roscoe law would calculate a UVA radiation setting of 30 mW/cm^2 applied for 3 minutes. In both examples, the amount of radiant energy is equivalent. In the CXL setting, both protocols deliver a total dose of 5.4 J/cm^2 of UVA radiation, but deliver this dose at very different rates to very different effects.

Not enough is known about the potential harmful effects of high UVA fluence on various eye structures such as the lens and retina,[101–104] epithelium (in transepithelial CXL),[105] and corneal stroma.[106–108] Some evidence exists to support the safety of A-CXL in terms of unchanged endothelial cell counts;[109,110] however, there are relatively few published clinical studies evaluating the effects of A-CXL on the corneal endothelium. Some suggest negative effects of high UVA fluence CXL on endothelial cells.[111–113] These studies differ in measuring parameters in addition to endothelial cell density, such as the percentage of hexagonal cells and the coefficient of variation. This may explain the contradictory results.

Kalkan et al[114] have reported no adverse effects on ocular surface and tear function after A-CXL, despite metaplastic changes and a reduction in the density of surface goblet cells. A small study using the speed of epithelial healing as an indirect indicator of limbal stem cell function demonstrated no significant difference between irradiated and control corneas.[115,116] Nevertheless, ultraviolet exposure, particularly UVA, is associated with a wide range of pathologies, including cataract, photokeratitis, pterygium, keratopathy, and neoplasias.[117] Limbal cell damage has been reported after standard CXL treatment[118] and may be exacerbated in A-CXL, despite the shorter exposure time. Assessment of genotoxic and long-term tissue changes would be helpful to complete the risk assessment of A-CXL.

On the other hand, corneal stiffening comparable to that after standard CXL has been recently demonstrated when UVA exposure times are shortened and intensities lower than 3 mW/cm^2 are used.[99,118] Our review suggests the A-CXL procedure (especially at very high fluences) may not be an attractive therapeutic option at the current time.

THE 400 μm RULE

To ensure that ocular structures deep to the cornea, such as the endothelium, lens, or retina, are not adversely affected by UVA irradiation, only patients with a central corneal pachymetry of at least 400 μm have been considered to be

eligible for the standard CXL treatment. The so-called 400 μm rule comes from the experiments by Wollensak et al using 3 mW/cm^2 as the UVA safety limit to avoid significant endothelial cell loss in CXL procedures[70,119] and was corroborated by Kymionis et al in 2012,[120] confirming a significant decrease in the endothelial cell count after performing standard CXL in thin corneas despite positive clinical outcomes. Adherence to this "rule" is clinically limiting because corneas thinner than 400 μm are common in patients with advanced-stage ectasia. Thinner corneas are also endemic in certain patient populations.[121,122]

Various approaches have been proposed over the years to circumvent the 400 μm limitation and expand the pool of patients who can benefit from CXL to include those whose eyes have thin corneas. Soaking de-epithelialized corneas with a hypo-osmolar solution can double the corneal thickness measured by pachymetry[67] This method has been used in corneas <400 μm thick with excellent clinical outcomes in terms of stabilization of keratectasia and no detectable stromal scarring.[123,124] Conversely, decreased endothelial cell density[125] and clinical failures have been associated with this protocol,[126] suggesting that hypo-osmolar riboflavin solutions are not the optimal solution for corneas <330 μm.

Epi-off CXL procedures induce keratocyte apoptosis[70] and corneal inflammation.[18] The use of hypo-osmolar agents and the resultant swelling may add additional inflammation and delay healing as corneal edema can interfere with re-epithelialization.[127,128] This increased corneal swelling produced by hypo-osmolar riboflavin solutions is variable and transient as assessed by intraoperative pachymetric readings,[129–131] limiting the rationale and applicability of this technique when dextran (with its hyper-osmotic properties) is present in the riboflavin formulation.[131,132]

A small pilot study introduced contact lens-assisted collagen cross-linking.[133] In this technique, corneal epithelium is removed, and riboflavin applied. Once the riboflavin has penetrated the corneal stroma, a soft contact lens, without ultraviolet blockers, soaked in riboflavin is placed on the cornea during the irradiation procedure. The contact lens/riboflavin layer was proposed to provide the extra thickness needed for a thin (<400 μm) cornea to attenuate the ultraviolet irradiation, protecting the endothelium. At 6 months, clinical results for the 14 eyes treated were good. No significant endothelial cell loss was reported,[133] and cytotoxicity (assessed by in vivo confocal microscopy) was similar to that found after standard CXL.[134]

The epithelial island CXL technique uses customized epithelial debridement to leave patches of epithelium over the thinnest corneal areas.[135] Although this study was conducted in a small series of patients (10) with a 1-year follow-up, good clinical outcomes were reported with no endothelial cell loss. Larger trials are in progress. The results of a similar protocol have been recently published by Cagil et al[136] in which significant endothelial cell loss was reported.

A unique approach to the treatment of thin corneas involves the use of customized protocols. These protocols are based on a mathematical elaboration of mean riboflavin corneal loading and consumption under UVA irradiation and are potentially able to predict the most effective and safe CXL UVA irradiation parameters (source intensity and exposure time) as a function of the patient's morphological parameters (corneal thickness).[137] Theoretically, stiffening thinner corneas should be safely achieved by decreasing average UVA fluences and exposure times.[137,138] Theoretical and in vitro results show promise, but clinical trials are needed.

Mooren et al[71] demonstrated a higher-than-expected resistance of human corneal endothelial cells to riboflavin-plus-UVA-irradiation damage. More recently, similar findings were reported for an in vivo study on rats, revealing that corneal stromal CXL occurs with reversible endothelial cell damage and transient declines in keratocyte viability when using higher UVA doses than the standard CXL safety limit.[139] These same findings were previously reported in a clinical trial.[112] This suggests that endothelial cell density is not the best, and should not be the sole, parameter assessed for safety concerns.[114,140–145]

A recent study using transepithelial CXL included eyes with corneas as thin as 302 μm, with no indication of damage to corneal endothelial cells.[47] Using the same CXL procedure, one of the authors (R.S.R.) successfully reduced his minimum corneal pachymetry inclusion criterion to 275 μm, with no cases of corneal edema or any indication of corneal endothelial cell damage (R. S. Rubinfeld, MD, MA, personal communication).

THE ROLE OF OXYGEN IN CXL

A second flawed photochemical assumption of CXL procedures invokes the Lambert–Beer law to describe a predictable exponential decline in UVA transmission as a function of corneal depth. This profoundly oversimplifies UVA absorption kinetics in CXL, which is not simply dependent on corneal thickness and stromal riboflavin loading, but on the interaction of UVA radiation with riboflavin.

When exposed to UVA radiation, riboflavin is rapidly photodegraded and converted to its oxidized derivatives, some of which are colorless, no longer block UVA radiation, and inactive in the CXL process.[99] The efficacy of the CXL process and protection of the endothelium rely on the active nondegraded riboflavin in the cornea. Thus, the current CXL procedures require intraoperative riboflavin application to replace the riboflavin that is degraded by UVA irradiation to inactive and non-UVA radiation blocking degradation products. This repeated application of riboflavin during UVA exposure profoundly and variably affects the transmission and dosing of UVA.[137]

The CXL photoreaction is driven by the interaction of corneal stromal oxygen, riboflavin, and UVA irradiation and can follow the type I or type II reaction pathway (Fig. 5).[146] The type I reaction pathway occurs in low-oxygen conditions. Riboflavin triplets act as a cross-linker, and toxic hydrogen peroxide is generated as a final product. In the aerobic (nonhypoxic) type II reaction, the riboflavin triplets react directly with oxygen to form the less toxic singlet oxygen that cross-links collagen.

This pivotal role for oxygen was initially proposed by McCall et al[147] who theorized that effective corneal cross-linking was directly related to the presence of singlet oxygen. In their study, sodium azide was used to quench singlet oxygen, resulting in decreased stiffening of shark and rabbit corneas after CXL. However, the study was confounded by the fact that sodium azide also protects riboflavin from photodegradation via an indirect mechanism involving singlet oxygen quenching.[148,149]

Another theory postulates that photoreaction mechanisms are based on the interaction between riboflavin triplets and stromal proteins,[150,151] although a subordinate role of singlet oxygen could not be excluded.[152,153] Oxygen was thought to restore stromal riboflavin concentrations by oxidation of its reduced and inactive derivatives.[154–157]

Currently, the most plausible mechanism for corneal CXL posits that during standard continuous UVA exposure, aerobic conditions exist for less than a minute of UVA exposure, generating safe CXL driven by singlet oxygen and other less toxic reactive oxygen species (type II mechanism).[158] After initial exposure, continuous UVA irradiation depletes stromal oxygen and the CXL reaction shifts to the type I mechanism, relying on the reaction of toxic hydrogen peroxide and riboflavin triplets.[158] This model explains the failure of A-CXL procedures when higher fluences are used as oxygen is rapidly depleted and its stromal rediffusion is prevented.[20,159–161] This may also explain why the DL depth actually decreases with increasing irradiation intensity despite having the same total energy exposure.[17,18,20,162]

Further evidence that oxygen, rather than irradiation power, is generally the critical limiting factor in corneal CXL comes from a paired-eye study of patients undergoing corneal CXL in conjunction with laser-assisted in situ keratomileusis (LASIK).[161] In the study, the only parameter varied was the duration of UVA irradiation (2 or 3 minutes). The irradiation intensity was held constant at 18 mW/cm^2 for a total energy exposure of 2.16 J/cm^2 in the 2-minute group and 3.24 J/cm^2 in the 3-minute group. The law of reciprocity would predict better outcomes from the 3-minute group. However, outcome measures (corneal haze, DL depth, and spherical equivalent) were nearly identical in the 2 groups. The authors postulate that corneal stromal oxygen concentration was the limiting factor in the treatment.[76,92,161,163,164]

Decreased rediffusion of oxygen may also explain why corneal haze, which is commonly seen with standard CXL (using continuous-wave UVA irradiation), is almost never seen when a pulsed UVA duty cycle optimized for reoxygenation is used.[47] Investigators are now testing supplemental oxygen CXL and/or ozone CXL to improve A-CXL.[165]

Oxygen diffusion may also be slowed by an intact corneal epithelium in I-CXL and epi-on procedures.[100,166] To address this problem, formulations have been developed that photostabilize riboflavin, maintaining its efficacy and UVA-blocking properties despite UVA exposure. One riboflavin formulation contains a novel excipient, sodium iodide, which stabilizes riboflavin in its excited state.[149,167] Iodide also promotes the immediate conversion of toxic hydrogen peroxide into oxygen and water by a redox reaction. This fuels the less toxic type II CXL reaction, protects cells from phototoxic damage,[46] and effectively counters UVA photodegradation of riboflavin in photostability testing (Fig. 6) (data on File 2018, Absorption Systems LLC, San Diego, CA). This novel TE-CXL formulation has also yielded excellent long-term clinical results in a large-scale clinical trial including 592 eyes in which periodic application of riboflavin was neither performed nor needed.[47]

Replacing continuous-fluence UVA irradiation with pulsed light, of correct cycle intervals, can allow oxygen stromal rediffusion during CXL.[168–170] In some epi-on systems, adequate UVA dose, stromal oxygen, and riboflavin are ensured in the presence of intact epithelium when the ocular surface and epithelium retain little or no riboflavin (Fig. 7). With all the riboflavin diffused into the corneal stromal layer, only 15% to 20% of the incident UVA light should be absorbed by the epithelium.[171] A variety of low fluence and customized irradiation protocols are being tested in clinical studies.[47,99,137]

CONCLUSIONS

When standard CXL was the only effective treatment, its drawbacks were relatively less important, with dramatically lower risk-to-benefit ratio compared with penetrating keratoplasty or no treatment. Since its introduction approximately 15 years ago, however, several technological innovations have been proposed and clinically tested to improve corneal CXL.

The ideal CXL procedure should be highly clinically effective, safe, and standardized and involve minimal patient discomfort and recovery time. Reviewing the literature, these requirements can be fulfilled, irrespective of the protocol used if 1) adequate homogeneous stromal loading of riboflavin is achieved, 2) adequate relatively unblocked transmission of UVA radiation through the stroma is maintained, and 3) adequate oxygen rediffusion is promoted. Improved safety and patient benefits accrue when the epithelium is not disrupted. A highly effective epi-on technique fulfilling these requirements would be a suitable and preferable protocol for corneal CXL.

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