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REVIEW

Corneal collagen crosslinking for corneal ectasias: a review

David P.S. O'Brart

Department of Ophthalmology, Keratoconus Research Institute, St. Thomas' Hospital, London - UK

ABSTRACT

Purpose: To review the published literature on corneal collagen crosslinking (CXL).

Methods: Importance has been placed on seminal publications, systemic reviews, meta-analyses, and randomized controlled clinical trials. Where such evidence was not available, cohort studies, case-controlled studies, and case series with follow-up greater than 12 months were examined.

Results: Corneal collagen crosslinking with riboflavin and ultraviolet A (UVA) 370 nm radiation appears to be capable of arresting the progression of ectatic corneal disorders, with most studies reporting significant improvements in visual, keratometric, and topographic measurements. Its mode of action at the molecular level is undetermined. Follow-up is limited to 5-10 years but suggests sustained stability and enhancement in corneal shape with time. Nearly all published long-term data and comparative studies are with epithelium-off techniques. Epithelium-on investigations suggest some efficacy but less than with epithelium-off treatments and long-term data are unavailable. Accelerated techniques with higher UVA fluencies and shorter treatments times, delivering the same UVA energy dosage, are the subject of recent investigation, with some laboratory and clinical studies suggesting reduced efficacy compared to the standard 3 mW/cm^2 for 30 minutes irradiation procedure. Combined methodologies of CXL with techniques such as photorefractive keratectomy and intrastromal rings show promise but long-term follow-up is indicated. Sight-threatening complications of CXL are rare.

Conclusions: Studies of epithelium-off CXL with irradiation at 3 mW/cm^2 for 30 minutes support its efficacy. Refinement in techniques may allow for safer and more rapid procedures with less patient discomfort but require further investigation.

Keywords: Cornea, Crosslinking, Keratoconus, Riboflavin, Ultraviolet light

Introduction

The concept of treating corneal ectatic disorders with riboflavin (vitamin B_2)/ultraviolet A (UVA) (370 nm) corneal crosslinking (CXL) was postulated by Spörl and colleagues at the University of Dresden (1-4). Physiologically, CXL occurs in tissues with aging via enzymatic pathways such as transglutaminase and lysyl oxidase. Wollensak et al (4) hypothesized that photochemical crosslinking of collagen within the corneal stroma could be achieved by utilizing the interaction between riboflavin and UVA to create oxygen free radicals, which then would activate the normal physiologic lysyl oxidase pathway. As well as acting as a photosensitizer, riboflavin could also prevent damage to internal ocular structures, such as the endothelium, lens, and retina, by absorbing the majority of the UVA energy, which is potentially cytotoxic and mutagenic, within the first 300 µm of the anterior stroma (4).

While Spörl and colleagues postulated the occurrence of lysine-based crosslinks following CXL, they have not been found chemically. Most research on riboflavin photochemical reactions has been undertaken in foods. They have been shown to be associated with the creation of singlet oxygen (5-7), with the crosslinking reactions involving tyrosine residues (8, 9), glycation end products (10), and alterations in secondary and tertiary protein structures (11). McCall et al (12) found that riboflavin/UVA CXL in vitro was inhibited by azide, which blocks singlet oxygen reactions (6-7, 13), and promoted by deuterium oxide (D_2O), which prolongs the half-life of oxygen radicals (13), and therefore postulated that singlet oxygen was central to the process. They further proposed that as CXL was prevented by the blocking of carbonyl groups with 2,4-dinitrophenylhydrazide/hydroxylamine but still occurred when the amine groups were blocked with acetic anhydride/ethyl acetimidate, it was not occurring via lysyl oxidase but other mechanisms, including imidazolone formation, which can attach to molecules, such as histidine, to form new covalent bonds, the triggering of endogenous populations of

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Corresponding author:
Professor David P.S. O'Brart
Department of Ophthalmology
Keratoconus Research Institute
St. Thomas' Hospital
London SE1 7EH, UK
davidobrart@aol.com

carbonyl groups in the extracellular matrix (ECM) (allysine, hydroxyallysine) to form crosslinks there, and/or the degradation of the riboflavin molecule itself, releasing 2,3-butanedione, which can react with the endogenous carbonyl groups of proteins (12). The significance of oxygen in riboflavin/UVA CXL was reinforced by Richoz et al (14), who treated corneas under 2 atmospheres: 1 with oxygen at 21% and the other at less than 0.1%. They found that under normal oxygen levels, there was a significant increase in extensiometry measurements, but not with corneas treated in a low-oxygen atmosphere and untreated controls.

Despite such results, the exact role of oxygen in the riboflavin/UVA CXL process is unclear. Kamaev et al (15) measured oxygen consumption in the stroma during CXL and discovered brisk oxygen depletion within 10-15 seconds of 3 mW/cm^2 UVA exposure. They proposed that aerobic conditions, allowing a type II photochemical reaction, are only present during the first seconds after UVA exposure and hypothesized that the majority of the riboflavin/UVA CXL process might be initiated by excited riboflavin triplets, with singlet oxygen playing only a transitory role. They observed that sodium azide, used in the study by McCall et al (12), also impairs the action of excited riboflavin triplets, as well as oxygen singlets (15, 16). It is of note that Kato et al (8) found that both azide and another singlet oxygen quencher, 1,4-diazabicyclo(2,2,2)octane, did not prevent riboflavin photodynamic crosslinking of collagen. They also noted that these photochemical crosslinking changes were associated with loss of tyrosine and histidine residues within the collagen molecules and that this tyrosine loss could be inhibited by oxygen (8). They recorded that dityrosine formation was seen with the loss of tyrosine and proposed that photodynamic modification of tyrosine may contribute to the riboflavin-sensitized CXL of collagen through the formation of dityrosine (8).

In addition to the uncertainty regarding the precise chemical interactions involved in riboflavin/UVA CXL, the location of the crosslinks at the molecular level is uncertain. Crosslinks cannot be formed between the collagen fibrils themselves, as the distance between individual fibrils is too large for any intramolecular bond to be possible. Hayes et al (17), in a series of experiments to investigate stromal ultrastructure using X-ray scattering, hydrodynamic behavior, and enzyme digestion, hypothesized that it was likely that the crosslinks were occurring on the surface of the collagen fibrils, rather than within them, and in the glycosaminoglycan protein network adjacent to the fibrils (17).

Laboratory studies: Biophysical changes

While the locations of the crosslinks between the proteins within the stroma created by CXL cannot be visualized, multiple laboratory studies have reported a number of alterations in the mechanical, physical, and chemical properties of the stroma consistent with their existence. In terms of mechanical changes, stress-strain (extensometry) measurements of stromal tissue are augmented (3-4, 18), both instantaneously as well as many months following CXL (19). Such modifications have been shown to mainly take place in the anterior 200 µm of the stroma, where most UVA absorption will occur (20). In addition, a greater thermal shrinkage temperature has been reported in the stroma with a larger effect documented anteriorly (21), while more recently, other novel techniques to measure corneal biomechanics such as ultra-high-speed Scheimpflug noncontact air pulse tonometry (22), scanning acoustic microscopy (23), and Brillouin microscopy (24) have demonstrated increased corneal stiffness following CXL.

Chemical changes include an increased resistance of stromal tissue to enzymatic digestion with a dose response in relation to UVA irradiation intensity (25). In particular, an augmented resistance to matrix metalloproteinase (MMP) degradation, especially subtypes MMP-1, 2, 9, and 13, following CXL has been reported (26). This increased resistance of collagen and proteoglycans from MMP degradation is likely to be important in the potential of CXL in averting progression in keratoconus, where increased activity of collagenases has been recognized (27).

Other biophysical changes after CXL include an increase in collagen fiber diameter in rabbit corneas (28) and a reduction in hydration behavior (29), which occur to a greater extent in the anterior compared to the posterior stroma. The increased resistance to hydration has led to the proposal that CXL might have a role in the management of corneal decompensation. However, recent investigations have suggested that such changes in hydration performance are just temporary alterations due to the effects of the osmolarity of the 20% dextran-containing riboflavin solutions used in the initial laboratory studies rather than consequences of CXL itself and unlikely to be permanent alterations (17, 30). Finally, there are modifications in the electrophoretic pattern of corneal collagen type I, with the occurrence of an extra polymer band, with a molecular size of approximately 1,000 kDa, resistant to mercaptoethanol, heat, and pepsin (31).

Laboratory studies: Safety

It must always be remembered that UVA is both cytotoxic and potentially mutagenic. Caution must be adopted with its usage in any proposed therapeutic intervention. It can cause keratocyte apoptosis and corneal endothelial cell damage/death as well as possible lens and even retinal injury (32-35). As might be anticipated, cell culture studies of keratocytes have demonstrated an enhanced cytotoxic irradiance point with UVA irradiation combined with photosensitizing riboflavin (32), which in the clinical setting occurs in human eyes to a depth of 300 µm (32). More importantly, with regards to irreversible endothelial cell damage, cell culture studies have shown a cytotoxic threshold level with irradiation levels greater than 0.35 mW/cm^2 (33), which should not be achieved, with a UVA dosage of 3 mW/cm^2 for 30 minutes (total dosage 5.4 J/cm^2) with corneal thickness greater than 400 µm (33). In vivo studies have substantiated these laboratory findings and confirmed that with the standard dosage more than 85% of the UVA is absorbed by the riboflavin in the anterior 400 µm of the stroma (34, 35), with the resultant irradiance at the endothelium being less than 0.18 mW/cm^2. This is half the cytotoxic level (34, 35). This situation is similar for the lens and retina, where the level of UVA radiation during CXL reaching these tissues is less than 3% of the cytotoxic limit (35).

With regards to limbal stem cell damage, which may take years to become apparent after UVA injury, a number

of studies have demonstrated potential adverse changes. Oxidative nuclear DNA damage has been found in cultured epithelial cell lines and ex vivo limbal corneal tissue (36), and damage to limbal epithelial cells with a drop in viable cells has been documented in human eyes ex vivo (37). In view of such changes, it is recommended that UVA limbal irradiation should be avoided during CXL.

Clinical studies (epithelium-off CXL)

Prospective cohort case series

The first published clinical application of riboflavin/UVA CXL was not to treat ectasia, but to address corneal ulceration and melting, with the procedure successfully arresting melting in a series of 3 out of 4 eyes (38). The first report of its use in keratoconus was published in 2003 (4). Wollensak et al (4), in a series of 23 eyes that underwent an epithelium-off CXL technique, reported stabilization of ectasia with up to 5 years follow-up. In 70% of eyes, an improvement was documented, with an average reduction in spherical equivalent refractive error of 1.0 D and maximum keratometry (Kmax) of 2.0 D. Endothelial counts were unchanged and no loss of transparency of the cornea or lens was documented (4). Since this seminal publication, other research groups have published multiple prospective, cohort case series of epithelium-off CXL with up to 24 months follow-up (39-50), including series of pediatric patients (51, 52) and advanced keratoconus (53). They have corroborated the results of Wollensak et al (4), with stabilization of keratoconus in the majority of treated eyes with few sight-threatening complications and statistically significant improvements in visual performance, topographic keratometry, corneal shape, and higher-order aberrations (39-53). Likewise, prospective case series of epithelium-off CXL to treat iatrogenic postlaser refractive surgery ectasia have documented stability of topographic parameters and improvements in vision with over 2 years follow-up (54-56) and up to 5 years follow-up in one case series (57). In addition, encouraging results have been seen in case reports of CXL in eyes with pellucid marginal degeneration (58-60). It should be noted, however, that while the results of these multiple clinical studies have been very encouraging and supportive of CXL, inclusion criteria have varied considerably, with differing follow-up parameters.

Randomized controlled studies

There is still a relative paucity of randomized, prospective clinical studies. O'Brart et al (61), in a randomized, prospective, bilateral study in 22 patients with documented keratoconic progression, in which one eye was treated with an epithelium-off technique and the other left untreated as a control, reported stabilization in all treated eyes with statistically significant improvements in corrected distance visual acuity (CDVA), keratometry, apex power, and higher-order aberrations, with progression in 14% of untreated eyes over an 18-month follow-up period. Similarly, Wittig-Silva and colleagues (62, 63), in a study of 48 untreated control eyes and 46 treated eyes with 3-year follow-up, documented a significant increase in Kmax and refractive cylinder with a reduction in uncorrected visual acuity (UCVA) in control eyes, while treated eyes showed a significant reduction in Kmax and improvement in UCVA and CDVA. Correspondingly, Vinciguerra et al (40), Chang and Hersh (64), and Greenstein et al (65) reported significant improvements in UCVA, CDVA, higher-order aberrations, and topographic indices in 66 eyes with keratoconus and 38 with iatrogenic ectasia with 12-month follow-up, and found better results in more severely affected eyes. More recently, Lang et al (66) documented a significant difference between treated and control eyes in terms of changes in corneal refractive power, which lessened in treated and increased in untreated cases over a 3-year follow-up period, while Seyedian et al (67) in a bilateral randomized study found a significant difference in Kmax and CDVA at 12 months, which improved in treated and worsened in contralateral control eyes. Most recently, Sharma et al (68) in a randomized trial with a sham treatment (riboflavin administration with no UVA exposure) in control eyes showed an improvement in 23 treated eyes in terms of uncorrected distance visual acuity (UDVA), refractive cylindrical correction, and Kmax, while the sham control group of 20 eyes showed no such changes. These randomized, controlled studies, while relatively few and limited in the number of subjects treated, continue to provide a growing body of evidence to support the efficacy of riboflavin/UVA epithelium-off CXL. Indeed, 2 recent meta-analyses have confirmed the consistent improvements in visual performance and the reduction in keratometry values seen in these randomized clinical trials, and support the use of CXL in as a therapeutic intervention to stabilize keratoconus, although further follow-up studies are necessary to determine the longevity of efficacy (69, 70).

Long-term follow-up

While prospective cohort, randomized controlled studies, and meta-analyses of epithelium-off CXL support its midterm efficacy and safety, there is scarcity of long-term data. Keratoconus characteristically progresses at an unpredictable rate for about 2 decades following presentation and then becomes stable, probably as a consequence of physiologic age-related crosslinking (71-73). The rate of molecular turnover of collagen and the ECM within the cornea is unknown. Given such considerations, the duration of effectiveness of CXL and the necessity to repeat the procedure is undetermined. Table I shows the outcomes from a number of published case series with follow-up of greater than 24 months. Raiskup-Wolf et al (74), in 33 eyes with over 3 years follow-up, reported stabilization of keratoconus with reduction of keratometry and improvements in vision with time, while Caporossi et al (75)documented stability of ectasia in 44 eyes after 48 months, with a reduction in keratometry and coma and improvements in vision. Similarly, O'Brart et al (76), studying 29 eyes, found stabilization in all, with improvements in refraction, vision, keratometry, and higher-order aberrations at 12 months, which showed statistically significant continued improvement at 4-6 years; likewise, Hashemi et al (77) demonstrated stabilization in 40 eyes of 32 patients with progressive keratoconus with continued improvement in corneal elevation measurements over 5 years of follow-up. More recently, in a group of 40 eyes of 40 pediatric patients aged between 10 and 18 years, Uçakhan et al (78) reported cessation of progression in all eyes with significant

TABLE I - Visual, refractive, and keratometric changes after epithelium-off riboflavin ultraviolet A (UVA) corneal crosslinking (CXL) in prospective case series in which all eyes have a reported follow-up of 24 months or more

First author	Year	No. of eyes	Follow-up, mo	UDVA change, logMAR	CDVA change, logMAR	MSE change, D	Kmax change, D	Pachymetry change, µm	Failure, %
Raiskup-Wolf (74)	2008	33	>36		-0.15		-2.57		6
Caporossi (75)	2010	44	48	-0.37	-0.14	+2.15	-2.26	+0.6	0
Kampik (47)	2011	46	24		-0.05		-1.23	-21.6	
Vinciguerra (52)	2012	40	24	-0.21	-0.19	+1.57	-1.27	+14.0	
Goldich (48)	2012	14	24		-0.08		-2.40		0
O'Brart (76)	2013	30	>48	-0.01	-0.1	+0.8	-1.06	+2.0	0
Uçakhan (78)	2016	40	48	-0.4	-0.3	+0.93	-1.4	-18.8	0
Theuring (80)	2015	34	120		-0.13		-3.64	-46.0	6
Poli (79)	2015	36	72	-0.08	-0.14		+0.11	-16.4	11
O'Brart (82)	2015	36	78	-0.14	-0.13	+0.78	-0.9	-3.0	0

CDVA = corrected distance visual acuity; UDVA = uncorrected distance visual acuity; MSE = mean spherical equivalent.

improvements in UCVA and CDVA and reduction in Kmax with continued improvements in topographic indices with continued follow-up. Such results in pediatric patients are particularly encouraging given the propensity for such young patients to progress.

Few studies have reported follow-up over 5 years. Poli et al (79) reported no evidence of keratoconic progression in 36 eyes of 25 patients 6 years after CXL with significant improvement in CDVA and no sight-threatening complications. Theuring et al (80) and Raiskup et al (81) documented significant improvements in vision and keratometry in 34 eyes of 24 patients at 10 years, with progression in only 6% of cases and no impairment in the transparency of the cornea or lens. O'Brart et al (82) reported no progression in any eyes in 36 eyes of 36 patients at a mean follow-up of 7 years with significant improvements in visual, topographic, and corneal wavefront parameters, which continued to improve between 1 and 5 years after surgery with stabilization of these parameters thereafter, although CDVA continued to improve even between 5 and 7 years. No sight-threatening complications were documented in their series, although during the 7-year follow-up 24% of untreated fellow eyes had an increase in Kmax of over 1.0 D and underwent CXL (82). This documented progression in fellow untreated eyes, seen in this and other studies, indicates that the improvements in visual and topographic parameters in treated eyes with long-term follow-up are unlikely to be due to any physiologic age-related changes but to the CXL treatment itself. Such long-term data from different investigators further support the efficacy and safety of epithelium-off CXL with up to a decade of follow-up. Continued follow-up will determine the nature of longer-term efficacy and the need if any to repeat the procedure. It will elucidate how long and to what degree eyes might continue with improvement in visual and topographic parameters even years after CXL. The recurrence of keratoconus following keratoplasty, which classically, albeit rarely, occurs 10-20 years following surgery (83), suggests that turnover of corneal collagen and ECM may be measured in decades and that CXL might be effective for at least this length of time if not longer given physiologic crosslinking changes with age.

Description of the standard epithelium-off CXL procedure

All long-term data over 5 years, together with the majority of prospective cohort case series with reported follow-up between 12 and 24 months and published randomized prospective studies, are with epithelium-off CXL using the standard UVA exposure of 3 mW/cm^2 for 30 minutes. As more has been published and is known about this CXL protocol and as its efficacy and safety is supported by the current scientific literature, it must be regarded as the gold standard technique. The following section describes this operative technique.

Prior to CXL, it is usually necessary to obtain documented evidence of progression of ectasia. The precise and best parameters to define progression are not agreed upon and remain undefined and undetermined. However, many published studies and surgeons define progression as an increase in topographic or tomographic Kmax and/or average keratometry and/or refractive astigmatism of over 1.0 D and/or decrease in pachymetry greater than 10% over the preceding 12-18 months (61-63). However, many surgeons will offer CXL in all adolescent and pediatric keratoconic patients and all patients with iatrogenic ectasia, even in the absence of documented progression, because of the high risk of progression in such eyes and the improvements in corneal shape and vision documented in most eyes after CXL with time (61-70, 74-80).

To minimize the risk of endothelial damage and corneal decompensation, it should be ensured that prior to surgery the corneal thickness is greater than 450 µm (400 µm with the epithelium off) at its thinnest point (33-40). Following fully

informed consent, CXL is typically performed under topical anesthesia such as tetracaine 1.0%. In the standard epithelium-off technique, the central 9.0 mm of the corneal epithelium is debrided to enable adequate stromal riboflavin absorption (4, 39-70). While the optimum riboflavin dosage and diffusion time required for sufficient CXL is undetermined, following epithelial removal, riboflavin 0.1% is typically applied every 2-3 minutes for 30 minutes. It is of note that Gore et al (84), using 2-photon fluorescence microscopy, have recently demonstrated that at least 30 minutes of riboflavin application time is required to allow sufficient and homogeneous stromal uptake prior to UVA exposure. In the initial studies, the riboflavin was suspended in a 20% dextran solution. While this is still used, many formulations now used clinically are isotonic to avoid stromal dehydration and inadvertent corneal thinning during the procedure and contain hydroxypropyl methylcellulose instead of dextran (85). Intraoperative pachymetry is advocated by many surgeons to monitor corneal thickness prior to UVA exposure and to administer hypotonic riboflavin drops if the cornea thins excessively (below 400 µm) during riboflavin administration. The central 8 to 9 mm of the cornea is then irradiated with UVA at 3 mW/cm^2 for 30 minutes. During this time, riboflavin 0.1% drops are applied to the stromal surface every 3-5 minutes during the 30-minute irradiation period. Following irradiation, topical antibiotics and corticosteroids are prescribed until corneal re-epithelialization. Systemic analgesic and bandage soft contact lenses can be used for pain management. Topical anesthetics in limited dosage, no more than 2 hourly and only for 48 hours, may also be of benefit (86). Significant ocular pain is typically experienced for the first 24 to 48 hours following surgery and vision is blurred for 1-2 weeks. Patients need to be carefully counseled concerning the expected occurrence of postoperative pain and slow visual recovery in the early postoperative period to avoid unnecessary distress. Contact lens wear can be resumed once the epithelium has fully healed, typically at about 3 weeks.

Confocal microscopic and ultrastructural wound healing studies following CXL

Wound-healing studies following CXL using confocal microscopy have identified edema, superficial nerve loss, reduced density of keratocytes in the anterior and midstroma, and isolated endothelial damage in the immediate postoperative period (87-89). During the first 3 months, there is keratocyte repopulation, which is usually complete by 6 months. An increased density of the ECM occurs to a depth of 300 to 350 µm, which forms the so-called demarcation line, which can be observed on slit-lamp examination (90). Regeneration of nerve fibers, with re-establishment of the subepithelial plexus, usually occurs within 12 months with return of full corneal sensitivity (91), although investigators have documented an increased occurrence of nerve looping, crossings, and tortuosity after CXL (92). Confocal investigations have confirmed the lack of endothelial damage, with no alterations of cell density changes or hexagonality (87-89). These in vivo confocal microscopic changes after epithelium-off CXL have been confirmed by histologic examination of corneal buttons after keratoplasty (87-95) and corroborate keratocyte loss and damage, which can be prolonged (95), with an increase in collagen fibril diameter (87-95).

More recent confocal microscopic investigations have highlighted different changes with varying CXL protocols. Reduced damage of corneal subepithelial nerves and anterior keratocytes has been demonstrated with iontophoretic transepithelial CXL compared to conventional epithelium-off treatments (96). Such findings of less keratocytes and nerve damage with transepithelial CXL compared to epithelium-off techniques have been confirmed by other investigators (97, 98). One study showed worse changes with accelerated CXL techniques (98).

Epithelium-on vs epithelium-off techniques

Riboflavin is a hydrophilic molecule and as such is unable to pass through the tight junctions of an intact epithelial barrier. This has been confirmed by a series of ex vivo laboratory investigations utilizing spectrophotometry to indirectly measure stromal riboflavin concentration (99-101). These demonstrated the necessity with standard 0.1% solutions to remove all layers of epithelium to achieve adequate stromal riboflavin concentrations. Superficial epithelial trauma, preoperative, multiple administration of topical tetracaine 1%, application of 20% alcohol solution, and grid pattern epithelial removal have all been found to be insufficient to attain homogeneous riboflavin stromal absorption at concentrations similar to that achieved by the epithelium-off technique (99-101).

Spörl et al (2, 3) suggested the necessity for full epithelial debridement to allow sufficient stromal uptake of riboflavin. This was based on their observations of no modifications in the biomechanical properties of corneal tissue when CXL was performed with the epithelium intact in their initial laboratory studies. On this basis, the epithelium was debrided prior to riboflavin administration in the first clinical investigations and epithelium-off CXL, as described above, must currently be regarded as the gold standard technique (4, 39-70, 74-82).

In an attempt to reduce postoperative pain, speed visual recovery, reduce risks of infection, reduce corneal scarring (by decreasing epithelial/stromal cytokine interaction), and limit potential endothelial damage (by having a greater overall corneal thickness and preventing perioperative stromal dehydration and thinning), investigators have postulated performing CXL with the epithelium on. Research has been directed at a number of methodologies to achieve transepithelial riboflavin absorption. Chemical enhancement of epithelial permeability has been attempted by using applications of topical anesthesia (102) and the addition of chemical additives to the riboflavin solution such as trometamol (Tris-[hydroxymethyl] aminomethane) (103), sodium ethylenediaminetetraacetic acid (EDTA) (103), benzalkonium chloride (BAC), and sodium chloride (104). Partial mechanical disruption has also been advocated (105), in addition to iontophoresis (106), increased riboflavin concentrations, reduced solution osmolarity (107), and/or increased application times.

Partial mechanical epithelial disruption

Partial mechanical epithelial disruption may be achieved using superficial scratches or specially designed surgical

instruments (108). Rechichi et al (108) used a specially designed epithelial disruptor to create pockmarks in the epithelium in 28 patients and reported an improvement in vision and to a limited extent in refraction and corneal topography at 12 months. Hashemi et al (109) in 40 eyes utilizing a technique with 3 to 4 vertical strips of complete debridement with intact islands of epithelium between reported significant improvements in CDVA and anterior and posterior corneal elevation at 5 years but no changes in Kmax, refraction, or pachymetry. However, 2 comparative studies, Hashemi et al (110) in a retrospective study of 80 eyes in 65 patients and Razmjoo et al (111) in a randomized controlled study in 44 eyes of 22 patients, showed that while visual outcomes in terms of CDVA may be better with partial disruption, improvement in topographic indices are superior with complete epithelial debridement (110-111). Such outcomes are supported by laboratory studies (99-101). Using spectrophotometry, authors showed that although partial epithelial disruption improved riboflavin absorption, uptake was only significant in the areas of complete epithelial debridement resulting in nonhomogeneous stromal absorption (99, 100). Such nonhomogeneous absorption might limit the efficacy of the procedure (99, 100). Long-term comparative studies are needed to compare these 2 approaches in terms of stability of outcomes and cessation of progression of ectasia before partial disruption can be considered as efficacious as the gold standard total epithelium-off technique.

Epithelium-on CXL: Chemical enhancers

The incorporation of trometamol and EDTA in riboflavin solutions to enhance transepithelial riboflavin absorption for epithelium-on CXL is ambiguous. Filippello et al (103) in a prospective case series reported rapid recovery, little postoperative pain, and outcomes in terms of reduction in Kmax comparable to epithelium-off CXL albeit with a shallower demarcation line. Similar outcomes were seen by Salman (112) in 22 eyes of pediatric patients, observing a 2.0 D decrease in keratometry, improved vision, and no progression at 12 months with a worsening of topographic parameters in untreated control eyes, and by Magli et al (113) in a retrospective comparative study that found little difference between epithelial-on and epithelial-off CXL. In contrast, Buzzonetti et al (114) reported that in 13 eyes although CDVA had improved, keratometry and higher-order aberrations were worse at 12 months. Similarly, Caporossi et al (115) recorded progression of ectasia and had to retreat 50% of cases at 24 months, suggesting little efficacy with the use of trometamol and EDTA as chemical enhancers.

The use of BAC and multiple administrations of topical anesthetics have also shown limited or no efficacy, despite some positive results in laboratory studies (104). Leccisotti and Islam (116), in a prospective, paired-eye study in 51 patients, with the eye with more severe keratoconus being treated and the fellow untreated eye acting as a control, showed an improvement in CDVA, refraction, and keratometry in treated compared to control eyes, but with less effect than that reported with epithelium-off CXL. Koppen et al (117), in 53 similarly treated eyes of 38 patients, showed an improvement in CDVA at 12 months, but with progression of Kmax and pachymetry. Gatzioufas et al (118) reported a high failure rate in epithelium-on treated eyes using riboflavin 0.25% with BAC. Not only did 24% of eyes in this series progress with an increase in Kmax greater than 1.0 D at 12 months, but almost 50% of eyes had epithelial defects on the first day due to epithelial toxicity from prolonged BAC application. Such findings of epithelial damage are consistent with the study by Yuksel et al (119), who found higher pain scores on day 1 and longer epithelialization times with epithelium-on treatments.

In terms of comparative studies, results are again equivocal. While a few have shown little difference between the 2 techniques, some clearly indicate better results with epithelial removal. Rossi et al (120), in a randomized, prospective study of 20 eyes, 10 per treatment group, utilizing an epithelium-on technique with EDTA and trometamol, reported no differences between the 2 treatments at 12 months. Similarly, Nawaz et al (121) in a nonrandomized study of 40 patients using an isotonic riboflavin solution found no differences in outcomes between epithelium-off or -on CXL at 6 months. However, Al Fayez et al (122), in a randomized study of 70 patients with 3-year follow-up, reported better results with epithelium-off CXL, with which there was no progression and an average reduction of Kmax of 2.4 D, while 55% of eyes with epithelium-on CXL showed progression and an average increase of Kmax of 1.1 D. Likewise, Soeters et al (123), in a randomized study of 51 eyes and utilizing a riboflavin solution containing EDTA and trometamol, documented better reduction of Kmax with epithelium-off CXL, with progression in 23% of epithelium-on treated eyes at 12 months, although improvement in CDVA with epithelium-on CXL was better and complications were less, while Kocak et al (124) in a retrospective study in 36 eyes with 12-month follow-up showed a greater reduction in cone apex power with epithelium-off CXL, with progression in 65% of epithelium-on treatments.

It is apparent that uncertainty remains concerning the efficacy of the currently commercially available epithelium-on CXL methodologies using riboflavin solution modifications in terms of osmolarity, concentration, as well as the addition of chemical enhancers, with many studies reporting high rates of treatment failure. This is likely to be due to limited stromal riboflavin penetration through the intact hydrophobic epithelial barrier as seen in photospectrometry studies (99-101) and corroborated recently by a series of published investigations conducted with 2-photon fluorescence microscopy to more directly measure riboflavin concentration within the stroma (125, 126). These 2-photon studies by Gore et al (126) show limited uptake with the use of epithelium-on CXL with chemical enhancers with at best only 20%-50% of the riboflavin concentrations achieved with the standard epithelium-off technique within the first 100 µm of the anterior stroma, which falls further at increasing depths. In addition with BAC-containing compounds, significant epithelial damage was observed after 30 minutes of solution application time and there appeared to be loading of the epithelium with a considerable amount of riboflavin with all solutions that would produce shielding of the stroma from UVA energy during irradiation (126). This shielding of UVA reaching the stroma is likely to further limit the efficacy of such epithelium-on treatments.

Iontophoretic epithelium-on CXL

In addition to novel formulations, laboratory investigations have shown enhanced transepithelial riboflavin absorption with iontophoresis (127-132). Riboflavin is an appropriate molecule for iontophoretic transfer as it is small, negatively charged at physiologic pH, and easily soluble in water. Mastropasqua et al (127) in human cadaver corneas not suitable for transplantation demonstrated a 40%-50% stromal riboflavin concentration after iontophoresis compared to epithelium-off treatment, which was 50% greater than noniontophoretic epithelium administration. Similarly, Cassagne et al (128) using 0.1% riboflavin and 1 mA current for 5 minutes reported 50% of the stromal concentration seen with epithelium-off CXL in a rabbit eye model with similar enhancements after UVA irradiation in extensiometry measurements and resistance in collagenase digestion between the 2 treatments. In a rabbit eye and human cadaver model, Vinciguerra et al (129) reported better riboflavin uptake and increased extensiometry measurements with iontophoresis compared to epithelium-on CXL without iontophoresis but with less marked alterations than epithelium-off treatments. Mastropasqua et al (22) documented increased stiffening of human cadaver corneas following iontophoretic CXL using noncontact air pulse tonometry and Lombardo et al (130) found comparable stiffness to that seen with epithelium-off CXL using an inflation methodology in human globes ex vivo.

Published clinical studies of iontophoretic CXL are few. Bikbova and Bikbov (131) treated 22 eyes using riboflavin 0.1% and 1 mA for 10 minutes with the standard UVA protocol of 3 mW/cm^2 for 30 minutes and reported a mean reduction of Kmax of 2.0 D at 12 months. Similarly, Vinciguerra et al (132) treated 20 eyes using riboflavin 0.1% and 1 mA for 5 minutes and showed an improvement in CDVA and stable keratometry, higher-order aberrations, pachymetry, and endothelial counts at 12 months. Likewise, Li et al (133) using the same protocol in 15 eyes documented improvement in visual and topographic parameters, with a demarcation line with an average depth of 288 µm at 6 months. More recently, Buzzonetti et al (134) using 1 mA for 5 minutes and an accelerated UVA protocol in 14 pediatric cases showed an improvement in CDVA and stability of refraction and topography at 15 months but with an average demarcation line depth of 180 µm and Magli et al (135) in a series of 13 pediatric patients documented stability of keratoconus 18 months after iontophoretic CXL with a similar protocol.

While such results are encouraging, there are at present no published comparative studies with epithelium-off CXL. Iontophoresis is currently being utilized to provide reduced application times of 5 to 10 minutes, instead of the usual 30-minute epithelium-off application time. In a series of laboratory investigations, Hayes and colleagues (136, 137) have shown that by increasing riboflavin concentration and iontophoresis application times and allowing short periods of time for the riboflavin, which is initially deposited only into the epithelium and anterior stroma, to diffuse deeper into the stroma, concentrations of up to 60%-80% of that achieved with epithelium-off application with a homogeneous distribution throughout the stroma can be achieved. With such improved transepithelial riboflavin penetration, it is hoped that results similar to epithelium-off CXL may be achieved. Comparative, randomized, prospective studies of this protocol with epithelium-off CXL are currently being undertaken (O'Brart, personal communication 2015).

Epithelium-on CXL: Other methodologies

Other methodologies under laboratory and clinical investigation to facilitate transepithelial riboflavin stromal absorption include the use of ultrasound (138), nanoemulsion systems (139), other epithelial permeation enhancers such as d-α-tocopherol poly(ethylene glycol) 1000 succinate (vitamin E-TPGS) (140, 141), and the creation of femtosecond laser intrastromal pockets (142, 143). At present, there are no large case series or comparative studies with epithelium-off CXL of these techniques, which are at an investigational stage, but might hold promise in the future.

Rapid (accelerated/high-fluence) CXL techniques

The UVA protocols in the initial case series, randomized controlled studies, and long-term follow-up studies utilized UVA energies of 3 mW/cm^2, requiring 30 minutes of UVA exposure to achieve the desired clinical effect (4, 39-70, 74-82). It has been hypothesized that by increasing the UVA fluence while simultaneously reducing exposure time (the Bunsen-Roscoe law of reciprocity), the same subthreshold cytotoxic corneal endothelial UVA dosage can be delivered (5.4J/cm^2), thereby maintaining efficacy and safety, but with a reduced treatment time. Reduced treatment time as well as improving case throughput may offer improved patient comfort and shortened keratocyte exposure time, which may result in less keratocyte damage and apoptosis.

Accelerated CXL: Laboratory studies

Initial preclinical ex vivo studies were encouraging, with similar biomechanical changes, measured by scanning acoustic microscopy and extensiometry, between the standard UVA exposure of 3 mW/cm^2 for 30 minutes (SCXL) compared with higher fluencies with shorter exposure times (23, 144-146), albeit with a sudden decrease in efficacy with very high intensity UV greater than 45 mW/cm^2 (146). However, recent studies have demonstrated conflicting results with reduced efficacy with higher fluence treatments. Hammer in ex vivo porcine eyes utilizing extensiometry found reduced efficacy with 9 mW/cm^2 for 10 minutes and 18 mW/cm^2 for 5 minutes compared to 3 mW/cm^2 for 30 minutes (147). Similarly, Aldahlawi et al (148) using a pepsin digestion model reported similar results using identical protocols to Hammer with less resistance to enzymatic digestion being seen with higher fluence treatments compared to SCXL.

Accelerated CXL: Clinical studies

Published clinical studies are somewhat limited at present. Cınar et al (149) in a study of 23 eyes showed that accelerated CXL (ACXL) produced a significant reduction in topographic keratometry values and improvement in corrected distance

acuity, albeit with a limited follow-up of 6 months. Shetty et al (150) in 30 eyes of 14 pediatric patients with a UVA dosage 9 mW/cm^2 for 10 minutes documented an improvement in vision and refractive cylinder at 24 months, while with the same ACXL protocol, Marino et al (151) in 40 eyes with postlaser-assisted in situ keratomileusis (LASIK) ectasia reported stabilization in all eyes at 2 years and Elbaz et al (152) documented stability in 16 keratoconic eyes at 12 months with an improvement in UDVA. Similarly, Ozgurhan et al (153) in a case series of 44 eyes of 38 pediatric patients receiving an ACXL protocol of 30 mW/cm^2 for 4 minutes (to give a 33% increased total UVA dose of 7.2J/cm^2) demonstrated cessation of progression and improvement in visual, keratometric, and wavefront parameters at 24 months.

Published comparative studies of ACXL and SCXL are conflicting. Ng et al (154) in a comparative study of 26 eyes found a greater reduction in Kmax and Kmean with SCXL at 6 to 18 months compared to ACXL (9 mW/cm^2 for 10 minutes), while Brittingham et al (155) in 131 eyes found a reduction of Kmax in SCXL but not ACXL at 12 months, with a similar ACXL protocol, and Chow et al (156) in 38 patients (19 per group) using an ACXL protocol of 18 mW/cm^2 for 5 minutes found more effective corneal flattening with SCXL. In contrast, Kanellopoulos (157), in a randomized, bilateral study of 21 eyes using a UVA power of 7 mW/cm^2 for 15 minutes, demonstrated similar results to SCXL at 18 to 56 months postoperatively. Similarly, comparable results between ACXL and SCXL were reported by Hashemian et al (158) in 153 eyes of 153 patients with 15-month follow-up and Hashemi et al (159) in 62 eyes with 6-month follow-up and an ACXL of 18 mW/cm^2 for 5 minutes. Likewise, Shetty et al (160) in 138 eyes of 138 patients with 12-month follow-up reported ACXL protocols of 9 mW/cm^2 for 10 minutes and 18 mW/cm^2 for 5 minutes had similar outcomes to SCXL, although 30 mW/cm^2 for 3 minutes was not as efficacious, and Sherif (161), in 25 eyes of 18 patients with a protocol of 30 mW/cm^2 for a 4 minutes 20 seconds (total UVA dose of 7.2J/cm^2), found comparative results with SCXL at 12 months. Such outcomes are somewhat confusing but cast doubt on ACXL protocols, especially with fluencies greater than 18 mW/cm^2. The reasons for this possibly reduced efficacy are uncertain but may be related to excessive oxygen consumption with higher fluencies of UVA and subsequent reduced oxygen availability, which has been shown to be crucial to the CXL process (12, 14).

This uncertain efficacy has led some investigators to postulate the need to increase UVA exposure time (153, 161, 162) or employ fractionated/pulsed treatments (163, 164). At present, there is little clinical data to support such protocols. Sherif (161) found comparable results with SCXL by increasing exposure time by one third with 30 mW/cm^2 and Kymionis et al (162) found the same depth of demarcation line by increasing exposure time by 40% from 10 to 14 minutes with the 9 mW/cm^2 protocol. Mazzotta et al (163) in a comparative nonrandomized study of 20 eyes found a greater reduction in keratometry with pulsed compared to nonpulsed treatments with a UVA fluence of 30 mW/cm^2 at 12 months and Moramarco et al (164) found significantly deeper demarcation lines with pulsed treatments using the same protocol. While such results with these ACXL protocols are interesting, they are still at an investigational stage and their efficacy is uncertain. Further randomized controlled long-term studies are indicated to ascertain their efficacy compared to the gold standard of SCXL.

Treatment of thin corneas

Due to potential endothelial toxicity, CXL is contraindicated with corneas thinner than 400 µm. It is not uncommon in the clinical setting to see eyes that meet the criteria for crosslinking, in terms of documented progression and good visual rehabilitation with contact lenses, whose corneas are less than 400 µm at their thinnest points. This has led a number of investigators to develop protocols to treat such eyes. Hypo-osmolar riboflavin solutions have been used to swell the cornea intraoperatively to over 400 µm. Using such a technique, Raiskup and Spörl (165) in a series of 32 eyes with corneas thinner than 400 µm showed stability of vision and keratometry after epithelium-off CXL with no adverse events at 12 months, while Nassaralla et al (166) in 18 eyes demonstrated swelling of the cornea with the intraoperative administration of hypo-osmolar 0.1% riboflavin with no postoperative complications. In one study of CXL in thin corneas, however, endothelial counts were shown to be reduced, although vision and keratometry improved (167), and in one case report, progression continued in an eye with a central thickness of less than 330 µm (168). In addition to hypo-osmolar riboflavin protocols, Spadea and Mencucci (169) demonstrated efficacy with no endothelial damage with epithelium-on CXL in corneas as thin as 331-389 µm. Other techniques in thin corneas include the use of riboflavin-soaked, non-UVA-filtering bandage contact lenses to be placed on the cornea during UVA irradiation (170), the placement of a refractive lenticule from patients who have undergone small-incision lenticule extraction over the host cornea (171), and the avoidance of epithelial debridement over the corneal thinnest point (172). All published case series of novel CXL techniques in thin corneas contain small numbers of treated eyes with limited follow-up. There is a need to crosslink such eyes and larger clinical series with long-term follow-up are required to establish if procedures in thin corneas are as effective and safe as SCXL.

Corneal crosslinking in combination with other treatment modalities

As well as an isolated treatment, CXL has been used in combination with other treatment modalities to optimize visual outcomes in keratoconic and postlaser ectasia. Excimer laser epithelial removal has been postulated as a more efficacious methodology than mechanical epithelial debridement in standard epithelium-off CXL. Reinstein et al (173), using high-resolution ultrasonic mapping, showed the epithelium to some extent masks the severity of any ectasia by showing hyoplasia/thinning over the cone apex and hyperplasia/thickening around the cone base. Hence phototherapeutic laser removal could theoretically improve outcomes as the cone would be slightly flattened due to superficial stromal tissue removal over its apex during laser ablation. Kapasi et al (174), in a comparative study of 34 patients comparing excimer laser with mechanical debridement, demonstrated better improvement in refractive error and astigmatism with laser

removal. Similarly, superior visual and refractive outcomes with laser removal were reported in a comparative study by Kymionis et al (175) in 38 eyes. Long-term follow-up utilizing this technique of up to 4 years has been published by Kymionis et al (176) and showed an average reduction in Kmax of 3.4 D in 23 eyes at this timepoint. Excimer laser epithelial removal prior to CXL shows promise and randomized, prospective studies to compare it to mechanical epithelial removal as well as long-term studies to ensure that progression rates are not increased by Bowman membrane and superficial stromal tissue removal in these already biomechanically unstable eyes are indicated.

Combined CXL and limited topography-guided photorefractive keratectomy (PRK) in selected eyes with moderate ectasia and adequate corneal thickness has been postulated and shown to be effective with marked improvements in visual, refractive, and topographic parameters and stabilization of the ectatic process in the majority of eyes (177-182). Such treatments have been shown to be associated with significant improvements in quality of life scores (183). Follow-up in these studies, however, is limited to only 3 years, so that long-term biomechanical stability has not been fully elucidated (184) and progression of ectasia has been reported (T. Seiler, personal communication 2014). In addition, significant corneal scarring has been reported following these combined treatments (185, 186). Undoubtedly, further follow-up studies, beyond 5 years, in large patient series and comparative studies would be of interest to establish this procedure.

Corneal crosslinking has been used after intracorneal ring segment (ICRS) insertion and even in one report in a 3-step procedure combined with both PRK and ICRS insertion (187). Some studies have suggested that CXL may have an additive effect when combined with ICRS (188), although this has not been demonstrated in all studies (189). The sequencing of the 2 treatments is as yet undetermined, with a single small randomized study suggesting that better results could be obtained with simultaneous rather than sequential treatment (190).

The use of thermokeratoplasty with radio or microwaves to flatten the keratoconic cornea followed by CXL to maintain the new flattened corneal profile has also been proposed (191, 192). However, despite initial dramatic improvements in corneal shape immediately following such thermal collagen shrinkage procedures, almost complete regression of effect within the first 3-6 months postoperatively has been documented and these treatments have not been widely adopted (191, 192).

Limited high-fluence CXL has been used in conjunction with keratorefractive procedures such as PRK and LASIK in an attempt to improve long-term stability and reduce the occurrence of postsurgery ectasia (193, 194). Such studies are limited in terms of numbers treated and long-term follow-up. However, results are encouraging, with a contralateral eye study by Kanellopoulos et al (195) of CXL after hyperopic LASIK in 23 eyes demonstrating less regression of correction over a follow-up of 23 months and a comparative study by the same author of high myopic LASIK corrections combined with CXL reporting better visual outcomes (196). While such studies are of interest, it should be noted that long-term studies of CXL have demonstrated continued flattening of the cornea and hyperopic refractive shift in some eyes for up to 5 years follow-up (81, 82) and longer follow-up over 5 years and randomized, prospective comparative studies are indicated.

Complications of CXL

While clinical studies indicate that CXL is a very safe procedure with few sight-threatening complications, adverse events can occur. Reported complications include haze and scarring, infectious and noninfectious keratitis, endothelial failure, failure of treatment with progression of ectasia, excessive corneal flattening with associated hyperopic shift, and potential long-term limbal stem cell anomalies.

Anterior corneal haze (the demarcation line)

Following CXL, a midstromal haze occurs in the majority of eyes. It typically appears at 2-6 weeks and generally clears by 9-12 months. It seems to be the result of an increased density of ECM and arises at a depth of 300 to 350 μm (88, 89) and forms the so-called demarcation line, which can be easily seen on slit-lamp examination (90). As this phenomenon is self-limiting, topical corticosteroids are not indicated.

The demarcation line has been shown to be shallower with ACXL (98) and epithelium-on treatments (103). It has been postulated that it represents the demarcation between crosslinked and noncrosslinked tissue and has been used by some investigators as a means of quantifying the efficacy of CXL (197-199). In one study it was shown to be shallower in older patients and in eyes with more advanced keratoconus receiving the same technique, with the depth of the line not being correlated to visual or keratometric outcomes at 6 months (177). It is generally thicker centrally and shallower paracentrally (198, 199), with a deeper depth of the line centrally being found in one study to be related to a larger decrease in corneal thickness within the first 12 months after CXL (200). While CXL is undoubtedly associated with the development of an anterior/midstromal haze during the first year after surgery, there is as yet no absolute evidence that it is the true delineation between crosslinked and uncrosslinked tissue and may only represent a natural wound-healing response. Until more evidence is forthcoming, it would be unwise to consider its depth as an accurate way to assess the efficacy of any CXL technique (201).

Corneal scarring

Permanent scarring over the axial cornea/cone apex rather than transient changes may also occur. Raiskup et al (202) reported stromal scarring at 12 months in 14 (8.6%) of a series of 163 eyes. Compared to eyes without such persistent changes, affected eyes in their series had a higher preoperative apex power, with an average power of 72.0 D, higher 3.00 mm keratometry values, and thinner central pachymetry compared to unaffected eyes. On the basis of these findings, they advised caution and careful counseling before CXL is undertaken in patients with advanced keratoconus (202). It is of note that scarring with an associated impairment of postoperative visual performance has been reported even in mild cases of keratoconus after CXL (203). Therefore all patients

need to be carefully counseled concerning this possible occurrence.

Stromal scarring may be more prevalent in eyes receiving simultaneous PRK followed by CXL. Kymionis et al (185) documented the occurrence of posterior linear haze formation persistent at 12 months in a series of 13 (46%) of 26 such treated eyes, while Güell et al (186) reported late-onset deep stromal scarring in a similarly treated patient that reoccurred after 2 years.

Failure of treatment: Progression

With the standard epithelium-off technique, utilizing riboflavin 0.1% and UVA at 3 mW/cm^2 for 30 minutes, the vast majority of patients achieving 2-year follow-up demonstrate no progression (74-82). Raiskup-Wolf et al (74) in a series of 241 eyes with a follow-up of over 6 months documented progression in only 2 cases (0.8%). Koller et al (204) in their series of 117 eyes, all of which reached 12-month follow-up, reported progression in 9 eyes (7.6%), while Ivarsen and Hjortdal (53), in 28 eyes with advanced keratoconus, all with a maximum keratometry greater than 55.0 D and a mean follow-up of 22 months, documented progression in only 1 eye (3.5%). Similarly, Sloot et al (205) in a series of 53 eyes with 12-month follow-up documented progression in only 5 eyes (8%), with little difference between advanced and mild keratoconic cases. Such results are encouraging and offer great hope for the control of this visually debilitating disease (206). Indeed, in the 102 eyes reported in the long-term follow-up studies of Theuring et al (80), Raiskup et al (81), O'Brart et al (82), and Poli et al (79), progression was evident in only 8% of cases at 5 to 10 years.

Sterile infiltrates

Sterile infiltrates occurring during the early postoperative period are not infrequent. Typically they present within the first days/weeks after CXL and resolve within a month with topical corticosteroid medication. Koller et al (204) reported sterile infiltrates in 8 eyes (7.6%) in a series of 117 cases, which resolved within 4 weeks with topical dexamethasone 0.1% treatment. Lam et al (207) reported a cluster of 4 cases of sterile keratitis and compared them retrospectively to 144 eyes their group treated with no such problem. They found that eyes with sterile infiltrates generally had advanced keratoconus with maximum keratometry values greater than 60.0 D and central corneal thicknesses less than 425 µm.

Noninfectious keratitis

While temporary, non-sight-threatening, sterile infiltrates are not uncommon, cases of noninfectious keratitis with corneal melt and resultant significant visual loss may also occur, albeit very rarely. Koppen et al (208) published 4 such cases occurring within 4 days of treatment. Two of their patients were atopic, 2 had permanent visual loss, and 1 underwent penetrating keratoplasty. Eberwein et al (209) reported a single case of corneal melting associated with activation of herpes simplex keratitis, which necessitated penetrating keratoplasty. While such episodes are rare, it is necessary to counsel patients preoperatively about such serious sight-threatening adverse events and the need in some cases for emergency keratoplasty. It is also prudent to control atopic eye disease prior to CXL, with topical and if indicated systemic medication, and give prophylactic systemic acyclovir to patients with previous herpetic eye disease.

Infectious keratitis

Infectious keratitis following CXL has infrequently been reported. This is to be expected as debriding the epithelium can expose the corneal stroma to microbial infection during the operative and early healing phases. Most cases reported have been bacterial in nature. Infections with *Staphylococcal epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and coagulase-negative *Staphylococcus* have been published with documented permanent visual loss (210-212). Some of these cases have been associated with postoperative bandage contact lens use and misuse (213). It is necessary to inform patients never to replace, remove, or clean these lenses themselves.

The precise incidence of microbial keratitis is undetermined. It would be expected to have a rarer occurrence than other operative procedures involving epithelial debridement given the role of CXL in the management of corneal microbial infections (214, 215). Shetty et al (216) reported 4 cases of infectious keratitis in a series of 2,350 patients (1,715 epithelium-off CXL, 310 epithelium-on CXL), giving an overall incidence of 0.0017%. All their cases were treated with an epithelium-off technique. All were due to methicillin-resistant *Staphylococcus aureus* (MRSA) and all had atopic dermatitis and conjunctivitis (216). Similarly, Fasciani et al (217) and Rana et al (218) reported post-CXL microbial keratitis due to MRSA, with an association with atopic dermatitis in 1 case and perforation in 2 eyes. Such reports, while anecdotal, reinforce the need to control atopic dermatitis and conjunctivitis prior to CXL and to counsel patients preoperatively about such rare sight-threatening complications.

In addition to bacterial keratitis, other pathogens have been implicated. Rama et al (219) reported *Acanthamoeba* keratitis in an individual who had rinsed his bandage contact lens in tap water postoperatively and then replaced it, while Al-Qarni et al (220) reported 2 cases of dendritic ulceration occurring within 2 weeks after CXL in patients with no history of herpetic keratitis that responded well to topical antiviral therapy.

These case reports, while few in number, highlight the possible exceptional occurrence of this sight-threatening complication and the need to inform patients to immediately report and seek urgent medical advice if there is any increasing pain and redness after the initial 12- to 24-hour period postoperatively or the occurrence of purulent discharge, so that if infectious keratitis is present it can be managed promptly and appropriately.

Endothelial failure

Endothelial failure has been reported occasionally after CXL resulting in corneal edema. Sharma et al (221), in a retrospective series of 350 patients treated with a standard epi-

thelium-off protocol in eyes with corneal thicknesses greater than 400 µm after epithelial removal, reported persistent problems in 5 patients (1.4%), 2 of whom (0.6%) required penetrating keratoplasty. Bagga et al (222) reported a single case with keratouveitis and endothelial failure that required keratoplasty. The etiology of such problems has not been fully elucidated but endothelial damage after CXL may occur even in corneas with adequate thickness, perhaps due to severe stromal thinning intraoperatively due to the use of hyper- and iso-osmolar riboflavin solutions and/or lack of homogeneity with hot spots in the UV beams associated with the use of diodes and limited focusing/alignment systems.

Excessive axial flattening and hyperopic shift

After CXL, a hyperopic refractive shift is typical. O'Brart et al (82) in a 7-year follow-up study of 36 eyes that underwent SCXL demonstrated continued flattening of corneal topographic parameters between 1 and 5 years, with an associated hyperopic refractive change. At 7 years, this had resulted in a mean hyperopic shift of almost +0.8 D in patients with a mean age at time of treatment of 28 years. Eight (22%) eyes experienced a hyperopic shift of over +2.0 D and 4 (11%) had more than +3.0 D of hyperopic refractive change over 7 years (82).

Such refractive changes with time need to be taken into consideration in the already hyperopic patient. In addition, as discussed above, the co-use of CXL has been postulated in the routine refractive surgery patient to improve postoperative refractive and corneal biomechanical stability in the so-called LASIK Extra procedure (193-196). Corneal crosslinking in these eyes might result in late and progressive corneal flattening and unwelcome long-term hyperopic refractive outcomes. Caution needs to be adopted with such treatments and potential patients counseled preoperatively concerning these possible changes with time.

Occasionally corneal flattening can be excessive. Santhiago et al (223) reported 2 cases, 1 a 28-year-old woman with flattening of greater than 14.0 D and the other a 14-year-old boy with flattening of 7.0 D at 12 months, while Kymionis et al (224) reported a 23-year-old woman with over 11.0 D of corneal flattening, with associated corneal thinning of over 220 µm during a 5-year follow-up period. The pathophysiology is unclear. Santhiago et al (223) postulated that such cases may be more apparent with a central cone location and more advanced disease resulting in a greater CXL and wound-healing effect. However, in their cases, there was no excessive corneal thinning, while in that reported by Kymionis et al (224), this occurred, suggesting perhaps variable mechanisms for this occurrence.

Potential limbal stem cell damage

Corneal crosslinking is typically undertaken on young individuals. Ultraviolet A radiation is known to have potential mutagenic and toxic cellular effects. Corneal limbal stem cells could in theory be adversely affected by UVA radiation, with any potential damage not being clinically evident for years or decades following CXL.

Moore et al (36) exposed cultured corneal epithelial cells and ex vivo corneal tissue to the SCXL protocol and found evidence of oxidative nuclear DNA damage in corneal limbal epithelial cells. Similarly, Vimalin et al (37) subjected cadaveric eyes to CXL and demonstrated damage to limbal epithelial cells with a drop in viable cells. Both investigators demonstrated that such changes could be easily avoided by avoiding UVA limbal irradiation and/or shielding the limbus at the time of UVA exposure.

Long-term clinical studies have shown no evidence of limbal dysfunction with up to 7 to 10 years of follow-up (64-66). However, such changes may take decades to occur. In a single case report, Krumeich et al (225) described a patient who presented with conjunctival intraepithelial neoplasia 2 years after CXL and deep anterior lamellar keratoplasty. While causation between CXL and the development of conjunctival intraepithelial neoplasia cannot be established with a single case report, it seems prudent to protect the limbus and avoid its irradiation during CXL.

Conclusion

Clinical studies of epithelium-off CXL using UVA irradiation at 3 mW/cm^2 for 30 minutes have shown great promise in stabilizing keratoconus and postrefractive surgery ectasia. While further randomized, prospective, and long-term follow-up studies are necessary, it is likely that in the future corneal ectasia can be halted at an early stage and perhaps the need for rigid contact lenses and keratoplasty avoided. Future refinement in techniques may allow for safer and more rapid procedures with less patient discomfort but require further investigation. Combined treatment with other methodologies to treat ectasia show promise but require further investigative and longer-term follow-up studies.

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