

Corneal Collagen Crosslinking: A Systematic Review

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Key Words

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Abstract

Keratoconus (KCN) is an ectatic disorder with progressive corneal thinning and a clinical picture of corneal protrusion, progressive irregular astigmatism, corneal fibrosis and visual deterioration. Other ectatic corneal disorders include: post-LASIK ectasia (PLE) and pellucid marginal degeneration (PMD). Corneal crosslinking (CXL) is a procedure whereby riboflavin sensitization with ultraviolet A radiation induces stromal crosslinks. This alters corneal biomechanics, causing an increase in corneal stiffness. In recent years, CXL has been an established treatment for the arrest of KCN, PLE and PMD progression. CXL has also been shown to be effective in the treatment of corneal infections, chemical burns, bullous keratopathy and other forms of corneal edema. This is a current review of CXL – its biomechanical principles, the evolution of CXL protocols in the past, present and future, indications for treatment, treatment efficacy and safety.

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Corneal Biomechanical Properties

Biomechanical Principles

Several biomechanical terms help characterize the function of biologic materials. Elasticity is the ability of a

substance to deform reversibly under stress. Viscous materials, on the other hand, flow when an external shear force is applied and do not regain their original shape when the force is removed. Viscoelastic materials exhibit characteristics of both viscosity and elasticity, resulting in energy dissipation when stress is applied. The energy lost in this process is called ‘hysteresis’. Young’s modulus, also known as the ‘tensile modulus’ or ‘elastic modulus’, is a quantitative measure of the stiffness of an elastic material. It is defined as the ratio of the stress along an axis over the strain along that axis. For many materials, Young’s modulus is essentially constant over a range of strains. Such materials are called ‘linear’. Nonlinear materials do not have a constant proportion of stress versus strain. Most materials are linear for a specific range of stress values and beyond this range lose their linear elasticity and become nonlinear.

Composite materials are materials made from two or more constituent materials with significantly different physical or chemical properties that, when combined, produce a material with characteristics different from the individual components. The individual components remain separate and distinct within the finished structure. ‘Anisotropy’ is the property of being directionally dependent, as opposed to ‘isotropy’, which implies identical properties in all directions. It can be defined as a difference, when measured along different axes, in a material’s physical or mechanical properties.

Biomechanical Properties of the Normal Cornea

In the terminology of mechanical science, the cornea is a complex anisotropic composite with nonlinear elastic and viscoelastic properties [1]. Understanding corneal macro- and microstructure allows for an understanding of its biomechanical properties. The cornea comprises 5 layers. The corneal stroma makes up 90% of corneal thickness and is the main contributor to the cornea's strength and transparency. This layer is composed of 250–400 stacked lamellae [2]. In the anterior one third of the stroma, the lamellae are more narrowly interwoven than in the posterior two thirds [3]. The lamellae are composed of type I/V collagen fibrils oriented in specific directions (depending on their location and depth). This structure, combined with the cornea's dome-like shape, converts the load created by the intraocular pressure (IOP) into a tangential tensile force carried by the stromal lamellae. While in the anterior third of the cornea collagen fibrils are more isotropic, in the central-posterior two thirds, collagen fibrils are arranged orthogonally to each other. The cornea's anisotropic tensile properties, including its resistance to the pull of the extraocular muscles, are also explained by this tridirectional fibrillar orientation [4, 5]. Proteoglycans within the stroma surround the 35-nm monodisperse collagen fibrils, thus creating uniform spacing of the collagen fibrils. The cornea's transverse material properties are determined primarily by the 36–48 mM of fixed charge density associated with the presence of these organizing proteoglycans [6, 7]. In the anteroposterior direction there is a relatively low structural resistance – contributing to the swelling properties of the cornea. The stroma is composed of 80% water, giving it viscoelastic properties [8, 9]. The viscoelastic property of the cornea is thought to be a major contributor to corneal hysteresis (CH; the cornea's ability to absorb energy) [10] and the corneal resistance factor (CRF; an indicator of its ability to resist external forces) [11]. Maintenance of a shape as maximally spherical as possible is essential for the cornea's refractive role. Therefore, it must distribute applied loads with great precision [12]. The biomechanical properties of the human cornea change with age. The cornea demonstrates considerable stiffening with age that could be attributed to age-related nonenzymatic cross-linking, affecting the stromal collagen fibrils [13, 14].

Biomechanical Properties in Keratoconus

Keratoconus (KCN) is an ectatic disorder with progressive corneal thinning and a clinical picture of corneal protrusion, progressive irregular astigmatism, corneal fibrosis and visual deterioration [15]. The preferred or-

thogonal fibril orientation of the normal cornea appears to be altered in KCN, possibly contributing to the mechanical instability [16]. It has been suggested that only the anterior 200 μm of the cornea are affected in KCN [17]. KCN may involve a disturbance of the balance between proteolytic breakdown and repair [18]. Both the concentration and the activity of the crosslinking enzyme lysyl oxidase have been shown to be significantly reduced in KCN corneas [19]. Mechanically, the KCN cornea shows a substantial reduction in stiffness [20]. A model-based analysis of a KCN cornea has shown that the shape distortion in KCN is affected by three factors: (1) localized thinning; (2) reduction in the tissue's meridian elastic modulus, and (3) reduction in the shear modulus perpendicular to the corneal surface [21]. The point of maximal stress on the KCN cornea was shown to be at the center of the corneal bulge – more specifically in areas of maximal thinning. The cornea's biomechanical properties can be measured *in vivo* using the Reichert Ocular Response Analyzer (ORA; Reichert, Inc., Buffalo, N.Y., USA). The device measures the corneal force/displacement relationship using an air jet to apply pressure to the cornea. During the first phase of measurement, the air jet causes the cornea to flatten and assume a concave shape. Shortly after, the air pressure decreases, causing the cornea to return to its basic formation. The measurement is performed during a very short time period (approx. 20 ms) to avoid the influence of momentary IOP (and other ocular parameter) changes. The changes in corneal shape throughout the measurement period are recorded using an electro-optical sensor which monitors the central 3 mm of the cornea. This enables *in vivo* measurement of CH and the CRF [10]. In KCN corneas, CH and the CRF have been shown to be significantly decreased when compared with non-KCN corneas [11, 22–24].

Biomechanical Properties in Other Keratoectatic Disorders

In addition to KCN, corneal ectasia is a feature of several other disorders, including: post-LASIK ectasia (PLE) and pellucid marginal degeneration (PMD).

Ectasia following LASIK is rare, with an incidence of approximately 0.66% [25]. It may be apparent immediately following LASIK surgery or years after, generally occurring within 2 years of surgery [26, 27]. Clinically, it is manifested as two distinct entities: the first is a central forward bowing with minimal irregular astigmatism, and the second is a KCN-like ectasia with paracentral thinning and resultant significant irregular astigmatism. Histopathologic analysis of eyes with PLE has shown features

similar to KCN. Changes included: forward protrusion of both anterior and posterior corneal surfaces, epithelial detachment, Bowman's membrane breakage and folding and irregular lamellae [28, 29]. Forward movement of the posterior corneal lamella appears to occur routinely following LASIK in a nonprogressive manner [30, 31]. Posterior corneal bulging following LASIK was suggested to be caused by IOP [31]. Other studies did not find such an effect of IOP [32]. Guirao [33] described a model used to examine the influence of myopic LASIK on corneal elastic properties. Based on this model, it was proposed that corneal thinning caused by ablation produces an elastic deformation of the posterior corneal surface. The degree of deformation was found to be dependent on intrinsic corneal parameters (curvature, Young's modulus and thickness) and extrinsic parameters (IOP and ablation profile).

PMD is a rare ectatic disorder which typically affects the inferior or superior peripheral cornea in a crescentic fashion. Histopathologically, the degeneration appears in a region close to the limbus. This region shows a replacement of Bowman's layer with a collagenous pannus. The anterior stroma contains degenerated collagen fibrils with very large proteoglycans. The lamellae are fused and keratocytes appear like fibroblasts [34]. This suggests that PMD could be related to a disorder in the synthesis of collagen fibrils. We were unable to find a report of PMD biomechanics in the literature.

Chemistry of Crosslinking

Crosslinking in Polymers

Crosslinking is the creation of bonds that connect one polymer chain to another. The bonds can be covalent or ionic. A polymer is defined as a chain of monomeric material – either a synthetic polymer or a biologic molecule (such as a protein). Crosslinking of polymers changes their physical properties. For example, crosslinking a rubber molecule will cause a decrease in its flexibility and an increase in its rigidity and melting temperature [35]. Crosslinking is used in bioengineering to strengthen materials, as well as in dentistry to harden filling materials [36, 37].

Early Applications of Crosslinking

In 1992, Hettlich et al. [38] investigated possible ways to perform lens refilling following phacoemulsification. They developed a method which included the injection of a monomer into the lens capsule, followed by intracapsular polymerization of the material by exposure to light

(400–500 nm). The substance did not seem to cause significant damage to surrounding tissues [38]. This method is an early example for the use of light energy to induce intraocular structural changes.

Riboflavin, also known as vitamin B₂, is an easily absorbed, colored micronutrient with a key role in maintaining health in humans and animals. Following exposure to ultraviolet (UV)A radiation, riboflavin molecules absorb energy and reach an excited state. In its excited state, riboflavin can either produce radicals or singlet oxygen molecules, depending on the availability of oxygen [39, 40]. These highly active molecules can induce covalent bonds, thus crosslinking collagen fibers (or other corneal molecules such as proteoglycans and nucleic acids) [41].

Riboflavin was initially described as an active component (together with fibrinogen) in a light-activated corneal tissue glue, excited using blue-green (488–514 nm) argon laser light [42, 43].

Evolution of Riboflavin: UVA Corneal Collagen Crosslinking

First Steps

In the 1970s, Siegel et al. [44, 45] discussed crosslinking reactions whereby lysyl oxidase catalyzed the formation of crosslinking aldehydes in collagen and elastin. In 1997, Spoerl et al. [46] used a similar principle to attempt an induction of corneal crosslinking (CXL), aiming to increase corneal stiffness. They had several porcine eye test groups treated with UV light alone/0.5% riboflavin alone/riboflavin with UV light or blue light or sunlight/glutaraldehyde and Karnovsky's solution. Examination of the stress-strain properties in each of the groups showed that riboflavin and UV light as well as glutaraldehyde or Karnovsky's solution led to increased corneal stiffness [46]. This technique was later studied in vivo by Wollensak et al. [47] on 22 progressing KCN patients. Following central removal of the epithelium, photosensitizing riboflavin drops were applied and the eyes exposed to UVA (370 nm, 3 mW/cm²) at a 1-cm distance for 30 min. Clinical follow-up showed that KCN stopped progressing in all eyes, and in 70% of the eyes a regression in keratometric and refractive values had been observed [47].

Duration of the Treatment Steps: The Dresden Protocol

The standard treatment protocol, commonly referred to as the 'Dresden protocol' (owing to the fact it was first

described by Wollensak et al. [47] from the Technical University of Dresden), includes the following steps:

- Anesthetizing the eye with a topical anesthetic
- Removal of the central 7–9 mm of the epithelium
- Application of a 0.1% riboflavin 5-phosphate and 20% dextran solution to the deepithelized surface every 5 min for 30 min
- Exposure to UVA (370 nm, 3 mW/cm²) radiation for a duration of 30 min with continued application of the above solution every 5 min
- Application of topical antibiotics and a soft bandage contact lens with good oxygen permeability

Accelerated CXL

An irradiance of 3 mW/cm² with a treatment zone of 9 mm for a duration of 30 min results in a total energy of 3.4 J or 5.4 J/cm². If one wants to make an effort to shorten the duration of the procedure, it is possible to use a higher-intensity light for a shorter period of time; an irradiance of 10 mW/cm² for a duration of 9 min was shown to have similar rigidity results in porcine corneas [48]. A large ex vivo study of porcine eyes examined the response to irradiances between 3 and 90 mW/cm² with illumination times between 30 and 1 min, respectively. It was shown that irradiation levels up to 45 mW/cm² produced significantly stiffer corneas when compared with nonirradiated controls. But levels of 50 mW/cm² and above (with their respective time periods) did not show significantly greater stiffness [49]. An ex vivo study compared ultrafast CXL (30 s of UVA exposure) using a custom CXL agent (by PriaVision Inc., Menlo Park, Calif., USA) with a standard 30-min riboflavin CXL in porcine eyes. Both groups showed similar stiffness changes (measured using surface wave elastometry) [50]. In human eyes, an ex vivo study compared CXL with standard (3 mW/cm² for 30 min) versus the accelerated (9 mW/cm² for 10 min) protocol. Corneal stiffness results were not different between the groups [51]. An evaluation of endothelial cell changes following accelerated CXL (18 mW/cm² for 5 min) in 36 KCN patients showed significant differences in endothelial cell density and morphology parameters. Those parameters returned to preoperative values at 3–6 months after CXL. This showed that while endothelial cells do recover safely following the accelerated procedure, there is clear evidence of endothelial effects following accelerated CXL [52]. A comparative clinical study included 21 patients with KCN, treated with accelerated CXL (7 mW/cm² for 15 min) in one eye and with standard CXL (3 mW/cm² for 30 min) in the fellow eye. A mean follow-up of 46 months showed no progres-

sion of KCN in any of the groups with similar improvement in visual acuity and keratometric parameters and no evidence of endothelial damage [53]. A series of 23 patients undergoing more rapid CXL (9 mW/cm² for 10 min) showed favorable outcomes with no evidence of endothelial cell density changes during a 6-month follow-up [54]. Currently, there is no uniform protocol for accelerated CXL and no large clinical trials investigating this method.

CXL Instruments

The initial in vivo CXL study by Wollensak et al. [47] in 2003 used 370-nm UV diodes (Roithner Lasertechnik, Vienna, Austria) with a potentiometer regulating the voltage. Three 1.3-volt accumulators were used as a power generator. Before each treatment, the desired irradiance of 3 mW/cm² was controlled with a UVA meter (LaserMate-Q; LASER 2000, Wessling, Germany) at a 1-cm distance and, if necessary, regulated with the potentiometer [47].

Several CXL instruments are in use today for standard collagen CXL. The XLink™ (Optos, Dunfermline, UK) has an intensity range of 0.5–5 mW/cm² and is used in standard 30-min CXL procedures. The CBM Vega XLink Crosslinking System (Carleton Optical, Chesham, UK), designed for standard 30-min CXL, was used effectively in several clinical studies [55, 56]. The LightLink CXL™ (LightMed, San Clemente, Calif., USA) has a wide range of irradiance between 0.5 and 30 mW/cm², allowing a choice of various treatment protocols from 3 to 30 min length. Several CXL instruments for accelerated CXL have been introduced. The UV-X™ 2000 Crosslinking System (IROC Innocross, Zurich, Switzerland) has a maximal intensity of 12 mW/cm² and can be used for a 10-min accelerated CXL procedure. The KXL™ System (Avedro, Waltham, Mass., USA) is able to produce a light intensity of 30 mW/cm², enabling ultrafast accelerated CXL with less than 3 min of UVA exposure. It appeared to be effective in a small group of KCN patients when combined with an LASEK procedure [57], and also for treatment of infectious keratitis in cats [58].

Epithelium On/Off

The standard CXL protocol includes large deepithelization of the cornea (epi-off CXL). This is due to the fact that riboflavin is a macromolecule with inadequate corneal penetration ability [59, 60]. Deepithelization could serve as a potential source of postoperative infections, a complication previously reported [61–65]. In addition, the period of epithelial healing is associated with intense

postoperative pain [66] and a delay in the return to daily activities. Performing a transepithelial CXL (epi-on CXL) could theoretically reduce these complications.

Animal studies have shown that epi-on CXL does produce increased corneal stiffness [67, 68]. When compared with epi-off CXL, epi-on CXL resulted in only one fifth to one third of the increase in corneal stiffness [67, 69]. It was also shown that the treatment depth in epi-on CXL is greatly reduced when compared with epi-off CXL [69]. Following epi-off CXL in rabbit corneas, measurement of maximal stress was 35.9% higher and Young's modulus 15.4% higher when compared with a similar epi-on CXL rabbit group [68]. These results raised the question whether the reduced effect of epi-on CXL is enough to maintain clinical stability. Human *in vivo* comparison of the two methods demonstrated an inferior effect of epi-on CXL. There were no significant stromal changes (such as a decrease in anterior keratocyte density, evident stromal edema and keratocyte activation) following epi-on CXL. All these changes were significantly evident following epi-off CXL [70]. A study performed on 20 eyes compared epi-off CXL with CXL performed using partial epithelial removal in a grid-like pattern. Evaluation of treatment depth using OCT showed that areas of an intact epithelium appeared to block penetration of riboflavin into the anterior corneal stroma [71]. Several small prospective studies evaluated the clinical value of epi-on CXL. Their results were variable. One prospective 20-patient series showed statistically significant improvement in uncorrected and corrected visual acuity and topography-derived keratometry, cone apex power and higher-order aberrations with no apparent progression of KCN [72]. A recently published 2-year prospective study of 26 eyes undergoing epi-on CXL showed that after relative improvement during the first 6 months after CXL, visual acuity parameters returned to baseline at 1 year. At 2 years after CXL, keratometric and pachymetric indices worsened compared with the pre-CXL values [73].

Several adjunctive methods were studied in an effort to increase riboflavin penetration through an intact epithelium. The use of benzalkonium chloride (BAC) and ethylenediaminetetraacetic acid (EDTA) as a means of weakening epithelial tight junctions has been evaluated. The use of BAC in rabbit eyes was shown to increase the riboflavin absorption coefficient through an intact epithelium. Nevertheless, the absorption coefficient achieved was only 37% that of epi-off absorption [74]. Slightly different results were shown in a very similar study, where the addition of BAC increased the riboflavin absorption coefficient in epi-on CXL to a level similar to a standard

epi-off CXL. Moreover, a biomechanical analysis of treated corneas showed no significant differences in resultant corneal stiffness in the two groups [75]. A prospective clinical study on 51 eyes, using epi-on CXL with the addition of BAC and EDTA, showed a limited but favorable effect of BAC/EDTA-assisted epi-on CXL on keratoconic eyes. The treatment effect was compared with the non-treated contralateral eyes for a duration of 12 months. While favorable, the treatment effect in this study appeared to be less pronounced than described in the literature after epi-off CXL [76]. Similar results were shown over a period of 18 months for 53 eyes undergoing BAC-assisted epi-on CXL [77].

Riboflavin is a water-soluble, negatively charged molecule with a molecular weight of 376.40 g/mol, which makes it a good candidate for iontophoresis. Iontophoresis appears to enhance transepithelial riboflavin penetration [78]. Epi-on CXL using iontophoresis showed good clinical results in terms of reduced astigmatism and keratometric values with improved best-corrected visual acuity (BCVA) in a prospective series of 22 eyes [79]. Other methods, investigated in rabbit corneas, include: phonophoresis (ultrasound-assisted drug penetration) and a biocompatible riboflavin-based nanoemulsion system. Both have shown potential in increasing transepithelial penetration of riboflavin [80, 81]. Lastly, the use of a hypoosmolar riboflavin solution (with 0.44% NaCl) was also investigated and appears to contribute to the transepithelial absorption of riboflavin [74].

Epi-on CXL is especially appealing for use in the pediatric population. Pediatric patients are more sensitive to the possible effects of epithelial debridement such as postoperative pain, temporary visual deterioration and increased risk of infection and haze. As in the adult population, there are no large-scale prospective trials evaluating epi-on CXL in children. Results of existing studies are conflicting. A recently published 1-year follow-up of 22 eyes of children treated with epi-on CXL showed significant improvement in visual acuity parameters and keratometric values [82]. A retrospective comparison of epi-on with epi-off CXL in 39 pediatric KCN patients (23 epi-off, 16 epi-on) showed no significant differences between the groups in any of the clinical parameters evaluated (visual acuity and topographic parameters). The epi-on CXL group showed reduced postoperative pain and no postoperative corneal edema [83]. Different results were shown in a prospective 18-month follow-up of 13 eyes of children, where epi-on CXL did not appear to halt KCN progression despite a significant improvement in corrected distance visual acuity (CDVA) [84].

Other CXL Methods in Corneal Collagen

Riboflavin and UVA is the universally accepted method today for CXL. However, photochemical CXL with other agents is under research. In one study, chemical derivatives of the photosynthetic pigments (chlorophylls and bacteriochlorophylls) introduced into rabbit corneas in vivo and ex vivo were excited using near-infrared illumination. The result was stiffening of the treated corneas. It is proposed that photoexcitation caused these materials to generate O_2^- and $\cdot OH$ radicals, which promoted protein crosslinking and the resultant stiffening [85]. Recently, the use of Rose Bengal dye excited by green light has shown to significantly increase rabbit corneal stiffness, using a rapid treatment protocol (12 min in total) with no apparent toxicity to keratocytes. It was suggested as a potential future option for CXL of corneas thinner than 400 μm [86]. The use of femtosecond laser to excite riboflavin molecules has shown a stiffening effect similar to UVA excitation when used in vitro to crosslink collagen hydrogels [87].

Other methods of purely chemical CXL were also investigated. These have potential advantages over Riboflavin-UVA, such as avoidance of radiation toxicity and a more simple delivery method of the active agent. The most widely used corneal crosslinkers are probably tissue fixatives such as formaldehyde, glutaraldehyde and Karnovsky's solution (discussed above). These agents cause tissue stiffening through CXL, thus fixating it for pathologic analysis. Unfortunately, these agents are toxic in vivo and are not used clinically [88]. Genipin is an active molecule derived from the plant *Gardenia jasminoides*. It was shown to produce a CXL effect similar to standard CXL in porcine eyes, with minimal endothelial toxicity and no need for irradiance [89]. In recent years, the use of β -nitroalcohols has been suggested as a promising CXL method. These agents function as both formaldehyde and nitrite donors under physiologic conditions, thus enabling CXL of collagenous tissue [90, 91]. Due to their widespread industrial use, their safety profile has been studied and was found to be favorable [92].

Results

Keratoconus

Histology and Morphology

In 2006, Seiler and Hafezi [93] described the formation of a stromal demarcation line following CXL at a depth of approximately 300 μm , representing the interface between treated and untreated cornea. They speculated the

demarcation line is a result of refractive and reflective differences between treated and untreated stroma [93]. Shortly after the application of a riboflavin plus dextran 25% solution, corneal thickness decreases [94]. This decrease in thickness may be the cause of the collagen fibril disorganization observed following CXL [95]. Histologic evaluation of rabbit corneas following CXL showed a pattern of extensive keratocyte loss throughout the entire stroma with accompanying endothelial loss – these were apparent in the central irradiated zone. The anterior stroma showed a pattern of lacunar edema in the space where apoptosed keratocytes used to be. The areas adjacent to the treated zone had diffuse edema. This difference in edema pattern between the treated zone and the areas adjacent to it can serve as an explanation for the formation of the above-described demarcation line. By week 6 after the operation, the cellular structure appears to normalize [96]. Others reported the keratocyte loss to be more prolonged – up to 30 months postoperatively [97]. In vivo studies using confocal microscopy showed similar findings following CXL, with keratocyte loss and stromal edema. In addition, a superficial nerve layer loss was observed [98]. Stromal edema persists for 4–6 weeks and then gradually resolves, with keratocyte repopulation and stromal collagen compaction. This transient edema causes the known decrease in visual acuity during the first postoperative months [99]. While central stromal keratocyte loss in the treated area persists at 36 months postoperatively, nerve regeneration starts at 1 month and continues past 36 months. A small percentage of patients demonstrate endothelial damage with gradual healing completed at 1 year postoperatively [98]. Collagen fiber diameter increases following CXL, with a reorganization of collagen fibrils in a parallel, lamellar structure similar to a non-KCN cornea (and opposed to a KCN cornea). This is accompanied by an increase in interfibrillar spacing, which also gives the cornea after CXL a structure more similar to a non-KCN cornea [100]. The increase in collagen fiber diameter is more pronounced in the anterior stroma, where a maximal treatment effect is achieved [101]. Macroscopically, the cornea shows significant flattening following CXL, with a reduction in K values [102].

Biomechanics

CXL stiffens the cornea. Increased corneal rigidity has been documented both in animal [46, 103, 104] and human ex vivo [105, 106] studies, showing an increase of 328.9% in rigidity and by a factor of 4.5 in Young's modulus on human corneas. Interestingly, in porcine corneas, rigidity and Young's modulus increased by only 71.9%

and a factor of 1.8, respectively [105]. This difference was attributed to the significantly greater thickness of porcine corneas and their reduced absorption of UVA [105].

As discussed above, the ORA instrument allows for noncontact *in vivo* evaluation of corneal biomechanic parameters. One single-center, prospective, randomized, controlled clinical trial evaluated 69 eyes after CXL *in vivo* (46 KCN and 23 PLE). CH and CRF were measured using the ORA and analyzed in a treatment, sham control and fellow eye control group during 12 months. Despite an increase in CRF at 1 month, there were no statistically significant changes in CH and CRF measurements 1 year after CXL [107]. Similar results were found in two other prospective *in vivo* studies of similar size, performed using the ORA [23, 108]. Several explanations were proposed for the lack of change in biomechanic parameters *in vivo* versus the obvious changes observed *in vitro*. One is the fact that different measuring systems were used (e.g. ORA vs. strip extensometry). Another is related to the fact that KCN cornea is extremely irregular and thus may exhibit great variability in resistance to deformation by the ORA depending on small location changes, and therefore averaging ORA measurements may not provide an assessment that is accurate enough [108]. Corneas following CXL also show an increased resistance to enzymatic digestion [109] and to swelling [110].

Clinical Results

As previously stated, in 2003 Wollensak et al. [47] were the first to describe the clinical effect of CXL on 22 KCN eyes during a 2- to 4-year follow-up period. All eyes experienced a halt in KCN progression. Visual acuity improved in 15 of the 22 eyes, and flattening of the maximum keratometry value (K_{max}) by 2 diopters was seen in 16 of the 22 eyes [47]. Since then, numerous studies have evaluated the clinical effect of CXL in the treatment of KCN. The only randomized, controlled clinical trial to date randomized 66 eyes of 49 progressive KCN patients into CXL treatment and control groups. The 1-year results showed that K_{max} had been significantly reduced at all follow-up periods, with an average decrease of 1.45 diopters at 12 months. A trend toward improvement in BCVA was also observed. Analysis of the control group showed a continuous deterioration in K_{max} and BCVA [111].

Other small prospective trials showed a significant improvement in visual acuity and keratometric parameters. Some also showed an improvement in refractive and topographic parameters. Their results are shown in table 1.

Long-Term Studies

The Siena Eye Cross Study, a phase II nonrandomized open trial, examined the effect of CXL on progressive KCN. The results for 44 study eyes completing a minimum follow-up of 48 months were analyzed. All eyes treated showed KCN stability; 65% of the fellow eyes showed a mean progression of 1.5 diopters in corneal power during the first 24 months of follow-up, and required CXL. In the treated eyes, the improvement in keratometric values, visual acuity and coma aberrations was maintained following 48 months of follow-up [123]. Two other prospective studies evaluated the longer-term effect of CXL. One was a 5-year study including 40 eyes [124] and the other a 3-year study including 55 eyes [125]. Both studies found CXL results to be completely stable through the follow-up period with no apparent safety issues.

Retrospective Studies

Two very large retrospective reports were aimed at analyzing the long-term effect of CXL. One included 241 eyes followed up for up to 6 years [126] and the other included 400 eyes followed up for up to 4 years [127]. It is important to note that the majority of patients in each of these trials had not reached a 2-year follow-up period. Only 9.15 and 1.75%, respectively, of the patients reached a 4-year follow-up period in each of the studies. Therefore, their ability to evaluate long-term effects is unclear. Despite the low number of long-term follow-ups, the results indicate long-term stabilization and improvement after collagen CXL [126].

Results of Combined Photorefractive Surgery and CXL

The addition of photorefractive surgery to the CXL procedure in patients with KCN can improve visual and refractive outcomes in addition to the prevention of KCN progression. A study comparing the use of same-day CXL and photorefractive keratectomy (PRK) with the use of sequential CXL and PRK performed 6 months later was conducted on 325 KCN eyes (127 eyes underwent the same-day procedure and 192 eyes underwent the sequential procedure). A follow-up period of 36 months (range: 24–68 months) showed that the same-day procedure was significantly more efficient in improving visual acuity, keratometric parameters, spherical equivalent and corneal haze scores [128]. Several smaller prospective series (ranging from 12 to 31 eyes each) showed combined PRK and CXL to be effective both in improving visual acuity and refractive error and in regressing KCN parameters [129–132]. One comparative contralateral eye series in-

Table 1. Prospective studies of CXL for KCN

Study	Eyes, n	Outcome measures	Duration	Results
Viswanathan and Males [112], 2013	76	K_{max} , BSCVA, astigmatism, SE, CCT	6–48 months	K_{max} improved by 0.96 ± 2.33 D ($p = 0.005$); logMAR BSCVA improved by 0.05 ± 0.13 ($p = 0.04$); other measures not changed significantly
Hersh et al. [113], 2011	71	UDVA, CDVA, K_{max}	1 year	LogMAR UDVA improved from 0.84 ± 0.34 to 0.77 ± 0.37 ($p = 0.04$); logMAR CDVA improved from 0.35 ± 0.24 to 0.23 ± 0.21 ($p < 0.001$); K_{max} improved by 1.7 ± 3.9 D
Lamy et al. [114], 2013	68	CS, BSCVA, K_{max}	2 years	Log CS improved $+0.16$ ($p < 0.001$); logMAR improved -0.16 ($p < 0.001$); K_{max} improved -0.61 D
Agrawal [115], 2009	37	BCVA, astigmatism, K_{max} , HOA	1 year	BCVA improved ≥ 1 line in 54%; BCVA unchanged in 28% ($p = 0.006$); astigmatism improved 1.20 D in 47% of eyes ($p = 0.005$); astigmatism unchanged in 42%; K_{max} improved 2.47 D in 54% ($p = 0.004$); K_{max} unchanged in 38%; HOA not changed significantly but for coma aberrations
Coskunseven et al. [116], 2009	38	SE, astigmatism, UCVA, BSCVA, K_{max} , CCT, EC	5–12 months	SE improved 1.03 ± 2.22 D ($p < 0.01$); astigmatism improved 1.04 ± 1.44 D ($p < 0.01$); UCVA improved 0.06 ± 0.05 ($p < 0.01$); BSCVA improved 0.10 ± 0.14 ($p < 0.01$); K_{max} improved 1.57 ± 1.14 D; no difference in CCT ($p = 0.06$) or EC ($p = 0.07$)
Guber et al. [117], 2013	33	CDVA, stray light, SE, CCT, K_{max} , K_{min}	1 year	CDVA improved -0.042 ($p = 0.021$); stray light value unchanged ($p = 0.215$); SE improved by 1.95 D ($p < 0.001$); CCT unchanged ($p = 0.175$); K_{max} and K_{min} : minimal change
Vinciguerra et al. [118], 2009	28	UCVA, BSCVA, SE, K_{max} , K_{min} , HOA, CCT, EC	2 years	UCVA improved ($p = 0.048$); BSCVA improved ($p < 0.001$); SE improved ($p = 0.03$); K_{max} and K_{min} improved ($p < 0.03$); HOA decreased ($p \leq 0.046$); CCT decreased ($p = 0.045$); EC unchanged ($p = 0.13$)
Arbelaez et al. [119], 2009	20	UCVA, BCVA, $K_{average}$, K_{apex} , sphere, astigmatism	1 year	UCVA improved 4.15 lines ($p = 0.001$); BCVA improved 1.65 lines ($p = 0.0004$); $K_{average}$ improved 1.36 D ($p = 0.0004$); K_{apex} improved 1.4 D ($p = 0.001$); sphere improved 1.26 D ($p = 0.033$); astigmatism improved 1.25 D ($p = 0.0003$)
El-Raggal [120], 2009	15	UCVA, BSCVA, refraction, K_{mean} , CCT	6 months	UCVA improved ($p < 0.05$); BSCVA improved ($p > 0.05$); refraction improved ($p < 0.05$); K_{mean} improved ($p < 0.05$); CCT unchanged
Goldich et al. [121], 2012	14	BCVA, UCVA, K_{max} , cylinder, EC, CCT, biomechanics (ORA)	2 years	BCVA improved ($p = 0.002$); UCVA stable ($p = 0.475$); K_{max} improved ($p = 0.001$); mean cylinder improved ($p = 0.001$); no change in EC, CCT and ORA measurements
Henriquez et al. [122], 2011	10	UCVA, BCVA, K_{max} , K_{min} , SE, EC	1 year	UCVA improved ($p < 0.001$); K_{max} improved 2.66 D ($p = 0.04$); K_{min} improved 1.61 D ($p = 0.03$); SE improved 2.25 D ($p = 0.01$); no change in EC

BSCVA = Best spectacle-corrected visual acuity; SE = spherical equivalent; CCT = central corneal thickness; logMAR = logarithm of the minimal angle of resolution; VA = visual acuity; UDVA = uncorrected distance visual acuity; CS = contrast sensitivity; HOA = high-order aberrations; UCVA = uncorrected visual acuity; EC = endothelial cell count.

cluding 17 patients with progressive KCN compared treatment with combined PRK and CXL with CXL alone. For each patient in the study, the eye with the greatest KCN progression was chosen to receive combined treatment, while the fellow eye received CXL alone. While, in both groups, keratometric parameters improved significantly, the improvement in visual acuity, refractive error and coma aberrations was more prominent in the combined PRK plus CXL group, showing statistical significance in all parameters [133]. It should be stated here that since the eyes in the combined PRK plus CXL group had greater KCN progression, the baseline characteristics were different between the groups and, therefore, the significant advantage shown for the PRK plus CXL group was also a result of those differences.

Results of Combined Intracorneal Ring Segments and CXL

While CXL has been shown to halt KCN progression, its effect on visual rehabilitation may be insufficient. Intracorneal ring segments (ICRS) produce rapid and substantial improvement in visual parameters but do not halt KCN progression [134]. Therefore, combining the two procedures can theoretically produce better results. A retrospective comparative analysis performed on 66 eyes showed that 1 year postoperatively, the 34 eyes treated with ICRS had better visual and keratometric results than the 32 eyes treated with a combination of CXL and ICRS [135]. A similar prospective study conducted over a 2-year follow-up period (39 eyes) showed no difference between patients treated with ICRS alone and patients treated with CXL followed by ICRS implantation 3 months later. Both groups showed similar results in terms of refractive, topographic, pachymetric, tonometric and corneal biomechanical parameters [136].

CXL in the Pediatric Population

Diagnosis of KCN at a younger age constitutes a poor prognosis regarding progression of the disease and the future need for a corneal transplant [137]. The prospective, nonrandomized, phase II open trial – the Siena CXL Pediatrics trial – included 152 patients aged 18 years or younger (10–18 years) suffering from progressing KCN [138]. A 3-year follow-up after epi-off CXL showed significant improvement in both visual acuity and keratometric values and a reduction in coma aberrations. Similarly, positive results were demonstrated in two smaller prospective pediatric trials [139, 140].

Other Results

The use of CXL for the treatment of KCN has been shown to significantly improve quality of life even 3 years following the procedure. This was shown using vision-specific quality-of-life questionnaires. CXL specifically improved the ‘mental health’, ‘driving’ and ‘dependency’ subscores in the vision-specific quality-of-life questionnaire [141].

Other Keratoectatic Disorders

Ectasia following Photorefractive Surgery

Progressive corneal ectasia is a well-known possible complication of photorefractive surgery [25, 142]. Morphological changes following CXL in corneas with PLE were found to be identical to changes in KCN corneas following CXL [143]. Several small, prospective clinical studies have evaluated CXL for PLE and found that visual acuity and keratometric measures either significantly stabilized or improved. All those studies had only 1 year of follow-up [144–147]. A retrospective study evaluated 26 eyes with PLE (23 eyes) and with ectasia after PRK (3 eyes) during a 2-year follow-up period. There was significant improvement in CDVA, K_{max} and several topographic indices of corneal regularity (such as index of surface variance, index of vertical asymmetry, KCN index and central KCN index) [148].

In 2011, Kanellopoulos and Binder [149] reported their data on a combination treatment using simultaneous CXL and PRK in patients with PLE. The goal was to use PRK to improve visual outcomes by normalizing the corneal surface, reducing irregular astigmatism and potentially reducing the refractive error – in addition to the corneal stabilization effect of corneal CXL. Twenty-seven of 32 eyes had an improvement in uncorrected distance visual acuity and CDVA to 20/45 or better at the end of follow-up; 4 eyes showed some topographic improvement but no improvement in CDVA; 2 of the 32 eyes had corneal ectasia progression after the intervention and 1 of the treated eyes subsequently required a penetrating keratoplasty [149]. This treatment protocol is commonly referred to as the ‘Athens protocol’.

Pellucid Marginal Degeneration

Being a rare condition, PMD has been evaluated much less than KCN, especially with regard to CXL treatment. A major difference between PMD and KCN is the location of maximal corneal thinning and steepening. In PMD, corneal thinning is more peripheral than in KCN [150]. A study examining the peripheral effect of CXL, using OCT evaluation of the stromal demarcation line,

showed that the depth of the CXL effect 3 mm away from the center of the cornea decreases to 65% of the central depth. Therefore, it was concluded that the intended depth of CXL using current light sources is achieved only within the central area of the cornea, and in order to provide CXL to the peripheral cornea, the ultraviolet beam either should have an improved intensity profile or may have to be decentered [151]. Two prospective studies evaluated the effect of CXL on the peripheral cornea. One was a randomized, controlled clinical trial examining the 1-year effect of CXL in 99 eyes with KCN and PLE. This trial showed a decreased flattening effect following CXL in peripheral cones when compared with flattening results for central cones [152]. The other study was a prospective 2-year series evaluating 68 KCN eyes. This study found a significant difference in visual acuity parameters between centrally and paracentrally located apices in favor of the central apices [114].

One series of 13 eyes with PMD following CXL procedures showed stable keratometric results and good visual acuity in all eyes except one [153]. A few case reports describing CXL for PMD have been published. All showed improvement and/or stabilization of visual acuity and keratometric parameters [154–156]. A study examining the effect of combined PRK and CXL on corneal ectasia included 6 eyes with PMD and 6 eyes with KCN. Results were only given for the entire cohort as a whole, and therefore they are less significant for evaluation of PMD. Nevertheless, the group as a whole showed a significant improvement in visual acuity, keratometry and astigmatism parameters [132].

Other Indications for Riboflavin-UVX Collagen CXL

Corneal Infections and Chemical Burns

Evidence of the antimicrobial activity of riboflavin and UVA was initially described by Tsugita et al. [157] in 1965. They demonstrated its ability to damage viral DNA in a virus infecting tobacco plants. The combination of riboflavin with UVA or the use of UVA by itself has the ability to damage nucleic acids and thus is directly antimicrobial [158]. In addition, CXL has been shown to increase the cornea's resistance to enzymatic digestion [109]. Thus, CXL treatment of corneas with infective keratitis can improve their ability to resist proteolytic enzymes secreted by infective microorganisms. The proteolytic enzymatic activity is the cause of clinical corneal melting during and following corneal infections. In vitro studies have demonstrated a good bactericidal effect of

combined riboflavin and UVA on several bacterial species which are known pathogens in bacterial keratitis [159, 160]. The effects on fungal and acanthamoebic activity in vitro were less favorable, showing no significant effect on viability [161, 162]. A different in vitro study showed that acanthamoebic growth was inhibited by UVA without any added effect following the addition of riboflavin [163]. An animal study showed a partial response of *Fusarium* keratitis to CXL [164], while a similar protocol for *Acanthamoeba* showed no response and possible worsening following CXL [165].

In 2008, Iseli et al. [166] published the first clinical series describing the resolution of corneal ulcers complicated by melting in 5 patients failing to respond to antibiotic therapy. A prospective study evaluated the adjunct use of CXL for infectious keratitis in 40 eyes (24 bacterial, 7 fungal, 2 acanthamoebic and 7 with no positive cultures). Recording of the infiltrate area showed good response to treatment in cases of bacterial origin, but in fungal and acanthamoebic cases, a poor response was seen. Infiltrate depth and size were found to be important factors in treatment success. Deep infections may avoid the effect of CXL, which is performed through no more than 400 μm of the anterior cornea [167]. In a different series of 16 patients with infective keratitis, the use of CXL as first-line treatment was evaluated. No antibiotics were given prior to CXL. In all eyes, the epithelium healed completely. Only 2 eyes required antibiotic treatment and 1 eye required an amniotic membrane transplant [168]. Smaller studies evaluated the use of CXL in cases of severe infectious keratitis that was refractory to antibiotics. In these studies (altogether 21 eyes in 3 studies), the majority of patients showed complete healing and scarring of the ulcer; the condition of 2 patients deteriorated: one required enucleation and the other a tectonic keratoplasty [169–171]. Fungal keratitis was evaluated clinically in an 8-patient series, where eyes with culture-proven fungal keratitis not responding to topical therapy were treated with CXL. All eyes showed complete healing of the ulcer following treatment [172].

Corneal melting caused by chemical burns may also respond to CXL. A study on rabbit corneas following alkali burn showed that 6 out of 10 control group corneas had melting after injury, with 2 corneas perforating, while in the CXL-treated group there was only 1 corneal melting (of 10 treated corneas) without any corneal perforation. A histological analysis also showed significantly less trauma in the CXL-treated group than in the control group [173].

Bullous Keratopathy and Other Causes of Corneal Edema

Failure of the endothelial pumping mechanism causes fluid accumulation in the extracellular space between the stromal lamellae [174]. CXL has been shown to increase the cornea's resistance to swelling [110, 175] and therefore was evaluated as a treatment for pseudophakic bullous keratopathy (PBK) and other causes of corneal edema. Edematous corneas treated with CXL demonstrated increased collagen fibril organization in the anterior stroma when compared with nontreated controls. This effect was less evident in corneas with advanced edema and/or fibrosis. The effect appeared to regress 3 months following CXL [176].

In 2008, Krueger et al. [177] described the results of an ex vivo study on 10 human eye bank corneas. Corneas treated with femtosecond laser-assisted pocket CXL showed improved clarity and a statistically significant improvement in corneal thickness. Nontreated controls showed no significant changes. This study also included a clinical case report where the use of CXL for PBK led to corneal thinning and a substantial improvement in visual acuity [177]. A combined clinicopathological study included 24 PBK patients treated with CXL. There was significant improvement in visual acuity, corneal thickness, pain score and corneal haze following CXL at 1 month postoperatively. However, all parameters had worsened by the 3-month postoperative examination [178].

Combined CXL and Photorefractive Surgery

The formation of ectasia following photorefractive surgery is currently one of its more serious postoperative complications [25, 142]. In an effort to prevent postoperative ectasia and to increase corneal stabilization following photorefractive surgery, a method of performing simultaneous photorefractive surgery and CXL has been investigated. Factors increasing the risk of postrefractive ectasia include high myopic correction, thin corneas and a small residual bed thickness [179, 180]. A study examining the effect of combined LASIK and CXL in patients with high myopia (≥ 6 diopters) included 44 eyes. During a mean follow-up period of 3.5 years (range: 1–4.5 years), none of the eyes developed ectasia despite their increased risk [181]. A comparative contralateral study included 34 hyperopic patients having combined LASIK and CXL in one eye and LASIK only in the fellow eye. Eyes undergoing combined LASIK and CXL demonstrated less regression of spherical equivalent during a 23-month mean follow-up period (statistically significant) [182]. The estimated incidence of PLE is up to 0.66% [25]. Therefore,

studies on a much larger scale are required to properly evaluate whether the addition of CXL reduces the risk of postoperative ectasia. There are no studies evaluating the addition of CXL to PRK for the prophylaxis of ectasia following PRK.

Corneal Wound Strengthening

An ex vivo study on cadaveric human eyes showed that the addition of CXL to an ex vivo model of either penetrating or anterior lamellar keratoplasty led to an increase in adhesion strength of the donor-recipient corneal interface, evaluated using burst IOP and tissue separation force measurements [183].

Safety

Several safety issues following CXL have been addressed over the years.

Corneal Infection

Epithelial scraping performed routinely during CXL exposes the cornea to possible infections. Other factors theoretically increasing the risk of corneal infection are the use of a bandage soft contact lens and topical corticosteroids in the immediate postoperative period. There are no large-scale studies evaluating the rates of infection following CXL. There are, however, case reports in the literature of bacterial, polymicrobial, acanthamoebic and even herpetic keratitis following CXL procedures [61–65, 184]. The cases of herpetic keratitis described were in patients with no previously known herpetic infections [65, 184]. For this reason, topical antibiotics are routinely used following the procedure [47].

Corneal Sensitivity and Tear Function

Following CXL, corneal sensitivity has been shown to significantly decrease and gradually recover during the first 6 postoperative months [185, 186]. No effect on tear secretion or tear film stability was observed [186].

Effect on Limbal Epithelial Cells

The integrity of limbal epithelial cells (LEC) is crucial for maintenance of a normal corneal epithelial structure. In vitro exposure of LEC to UVA levels similar to the levels used during CXL promoted expression of genes involved with apoptosis. The addition of riboflavin reduced the damage caused but did not prevent it completely [187]. The combination of riboflavin and UVA was also shown to inhibit the growth and expansion of LEC [188,

189]. Enucleated human eyes undergoing a CXL procedure with half of the limbus protected by a metal shield showed a significant drop in viable LEC count and a lack of LEC growth in the area not protected by the metal shield [190]. Different results were shown for rabbit corneas, where exposure to CXL did not appear to cause any significant histological limbal changes [191]. There are no *in vivo* studies evaluating the effect of CXL on LEC. Therefore, the use of limbal protection during CXL should be considered. This is especially true for treatment of PMD, since the irradiated area in CXL for PMD may be decentered and thus positioned closer to the limbus [151].

Stromal Haze and Sterile Infiltrates

The formation of significant stromal haze following CXL is a potential complication that can affect visual acuity. A prospective series of 50 eyes, treated with CXL either for KCN or PLE, examined the degree of stromal haze both quantitatively and qualitatively throughout a 1-year follow-up period. Stromal haze peaked 1 month after CXL, plateaued 3 months after CXL and decreased up to the 1-year follow-up point. At this point, stromal haze values returned to baseline in the PLE group but not in the KCN group [192]. A retrospective analysis of 163 eyes having undergone CXL showed the development of significant stromal haze in 14 of them (8.6%), which persisted through the 1-year follow-up period. While the 149 eyes (91.4%) that did not develop corneal haze showed significant improvement in visual acuity, the 14 eyes with corneal haze showed a significant deterioration in visual acuity. Preoperative corneal thickness was significantly reduced, and preoperative mean keratometry significantly increased, in the group of eyes developing haze when compared with those that did not. This may indicate that keratometry and corneal thickness may be factors predictive of the formation of stromal haze [193].

Few small patient series described the formation of sterile stromal infiltrates following CXL. In one series, 7 eyes developed peripheral sterile ring infiltrates, resolving completely following the instillation of topical steroids [194]. Another case series described the late formation of deep paracentral stromal infiltrates, persisting at 6 and 12 months following CXL. These did not cause a reduction in visual acuity due to their noncentral location [195]. Sterile infiltrates were also described as part of an inflammatory response after CXL – including keratitis, anterior chamber cells and keratic precipitates – appearing in 4 patients. These responded rapidly to topical and periocular steroids, with stromal opacity persisting in some of the

eyes [196]. These infiltrates differ significantly from the previously described stromal haze, and probably do not represent the same pathologic process [194].

Endothelial Toxicity and Thin Corneas

The standard irradiance of 3 mW/cm² combined with the application of riboflavin 0.1% results in a significant and relatively sharp drop in UVA light of up to 95% and a resultant irradiance of the corneal endothelium (in a 500- μ m-thick cornea) of only 0.15 mW/cm² (= 0.27 J/cm²) [197]. Thus, it can be concluded that the endothelial cytotoxic threshold is far from being reached in eyes with sufficient corneal thickness. Wollensak et al. [198] published two studies in 2003, investigating the threshold dose and depth for endothelial toxicity in animal eyes. In rabbit corneas with a thickness of less than 400 μ m, the endothelial UVA dose crossed the cytotoxic threshold level of 0.65 J/cm² (0.36 mW/cm²) following the standard surface CXL UVA dose of 5.4 J/cm² (3 mW/cm²) [198]. *In vitro* analysis of porcine endothelial cells exposed to UVA and riboflavin showed similar results [199]. It was concluded that pachymetry should be routinely performed before CXL, and in corneas thinner than 400 μ m, irradiation should not be performed, because of the cytotoxic risk to the endothelium [198, 199]. However, additional thinning of corneas thicker than 400 μ m can take place during the CXL procedure itself, causing them to become less than 400 μ m thick during the course of the procedure. A reduction in thickness of 75–87 μ m during different stages of the CXL procedure has been demonstrated [200, 201]. This transient thinning can be related either to evaporation through the deepithelized surface or to the oncotic effect of 20% dextran used to form an isoosmolar riboflavin solution. This intraoperative thinning can theoretically increase the risk of endothelial damage even in corneas with apparently sufficient preoperative thickness. A retrospective analysis of 350 patients having undergone CXL found postoperative corneal edema in 10 of them (2.9%). In 5 of the 10 patients, the corneal edema resolved, but in the remaining 5 patients edema change plateaued at 3 months after CXL and persisted. The patients were offered penetrating keratoplasty, with 2 of them undergoing this procedure. While it was not possible to conduct specular microscopy on those eyes, the mechanism suggested for persisting corneal edema was one of significant endothelial damage [202].

Several clinical studies examined the effect of CXL in thin corneas (<400 μ m). A clinical evaluation of 14 eyes with minimal corneal thickness <400 μ m undergoing

CXL using the Dresden protocol showed that 1 year following the procedure a significant endothelial decrease in cell density was present. While the procedure appeared effective, it resulted in significant endothelial damage [203]. The use of epi-on CXL in thin KCN corneas appeared to cause no endothelial changes postoperatively, but showed only moderate efficacy [204]. The use of hypoosmolar riboflavin solutions during CXL of thin corneas can increase intraoperative corneal thickness to safer levels. This has been evaluated clinically, showing an increase in intraoperative corneal thickness from 337 μm preoperatively to 452 μm intraoperatively. KCN showed stabilization following the procedure. However, endothelial cell integrity was not evaluated in this study [205]. A similar study of hypoosmolar riboflavin in CXL of thin corneas showed no apparent endothelial changes following the procedure [206]. The efficacy of hypoosmolar CXL seems to be reduced when compared with standard CXL [207]. A second problem with the use of hypoosmolar riboflavin is its short-term effect on corneal swelling, which was shown to last no more than 10–30 min [208].

Corneal Drug Penetration and IOP Measurements

Corneal permeability has been shown to decrease in rabbit eyes following CXL both *ex vivo* as a reduction in mean permeability coefficient and *in vivo* as a reduction in pupillary response following instillation of pilocarpine drops [209]. A reduction in aqueous concentrations of ofloxacin and voriconazole following topical instillation was seen in CXL-treated eyes when compared with nonirradiated controls. This was shown in an *ex vivo* porcine model [210].

IOP measurements following CXL show overestimation, demonstrated using several tonometers including applanation tonometry (Goldmann), indentation/applanation tonometry (Tono-Pen; Reichert Technologies, Depew, N.Y., USA) and noncontact dynamic tonometry (ORA). This is attributed to the change in corneal biomechanics, leading to increased corneal rigidity. The difference in IOP measured following CXL ranged from 1.2 to 3.1 mm Hg, depending on the tonometer used [211, 212].

Changes in Corneal Thickness

Corneal thickness shows a significant reduction during and shortly after CXL. In one study, corneal thickness decreased by a mean of 87 μm during the first 60 min [201]. Corneal thinning gradually resolves over the first 6 months following CXL and by 1 year returns to baseline values [213, 214].

Postoperative Pain

The epithelial debridement performed routinely in CXL is associated with postoperative pain. A prospective study including 178 KCN eyes undergoing CXL evaluated pain during the first 5 postoperative days. Pain was evaluated using documentation of the need for analgesia and patients' subjective evaluation on the Wong-Baker FACES Pain Rating Scale. It was concluded that pain following CXL can be intense, especially in the first 3 days, even with an aggressive pain control regimen. All pain evaluation parameters decreased rapidly with each day following CXL. Pain was significantly correlated with the patient's age [66].

Conclusion

More than a decade after being introduced, collagen CXL is still changing. Indications are evolving, and different protocols are being developed and tested. Collagen CXL has changed the clinical approach to keratoectatic disorders dramatically. To the defensive approach – aiming to maximally improve current vision by different modalities (eye glasses, contact lenses and, finally, surgery) – the ophthalmologist adds an offensive approach, changing the course of the disease and preventing further loss of vision and functionality.

It is now understood that this modality is safe and effective. Future studies will lead to a better understanding of the risks and benefits. With this new experience, eventually the present relative disorder will probably be distilled down to a few treatment variants or protocols, appropriate for an exact list of rigid indications.

Disclosure Statement

None.

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