Corneal collagen cross-linking and keratoconus

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Abstract

Keratoconus is a condition resulting in corneal ectasia. Abnormal protrusion of the cornea leads to reduced visual acuity. Riboflavin induced collagen cross-linking (CXL) has received significant attention the last few years and has shown to stop the progression of keratoconus. The original CXL procedure involves epithelial debridement, application of topical riboflavin drops and UVA exposure at 370 nm for approximately 30 minutes. However, not all patients are suitable for this treatment. Collagen cross-linking is a relatively new method and several complications of the treatment have been reported. The treatment may greatly reduce the need for corneal transplantations. This article discusses CXL. Current studies show that the stiffening effect of CXL appears to remain stable after 6 years. However, the exact duration of the corneal stiffening effect is as yet unknown, and more prospective randomized controlled trials in the future are desirable.

Introduction

Cornea

The light passing through the main refractive media of the human eye, consisting of the cornea, crystalline lens, and vitreous, must focus on the retina in order to create a clear image. The cumulative refractive power of the whole eye equals 59 diopters (D), two thirds of which is provided by the cornea, making its normal physiological shape and curvature essential for normal vision. Any minor morphological irregularity of the corneal surface will therefore lead to optical distortion and will affect the vision. Hence, a healthy cornea with a regular shape and biomechanical stability is essential for normal stable vision.

The cornea is made of transparent, avascular tissue that measures 11-12.5 mm horizontally and 10-11.5 mm vertically. The corneal stroma consists of 200 to 500 layers of flattened collagenous lamellae extending from limbus to limbus (1). The collagen structure in the stroma provides the cornea with biomechanical strength, and thus is responsible for the curvature and shape of the cornea. It appears that stiffness of a keratoconic cornea is only 60% of that of the normal cornea, and the conical shape assumed by a keratoconic cornea is the result of decreased biomechanical stability (2).

Keratoconus

Keratoconus is a degenerative non-inflammatory disease of the cornea, leading to in distortion of the cornea and apical thinning. These corneal changes result in decreased vision due to high irregular astigmatism and less frequently, central corneal scarring. The condition usually begins at puberty, but tends to progress during adolescence. In brief, treatment consists of glasses, rigid contact lenses and intracorneal rings early in the disease, however none of these modalities affect progression of the condition. Eventually, penetrating keratoplasty may be required in advanced cases to restore vision. A recent technology, corneal collagen cross-linking, might stop the progression of keratoconus, thereby reducing the need for penetrating keratoplasty.

Demographics: An equal distribution between men and women is found, and all ethnic groups may be affected (3). However, incidences of keratoconus in western population are found to be lower compared to incidences in the Asian and Arabic populations (table 1.1). It typically affects the young, presenting in adolescence (4).

Table 1: The table gives a distribution of keratoconus incidences based on ethnicity (per 100 000).

Region/country	Ethnicity	Number of incidents (average)
UK (5)	Western	1-3.5
Japan (5)	Asian	7.6
Arabia (6)	Arab	20
UK (3)	Indian	19.6

Symptoms/findings: Keratoconus usually affects both eyes, though one eye may be more affected than the other (7). The disease is usually diagnosed during the second and third decade of life. Progression varies, although it may progress more rapidly at a younger age. The condition is often caught by the optician when worsening of the visual acuity occurs that is no longer correctable by spectacles (8). The disease is diagnosed by clinical signs, topography, slit-lamp biomicroscopy and pachymetry (9).

Pathogenesis: Keratoconus is considered a non-inflammatory (7) degenerative disorder of cornea, characterized by mild to severe progressive conical protrusion with irregular astigmatism, apical thinning and myopia. Complications of central scarring, hydrops, perforation or blindness may occur with time (3;10;11). The pathogenesis is not known. The main function of the collagens is to act as a supporting tissue. Physiological cross-linking occurs as a part of maturation, but also in ageing and disease, for example diabetes (8). The keratoconic corneas undergo changes in the collagen structure and organization as well as changes in the extracellular matrix (12-15). In keratoconic corneas, there are possibly a reduced number of intra- and inter-lamellar cross-links compared to normal corneas, which is illustrated by a significantly higher pepsin digestion of keratoconic corneas compared to a normal cornea (16;17). The modifications, along with keratocyte apoptosis and necrosis, lead to 50 % reduction in the biomechanical resistance of the cornea (16;17).

There is found strong associations with atopy and with eye rubbing (7). 6 - 8% of reported cases have a positive family history or show evidence of familial transmission and genes playing a major role in the etiology. Keratoconus is usually an isolated condition. However, it is commonly associated with Down syndrome, Leber congenital amaurosis, and connective tissue disorders (4;10). Recent biochemical assays and immunohistological studies of corneas with keratoconus indicate that the loss of corneal stroma, after digestion by proteolytic enzymes, can be caused by increased levels of proteases (matrix metalloproteinase) and other catabolic enzymes, or decreased levels of proteinase inhibitors (TIMP-1) (4).

Treatment options: In the early stages of the disease the patients are typically asymptomatic and may only need corrective glasses or contact lenses (18). However, as the corneal protrusion and associated astigmatism progresses, first- line treatments is to fit rigid gas-permeable contact lenses (19). For patients where the disease has advanced, and spectacles or lenses are no longer efficient in improving visual acuity, intacs inserts may be indicated. Intacs are small plastic rings implanted in the stroma of the cornea to correct vision. Intacs are a minimal invasive surgical procedure and have been reported to be effective in decreasing the corneal steeping and astigmatism, and enhance visual acuity (20-22). However, neither the gas-permeable lenses nor the intacs-treatment can stop further progression of ectasia. The progressive changes are the result of diminished collagen which leads to the protrusion of the cone (23-26). The progression continues and it is estimated that approximately 20% of all patients with keratoconus will require corneal transplantation (27). Corneal transplantation is a major ophthalmic surgical procedure with several potential complications. Corneal collagen

cross-linking with riboflavin is a relatively new treatment that shows good results in preventing progression of keratoconus (8). Corneal collagen cross-linking targets the biochemical properties (28), prevents visual loss and significantly reduces the number of patients requiring surgical treatments (8).

Methods

This literature study is based on selected articles on CXL and keratoconus. The information is provided by PubMed with search words "keratoconus" and "corneal collagen Cross-linking". The search of articles has not been limited by year of publication, but papers in other languages than English have been excluded considering publish-year.

Corneal Collagen Cross-linking and Keratoconus

1. Corneal collagen cross-linking

Corneal collagen cross-linking is a treatment designed to increase the rigidity and the structural integrity of the cornea. This prevents further progressive ectasia (4). Collagen cross-linking is indicated in progressive keratectasia, such as keratoconus (29) and the associated variants (post-LASIK ectasia (LASIK = laser-assisted in situ keratomileusis), corneal melting conditions or infectious keratitis) (18).

Spoerl and Huhle et al. (30) were the first to prospect the use of riboflavin and UVA to obtain cross-linking of corneal collagen. By using this method, Wollensak (31) and Kohlhaas et al. (32) demonstrated a clearly positive effect of cross-linking of both porcine and human corneal tissue. The results showed that CXL increased the rigidity of corneal tissue (31). It is thought that the riboflavin has double function; it acts as a photosynthesizer for the production of oxygen-free radicals, in addition to absorbing the UVA irradiation and preventing damage to deeper ocular structures such as the corneal endothelium, the lens, and the retina (33). When riboflavin is excited into a triplet state by UV exposure it releases highly reactive oxygen species into the stroma (34) These oxygen species react with surrounding molecules and amongst several non-specific interactions also result in the formation of cross-links consisting of intra- and intermicrofibrillar covalent bonds (35). The process is dependent on oxygen, and enhanced by deuterium oxide (36), which differs it from the formation of cross-links that are made with increased age and diabetes (37). The cross-links cause increase in the collagen diameter and in spacing between collagen fibrils (34;38).

Corneal collagen cross-linking was initially thought to increase the biomechanical stability of the corneal stroma and its resistance to enzymatic digestion, by inducing cross linkage between the stromal collagen molecules. However, Meek et al. (39) suggested as a result of x-ray diffraction studies, that CXL is instead strengthening the interlamellar collagen fibril adhesion of adjacent lamellae originally weakened by keratoconus. The technique has shown to be safe with no endothelial damage, presupposed that the cornea is thicker than 400 μm, with no loss of corneal stromal transparency and no damage to deeper ocular structures (40;41). In several studies, riboflavin /UVA cross linkage has been shown to increase stress-strain measurements, reduce the swelling rates, increase shrinkage temperature and increase the resistance against enzymatic degeneration (pepsin) of corneal stromal tissue (31;42;43). Morphometric computer software has shown increased diameter of the collagen fibers with most changes occurring in the anterior 200 μm without any damage to the corneal endothelium (32;34). Recent data show an actual statistically significant modification in the modulus of elasticity, increased by 4.5 times, and 328 % increase in tensile rigidity, in the human cornea after Riboflavin/UVA CXL (41;44). Corneal cross-linking is the latest treatment which may be a potential intervention slowing down the progression of the disease (8).

Individuals who are in "early-to-moderate keratoconus-phase" and are showing signs of progression are better candidates for CXL than those at the end-stage of the disease (45). Corneal cross-linking is also indicated in patients with keratectasia after corneal laser corrective surgery (29). Recommended limitations in order to perform CXL are maximum keratometric (K) readings less than 60 diopters (D) and a central corneal thickness (CCT) of at least 400 μm. The latter concerns the thickness of the deepithelialized cornea when epi-off CXL is used. Also, CXL done on patients with keratoconus younger than 18 years have shown positive results 3 years post-treatment(46).

2. History

Collagen cross-linking, involving corneal collagen was first investigated in diabetics in the 1990s (45). Researchers in Dresden noted that this group reduced the incidence of keratoconus because of glycosylation-mediated cross-linking which strengthened the tissue (37;45;47;48), and found that diabetes may be a protective factor against the development of keratoconus (48). To study and show this, Maillard reactions (addition of an aldehyde sugar to amino group of protein chains), were investigated in the corneas of diabetics compared to controls (37). It was found that diabetics accumulate more and more glycation end-products (AGEs) and collagen modification by AGEs results in covalent cross-linking. Another study found that diabetics had increased AGEs and increased cross links from pentosidine, which is one of the AGEs formed (37).

In addition to the AGEs related CXL, cross linking has been found to occur in the cornea and crystalline lens with increasing age (37;49). In a normal cornea it exist covalently bonded molecular bridges or cross-links between nearby collagen-helices and between microfibrils and fibrils along their length (50;51). Corneal fibril diameter increases with 4,5 % through life (52) and the types of cross-linking seen in aging has been shown to be due to enzyme lysyl oxidase, which leads to aldehyde formation and further post-translational modifications (37). With aging, the range of natural cross-linking increases (52;53) with modifications in the biomechanical properties in the tissue and a measurable enhance in Young's modulus (54). This may explain the flexibility of the infant cornea compared to adult tissue and the slowing progression of keratoconus observed with increasing age (27).

The concept of photopolymerisation to strengthen tissues has been around for some time in industry, as a means to harden various plastic and other long chain molecules (35). In 1968 Christopher Foote published the mechanisms by which photosensitized oxidation happens in biological systems (55). In 1988 Fujimori disclosed a third mechanism of cross-linkage in type I collagen involving either oxidation by ozone or photo-oxidation by UV-light (56). It is also the same technology that has been used to harden bioprosthetic materials (57), as for example artificial heart valves that are stiffened using glutaraldehyde (8), and as a component in dental filling materials (57). Indeed a visit to a dentist who used UV irradiation to harden filling material led to the inspiration to stiffen the cornea (58).

Sporl et al. (30) published the result of their study that induced cross-linkage in porcine eyes in German in 1997 (followed by in English in 1998 (49)). Their study included 8 test groups with 10 eyes in each group. The eyes were de-epithelialized and the cornea was treated with

UV light (254 nm), 0.5 % riboflavin, 0.5 % riboflavin and UV light (365 nm), blue light (436 nm), sunlight, glutaraldehyde (0.1 %, 10 min), glutaraldehyde (1 %, 10 min) or Karnovsky's solution (a fixative used in electron microscopy which contains paraformaldehyde, sodium hydroxide and glutaraldehyde) (0.1%, 10 min). A ninth group served as control. The results of this study showed; riboflavin and UV, glutaraldehyde and Karnovsky's caused an increase in stiffness of the treated cornea compared to the control group (p<0.05) (49), and ultraviolet at 365 nm was found to strengthen the cornea best compared to ultraviolet at 254 nm and 436 nm (18). The toxicity and the inability to control depth and reach of diffusion of glutaraldehyde makes it impractical in clinical content (35). While the riboflavin-UVA showed promising results for the next stage of human studies (59).

All studies thereafter were done in context of this, with the exception of the riboflavin concentrate that was changed to 0,1 % in all future studies (18). The next big experiment consisted of 20 porcine eyes treated with 0.1 % riboflavin and ultraviolet light 370 nm, 3 mW/cm2 for 30 minutes, compared to 20 untreated porcine eyes (32). In a similar manner 5 human donor corneas were treated the same way. The experiment concluded that the stiffening effect was marked in the treated eyes (32). The stiffening effect was increased by a factor of 4.5 compared to1.8 in the porcine cornea (31). In addition they discovered that 65-70 % of the UV light was absorbed in the anterior 200 μm of the stroma and only about 20 % reached the next 200 μm (32), which was later used to determine the effect on deeper structures for in-vivo studies (18).

In 2004 Seiler et al. (34) published results of increase of collagen fiber diameter in rabbit corneas. They found that in the treated anterior stroma the collagen fiber was significantly increased by 12.2% compared to control eyes, while in the treated posterior cornea it was increased by 4.6 %. Enzymatic digestive studies and electrophoresis were done to analyze the biomechanical aspects of the CXL (42;60). The former study compared 60 riboflavin-induced cross linked porcine eyes to control group, to investigate their time to be digested fully by pepsin, trypsin and collagenase. The results concluded that it took 13 days to digest pepsin in treated eyes vs. 6 days in the control eyes (42). The results for trypsin and collagenase were similar and the conclusion was a marked resistance to enzymatic digestion, suggesting high numbers of cross linked bonds requiring digestion (18). In a study performed by Wollensak et al.(60), the type I collagen was extracted from the corneas, run on sodium deodecyl sulfate-polyacrylamide gels and separated by electrophoresis (60). In the cross linked cornea-extracts they found another much larger band (1000 kDa in contrast to usual band sizes of 130 kDa, 200 kDa, and 300 kDa) compared to the control corneas that only showed the usual type I collagen pattern. This larger band was further found to be chemical stable; resistant to heat denaturation, pepsin treatment and mercaptoethanol treatment.

Another study by Wollensak et al. (61), before he did in-vivo human studies, was a hydration study and second analysis of the depth of treatment using optical coherence tomography (OCT). Wollensak et al. treated 20 porcine eyes with the riboflavin ultraviolet protocol. The eyes were incubated for 24 hours in a moist chamber, then 15 eyes were examined by biomicroscopy and OCT and 5 eyes were examined by light microscopy. The results of this study were concluded with a characteristic swelling pattern that affected the anterior cornea

portion greater than the posterior portion. The cornea was divided into 3 zones; anterior zone (242 μm), intermediate zone (next 238 μm) and a posterior zone (The remainder of the tissue, 1355 μm for porcine corneas). The swelling was found to be increased by a factor of 2.2 in the intermediate zone and by a factor of 2.7 in the posterior zone (61). The light microscopy concluded cross linking pattern. These results were profitable in analyzing the amount of swelling following treatment and the treatment depth (18).

Wollensak et al. (41) began in 1998 to treat patients using the riboflavin UVA cross-linking technique, with the support of all of the in-vivo and ex-vivo animal and human corneal studies. The results of the pilot study were published in 2003, with a follow-up ranging from 3 months to 4 years. The study showed that all treated eyes had an obstacle of the progression of the keratoconus and that 70 % had regression and a reduction in the maximal keratometry-readings by an average of 2 diopters (41). Since then the idea spread to other centers and resulted in patients with keratoconus being treated worldwide (18).

In the Dresden study they found statistically significant changes after the first year post-operatively and remained stable for the remainder of the follow-up, which extended up to 6 years (62). The explanation to the observation that many of the patients had improvement in visual acuity felt to be a combination of a decrease in the amount of irregular astigmatism, a decrease in the corneal curvature, and a secondary improvement in the ability to fit contact lenses. They found no statistically significant change in IOP (intra ocular pressure) at one year (62). Small differences were found in the effect of the central corneal thickness (CCT) change (44).

3. Technique

3.1. Before the treatment

Before the treatment the patient needs to go through an eye examination including refraction, keratometry, topography and regional pachymetry (33;63;64). It is also by some recommended to discontinue the use of contact lenses 1 month before treatment (65-67) and use broad-spectrum antibiotics 1 to 3 days before treatment.

3.2. Treatment

The CXL treatment protocol most widely used is based on the work of Wollensak, Spoerl, Seiler and co-workers. It is optimized based on extensive laboratory data to maximize the crosslinking effect and minimize any damages to ocular tissue. The CXL procedure is conducted under sterile conditions, after the patient having 4 drops of Ofloxacin 0.3 % and 2 drops of Pilocarpine 2% instilled in the eye to be treated. Then the patient's eye is anesthetized with Oxybuprocaine 0,4% drops (4). The central 7-9 mm of the corneal epithelium are removed to allow better diffusion of riboflavin into the stroma (68). The epithelium acts as a barrier and without epithelial removal the biomechanical effect is reduced to be under 50 % of the standard cross-linking procedure (28). Baiocchi et al. (69) showed that the necessary stromal concentration of riboflavin is only obtained after epithelial removal.

Iso-osmolar riboflavin 0, 1 % solution with dextran 20 % is then applied to the cornea every 2 to 3 minutes for 30 minutes (68). After this time an ultrasound pachymetry (5 repetitive measurements) on the cornea at the thinnest is done to confirm that it is at least 400 µm in depth (68). If the cornea is too thin, riboflavin 0, 1 % solution in hypo-osmolar saline is used instead. Ultrasound pachymetry has to be performed to make sure that the thickness is at least 400 µm (4). Successful penetration of riboflavin through the cornea is certified by visualization of riboflavin in the anterior chamber by slit-lamp biomicroscopy, using cobalt blue light, before irradiation (4).

UVA irradiation is commenced using wavelength of 370 nm for 30 minutes at working distance of 54 mm (68). There are at least three widely available UV light sources for CXL; the IROC UV-X system (IROC AG, Switzerland), the CSO VEGA system (CSO Italy) and OptoXlink (Opto Global Pty Ltd, Australia). The three devices come with separate luminescence-measuring device and have somewhat different operating instructions and focusing distances (70). Through the irradiation riboflavin solution is applied every 2-3 minutes while ensuring the stroma is thick enough (68).Topical anesthetic agent (Oxybuprocaine 0,4 %) is applied whenever necessary (4).

3.3. After the treatment

Antibiotic drops (Ofloxacin 0,3 %) are applied and a bandage contact lens is inserted until the reepithelialization is complete (4). Antibiotic drops (Ofloxacin 0,3 %) 4 drops a day for a week were administered and analgesics by mouth as necessary (4). Corticosteroids are used to minimize the inflammatory response (58). The patient should be closely examined in the early postoperative period, typically at day 1, 3 and further as required to ensure epithelial healing (70). Later examinations were performed in month 1, 3, 6 and 12 (45;66;67;71) postoperatively. Patients wearing gas permeable contact lenses may interpret the results of CXL since lens wear improves the topographic parameters (72).

4. Contraindications

4.1. Absolute contraindications for treatment with CXL

Corneal thickness of less than 400 µm: Patients with corneas thinner than 400 µm should not undergo CXL because of possible endothelial cell density decrease postoperatively (29;73). In advanced keratoconus progressive corneal thinning often leads to thinning of remaining stromal thickness by less than 400 µm. Hafezi el al. (74) changed the current treatment protocol by preoperatively swelling thin corneas to a stromal thickness of at least 400 µm using riboflavin 0,1 % solution in hypo-osmolar saline. This treatment protocol was performed in case series of 20 patients, and no complications were observed (74).

Central corneal opacities and severe dry eye; can be a hinder to re-epithalization (29).

Pregnancy, nursing, having systemic collagen vascular disease (29), concomitant autoimmune disease (28), history of herpetic keratitis, corneal scarring or concomitant autoimmune diseases are all conditions were undergoing CXL should be avoided since these populations have not been appropriately tested (29).

4.2. Relative Contraindications

Maximum K reading more than 58 D: the efficiency of CXL would likely increase if the treatment was limited to eyes with a maximum K reading of less than 58.00 D (75).

Comprehensive corneal scarring is associated with poor existing best-corrected visual acuity: Patients with an already limited vision may not advantage from CXL unless it is combined with a refractive treatment option (for example INTACS). Alternative treatments should then be considered (for example graft surgery) (4).

Patients with primary or secondary keratectasia, especially corneal guttata or other endothelial irregularities, history of recurrent erosions, ocular surface disorders, and connective tissue disease: should be advised and the risks ought to be discussed with the patient (4).

5. Complications

Blurry vision, lacrimation and foreign body sensation: Can be expected for 24-48 hours (34). Blurry vision can last up to 1 month or longer depending on the corneal edema.

Stromal haze: Haze occurring after collagen cross-linking usually subsides completely the first postoperative year. It reaches up to approximately 60 % depth in the anterior stroma (equal to absolute depth of 300 mm) (64;76). The nature of mild stromal haze is related to transient corneal fibroblast generation rather than more persistent haze due to generation of myofibroblasts (77). The demarcation line was described by Seiler and Hafezi to represent the transition zone between the CXL-treated stroma and the untreated posterior layers (64).

During healing phase: The cornea is vulnerable to infection and melting. Several studies have published case reports, of infectious keratitis and limited corneal melting, associated with riboflavin/UVA CXL (78-85). It is more likely that corneal infections after CXL break out during the early postoperative period rather than during surgery because CXL kills bacteria and fungi (86-89).

Limbal epithelial cells: Riboflavin-UVA should not be used on the limbus during CXL since the oxygen radicals can have toxic effect, and reduced cell expansion of limbal epithelial cells has been reported (90).

Postoperative pain: Pain can be seen the first 3 days after treatment, and can be intense. The pain has been found to be more intense the younger the patient is (91;92).

IOP: Higher IOP-values are found after CXL when measured by tonometry. These are probably due to increased stiffness of the cornea after treatment (93).

6. Results

Table 2: The table presents an overview of some of the CXL-trials on progressive keratoconus.

	Number of treated eyes	Type of study	Description	Follow-up	Results
Wollensak et al. 2003 (41).	23 eyes	Prospective, non-randomized clinical pilot study.	23 eyes of 22 patients treated with standard CXL-protocol.	3 months to 4 years	Progression stopped in all eyes. Regression with reduction of the max keratometry readings by 2.01 D and of the refractive error by 1.14 D in 16 eyes. No changes in corneal and lens transparency, endothelial cell density, and IOP. Visual acuity improved slightly in 15 eyes.
Wollensak et al. 2006 (35).	60 eyes	Clinical study	60 eyes of 48 patients.	Up to 5 years	Progression of keratoconus was stopped in all eyes. Reversal and flattening of the keratoconus by up to 2.87 D in 31 eyes. BCVA improved by 1.4 lines. The trial revealed that the maximum effect of the treatment is in the anterior 300 mum of the cornea.
Hafezi et al. 2007 (94).	10 eyes		10 patients treated with standard CXL after LASIK.	Up to 25 months	Stopped and/or partially reversed keratectasia. Reduction in maximum keratometric readings.
Raiskup-Wolf et al. 2008 (62).	241 eyes	Long-term, retrospective	Long-term dampening effects of CXL.	6 months to 6 years	Long term stabilization and improvement were indicated with decreasing in steeping by 4.84 D after 3 years. BCVA improved significantly (> or =

					1 line), 57% of 66 eyes or remained stable in 24% after 2 years. Two patients had to repeat CXL.
Wittig-Silva et al. 2008 (95).	33 eyes	Prospective, randomized, controlled	The first randomized trial. Included 66 eyes of 49 patients	3-12 months	After 1 year treated eyes showed a flattening of the steepest simulated K-max by an average of 1.45 D (P = .002). Improvement in BCVA was observed. No statistically significant found for spherical equivalent or endothelial cell density.
Grewal et al. 2009 (96).	102 eyes		Evaluated changes in corneal curvature, corneal elevation, corneal thickness, lens density, and foveal thickness after CXL.	Up to 1 year	No significant differences in mean values between preoperatively and 1 year postoperatively was found.
Coskunseven et al. 2009 (97).	19 eyes	Comparative study	19 patients. The worst eye was treated, while the other worked as control.	5-12 months	The group treated with CXL showed an increase in UCVA and BCVA (P < .01). The treatment did inhibit the progression of keratoconus by reducing the corneal curvature, spherical equivalent refraction, and refractive cylinder in eyes after 9 month follow-up. The untreated group showed no statistical difference except

					UCVA and BCVA which decreased.
Koller et al. 2009 (67).	21 eyes	Non randomized	21 patients. One eye treated, while the other worked as control eye. Compared geometrical shape factors after CXL.	Up to 12 months	No treated eyes showed topographic progression (in untreated group 8 eyes experienced significant progression. Minimal curvature radius increased significantly after 1 year (in the untreated fellow eye, it significantly decreased. Minimal corneal thickness was significantly reduced after treatment (P < 0.002 at 12 months). The cornea showed an evolution toward a more regular shape as indicated by a significant reduction in 4 of 7 keratoconus indices. No complications were found.
Hoyer et al. 2009 (98).	153 eyes	Long-term, retrospective	153 eyes of 111 patients. Studied the long-term halting effects of CXL.	12 months to 7.5 years	Keratectasia significantly decreased in the third year by 4.34 D. Visual acuity improved significantly in at least 1 line or remained stable after 3 years in 60.6% and 36.4%. No side effects were seen. Three patients had to repeat CXL treatment.
Vinciguerra et al. 2009 (99).	28 eyes	Non randomized	Refractive, topographic, tomographic and aberrometric	Upto 12 months	After 1 year: mean UCVA and BCVA improved (P<0.05). Significantly reduced corneal

			outcomes 12 months after CXL.		average papillary power (APP), apical keratometry (AK), and corneal and total wave front aberrations. No changes in endothelial cell counts were seen.
Hafezi et al. 2009 (74).	20 eyes	Case series study	Study with 20 patients. CXL concerning patients with keratoconus where the cornea is thinner than the required 400 µm.	Up to 12 months	Preoperative swelling of the cornea makes it possible to treat patients with thin corneas that would otherwise not be eligible for treatment. No complications were found.
Vinciguerra et al. 2010 (100).	13 eyes		9 patients. Results of CXL after excimer laser refractive surgery	Up to 12 months	Eyes with ectasia seemed to stabilize. BCVA improved statistically significant (P < .05) beyond 6 months after surgery. (After 1 year improved of 0.1 logMAR).
Caporossi et al. 2010 (101).	44 eyes	Non randomized	363 eyes treated with standard CXL, of these 48 eyes underwent long-term follow-up.	4-5 years	Stability of disease was found in 44 eyes. Mean BCVA improved by 1.9 Snellen lines, and UCVA improved by 2.7 Snellen lines.
Asri et al. 2011 (102).	142 eyes	Case series study. Retrospective, uncontrolled		Upto 12 months	At 12 months; Keratoconus progression had stopped in 42 eyes (68.8 %). CDVA stabilized in 31 eyes (47.6%), improved in 26 eyes (40.0%), and decreased in 8 eyes (12%). and the maximum K value had decreased by more than 2.0 D in

					13 eyes (21.3%). Complication rate with loss of vision; 3.5%.
Filippello et al. February 2012 (103).	20 eyes	Cohort study	20 patients with bilateral progressive keratoconus. Worst eye treated with trans-epithelial CXL while the other eye worked as control.	18 months	Transient hyperemia and mild foreign-body sensation occurred in 8 eyes which resolved within 24 hours. Stop in keratoconus progression were seen with improvement in visual and topographic parameters.
Goldich et al. June 2012 (104).	14 eyes	Clinical controlled study	14 patients. Tested the effects and safety of treatment with CXL.	Up to 2 years	After 2 years: Progression of keratoconus was stabilized; UCVA was stable, BCVA was improved, and reduced keratometry. Unchanged corneal thickness, endothelial cell density, and foveal thickness suggest the long-term safety of this procedure.
Lamy et al. September 2012 (105).	34 eyes	Prospective study	34 patients (68 eyes), one eye treated while the other worked as control. Investigated appraise on the effects of CXL.	40 days to 2 years	Progression of keratoconus was inhibited and corneal sensitivity was improved.

*BCVA = Best corrected visual acuity. UCVA = Uncorrected visual acuity. CDVA = Corrected distance visual acuity. D = Diopters. IOP = Intraocular pressure.

Discussion

The primary challenge in identifying a permanent treatment for keratoconus is the pathophysiology of the disease not being fully understood. The corneal changes of keratoconus can vary across a wide spectrum from stability to rapid progression. Thus variation in progression can be seen in the same patient at different points, and can even vary significantly between each eye in the same patient.

When it comes to the safety and long-term efficacy of the treatment, there are areas of concern. The treatment criteria for patients who should be treated have to be specified accurately. More studies are necessary to assemble a list of indications regarding patients' age, diagnosis and the stage of keratectasia. In future studies the effects of other ocular parameters should be addressed in further detail: parameters like tear function, corneal sensitivity, alterations in conjunctival epithelium and goblet cells, the long-term effect on direct UVA irradiation on ocular surface limbal stem cells, and potentially occurrence of metaplastic disorders of the ocular surface.

The clinical studies of CXL have mostly been limited to small uncontrolled retrospective series with relatively short follow up. However, the last few years there has been a rapid growth in interest in this treatment worldwide, with clinical outcomes now reported from several countries, and the findings of all studies being remarkable consistent.

A disadvantage of CXL is that complete epithelial debridement prolongs early postoperative discomfort. In addition it prolongs epithelialization, thus increasing the chance of bacterial and or fungal infiltration. However, adequate stromal absorption of riboflavin may not be achieved without epithelial debridement. Even if the secondary infection is treated it may cause corneal opacification and decrease the corrected distance visual acuity (106-108). There are also concerns about an important aspect of the CXL-technique. By actually increasing rigidity, the cornea is being aged, and the long-term results of that might not be beneficial. The induced rigidity of the cornea may promote premature ocular rigidity in general, creating future severe ocular problems for example age related macular degeneration (109).

Regarding safety considerations, riboflavin, which is also known as vitamin B2, is considered to be non-toxic. It is water-soluble and is able to penetrate corneal stroma after the removal of epithelium. There has not been any evidence of riboflavin toxicity in human-studies with oral intake up to 400 mg/day (49). Given that all studies have shown minimal effect on intraocular pressure and the optic nerve, it is assumed that riboflavin is filtered efficiently through the trabecular meshwork (18). Other safety considerations have been the effects on the keratocytes. Studies have shown that keratocyte loss could be seen down to a depth of 340 μm in the entire CXL treated corneas (110) compared to the kerotoconus corneas that have not been treated with CXL and the normal corneas. Corneal cross-linking can cause morphologic corneal changes up to 30 months after treatment, especially a long-lasting, maybe permanent keratocyte loss in the anterior and middle corneal stroma involving the central and peripheral cornea. (111). However, it is suggested that corneal cross-linking does not induce significant cellular epithelial damage (112) CXL with epithelial debrievement have resulted in nerve

fiber damage and reduced sensitivity, but the nerves have in the mentioned complications regenerated and appeared normal by 180 days after treatment (113). Changes in the structure and cellularity of the cornea have been observed up to 3 years after CXL treatment with confocal microscopy. The alterations can be related to increased cross-link formation, synthesis of well-structured collagen, or other lamellar interconnections (110). When it comes to photochemical damage of keratocytes the endothelial thresholds are 0.45 and 0.35 mW/cm2. In a 400 μm thick cornea saturated with riboflavin, the irradiance at the endothelial level is calculated to be 0.18 mW/cm, which is a factor of 2 smaller than the damage threshold (33). This is the reason why a cornea less than 400 μm is recommended not to be treated (1).

If the patient is less than 35 years old and the best corrected visual acuity is worse than 6/7,5 CXL seems to be a safe treatment that would yield a complication rate of approximately 1 % (75). A temporary loss of visual acuity can be observed in some cases. A permanent loss of 2 or more Snellen lines at an appropriate time after surgery (6 months or 1 year) is considered a complication. Koller et al, found a permanent loss in 2.9 % of their patients. Other studies show a rate of less than 1 % assumed the patient age is above 35 years and best spectacle corrected vision better than 6/7,5 (75).

Publication of the results of the randomized controlled trials currently in progress will enable a more confident assessment of the efficacy of this procedure. Also the safety of CXL will take time to fully ascertain and long-term observations will be required. Corneal collagen cross-linking may represent a breakthrough in the treatment of keratoconus, significantly reducing the need for corneal transplantation.

Conclusion

Collagen cross-linking with riboflavin and UVA have shown to be a very promising method of treating keratoconus. It is the first therapeutic modality that is shown to halt the progression of the ectatic process in keratoconus. The treatment is a minimal invasive technique, promising therapeutic intervention for tissue stabilization.

This treatment appears to have the potential to reduce incidence of progressive forms of keratoconus. There is also a theoretical basis for the use of CXL in several other corneal diseases. Both factors may ultimately reduce the need for corneal transplantations. It is approximated that 20 % of patients with untreated keratoconus will eventually end up having corneal transplantation. In addition to the clinical benefits, there are also economic and psychosocial benefits of CXL.

Clinical trials of CXL on progressive keratoconus have shown interesting results. The results presented shows that moderate to advanced progressive keratoconus is effectively halted, often with some regression of the corneal steepness resulting in improvement in vision. Only a few cases needed to repeat treatment. In particular no damage to corneal endothelium, lens or retina was noted in any of the clinical trials. The data also indicates that the treatment should only be performed in patients with documented progression of keratoconus in the preoperative months. A Few cases of keratitis after CXL have been published. Infection occurs early in the immediate postoperative period and requires strict vigilance during this time.

Current studies indicate the stiffening effect of CXL appears to remain stable after 6 years, and long term data are awaited. However, the exact duration of the corneal stiffening effect is still unknown due to the relative novelty of the treatment, and long term data are yet to be evaluated. More prospective randomized controlled trials in the future are desirable.

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