Cross-linking: Indications and effective timing

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Cross-Linking Indications and Effective Timing

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Introduction

About 10 years ago, a new technique- that was able to change the intrinsic biomechanical properties of the cornea - opened a new perspective. There is now hope, validated by several studies, that keratoconus may have a specific treatment, which can be extended to pellucid marginal degeneration and post-LASIK ectasia.

The Beginnings

The idea to crosslink the collagen in the cornea was first developed and put into practice by Theo Seiler, MD, who at the time was a Professor of Ophthalmology at Dresden University, Germany. The suggestion came from previous applications of this chemical-physical process in different fields.

Preclinical studies on corneal cross-linking began in Dresden in 1993. Several chemical cross-linking substances and light irradiation with different photosensitizing agents were investigated, and in 1998, the first patient was treated with riboflavin and UV light. Because no side effects were reported, a pilot study was started 1 year later.

Prospective trials began in Europe in 2003-2004. In the meantime, the technique has been adopted by an increasing number of surgeons, the technology has evolved, the protocols for treatment have come to a better definition and indications are slowly becoming clearer.

The first clinical results of collagen cross-linking in keratoconus were reported by a research group from Dresden University in 2003. In this study, 23 eyes of 22 patients with keratoconus were included and only one eye of each patient was treated except one case that was treated bilaterally. The follow-up period was from 3 to 47 (23,2 ± 12,9) months. The BCVA improved in 65% of the patients by an average of 1.26 lines. The refractive correction improved by an average of 1.14D. In 70% of the patients, maximum K was flattened by an average of 2.01D. K value remained stable in 5 patients and in 1 patient an increase of 0.28D was present. In 22% of the fellow control eyes, however maximum K value increased by an average of 1.48D. The same research group later published their long-term results in 2008 on 153 eyes of 111 patients. The minimal follow-up time was 12 months and the maximum follow-up was 7,5 years.

The Outcomes

A few main points have emerged from this 10-year experience with the procedure. In Dresden, where now more than 400 eyes have been treated, it was demonstrated that cross-linking has an impact on corneal biomechanics, as it strengthens and stiffens the cornea. It was also shown that keratoconus progression is halted in 97% of the cases; maximum keratometry readings decreased and all Pentacam-measured keratoconus indexes are reduced. These results were confirmed by controlled prospective trials.

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Between 2006 and 2007, complications and contraindications were prospectively evaluated by the Dresden group. It was shown that the procedure induces keratocyte depopulation about 300-µm deep within the stroma and that repopulation requires up to 6 months. Therefore, a minimum corneal thickness of 400 µm (as previously recommended) was confirmed as the safety threshold for the treatment to avoid damage to the endothelium and deeper structures such as the lens and retina.

In a recent publication, however, Prof. Seiler and co-authors showed that preoperative swelling of the cornea using hypo-osmolar riboflavin solution safely broadens the spectrum of corneal cross-linking indications to thin corneas that would otherwise not be eligible for treatment.

Indications

The major indication for the use of CXL is to inhibit the progression of corneal ectasias, such as keratoconus and pellucid marginal degeneration (PMD).[1-10] CXL may also be effective in the treatment and prophylaxis of iatrogenic keratectasia, resulting from laser in situ keratomileusis.[11-12] This treatment has also been used to treat corneal melting, bullous keratopathy and infectious corneal ulcers with apparently favorable results.[13-20] CXL has also been used in combination with other treatments, such as intra-corneal ring segment implantation[21-23] and limited topography-guided photoablation, with some success.[24-25]

Until recently, there was no successful way of treating keratoconus. The CXL treatment was likely just halting or reducing the progression of keratoconus. Mild regression that occurs may be explained as an effect of the rearrangement of corneal lamellae and the surrounding matrix.[1] Due to an increased number of cross-linking sites within the collagen molecule after CXL, stiffer fibrils and lamellae are probably generated.

Even though the treatment of corneal CXL mediated by riboflavin and UVA shows signs of success and is considered as safe, the possible complications that could arise during the procedure – cases having not met the minimum criteria and/or are not suitable for the treatment - should be taken under serious consideration.

In a recent retrospective study of 163 eyes of 127 patients with stage 1-3 keratoconus, 8.6% developed a clinically significant haze after 1-year follow-up.[26]

Therefore, advanced keratoconus should be considered at higher risk of haze development after CXL due to low corneal thickness and high corneal curvature.

Additional case reports describe diffuse lamellar keratitis.[27] And a reactivation of herpetic keratitis.[28] On another complication, Koppen et al. reported four cases of keratitis and corneal scarring from a total of 117 eyes treated with CXL where patients experienced delayed (more than 24 h) symptoms and signs of inflammation. The eyes showed pronounced ciliary redness with cells in the anterior chamber and central keratic precipitates; multiple white infiltrates had developed at the edge and within the area of CXL.[29]

Indications of CXL

- Forme fruste keratoconus.
- Keratoconus.
- Pellucid marginal degeneration (PMD).
- Iatrogenic keratectasia.
- Corneal melting.
- Bullous keratopathy.

CXL in Forme Fruste Keratoconus

Especially in forme fruste keratoconus it is very important to determine the degree of progression of

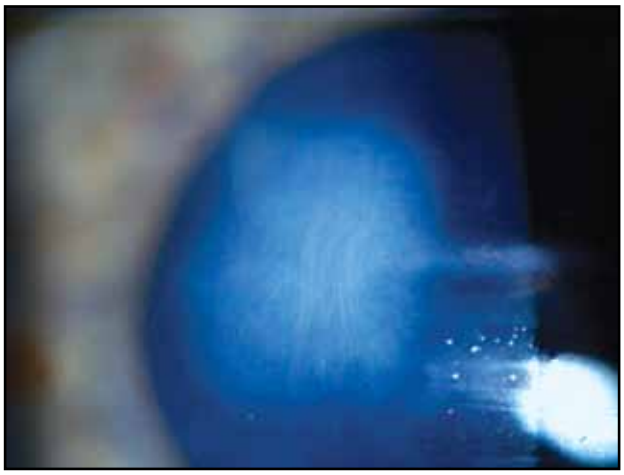


Figure 1: Corneal scar.

keratoconus. This is why we have to keep track of the patient's progression. If the K max values are increasing by 1 D in a time span of 12 months, we have to evaluate the progression of the disease.

We have to keep into account that some of the major factors of the progression of keratoconus are determined by the following status: age, smoking, pregnancy and massive eye rubbing. Forme fruste keratoconus in patients under the age of 20 should undergo topographic examinations and follow-up control schedules more frequently than older patients.

Patients diagnosed with the forme fruste keratoconus disease can be treated even after the first visit with CXL, if the patient is under the age of 20, complaining about massive eye itching and rubbing and if the patient cannot attend follow-up control schedules due to problems with transportation and/or accommodation.

Figure 2: Riboflavin solution.

CXL in Keratoconus and PMD

A study by Jankov et al. found that progression of keratoconus stopped in all patients who were actively progressing 6 months prior to treatment.[7] Max K decreased by more than 2 D (from 53.02 ± 8.42 D to 50.88 ± 6.05 D), SEQ decreased from 3.27± 4.08 to 2.68 ± 3.02 D, while refractive cylinder decreased by ± 0.5 D (from 2.29 ± 1.77 to 1.86 ± 0.92 D).[7] After treatment, no eyes lost lines of best spectacle-corrected acuity (BCVA), 12 maintained BCVA, one gained one line of BCVA, and five gained two lines of BCVA and one patient gained three lines of BCVA.[7]

Results from a study by Coskunseven et al. confirm Wollensak et al.'s findings; the group treated with CXL showed a similar mean decrease in SEQ of 1.03 ± 2.22 D (5.25 D to ± 3.75 D), decrease in cylinder by 1.04 ± 1.44 D (2.00 D to ± 4.00 D) and decrease in max K by 1.57 ± 1.14 D (0.00 D to 3.90 D).[6]

In the Coskunseven et al. study, the non-treated group showed progression of all corneal parameters under study.[6] On another study by Wollensak et al., which included 23 eyes with moderate or advanced progressive keratoconus, showed that CXL was effective in halting the progression of keratoconus over a period spanning 4 years.[2] In this study, a mean pre-operative progression of keratometry (max K) by 1.42 D in 52% of the eyes over a 6-month period immediately prior to the treatment was followed by a postoperative decrease in 70% of the eyes.[2] The statistics also revealed a reduction of the max K by 2.01 D, while the SEQ was reduced by an average of 1.14 D.[2] In contrast, 22% of the untreated fellow control eyes had a post-operative progression of keratectasia by an average of 1.48 D.[2] Vinciguerra et al. found that CXL treatment was effective in reducing corneal and total wavefront aberrations 1 year post-operatively.[1] In their preliminary results, Wittig-Silva et al. found similar results of BCVA and K readings, with no difference in spherical equivalent and endothelial cell density between treated and control eyes 12 months after CXL.[9]

Agarwal found similar results in 37 eyes of Indian subjects 1 year after treatment.[8] Agarwal reported that 54% of the eyes gained at least one line of BCVA, astigmatism decreased by a mean of 1.2 D in 47% of the eyes, the keratometry value at the apex decreased by a mean of 2.73 D in 66% of the eyes and the maximum K value decreased by a mean of 2.47 D in 54% of the eyes.[8]

CXL in Iatrogenic Keratectasia

In a recently published study, CXL was performed in 10 patients with previously undiagnosed forme fruste keratoconus or pellucid marginal corneal degeneration that underwent LASIK for myopic astigmatism and subsequently developed iatrogenic keratectasia.[11] CXL led to an arrest and/or even a partial reversal of keratectasia over a post-operative follow-up period of 1 year[1] to 25 months.[11] The differences were demonstrated with pre-operative and post-operative corneal topography and a reduction of max K.[11]

CXL in Corneal Melting

The CXL is a promising technique for treating corneal melts or infectious keratitis because cross-linking would strengthen a collagenolytic cornea while UVA irradiation eliminates the infectious agent.[13]

Although all plates exposed to riboflavin alone showed no bacterial death and two of five *P. aeruginosa* plates exposed to ultraviolet light alone showed minimal inhibition, all plates exposed to the combination of riboflavin and ultraviolet light showed bacterial death. In an experimental study, Schrier et al. tested the antibacterial action of riboflavin alone, ultraviolet light alone and the combination of riboflavin and UV light on *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa*.[14]

CXL in Bullous Keratopathy

Ehlers et al, presented that CXL of the cornea has been shown to have an anti-edematous effect in bullous keratopathy after i.e. cataract surgery. Hafezi et al, came to the conclusion to reduce visual fluctuations due to changes in CCT in early Fuchs dystrophy. Wollensak et al. presented a case series of three eyes with bullous keratopathy due to pseudophakia, corneal transplant rejection and Fuchs' endothelial dystrophy that underwent CXL.[15] After dehydration for 1 day using 40% glucose, the standard CXL technique was used by Wollensak et al. for treatment. Corneal thickness was reduced by 90.33 ± 17.04 microns three days after cross-linking and by 93.67 ± 14.22 microns 8 months after CXL. The bullous changes of the epithelium were markedly improved, with the patients reporting no pain or discomfort after CXL.[15-17] In such cases, CXL is primarily suited for patients with pain symptoms, restricted visual prognosis or to extend the time interval for an upcoming corneal transplantation. Visual acuity was significantly improved in a case without prior stromal scarring.

Prophylactic Indications to Prevent Iatrogenic Ectasia

In some cases, as a prophylactic treatment is recommended, to have a thin cornea and underwent a high ablation Lasik procedure, even if the patient has not been diagnosed with forme fruste keratoconus or keratoconus.[11-12]

On forme fruste diagnosed patients with low myopia and astigmatism, first a CXL treatment followed by a trans-epithelial topo guided PRK operation can be performed for the refractive correction, in the same time or the mean interval between both treatments is 6 months.

Age Related Cross-Linking

From here on after, exclusion and inclusion criteria's had evolved since 2003. Patients can now be treated even at the age of 18 and below, while this was not the case before, with patients younger than 18.

Other areas of this group's research are the indications in relation to age. According to Prof. Aldo Caporossi only after "following approval from the ethic committee, [we] have started applying the technique to very young, 10- to 16-year-old patients, and now more than 60% of our treatments are performed in this age group."

When keratoconus develops at such a young age, it is particularly severe, evolves quickly and inevitably leads to early corneal transplantation. Prof. Caporossi's study and evaluation of his results in terms of efficacy and stability and comparing them to those of different age groups will confirm his previous conclusion: In all cases of progressive keratoconus, cross-linking should be performed as early as possible. As he pointed out numerous times, that on average, the time of maximum evolution of the disease is between 12 and 26 years, and it is at this stage that we should crosslink the cornea."

Dr. Albé and her colleagues treated 66 eyes with documented progressive keratoconus in patients who ranged in age from 9 to 18 years (mean age, 15 years). To be eligible for the study, the patients' corneal thickness had to be no less than 400 µm at the thinnest point, the standard cutoff considered necessary to ensure that the retina does not absorb UV-A radiation. [30]

CXL Combined with Intracorneal Rings

We have previously conducted a prospective, comparative study that comprised 48 eyes of 43 patients with progressive keratoconus. In group 1,

Figure 3: UV light radiation.

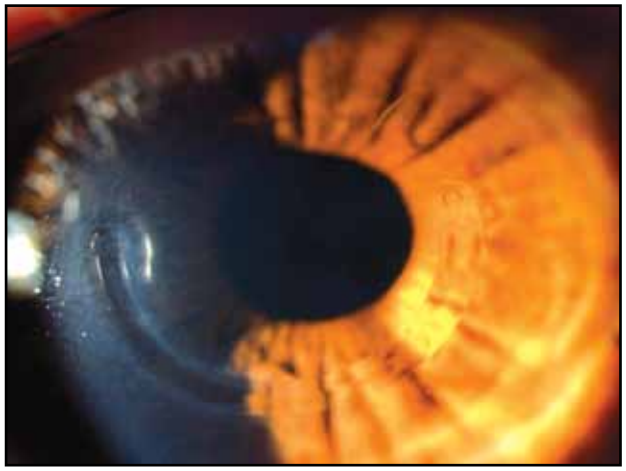


Figure 4: CXL+ICR implantation.

CXL was performed first, followed by intracorneal rings (ICR) implantation. In group 2, intracorneal ring implantation was performed first, followed by CXL. Implantation of ICR followed by the CXL resulted in greater keratoconus improvements than the CXL procedure followed by ICR implantation.[21] Combination of ICR implantation with ultraviolet/riboflavin-mediated corneal CXL procedure seems to have a synergic effect for reverting the progressive irregular astigmatism due to keratoconus or iatrogenic ectasia.

Corneal Collagen Cross-Linking Combined with Limited Topo Guided Photorefractive Keratectomy

One of the most promising uses of the CXL procedure is in combination with a modified version of PRK. Kymionis et al. reported favorable results of 14 eyes with progressive keratoconus that were treated with customized topography-guided PRK with the Pulzar Z1(wavelength 213 nm, CustomVis) immediately followed by CXL.[25] It is important to emphasize that combined treatment of CXL/PRK is a specialized intervention with the goal of normalizing the cornea as much as possible to increase BCVA rather than treating the refractive error itself. Therefore, the primary treatment target is cylinder in order to improve the irregular astigmatism and the secondary target is correcting some of the sphere. Most importantly, the eye may not require a corneal transplant.

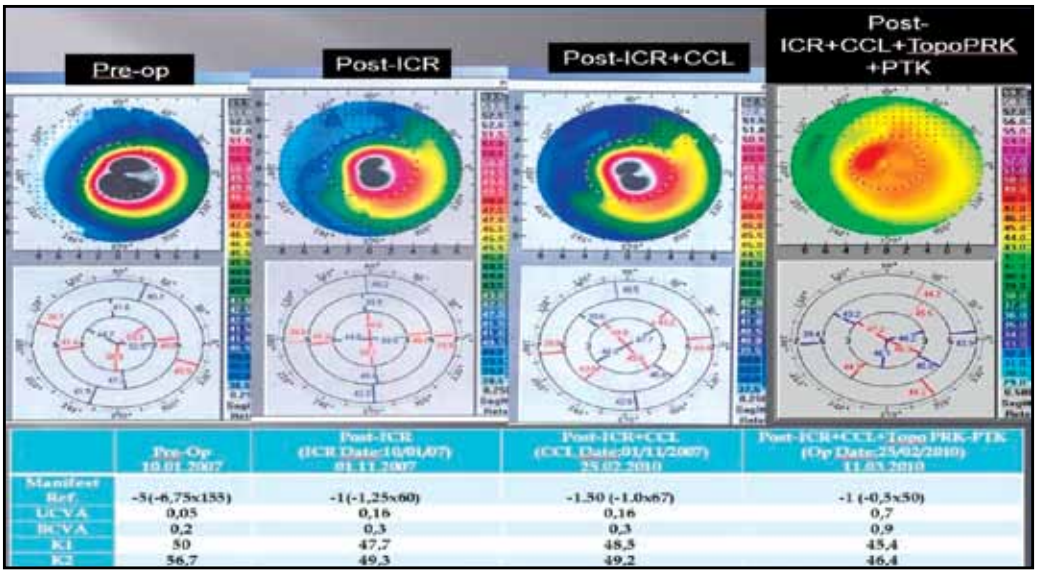
Conclusions

Corneal CXL appears to be a safe and effective procedure in stopping the progression of forme fruste keratoconus, keratoconus and PMD and iatrogenic Ectasia. Also, the CXL treatment is successful in treating corneal melting conditions or infectious keratitis and bullous keratopathy. However, CXL reduces minimally the corneal curvature, spherical equivalent refraction and refractive cylinder in eyes with corneal instability and progressive irregular astigmatism due to keratoconus and ectasia.

In keratoconus treatments we need combined treatments to stop the progression and to improve the visual acuity. Cross-linking can be combined with contact lenses and different refractive procedures, such as intracorneal rings implantation and limited trans-epithelial topography-guided PRK, phakicintra-ocular lenses. Combination treatments seem to have a synergistic effect for reverting the progressive irregular astigmatism due to keratoconus or iatrogenic ectasia.

Effective Timing of CXL

In vitro studies have shown that the cornea absorbs approximately 30% of UVA light while an additional 50% of UVA absorption occurs in the lens.[1] Corneal UVA absorption can be considerably increased with a photosensitizer such as riboflavin. With an irradiance of 3 mW/cm^2 of UVA and 0.1% riboflavin, as much as



	Pre-Op 10/01/2007	Post-ICR (ICR Date:10/01/07) 01/11/2007	Post-ICR+CCL (CCL Date:01/11/2007) 25/02/2010	Post-ICR+CCL+Topo PRK-PTK (Op Date:25/02/2010) 11/03/2010
Manifest Ref.	-5(-6,75x155)	-1(-1,25x60)	-1.50 (-1.0x67)	-1 (-0,5x50)
UCVA	0,05	0,16	0,16	0,7
BCVA	0,2	0,3	0,3	0,9
K1	50	47,7	48,5	45,4
K2	56,7	49,3	49,2	46,4

Figure 5: Triple procedure: CXL+ICR+Trans-epithelial Topo guided treatment.

95% of UVA light will be absorbed within the cornea. This results in a 20-fold reduction of the original irradiance of 3 mW/ cm^2 of UVA (at the corneal surface) down to 0.15 mW/ cm^2 (at the endothelial level), which is well below 0.36 mW/cm^2, the threshold considered cytotoxic for the endothelium.[31-33]

By comparison, the same UVA irradiance at the corneal surface as used in the aforementioned studies can be measured at noon during an average sunny summer day in the tropics (23 of latitude and 800 m above sea level). Despite the expected reduction of irradiance from the corneal surface toward the deeper layers of corneal stroma, the irradiation levels still exceed the threshold down to a depth of approximately 300 microns. Therefore, keratocyte apoptosis in the anterior stromal layer has been described and a demarcation line between the treated and untreated cornea has been clearly shown in both in vitro and in vivo studies.[31-34]

Using a wavelength of 360-370 nm with an accumulated irradiance of 5.4 J/cm^2 ensures that the exposure of all structures is below harmful levels.[1] However, a non-homogenous irradiation field may create localized hot spots of increased radiance with potentially harmful consequences. Therefore, for clinical use, a uniformly emitting irradiance source is required, and must be continuously evaluated. The riboflavin in the cornea itself also serves as a further protective layer, which has been reported to reach more than 400 µm after 30 min of application, penetrating the anterior chamber, where it is visible with the slit lamp as a yellow flare. Therefore, there is an UV absorption coefficient that shields the more posterior structures such as the endothelium, the crystalline lens and the retina.[1]

Confocal microscopy shows the repopulation of keratocytes by 1 month after treatment, reaching their pre-operative quantity and quality in terms of functional morphology within 6 months after treatment.[35]

Keeping the total UVA energy exposure equivalent at 5.4 mJ/cm^2 facilitates effective cross-linking and appears to maintain safety. We can safely reduce the UVA exposure time for CXL.

Ronald R. Krueger, and Eberhart Spoerl, presented a study on this subject. Since ROS generation is linearly related to the fluence of UVA light, they propose that high irradiance and short exposure with the equivalent energy dosing will be as effective and safe as standard UVA CXL.

We need 5.4mJ/cm^2 radiant exposure. Eberhard Spoerl, et al showed safety of UVA–riboflavin cross-linking of cornea in their study. Total energy should be 5.4 mJ/cm^2. Standard treatment timing is 3.0 mW/cm^2 X 30 min. Rapid treatment timing is, 6.0 m W/cm^2 X 15 min, 9.0 m W/cm^2 X 10 min, 15.0 mW/cm^2 X 6 min.

Slow treatment timing is 2.0 m W/cm² X 45 min. And for the riboflavin vehicle: Dextran vs Carboxymethylcellulose[1] using dextran RF solution: 15mW/cm² for 10 minutes qty 3,13 mW/cm² for 7 minutes qty 2,9mW/cm² for 10 minutes qty 3,6mW/cm² for 14 minutes qty 3, using carboxymethylcellulose (non-dextran) RF solution, 15mW/cm² for 10 minutes qty 3, 13m W/cm² for 7 minutes qty 2, 9 mW/cm² for 10 minutes qty 3, 6mW/cm² for 14 minutes qty 3, we saw no statistically significant differences between two groups.

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