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Overview

Corneal collagen crosslinking: from basic research to clinical application

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Abstract

Corneal collagen crosslinking is a promising new treatment of progressive corneal ectasia. Its clinical use has been rapidly increasing since it was originally introduced in 1997 as the first treatment that can improve biomechanical stability of the weakened cornea. The method is based on the combined action of photosensitizer riboflavin (vitamin B2) and ultraviolet A light, which induce the formation of new covalent bonds between the collagen fibers.

Our systematic search of literature in English has yielded only eight prospective trials with the efficiency and safety data published to date. However, all of the published studies reveal a halt in the progression or a slight improvement of corneal ectasia with the low complication and failure rates after the treatment. In this review we are highlighting the method's history, scientific basis and its current clinical application in order to provide clinicians with the recent data on its benefits and potential risks.

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INTRODUCTION

The term »crosslinking« refers to the formation of bonds between synthetic or natural polymer molecules. Collagen is the most common natural polymer and, until recently, there were only a few existing applications of collagen crosslinking in medicine, such as bioprosthetic heart valves *(1)* or formaldehyde tissue fixation *(2)*.

Collagen crosslinks can be achieved through three basic pathways *(3)*. The enzymatic crosslinking of collagen fibrils occurs as a part of their natural maturation. A prolonged exposure to monosaccharides during aging or in diabetes mellitus can result in a non-enzymatic reaction named glycation. The glycation-induced crosslinks are most likely responsible for the protective effect of diabetes or aging on the development and progression of keratoconus *(4)*. Another example of collagen crosslinking by glycation is the application of glyceraldehydes in order to increase the corneal and scleral rigidity in tested animals *(5, 6, 7)*. Ozone-mediated or UV-mediated oxidation represents a third mode of crosslinking formation *(8)*. Since the introduction of corneal collagen crosslinking (CXL) by Spoerl and his co-workers at Dresden University in Germany in 1997, the use of UV-mediated photooxidation became a widely accepted new approach to strengthening the cornea weakened by the ectatic disease or the iatrogenic intervention *(5, 6)*.

The most common ectatic disorder is keratoconus. Keratoconus is characterized by bilateral, mostly asymmetric, non-inflammatory and progressive corneal thinning and protrusion, caused by the biomecha-

nical instability of the cornea. It affects approximately 1 in 2000 people and usually begins in puberty and progresses at variable rate until the patient's mid thirties *(9)*. The recent advances in diagnostic devices resulted in the detection of more subtle corneal changes, suggesting that the subclinical forms are more common than the fully developed keratoconus. The iatrogenic post-LASIK ectasia is the second most common corneal ectasia. It usually appears as a result of the refractive surgery performed in predisposed individuals or in patients with an undetected early form of keratoconus. Before the introduction of CXL, the only treatment that could halt the progression of keratoconus in most cases was the transplantation of cornea. Corneal transplantation is performed in up to 20% of keratoconus patients with the significant progression, resulting in the deterioration of their best corrected visual acuity *(10)*. Despite the low rate of graft failure, corneal transplantation is still associated with the significant risks that could result in a permanent loss of vision. In addition, it often causes changes in patient's lifestyle, during and after the long period of surgical recovery.

The minimally invasive technique of corneal collagen crosslinking improves biomechanical properties of the ectatic corneas by forming new covalent bonds between collagen molecules. Studies which have been conducted so far demonstrated the beneficial effect in halting the progression of the diseases with a very low complication rate *(11, 12)*.

The aim of this review is to present the new treatment and highlight its history, scientific basis and current clinical application in order to provide clinicians with the recent data on the benefits and potential risks involved.

METHOD

The original crosslinking technique described by Wollensak and co-workers is still being used today with the minor modifications *(13)*. The photopolymerizing effect is induced by the combined action of riboflavin as a photosensitizer and long wavelength ultraviolet (UVA) light of 370 nm. This particular wavelength was chosen so that riboflavin can achieve a maximal absorption while still remaining bellow harmful radiation levels. During the exposure, riboflavin is excited into a triplet state generating the so called reactive oxygen species which in turn induce the formation of new covalent bonds between the amino acids of neighboring collagen fibers *(4)*.

The treatment is performed in sterile environment. After a local anesthetic had been applied, lid speculum is inserted and a central corneal abrasion up to 9.0 mm in diameter is made for a better diffusion of riboflavin into the stroma. According to the protocol of the Institute for Refractive and Ophthalmic Surgery in Zurich, Switzerland, 0.1% riboflavin solution containing 10 mg of ribloflavin-5-phosphate diluted in 10 mL of 20% dextran solution is instilled every 3 minutes for 30 minutes. After that, central corneal thickness is measured by an ultrasound pachymetry device. For safety reasons, additional riboflavin 0.1% hypoosmotic drops without dextran should be applied in corneas whose thickness is less than 400 μm. Otherwise, UVA light should not be applied. Also, eyes are inspected with the slit lamp to ensure that riboflavin has penetrated into the anterior chamber of the eye. The cornea is then irradiated for 30 minutes with an UVA illumination device. The device must provide a homogenous UV radiation with irradiance of 3 mW/cm^2 at the working distance of 5 cm. During irradiation, the cornea is moistened every 3 minutes with the riboflavin 0.1% drops. The total dosage delivered to the cornea in that way is 5.4 J/cm^2. At the end of the procedure antibiotic ointment is applied and bandage lens inserted to facilitate the epithelial healing. After the healing is complete, the patients are instructed to use steroid drops in order to prevent haze formation.

The modifications of the standard protocol include the use of a 9 mm marker, shorter initial riboflavin application and the optional Pilocarpine 1% preoperative use in Sienna protocol *(14, 33),* working distances of up to 10 cm *(15, 16)* or the less efficient transepithelial (C3-R) crosslinking that could be utilized in cases of thin cornea *(12, 17, 18)*.

The effect of CXL on the corneal biomechanics

The human cornea has 5 layers. Only two corneal layers, Bowman's layer and stroma, contain collagen fibrils. While Bowman's layer is only 8 to 10 μm thick, stroma accounts for up to 90% of the corneal thickness and is responsible for the most of the cornea's tensile strength *(19, 20)*. Collagen fibrils form bundles which are woven into three to five hundred equidistant lamellae that run from limbus to limbus. Collagen lamellae are inserted into a network of dendriform keratocyte cells which are responsible for the development and the maintenance of the stromal structure. The more oblique interweaving and branching of bundles and lamellae occurs in anterior and peripheral stroma. The interweaving and branching, together with the circumferential orientation of fibrils in the peripheral stroma, *(21)* could be one of the reasons why only very few pathological changes in cases of ectatic diseases, like keratoconus, occur near the corneal limbus *(19)*.

Because of the very complex 3-dimensional structure of the cornea, it is necessary to reduce it to a linear and directionally uniform system in order to perform comparable calculations. Thus, changes in the biomechanical properties of the cornea can be assessed through more reliable and comparable parameters, like Young's modulus of elasticity. Young's modulus is usually experimentally determined during the from the slope of the stress-strain curve.

In the ectatic diseases like keratoconus, the tensile strength of the cornea is reduced by about 36% *(22)*. On the other hand, the stress-strain measurements of the human corneas treated with CXL showed an increase in biomechanical rigidity by up to 329%, as well as an increase in Young's modulus by the factor of 4.5 *(23)* This

increase is much more significant than the increase in the crosslinked animal models *(23, 24)*. The reduced biomechanical effect in rabbits' corneas is explained by the higher degree of baseline cross-linking. The reason for the lesser effect in porcine corneas could be a significantly higher average corneal thickness. Namely, the main effect of CXL is observed in the anterior 242 μm of the cornea, with the maximum depth of approximately 350 μm *(24, 25)*. Therefore, in porcine corneas, with the average thickness of approximately 850 μm, the crosslinked anterior section is counterbalanced by the larger posterior stroma where no crosslinks were formed.

It has also been shown that the collagen fibril diameter in the treated portion of rabbits' corneas was increased by 12.2%, probably due to the molecular spacing of the crosslinked collagen *(26)*. The long-term effects of CXL in rabbits showed a significant and variable increase in the stress measurements which remained stable over the 8 month period. The increase of 79% in Young's modulus remained unchanged when measured immediately after, one month and 8 months after the treatment *(24)*.

Clinical efficacy and safety

The first prospective, nonrandomized clinical pilot study on humans was published by the Dresden research group in 2003 *(13)*. The study included 23 eyes of 22 patients with the moderate or advanced progressive keratoconus. The progression of keratoconus was halted in all treated eyes. Regression was detected in 70% of the eyes, with the reduction of the maximal keratometry readings by 2.01 diopters and of the refractive error by 1.14 diopters. There was a slight improvement in the visual acuity in 65% of the eyes. No adverse reactions were noted.

Since then, eight prospective studies were published. Four prospective studies included less than 30 eyes with a follow-up period of 4 to 12 months *(27, 28, 29, 30)*. All four studies reported varying degrees of improvement and a very good safety profile. The preliminary results of the first randomized prospective clinical trial of 66 eyes of 49 Australian patients treated with CXL were also favorable *(31)*. In more than half of the treated eyes a notable reduction in maximal keratometry readings was noted after one year. While there was no statistically significant improvement in the best corrected visual acuity, a positive trend was observed. A degree of postoperative haze, which resolved during the follow-up, was noticed in all treated patients. There were no vision-threatening complications and no significant changes in the endothelial cell density. The investigators have indicated a relatively poor reproducibility of the visual acuity, subjective refraction and scanning-slit topography. The scanning-slit topographers, like the Orbscan device used in their study, were shown to have a lower repeatability and reproducibility than the rotating Scheimpflug cameras *(32)*.

The scanning-slit topography also showed some underestimation of the corneal thickness compared to the ultrasound and OCT pachymetry during the first 3 months after the CXL in the Italian non-randomized prospective trial. The authors attribute the difference in Orbscan pachymetry measurements to the presence of a demarcation line between the treated and the untreated stroma. Other studies have observed a decrease in the number of keratocytes in the treated anterior and intermediate stroma, in front of the demarcation line, during the first two to three months after which the repopulation by activated keratocytes took place *(25,16)*. The 2-year results of the Italian study showed the stability of 44 keratoconic eyes and the improvement in the best corrected visual acuity without any relevant side effects *(33)*.

The researchers in the other two prospective studies used the Scheimpflug cameras for corneal measurements *(11, 34)*. Along with the Scheimpflug measurements, the Indian researchers also used the optical coherence tomography in order to obtain more reproducible results. They have found no change in the best corrected visual acuity and the corneal curvatures 1 year after crosslinking was made, thus indicating a halt in keratoconus progression. The study has also shown no lens and macula changes after the UVA exposure.

The Swiss prospective study of a large group of 117 eyes with primary ectasia evaluated the complication and failure rates after CXL with a 1-year follow-up *(11)*. The complication rate, defined as a percentage of eyes loosing two or more lines on the Snellen visual acuity chart, was 2.9%. The identified risk factors for visual loss were age over 35 years and the best spectacle-corrected visual acuity of 20/25 or better. With the established upper age-limit of 35 years, the complication rate in their study dropped to 1.04%. The failure rate, defined as the percentage of eyes with continued progression, was 7.6%. The only identified risk factor for failure in this study was a maximal corneal curvature greater than 58.00 diopters. For the eyes bellow that limit, the failure rate was 2.8%. The failure rate reported in most other studies is even lower, ranging between 0 to 1%. More information is expected from the nine FDA approved phase II, III and IV trials on the crosslinking treatment, which are at the moment being conducted in the United States.

Regarding the use of corneal collagen crosslinking in patients with iatrogenic ectasia, a small series of ten patients who developed a post-LASIK ectasia was published *(15)*. The follow-up demonstrated a halt or partial improvement of keratectasia in all patients after treatment.

Despite the fact that no sight-threatening complications were recorded in the large prospective studies, there are some sporadic reports in that regard in the literature. In two cases, a stromal haze resistant to topical steroid treatment appeared 2 to 3 months after the CXL. The best corrected visual acuity improved despite the haze *(36)*. Kymionis and co-workers reported one case of a diffuse lamellar keratitis after the corneal crosslinking was performed in a patient with the post-LASIK ectasia *(37)*. He also reported a case of herpetic keratitis and iritis induced by the CXL treatment which responded well to the therapy *(38)*. An unfortunate case of Acanthamoeba

keratitis with corneal perforation in a patient who was repeatedly rinsing his face with tap water, unaware that he was wearing a bandage contact lens, was also reported *(39)*. In this patient a therapeutic keratoplasty was performed. Several other reports of microbial keratitis in CXL patients were also reported *(40, 41)*. The patient treated in Sydney acquired a severe keratitis after cleaning his bandage contact lens in his mouth before putting it back in his eye treated with CXL *(41)*.

Final remarks

In recent years, corneal collagen crosslinking has become a standard treatment for the progressive corneal ectasia in numerous centers throughout the world. Additional basic and clinical research is necessary in order to establish more precise indications and to demonstrate the permanence of the treatment. The reliable data on the turnover rate of the collagen fibers are still insufficient due to the almost complete absence of corneal remodeling *(24)*. However, data such as the increased resistance to the enzymatic degradation in the crosslinked corneas, support the theory of the long-lasting effect of the treatment *(42)*.

Other potential indications for the collagen crosslinking treatment are being investigated. There are several reports on the use of CXL in patients with the bullous keratopathy and the corneal ulcers resistant to conservative therapy *(43, 44)*. Collagen crosslinking of sclera using riboflavin and UVA or glyceraldehyde was suggested as a potential treatment for progressive myopia *(7)*.

Despite the evidence in support of the safety of new procedure, future studies are necessary to define its limitations and its long-term efficacy. Every deviation from the standard technique can cause irreversible damage to the corneal endothelium or other eye structures *(45)*. Until a more complete list of indications and contraindications is established, this useful procedure should be performed only by the well informed surgeons that can identify and diminish all the potential risks.

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