Collagen Cross Linking in Keratoconus: A Review

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Abstract: Corneal collagen cross-linking (CXL) is a therapeutic procedure that helps in increasing the corneal stiffness in the keratoconus eyes. It increases the collagen cross linking within the extracellular matrix (ECM). Ultraviolet-A (370 nm) irradiation of the cornea after saturation with the photosensitizer riboflavin is used. A minimum deepithelialized corneal thickness of 400 μm is recommended to avoid potential irradiation damage to the corneal endothelium but in advanced cases this is not achieved which limits the application of CXL in that category. Modifications have been done in the conventional CXL procedure to be applicable in thin corneas. The current review discusses different techniques employed to achieve this end and their results. The modifications in CXL halt the progression of keratectasia without postoperative complications. However, the evidence of safety and efficacy in the use of modified CXL protocols is still limited to few studies with few patients involved. Controlled studies with long-term follow-up are required to confirm the safety and efficacy of the modified protocols.

Keywords: Keratoconus, Collagen Cross Linking, Thin Cornea

1. Introduction

Keratoconus is a degenerative disorder with onset at puberty. It progresses for 10-20 years and after that it stabilizes. It affects the paracentral cornea resulting in thinning and ectasia. This causes irregular astigmatism resulting in impairment of vision and polyopia. It is often bilateral but asymmetrical in nature. There is no gender predisposition [1, 2].

Its exact etiology is not fully understood and includes genetic, biochemical, and physical factors, with no sole proposed theory. It usually appears as an isolated condition. Ocular and systemic associations include vernal disease, retinitis pigmentosa, blue sclera, atopy, magnesium deficiency, Down's syndrome, Turner syndrome and connective tissue disorders such as Marfans syndrome, Ehlers–Danlos syndrome, osteogenesis imperfecta and pseuodoxanthoma elasticum. In vernal disease, hard contact lens there is repeated ocular trauma caused by eye rubbing ultimately leading to keratoconus [1, 2]. Family history is positive in 10% of the cases [3].

The stiffness of keratoconic cornea is approximately 60% of the normal cornea and the development of conical shape is the result of decreased biomechanical strength. There is increased stromal protein digestion which decreases the biomechanical stability and causes keratoconus. This is due to increased activity of proteinase enzymes and decreased activity of proteinase inhibitors [4]. Reduced mean diameter and interfibrillar spacing of the collagen fibrils, slippage of collagen lamellae, as well as a loss of the normal interwoven structure of the lamellae, have been reported

Mild keratoconus is correctable with spectacles and soft toric contact lenses. However, with progression, the cornea becomes more irregular and rigid gas permeable lenses are required. Keratoplasty is indicated in 15–20% of patients because of contact lens intolerance, corneal scarring and thinning. The 10-year graft survival after penetrating keratoplasty for keratoconus was reported to be 89%.

These interventions are helpful in terms of visual rehabilitation but do not treat the underlying causes of kerectasia and its progression. It is only with the advent of corneal collagen cross-linking (CXL) that we can hope to slow, stop or even to a limited extent reverse keratoconus.

2. Conventional Collagen Cross Linking

It was developed at the University of Dresden by Spoerl and Seiler [5]. This procedure requires minimal corneal stromal thickness of 400 μm, and involves the removal of the central 7–9 mm of corneal epithelium followed by installation of isoosmolar riboflavin 0.1% solution in 20% dextran. UVA (370 nm) irradiation with 3 mW/cm^2 of UVA for 30 minutes (5.4 J/cm^2) over 8 mm diameter of central cornea is initiated after stromal saturation with riboflavin. This procedure causes cross linking of the collagen by riboflavin absorbing UVA to act as a photo sensitizer to produce free radicals (oxygen singlets) that activate the natural lysyl oxidase pathway. Riboflavin prevents damage to deeper ocular structures like endothelium, lens and retina due to absorption of UVA [6].

According to a more recent paper, the cross linking caused by singlets are not due to lysyl oxidase but possibly by three other mechanisms: imidazole production which attaches to histidine forming new covalent bonds; formation of cross links due to triggering of endogenous populations of carbonyl groups in the extracellular matrix (allysine, hydroxyallysine), and / or the degradation of the riboflavin molecule itself releasing 2,3-butanedione, which can react with the endogenous carbonyl groups of the stromal proteins [7].

The bonds produced by CXL are not seen microscopically but ex vivo studies have reported changes in physico – chemical properties of the stroma following CXL. The corneal stroma have shown increased in stress strain measurements both immediately as well as several months following the procedure. These changes are found mainly in the anterior 200 microns of the stroma as most of the UVA absorption occurs here. The following changes are seen after CXL: Increased shrinkage temperature, increased resistance of stroma to enzymatic digestion and increased resistance to matrix metalloproteinase. Recent reports have suggested that the above changes are short term alterations due to the effects of osmolarity of riboflavin solutions rather than actual effects of cross linking [8].

The efficacy of the Dresden protocol is supported by numerous studies since its introduction in 2003. Kymionis et al. [9] applied conventional CXL procedure in 14 thin corneas with minimum corneal thickness of less than 400 μm (range 340–399 μm) after epithelial removal. Improvement in uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), and reduction in mean keratometry readings were recorded during the 12 months follow-up. However, despite the absence of clinically evident complications, significant reduction of endothelial cell density from 2733 to 2411 cells/mm^2 was observed postoperatively. The film of 0.1% isoosmolar riboflavin with 20% dextran was measured to be approximately 70 μm thick after 1 minute of instillation and remained stable for 22 minutes [10]. With the riboflavin-dextran film, the UVA irradiance in human corneal stroma at 400 μm was measured to be 0.21 mW/cm^2, which is much lower than the previously mentioned cytotoxicity level on which the set limitation of minimal deepithelialized stromal thickness of 400 μm is based. Hence, the absorption and shielding of UVA by the riboflavin film may have prevented the damage to the endothelium. Nevertheless, longer follow-up and larger patient series is essential to evaluate the safety and efficacy of conventional CXL in clinical application in thin corneas.

3. Hypoosmolar Riboflavin Solution

There is an inert swelling pressure in the cornea that is the cornea has a tendency to swell in an isooncotic environment. The deepithelialized cornea can swell to double its normal volume when irrigated with a hypoosmolar solution. Hafezi et al. [11] applied this method in thin cornea to increase the corneal thickness before CXL. After epithelial removal, 0.1–20% dextran isoosmolar riboflavin was applied to the cornea for 30 minutes. The 0.1% dextran-free hypoosmolar riboflavin was then administered until the corneal thickness at the thinnest point reached 400 μm, before the initiation of UVA irradiation. The authors reported a stabilization of keratectasia in 20 eyes treated with this approach.

In a study by Raiskup et al. [12] 0.1% hypoosmolar riboflavin was applied after epithelial debridement until the riboflavin saturated cornea reached the minimum of 400 μm. In this study, one year after the treatment, CDVA and keratometric value remained unchanged and no damage to the cornea in the form of detectable scarring lesions in the stroma was registered. Similar results were reported by Wu et al. [13] On the contrary, in eyes treated with isoosmolar riboflavin solution, a permanent stromal scar tended to develop in thin corneas after CXL. Gu et al. [14] used 0.1% hypoosmolar riboflavin solution as saturation and swelling solution in 8 thin corneas that underwent CXL procedure. They reported a slight decrease of endothelial cell density 3 months after the treatment.

The preoperative swelling of the cornea broadens the spectrum of CXL indications to thinner corneas. However, Hafezi et al. [11] reported a case where CXL could not stop the progression of keratoconus in a very thin cornea (minimal thickness of 268 μm after removal of the epithelium), despite the fact that swelling with hypoosmolar riboflavin solution increased the thickness to 406 μm and no adverse endothelial reaction was observed postoperatively. The authors, therefore, hypothesized that there is a minimal, yet to be determined stromal thickness necessary for effective CXL to occur. They suggested a minimal stromal thickness of 330 μm or more before swelling, when using hypoosmolar riboflavin solution.

Intraoperative corneal thickness measurements during CXL with hypoosmolar riboflavin solution in thin corneas has been done by Kaya et al. [15] and Soeters et al. [16]. They found that the artificial swelling effect was transient, and the thinnest pachymetric readings decreased significantly after 10 and 30 minutes of isoosmolar riboflavin (with dextran) application, with or without UVA irradiation. Thinning of deepithelialized cornea after instillation of 0.1–20% dextran riboflavin isoosmolar solution has also been

reported in other studies. According to the authors the reduction of the corneal thickness was induced by the hyperoncotic effect of the dextran. Vetter et al. [17] evaluated the modulatory effect of various riboflavin 0.1 and 0.2% compositions on the central corneal thickness in fresh postmortem porcine eyes. No correlation between the osmolarity of the composition and the swelling behavior of the treated corneas was observed, whereas an inverted correlation was verified between the dextran concentration and the swelling effect. Concurrently, lower absorption and shielding effect of the thinner hypoosmolar riboflavin film on the cornea, by application of the hypoosmolar riboflavin without dextran alone causes increase in irradiance level in the stroma and increases damage to the endothelium [10]. Therefore, the cornea should be swollen to a thickness greater than 400 μm or concentration of riboflavin in the hypoosmolar solution could be increased. It was therefore suggested that development of new riboflavin solutions with isooncotic properties to create a stable film could increase the safety of CXL. Moreover, lack of the evaporation resistance provided by the corneal epithelium, and/or an increase in endothelial pump activity may also contribute to corneal thinning. It was proposed that removal of the lid speculum during riboflavin saturation, and use of irradiating devices with shorter irradiation time (and higher power) might be advantageous. Monitoring the corneal thickness throughout CXL treatment could also be important. CXL can be expected to have less effect on biomechanics of artificially swollen corneas due to the lower relative concentration of collagen in the hydrated stroma. Long-term follow-up studies addressing this issue are warranted.

4. Transepithelial Collagen Cross Linking

Substances such as benzalkonium chloride, ethylenediaminetetraacetic acid (EDTA) and trometamol, especially when combined, enhance epithelial permeability of hydrophilic macromolecules, such as riboflavin [18-21]. By adding the enhancers to help riboflavin penetrate the corneal stroma through the intact epithelium, CXL can be performed without epithelial debridement (transepithelial CXL). Transepithelial CXL has been proposed (but not proven) to reduce early postoperative pain, temporary worsening of vision, as well as complications such as infectious keratitis after conventional CXL. Additionally, thinner corneas may be treated safer by transepithelial compared to the conventional CXL, since the endothelium is better protected by UVA-filtering effect of the intact epithelium.

Filippello et al. [22] used trometamol and sodium EDTA as enhancers and applied transepithelial CXL in 20 keratectatic eyes with a mean corneal thickness of 412 ± 21 μm. The transepithelial CXL treatment appeared to halt the progression of keratoconus in all treated eyes over 18 months follow-up. It also yielded statistically significant improvements in all visual and topographic outcome measures, whereas the contralateral untreated eyes demonstrated worsening of all parameters. Spadea et al. [23], who used a similar protocol in thin corneas, confirmed its effect in stabilization of the keratoconic eyes. However, the visual and topographic improvement was minimal. No endothelial cell damage was observed in either of the studies.

Wollensak et al. [24] reported an increase in corneal rigidity up to 64% in human corneas with transepithelial CXL using topical anesthetics and benzalkonium chloride as enhancers, versus a 320% increase when using CXL with de-epithelialization. The postoperative demarcation line depth in the study by Filippello et al. [22] was only approximately 100 μm, in contrast to about 300 μm in conventional CXL with epithelial debridement, so the safety and reproducibility of the study is questionable. Seiler and Hafezi [25] first reported the demarcation line after CXL and related the depth of the line to that of keratocyte death after CXL as measured by confocal microscopy. They suggested that the line represented the transition zone between cross-linked anterior and untreated posterior stroma. It is unclear whether the shallower demarcation line using the transepithelial approach was due to limited penetration of riboflavin into the stroma or that it was a result of reduced UVA-light penetration by shielding from riboflavin-impregnated intact corneal epithelium. Iontophoresis-assisted transepithelial CXL, using a noninvasive delivery system based on a small electric current, was recently designed to enhance the penetration of riboflavin into the corneal stroma [26]. Preclinical results showed that the iontophoresis was able to increase the concentration of riboflavin in the corneal stroma when compared to enhancer-assisted transepithelial CXL, but did not reach concentrations previously reached with conventional epithelium-off CXL. Demarcation line after iontophoresis-assisted transepithelial CXL appeared to be less easily distinguishable and shallower than in conventional CXL, however, it demonstrated features more similar to that after conventional CXL in terms of depth and visualization, compared to enhancer-assisted transepithelial CXL [24]. In general, there is consensus within the scientific community that current transepithelial CXL protocols are not as effective as conventional epithelium-off CXL.

5. Contact Lens Assisted Collagen Cross Linking

Jacob et al. [27] introduced contact lens assisted CXL (CACXL). A daily disposable soft contact lens of 90 micron thickness was used. It was of 14 mm diameter and was made of hilafilcon without UV filter. The contact lens was applied on to the deepithelialized riboflavin saturated cornea after immersing it in isoosmolar riboflavin 0.1% in dextran for 30 minutes. The UVA radiation of 3.0 mW/cm^2 was applied for 30 minutes after confirming that the minimum corneal thickness including contact lens and riboflavin film is greater than 400 microns. The riboflavin solution was instilled every

3 minutes during the UVA radiation to maintain corneal saturation and to keep pre corneal and pre contact lens riboflavin film uniform. The pre-corneal riboflavin film with contact lens created an absorption medium in the pre-corneal space by artificially increasing the thickness of the "riboflavin-filter".

Jacob et al. [27] treated 14 eyes with contact lens assisted CXL and the patients were followed up for 6 months. At the end of follow up no significant endothelium loss or signs of post operative endothelial damage were observed. No significant changes in CDVA or mean maximum keratometric value was detected post operatively although 1 D decrease of mean K value was observed in 4 eyes (28.5%).

The advantage of CACXL is the absence of descemet's membrane folds and endothelial damage as it is not dependent on the swelling properties of the cornea. However the surface irradiance at level of cornea stroma is reduced by 40-50% in CACXL due to absorption by riboflavin film and soaked contact lens. Furthermore the effect of CXL is reduced in CACXL as oxygen diffusion is hindered by contact lens. The limitations of the study are the small patient population, short follow up and absence of control group.

6. Conclusion

In conventional CXL a minimum corneal thickness of 400 μm is recommended. Due to the improvement in screening technique of keratoconus most of the keratoconus eyes would be treated by this protocol. However some cases are diagnosed lately and have values below this threshold. To offer CXL to this group of patients several modifications have been proposed. The overall safety of the presented protocols for CXL in thin cornea is good. Modification in tonicity and concentration of riboflavin as well as UV energy and/or power have been proposed. Iseli et al. [28] have suggested a higher riboflavin concentration for improved protective screening of endothelium in thin cornea. Accelerated CXL (UVA irradiation at 30 mW/cm^2 for 3 minutes) has recently been applied and shown to stabilize the progression of keratoconus in 34 thin cornea, without endothelial cell density loss during the 12 months of follow up [29].

The efficacy in accelerated CXL is more than continuous UV light due to optimization of oxygen availability. Thus the pulsed mode used during UVA radiation is more effective. There is a need of introduction of a comprehensive mathematical model for the calculation of optimal parameters such as concentration and tonicity of riboflavin, as well as UV light duration, power and dose for any given corneal thickness. This way the CXL may be tailored according to the patient's corneal thickness. Presently, only laboratory research can be found on the subject [30, 31].

The proof of safety and efficacy regarding the modification in CXL protocols is still limited to a handful of studies. Further long term follow up studies with a large number of patients is required.

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