Collagen cross-linking in thin corneas

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Collagen cross-linking (CXL) has become the standard of care for progressive keratoconus, after numerous clinical studies have established its efficacy and safety in suitably selected eyes. The standard protocol is applicable in eyes which have a minimum corneal thickness of 400 µm after epithelial debridement. This prerequisite was stipulated to protect the corneal endothelium and intraocular tissues from the deleterious effect of ultraviolet-A (UVA) radiation. However, patients with keratoconus often present with corneal thickness of less than 400 µm and could have otherwise benefited from this procedure. A few modifications of the standard procedure have been suggested to benefit these patients without a compromise in safety. Transepithelial cross-linking, pachymetry-guided epithelial debridement before cross-linking, and the use of hypoosmolar riboflavin are some of the techniques that have been attempted. Although clinical data is limited at the present time, these techniques are worth considering in patients with thin corneas. Further studies are needed to scientifically establish their efficacy and safety.

**Key words:** Corneal collagen cross-linking, hypoosmolar riboflavin, keratoconus, thin cornea, transepithelial collagen cross-linking

Keratoconus is a disease characterized by progressive thinning and ectasia of the cornea which induces irregular astigmatism resulting in impairment in quality of vision. Until the turn of this century, treatment consisted of various methods that could provide optical, refractive, or tectonic rehabilitation without altering the natural history of the disease. Recently, corneal collagen cross-linking (CXL) has been introduced as a treatment that for the first time addresses the pathophysiology of ectasia and aims at retarding or halting the progression of disease. Introduced for human use by Wollensak et al,[1] CXL was shown to increase the mechanical strength and biochemical stability of the corneal stromal tissue.

Rigorous *in vitro* and *in vivo* studies preceded the preparation of the “Dresden protocol” which prescribes the safety guidelines for this procedure.[2] One of the important prerequisites for safety was that the corneal stroma, after epithelial debridement, should have a minimum thickness of 400 µm. This would limit the UV irradiance to 0.18 mW/cm² at the endothelial level, which was at least a factor of 2 smaller than the damage threshold of 0.35 mW/cm², and very much less than the damage threshold for the lens (70 J/cm²) and the retina (4.3 mW/cm²). This would hold true provided the cornea was photosensitized with isoosmolar riboflavin 0.1% solution in 20% dextran for 30 min and exposed to UVA radiation of 370 nm, at 3 mW/cm² for 30 min. This method has been accepted as the “standard protocol” and is believed to cause and restrict the morphological effects of CXL to the anterior 250-350 µm of corneal stroma. Adhering to this protocol, several studies have confirmed the efficacy and safety of CXL, making it, today, the standard of care for progressive keratoconus.

Dextran 500 is a 500-kDa polyglucose biopolymer with a high affinity to water because of its abundant hydrophilic hydroxyl groups.[3] The onotic effect of a 20% concentration, which is used in the preparation of isoosmolar riboflavin would lead to corneal deswelling. It is therefore not surprising that intraoperative ultrasonic pachymetric measurements during CXL with isoosmolar riboflavin showed a mean decrease of 75 µm in central corneal thickness[4] which was statistically significant and has important clinical implications.

Holopainen and Krootila[5] in a similar study found a mean corneal thinning of 87 ± 40 µm, most of which occurred during the UV irradiation process, suggesting evaporative losses from a deep epithelialized cornea to be a contributing factor. These studies serve to remind us that eyes with a corneal thickness of >400 µm could still cross the borders of safety during the CXL procedure becoming “thin corneas”, as possibly occurred in the case reported by Gokhale.[6] Such corneas call for modifications in our approach.

The risks of using the standard CXL protocol in corneas less than 400 µm after epithelial debridement, or those in danger of falling into this category during treatment, are borne out by Kymionis et al.[7] The authors reported a significant decrease in endothelial cell count when they used the standard isoosmolar CXL protocol on 14 eyes with a minimum corneal thickness of <400 µm. This is a cause for concern, especially considering the fact that most of these patients are young and that the very purpose of this treatment is to avoid the need for a corneal transplant.

**Dextrans**

- 500 kDa
- High affinity to water
- Hydrophilic hydroxyl groups
- Onotic effect

**Clinical Implications**

- Mean decrease in central corneal thickness of 75 µm
- Statistically significant

**Modifications**

1. Modification in parameters, for example, riboflavin concentration, intensity and/or wavelength of UVA, duration of treatment, etc.
2. Transepithelial CXL
3. Customized pachymetry: guided epithelial debridement
4. Iatrogenic corneal swelling before CXL
Modification in Parameters

The currently prescribed concentration of riboflavin, the wavelength of UVA radiation and duration of treatment were established after a series of time and dose-response assays in animal models over several years of studies. These would need to be repeated of any alteration is envisaged, before human application.

Transepithelial Collagen Cross-linking

Transepithelial CXL was introduced to prevent the adverse events associated with epithelial debridement (postoperative pain, infectious keratitis, stromal haze, etc.) as well as for its possible role in treating thinner corneas. Initial experimental studies in porcine corneas demonstrated that complete epithelial removal was necessary for riboflavin permeation. Superficial epithelial trauma or tetracaine administration or grid-like epithelial removal were not sufficient to achieve adequate stromal riboflavin concentration and may impair the efficacy of cross-linking.[8,9] Wollensak and Iomdina, showed in rabbit models that corneal cross-linking without epithelial debridement (using benzalkonium chloride containing proparacaine eyedrops) reduced the biomechanical effect by approximately one-fifth compared with standard cross-linking. The cytotoxic damage was restricted to 200 µm stromal depth, which can be beneficial in corneas <400 µm.[10] Transepithelial CXL in 20 patients with bilateral progressive keratoconus using enhanced riboflavin solution, containing trometamol and ethylenediaminetetraacetic acid (EDTA) sodium salt, was undertaken by Filippello et al. They reported a statistically significant improvement in visual and topographic parameters and concluded that the treatment appeared to halt keratoconus progression.[11] Trometamol is a biologically inert low-toxicity amino alcohol used as buffering solution and sodium EDTA is a well-known chelator of calcium and magnesium ions. Their combination breaks intercellular bonds, thus facilitating the penetration of riboflavin through the intact epithelium.[11,12] Applying a similar technique of transepithelial CXL in ultrathin corneas (thinnest pachymetry 331-389 µm) moderate efficacy was reported by Spada et al.[12] Though both these studies reported no endothelial toxicity, it still remains a concern because improper stromal concentration of riboflavin may not be effective in absorbing all the UV radiation.

Pachymetry Guided Epithelial Debridement Before CXL

Another method for cross-linking in thin corneas, proposed by Kymionis et al., is customized pachymetric guided epithelial debridement. The technique involves mechanically removing 8.0 mm diameter of corneal epithelium, while preserving a small localized island corresponding to the thinnest area or the area of maximum topographic steepening. They cross-linked 2 patients with this technique with thinnest pachymetry of 380 and 375 µm. Postoperative results showed stabilization of ectasia with no endothelial cell density reduction.[13] Preservation of epithelium over the thinnest area also has possible advantage of prevention of local stromal dehydration apart from blocking excessive UV radiation in this susceptible area. The effect of cross-linking with this technique was studied with anterior segment optical coherence tomography (AS-OCT) and confocal microscopic imaging by Kaya et al., Haze formation and demarcation line were present only in the deep epithelialized stroma and were not evident under areas which had an intact epithelium. Further confocal microscopy revealed keratocyte loss with intense stromal edema only in the deep epithelialized areas.[14] For CXL to be effective, it is vital for the thinnest areas to achieve cross-linking. But here this area seems to be spared from the effect of CXL, putting the efficacy of the whole procedure in doubt. Even after partial epithelial removal in a grid pattern, the riboflavin uptake is limited and nonhomogenous, which might compromise the efficiency of cross-linking procedure.[9]

CXL with Hypoosmolar Riboflavin

To treat corneas thinner than 400 µm, Hafezi et al., modified the technique of CXL by swelling the corneas to increase stromal thickness before UV irradiation. The deep epithelialized cornea can swell to double its normal thickness when irrigated with a hypoosmolar solution, because of the hydrophilic property of the stromal proteoglycans. Their technique involved applying isoosmolar riboflavin 0.1% solution with 20% dextran first, every 3 min for 30 min, on 9.0 mm of deep epithelialized cornea. Ultrasound pachymetry is then performed at the thinnest point. This was followed by application of hypoosmolar riboflavin (riboflavin 0.5% with 0.9% sodium chloride solution) every 20 s for 5 more min or till the minimum corneal thickness reaches 400 µm. During irradiation isotonic riboflavin 0.1% is administered every 5 min. They treated 20 patients with minimum stromal thickness 320-400 µm post epithelial removal and observed a swelling of between 36-110 µm after using hypoosmolar riboflavin. They reported stabilization of ectasia in 12 patients and regression in eight patients.[15] No clinical signs of endothelial damage or other side effects were seen. Raiskup and Spoerl published 1 year results of hypoosmolar CXL in 32 eyes. Their technique differed as they applied hypoosmolar riboflavin 0.1% solution every 2 min for 30 min. During irradiation also hypoosmolar riboflavin drops were applied every 2 min. They reported stabilization of ectasia in terms of mean K-value and best corrected visual acuity (BCVA). No side effects were observed with no stromal scarring.[16] Our own experience with hypoosmolar riboflavin followed by CXL also seems to indicate that while stabilization of the ectatic process is most often achieved, the flattening of the steepest keratometry is more modest than what is seen with isoosmolar riboflavin in the standard CXL. Fifty eyes that underwent hypoosmolar CXL were compared with 50 eyes that underwent isoosmolar (standard) CXL in the same period. At 1 year follow-up, the steepest keratometry (Pentacam) had decreased by an average of 0.88 ± 2.26 D in the isoosmolar group and 0.18 ± 3.23 D in the hypoosmolar group. This poses a doubt whether artificially swollen corneas behave in the same way as nonswollen keratoconus corneas. CXL might be expected to have a smaller effect on the biomechanics of an artificially swollen cornea because of lower relative concentration of collagen in the hydrated stroma.[17] Small angle X-ray scattering study, post CXL (iso- and hypotonic) in postmortem and and posttransplant corneal button has been done to evaluate the stromal collagen ultrastructure. It showed that some fluid enters the collagen fibrils to increase the fibril diameter, but majority of swelling occurs between the fibrils. Collagen D periodicity was not altered post treatment.[18] Another concern was raised by Kaya et al., when they demonstrated through their study that the artificial swelling effect of hypoosmolar
riboflavin is transient. They followed the technique described by Hafezi et al.[15], and found that thinnest pachymetric readings decreased significantly after 10 and 30 min compared with the readings at the end of hypoosmolar riboflavin application.[16] This can probably be avoided by repeated application of hypoosmolar riboflavin during the irradiation process also, rather than isoosmolar riboflavin. Considering this risk, intraoperative pachymetry is essential during the procedure. Optical pachymetry does not provide reliable data in swollen corneas because of excessive light scattering and absorption. So ultrasound pachymetry is recommended for the purpose. But identifying the thinnest area is neither easy nor accurate while the procedure is going on, even if it has been marked previously on the basis of corneal topography. Thus, there is still some ambiguity regarding the correct way of measuring intraoperative corneal thickness. The behavior of different riboflavin films was studied by Wollensak et al.[17] The hypoosmolar riboflavin film is very unstable with a breakup time of 90 s as compared to isotonic (with dextran) riboflavin film which has a breakup time of 22 min. The corneal absorption coefficient of the combined stroma-riboflavin film system was 56.36 cm⁻¹ using dextran-riboflavin and 48.19 cm⁻¹ using hypoosmolar riboflavin. This poor shielding effect and lower absorption of hypoosmolar riboflavin film can cause irradiance levels at endothelial level to reach the toxicity threshold of 0.36 mW/cm². Thus the safety and efficacy of hypoosmolar CXL still needs to be established by detailed studies. Failure of hypoosmolar riboflavin in a case with an extremely thin cornea has been reported.[20] The patient with a post epithelial application of hypoosmolar riboflavin during the irradiation procedure. Optical pachymetry does not provide reliable data in swollen corneas because of excessive light scattering and absorption. So ultrasound pachymetry is recommended for the purpose. But identifying the thinnest area is neither easy nor accurate while the procedure is going on, even if it has been marked previously on the basis of corneal topography. Thus, there is still some ambiguity regarding the correct way of measuring intraoperative corneal thickness. The behavior of different riboflavin films was studied by Wollensak et al.[17] The hypoosmolar riboflavin film is very unstable with a breakup time of 90 s as compared to isotonic (with dextran) riboflavin film which has a breakup time of 22 min. The corneal absorption coefficient of the combined stroma-riboflavin film system was 56.36 cm⁻¹ using dextran-riboflavin and 48.19 cm⁻¹ using hypoosmolar riboflavin. This poor shielding effect and lower absorption of hypoosmolar riboflavin film can cause irradiance levels at endothelial level to reach the toxicity threshold of 0.36 mW/cm². Thus the safety and efficacy of hypoosmolar CXL still needs to be established by detailed studies. Failure of hypoosmolar riboflavin in a case with an extremely thin cornea has been reported.[20] The patient with a post epithelial removal corneal thickness of 268 µm still showed progression of 1.9 D after 3 months of CXL, though no endothelial adverse events were noted. The author concluded that in order to prevent ectasia, a minimum stromal thickness of 330 µm should be present so as to achieve a minimum cross-linked stromal thickness of 250 µm (75% of 330 µm). Clinically, there are distinct interindividual variations in the stromal swelling response of different corneas, making it difficult to formulate a fixed protocol for the treatment of such eyes.

In summary, finding ways of treating eyes with thin corneas is both relevant and challenging.

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