Recent Advances in Corneal Collagen Crosslinking in thin Corneas

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Abstract
Corneal collagen crosslinking has been a breakthrough in keratoconus management since its inception as it offers stabilization of the progressive ectasia. However, the limitation of its use in thin corneas (< 400 microns) is a limiting factor and various methods have been devised to overcome this shortcoming. Modifications like application of hyposmolar riboflavin, transepithelial collagen cross-linking (CXL) and customized epithelial debridement have been tried but have limited clinical data. Contact lens assisted and SMILE lenticule assisted CXL are new entrants for application on the thin corneas. The article highlights the various modifications employed in tackling thin corneas in CXL.

Keywords: collagen, crosslinking, SMILE, thin cornea

Introduction
Keratoconus is a complex disease with a genetic component. It is a slowly progressive, usually bilateral, non-inflammatory corneal thinning disorder characterized by changes in the structure and organization of corneal collagen. Until recently, treatment consisted of various methods that could provide optical, refractive, or tectonic rehabilitation without altering the natural history of the disease. Corneal collagen cross-linking (CXL) was introduced as a treatment that for the first time addressed the pathophysiology of ectasia and aimed at retarding or halting the progression of disease. Introduced by Wollensak et al1 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 thickness of corneal stroma, which after epithelial debridement, should be at least 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 was feasible if 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.

In developing countries like India where the onset of disease is early and progression faster, it is not uncommon to find keratoconus patients with pachymetry below 400 microns. In younger age group if penetrating keratoplasty is performed, the rate of graft failure is higher and the visual prognosis is worse than adults. Moreover, vernal keratoconjunctivitis (VKC) is more frequently found in these children which also reduces the chances of a successful corneal graft. Hence it is important to have modifications in CXL to enable its usage in thin and ultrathin corneas. Another concern is desiccation of the cornea during the procedure. Dextran 500 is a 500-kDa polyglucose hydrophilic biopolymer with a high affinity towards water.3 The oncotic effect of a 20% concentration, used in the preparation of isoosmolar riboflavin leads to corneal deswelling. Intraoperative ultrasonic pachymetric measurements during CXL with isoosmolar riboflavin showed a mean decrease of 75 µm in central corneal thickness which was statistically significant and had important clinical implications.4 Another study found a mean corneal thinning of 87 ± 40 µm, most of which occurred during the UV irradiation process, suggesting evaporative losses from a de epithelialized cornea to be a contributing factor.5 Hence, patients with pachymetry of >400 µm could still cross the borders of safety during the CXL procedure becoming “thin corneas”. Such corneas call for modifications in approach while dealing with them.

This article aims to describe various modalities used to perform CXL in thin corneas and their current status.

CXL Using Hyposmolar Riboflavin
To treat corneas thinner than 400 µm, Hafezi et al6, modified the technique of CXL by swelling the corneas to increase stromal thickness before UV irradiation. Their technique involved application of isoosmolar riboflavin 0.1% solution with 20% dextran first, every 3 min for 30 min, on 9.0 mm sodium chloride solution) every 20 s for 5 more min or till the minimum corneal thickness reached 400 µm. During irradiation isotonic riboflavin 0.1% was administered every 5 min. They treated 20 patients with minimum stromal thickness 320-400 µm post epithelial removal and observed

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a swelling of between 36-110 µm after using hypoosmolar riboflavin. They reported stabilization of ectasia in 12 patients and regression in eight patients No clinical signs of endothelial damage or any other side effect was seen. Raiskup and Spoerl published 1 year results of hypoosmolar CXL in 32 eyes. They applied hypoosmolar riboflavin 0.1% solution every 2 min for 30 min. During irradiation also hypoosmolar riboflavin drops were instilled every 2 min. They reported stabilization of ectasia in terms of mean K value and best corrected visual acuity (BCVA) with no side effects. In a comparative study it was demonstrated that although there was no stromal scarring on 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 posed a doubt whether artificially swollen corneas behaved 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. 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, and found that thinnest pachymetric readings decreased significantly after 10 and 30 min compared with the readings at the end of hypoosmolar riboflavin application. The transient effect was also demonstrated by Schmidinger et al in their study in which they have compared the pachymetry changes between three groups at various intervals. Group 1 underwent standard CXL with open eyelids with speculum, in Group 2 speculum was removed during instillation of riboflavin and in Group 3, hypoosmolar riboflavin was used throughout the procedure. Hyposmolar riboflavin was instilled in Group 1 and 2 to increase the corneal thickness prior to U V irradiation if less than 400 microns. It was noted that corneal thickness reduced (<350 microns) in all three groups but the steepest reduction was in Group 3. Therefore it is mandatory to do ultrasonic pachymetry in all the patients at regular intervals. 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 film in a case with an extremely thin cornea has been reported. The author concluded that in order to prevent ectasia, a minimum stromal thickness of 330 µm should be present.

Transepithelial CXL

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. Wollensak and Iomdina, showed in rabbit models that CXL without epithelial debridement (using benzalkonium chloride containing proxygenetacue 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. 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. 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. Applying a similar technique of transepithelial CXL in ultrathin corneas (thinnest pachymetry 331-389 µm) moderate efficacy was reported by Spada et al. However, long term results by Caporrosi et al failed to support this technique. In their 24 months follow up they found keratoconus instability and functional regression particularly in patients less than 18 yrs. Eren et al had made a unique conclusion in their comparative study that although transepithelial CXL seemed to have reduced efficacy in terms of topographic indices, its effect on visual acuity could actually be similar to epi – off CXL.

Customised Pachymetry 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 crosslinked two patients with this technique with thinnest pachymetry of 380 and 375 µm. Postoperative results showed stabilization of ectasia with no endothelial cell density reduction. 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 (ASOCT) and confocal microscopic imaging by Kaya et al, and they reported that the CXL effect in terms of keratocyte loss and hyperreflectivity was limited to anterior 150 microns as compared to 250 microns in traditional CXL.
**Contact Lens Assisted CXL**

Jacob et al\(^2\) describe the use of a riboflavin-soaked bandage contact lens of negligible power to artificially increase the corneal thickness for CXL. Both the contact lens and de epithelialized cornea is soaked in Riboflavin 0.1% solution for 30 min, at the end of which the riboflavin soaked contact lens is applied over the cornea and collective pachymetry is measured. If it is more than 400 microns then cornea is further exposed to UV irradiation. Long-term results of this procedure are not available. The inability to customize the thickness of the contact lens, varied hydration states of different contact lens materials, difference in UV-light transmission properties of contact lenses and corneal stroma, and uneven adherence to the underlying stromal bed causing irregular interface and subsequent pooling of riboflavin are some drawbacks of this innovative technique. Intraoperative buckling of the contact lens might also create an uneven precorneal riboflavin film and consequently lead to hot or cold spots.

**Smile Assisted CXL**

This is the most recently published technique by the authors.\(^3\) It involves application of refractive lenticule extracted from the patients undergoing small incision femtosecond lenticule extraction (SMILE) procedure for myopia. SMILE involves the extraction of a femtosecond laser-constructed corneal lenticule through a single small incision without raising a flap.\(^4\) The lenticular thickness depends on the refractive error of the patient. Also, the thickness of the lenticule is maximum in the center and decreases toward the periphery, as described in a study by Tay et al.\(^5\) In our technique of CXL using the refractive lenticule, the lenticule is placed over the deepithelialised surface of the cornea so that the thickest portion of the lenticule corresponds to the thinnest portion of the cornea. Therefore, thickness of the cornea is increased in the most physiologic manner by adding stromal tissue, with biologic and absorptive properties similar to the cornea that has to be treated. Refractive lenticules of variable thickness (20 to 140 \(\mu\)m) can be obtained following femtosecond lenticule extraction depending on the extent of the refractive error to be corrected. Placing the central lenticule over the apex of the cone enabled the surgeon to augment the corneal thickness where required while sparing the remaining stroma to be crosslinked normally. Moreover, the relatively rough host stromal surface made it easy to spread the lenticule and prevent buckling. Also, after the application of riboflavin 0.1% for 30 minutes, the lenticule got reasonably attached and had to be peeled off the bed after the procedure. An even demarcation line indicative of CXL was seen in all cases. In our series, both femtosecond lenticule extraction and CXL were performed in the same sitting in adjacent operation rooms, thereby maintaining sterility. Long-term preservation of myopic lenticules would enable more widespread use of this technique.

**Modification Of Parameters During CXL**

Safe CXL in thinner corneas can also be achieved by making alterations in riboflavin concentration, UV exposure time and UV wavelength or intensity. But these still await human trials and therefore cannot be adopted as yet. Apart from these it has been now suggested that usage of HPMC (Hydroxypropyl methylcellulose) riboflavin\(^6\) helps prevent corneal dessication induced by dextran and could be considered in borderline cases. Other methods by which dessication could be reduced are using accelerated CXL\(^7\) and eyelid closure\(^8\) during the initial riboflavin instillation. All of these combined can work in borderline pachymetry without any specialized technique.

To summarize, CXL in thin corneas should be performed only by practitioners having considerable experience and understanding of corneal biomechanics. Each procedure is fraught with its limitations and complications which should be kept in mind before performing CXL.

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**References**

1. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol* 2003; 135:620-7.

2. Spoerl E, Mrochen M, Sliney D, Trokel S, Seiler T. Safety of UVA-riboflavin cross-linking of the cornea. *Cornea* 2007; 26:385-9.

3. Zhang ZY. Unstable corneal thickness during hypoosmolar riboflavin irrigation for collagen cross linking. *Cornea* 2013; 32:110.

4. Kymionis GD, Kounis GA, Portalidou DM, Grentzelos MA, Karavitaki AE, Coskunseven E, et al. Intraoperative pachymetric measurements during corneal collagen cross-linking with riboflavin and ultraviolet A irradiation. *Ophthalmology* 2009; 116:2356-9.

5. Holopainen JM, Krooluta K. Transient corneal thinning in eyes undergoing corneal cross-linking. *Am J Ophthalmol* 2011; 152:533-6.

6. Hafezi F, Mrochen M, Iseli HP, Seiler T. Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. *J Cataract Refract Surg* 2009; 35:621-4.

7. Raiskup F, Spoerl E. Corneal cross-linking with hypo-osmolar riboflavin solution in thin keratoconic corneas. *Am J Ophthalmol* 2011; 152:28-32.

8. Hayes S, Boote C, Kamma-Lorger CS, Rajan MS, Harris J, Dooley E, et al. Riboflavin/UVa collagen cross-linking-induced changes in normal and keratoconus corneal stroma. *PLoS One* 2011; 6:e22405.

9. Kaya V, Utine CA, Yilmaz OF. Intraoperative corneal thickness measurements during corneal collagen cross-linking with hypoosmolar riboflavin solution in thin corneas. *Cornea* 2012; 31:486-90.

10. Schmidinger G, PachalaM, PragerF. Pachymetry changes during corneal crosslinking:Effect of closed eyelids and hypertonic riboflavin solution. *J Cataract Refract Surg* 2013;
Recent Advances

11. Wollensak G, Aurich H, Wirbelauer C, Sel S. Significance of the riboflavin film in corneal collagen crosslinking. J Cataract Refract Surg 2010; 36:114-20.

12. Hafezi F. Limitation of collagen cross-linking with hypoosmolar riboflavin solution: Failure in an extremely thin cornea. Cornea 2011; 30:917-9.

13. Samaras K, O’Brart DP, Dought J, Hayes S, Marshall J, Meek KM. Effect of epithelial retention and removal on riboflavin absorption in porcine corneas. J Refract Surg 2009; 25:771-5.

14. Hayes S, O’Brart DP, Lamdin LS, Dought J, Samaras K, Marshall J, et al. Effect of complete epithelial debridement before riboflavin–ultraviolet-A corneal collagen crosslinking therapy. J Cataract Refract Surg 2008; 34:657-61.

15. Wollensak G, Iomdina E. Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. J Cataract Refract Surg 2009; 35:540-6.

16. Filippello M, Stagni E, O’Brart D. Transepithelial corneal collagen crosslinking: Bilateral study. J Cataract Refract Surg 2012; 38:283-91.

17. Spadea L, Mencucci R. Transepithelial corneal collagen cross-linking in ultrathin keratoconic corneas. Clin Ophthalmol 2012; 6:1785-92.

18. Caprrossi A, Mazzota C, Paradiso AL, Baiocchi S, Marigliani D, Caprrossi T. Transepithelial corneal collagen crosslinking for progressive keratoconus: 24 months results. J Cataract Refract Surg 2014; 49:450-8.

19. Cerman E, Toker E, Ozcan DO. Tranepithelial versus epithelium off crosslinking in adults with progressive keratoconus. J Cataract Refract Surg 2015; 53:236-9.

20. Kymionis GD, Diakonis VF, Coskunseven E, Jankov M, Yoo SH, Pallikaris IG. Customized pachymetric guided epithelial debridement for corneal collagen cross linking. BMC Ophthalmol 2009; 9:10.

21. Kayy V, Utine CA, Yilmaz OF. Efficacy of corneal collagen cross-linking using a custom epithelial debridement technique in thin corneas: A confocal microscopy study. J Refract Surg 2011; 27:444-50.

22. Jacob S, Kumar DA, Agarwal A, Basu S, Sinha P, Agarwal A. Contact lens-assisted collagen cross-linking (CACXL): a new technique for cross-linking thin corneas. J Refract Surg 2014; 30:366-72.

23. Sachdev M, Gupta D, Sachdev G, Sachdev R. Tailored stromal expansion with a refractive lenticule for crosslinking the ultrathin cornea. J Cataract Refract Surg 2015; 41:918-23.

24. Shah R, Shah S, Sengupta S. Results of small incision lenticule extraction: all-in-one femtosecond laser refractive surgery. J Cataract Refract Surg 2011; 37:127-37.

25. Tay E, Li X, Chan C, Tan DT, Mchta JS. Refractive lenticule extraction flap and stromal bed morphology assessment with anterior segment optical coherence tomography. J Cataract Refract Surg 2012; 38:1544-51.

26. Mazzota CA, Baiocchi S, Caparrossi T, Craquilli S, Paradiso AL, Caparrossi A. Riboflavin0.1%(vibex) for the treatment of keratoconus. Expert opinion on orphan drugs 2013; 1:223-40.

27. Ozgurhan EB, Ackay BI, Kurt T, Yildrim Y, Demirok A. Accelerated corneal collagen crosslinking in thin keratoconic corneas. J Refract Surg 2015; 31:386-90.

28. Soeters N, Busse E, Valk RV, Lelij AV, Tahzip N V. Effect of the eyelid speculum on pachymetry during corneal collagen crosslinking in keratoconus patients. J Cataract Refract Surg 2014; 40:575-81.