10.5005/jp-journals-10025-1034

REVIEW ARTICLE

Collagen Corneal Cross-linking and the Epithelium

Ramez Barbara, Lamis Abdelaziz, Ankur Barua, Hanna Garzozi, Adel Barbara

ABSTRACT

Collagen corneal cross-linking is an acceptable method for halting the progression of keratoconus and post-Lasik ectasia. The traditional method advocates the removal of epithelium for proper saturation of riboflavin within the stroma. The postoperative complications associated with epithelial removal have necessitated the search for a transepithelial method of riboflavin delivery. Controversies aroused with regard to the effectiveness of this approach. In this article, we review the up-to-date literature on this controversial subject.

Keywords: Transepithelial cross-linking, Collagen corneal cross linking epithelium off, Collagen corneal cross-linking epithelium on keratoconus, Ectasia.

How to cite this article: Barbara R, Abdelaziz L, Barua A, Garzozi H, Barbara A. Collagen Corneal Cross-linking and the Epithelium. Int J Kerat Ect Cor Dis 2012;1(3):179-184.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

Keratoconus is a noninflammatory degenerative disorder of the cornea. It manifests as progressive thinning, steepening and biomechanical instability of the cornea. This abnormal curvature changes its refractive power, causes irregular astigmatism which cannot be corrected with spectacles or can be corrected only partially. The irregular astigmatism and myopia impair quality of vision.

The only known treatment for arresting the progression of the disease is collagen corneal cross-linking (CXL). CXL has become a widely used procedure. It improves visual acuity (VA), reduces astigmatism and K max in almost two-thirds of treated eyes. Long-term safety and efficacy has been reported.[1-3]

The standard CXL or what is referred to as the Dresden protocol involves the instillation of 0.1% riboflavin solution in 20% dextran for 30 minutes and NaCl 0.9% drops every 3 to 5 minutes during treatment, followed by 365 nm ultraviolet A (UVA) illumination with 3 mW/cm^2 for 30 minutes for a total dose of 5.4 J/cm^2 with continued instillation of riboflavin drops every 5 minutes.[1] CXL treatment is indicated for corneas thicker than 400 µm in order to prevent endothelial damage. Hafezi et al reported on the safety of hypo-osmolar riboflavin without dextran used in thin corneas (less than 400 µm) in order to swell the cornea prior to treatment to 400 µm and above with no reported complications. There was stabilization of the keratoconus at 1 year post-CXL for thin corneas using hypo-osmolar riboflavin.[4]

Several new protocols using different riboflavin formulas, different frequencies, durations of riboflavin instillation and different UVA illumination irradiance levels (accelerated cross-linking) are being developed. The wide variety of these protocols have raised controversies and caused confusion with regard to the safest and most efficacious way to perform CXL.

Different Ways of Epithelial Removal

Various modifications of epithelial removal were reported. One-way discusses partial removal of the epithelium by excimer laser. This method proved to be inferior in its effectiveness when compared to mechanical removal. It resulted in prolonged application time of riboflavin to achieve corneal saturation and more postoperative pain.[5] On the other hand, complete epithelial removal using excimer laser (50 µm) proved to yield superior results compared to mechanical removal.[6,7] As corneal epithelium is thinner at the apex in keratoconic eyes[8,9] and laser 50 µm depth ablation removes few microns from the stroma at the apex of the cone which results in flattening of the surface, leading to reduction in the refractive error. Kymionis et al reported on customized epithelial removal in thin corneas in two cases of post-Lasik ectasia, the epithelium was removed leaving a small localized inferior area of corneal epithelium corresponding to the thinner-area of topographic steepening,[10] the treatment was safe and effective.

Seiler reports less CXL effect when strips of epithelium were removed by inducing scratches in the epithelium. At 1 month follow-up, stromal haze was detected under the strips of removed epithelium but none under the epithelial islands which indicates less CXL under the epithelial islands (round table discussion on the future of CXL, January 2011). Available from: http://www.bmctoday.net/crstodayeurope/2011/01/article.asp?f=the-future-of-corneal-collagen-cross-linking).

Malhotra et al examined the penetration of riboflavin in a prospective study using spectral domain optical coherence tomography (OCT) in two groups of patients. In the first group (20 patients) the epithelium was removed completely, while in the second group (10 patients) a grid-like epithelial removal was performed. The OCT demonstrated a homogenous penetration in the first group, while the second group had uneven penetration with significantly more riboflavin presence in the areas where the epithelium was off.[11]

An alternative way (which may be called the hybrid technique) utilizes a device developed by Sheraz Daya, a disruptive device, (the Daya Disruptor; Duckworth and Kent, Hertfordshire, England) to create pockmarks in the epithelium and enhances the riboflavin absorption into the corneal stroma. Encouraging results were reported at the ESCRS in Milan in September 2012 and a deep demarcation line was reported by Sheraz Daya (round table discussion on the future of CXL, January 2011; Available from: http://www.bmctoday.net/crstodayeurope/2011/01/article.asp?f=the-future-of-corneal-collagen-cross-linking).

A stromal demarcation line was described by Seiler et al at a depth of 300 µm following CXL. It is presumed that the line may be due to differences in refractive index between the anterior cross-linked part and the posterior uncross-linked part of the cornea. This difference creates alteration in the reflection properties between these corneal layers.[12] The presence of demarcation line following CXL and detection of keratocytes apoptosis are considered to be an indication of the efficacy of CXL. The significance of these changes and their impact on the results need further investigation.

Epithelial removal prior to CXL carries disadvantages: Pain, tearing, photophobia, stromal haze, increased risk of infection and possibly delayed epithelial healing and corneal melting.[13-16]

Avoiding Epithelial Removal

Kanellopoulos et al reported favorable results by injecting riboflavin into intrastromal pocket created by femtosecond laser and then performing CXL.[17] Alio et al evaluated two-ways of CXL in keratoconic corneas with intrastromal corneal ring segment (ICRS); CXL following epithelial debridement (the classic group) and CXL with riboflavin injection in the intrastromal pocket (the pocket group). No statistically significant differences between the classic and pocket groups were found with regard to postoperative visual acuity, refraction, keratometry, corneal aberrations or central pachymetry.[18] Kilic et al reported on 131 eyes of 105 patients who underwent combined ICRS (Intacs) implantation and transepithelial CXL. They used 20% alcohol application to the cornea to increase riboflavin penetration and riboflavin injection into the corneal channels. Adding to the safety of this method, the authors report improvement in uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA), mean K readings, manifest spherical refraction and cylinder.[19]

Epithelium and CXL

Transepithelial CXL enables treating thin corneas and spares patients from pain and eye irritation. It fastens patient's rehabilitation and may prevent complications reported, following epithelium-off CXL.

On one hand, riboflavin is a photosensitizer inducing collagen cross-linking after its exposure to UVA light, and absorber of the UVA irradiation preventing damage to deeper corneal and ocular structures on the other hand.[25,26] Proper corneal stromal saturation with riboflavin is therefore essential in CXL. The high molecular weight of riboflavin (376.37 gm/mol) and its hydrophilic properties prevent it from penetrating into the stroma through an intact epithelium. Various ways have been tried to enhance the epithelial permeability to riboflavin; partial grid-like pattern epithelial removal, excimer laser superficial epithelial removal,[5] hypotonic riboflavin solution[27] and chemical enhancers, such as benzalkonium chloride (BAC), ethylenediaminetetraacetic acid (EDTA), gentamycin, tetracaine and 20% ethanol.[28,29]

Epithelium absorbs part of the UVA and may prevent certain amount of UVA from reaching the stroma, consequently reducing CXL effect. Kolozsvári et al reports that corneal epithelium has a significantly higher absorption coefficient for UV rays with wavelengths shorter than 300 nm, but not for wavelength, such as 365 nm which is used in CXL; these rays are absorbed by the stroma.[20] An intact epithelial basement membrane is a barrier to riboflavin absorption.[21] Samaras et al reported on inadequate absorption of riboflavin even after using 20% alcohol to loosen the epithelium or using the grid-like pattern of epithelial removal.[22] Baiochi et al demonstrated that stromal concentrations of riboflavin increased with time only if the epithelium was removed.[23]

This raises the question: Is the effect of transepithelial CXL reduced because of the UVA absorption by the epithelium or is it due to barrier properties of the epithelium to riboflavin. Bottos et al tried to answer this question by performing transepithelial CXL in porcine corneas using two different ways. In the first group they used riboflavin with tetracaine drops; in the second group, riboflavin was injected into the anterior chamber. They tested stromal saturation of riboflavin by immunofluorescence microscopy and demonstrated stromal staining with riboflavin in the second group indicating absorption of riboflavin through the endothelium. After performing CXL there was higher organization of the collagen fibers in this second group compared to corneas with intact epithelial basal membrane.[24] This article proves that the epithelium acts as a barrier to riboflavin absorption, preventing stromal saturation but not a barrier to UVA radiation used in CXL.

Enhancing riboflavin penetration through intact epithelium is a hot topic. Many researchers and companies are working on new formulas of such riboflavin.

Raiskup et al investigated the influence of osmolarity on transepithelial permeability of riboflavin solutions. Several riboflavin 0.1% solutions that contained different NaCl and BAC concentrations were applied to 36 rabbit eyes for 30 minutes. They demonstrated that transepithelial riboflavin solution should contain 0.01% BAC and 0.44% NaCl with no dextran to enhance its permeability through intact epithelium and get enough saturation for effective CXL.[30]

While good results have been reported with transepithelial CXL for the treatment of keratoconus,[31,32] other clinical and laboratory studies have reported weaker or no effect of transepithelial CXL and that was justified by insufficient and inhomogeneous saturation of the stroma with riboflavin.[24,34-36] Wollensak et al, in an animal model, demonstrated corneal biomechanical stiffening following epithelium-on CXL of about one-fifth compared to the epithelium-off CXL.[33] Spadea et al reported the results of transepithelial CXL with modified riboflavin in 16 patients with thin corneas (from 331 to 389 µm). The thin corneas could not be treated using the standard Dresden protocol. Riboflavin 0.1% solution in 15% dextran T500 containing EDTA 0.01% and trometamol was used. All patients showed slight improvement in UCVA and best spectacle corrected visual acuity (BSCVA). There was reduction in keratometric astigmatism (up to 5.3 D) and K_{max} values (up to 4.3 D). Endothelial cell density was unchanged after 1 year of follow-up.[37]

Filipello et al analyzed modifications of corneal layers following transepithelial CXL in 20 eyes using confocal microscopy. Patients were followed up for 18 months. A dense demarcation line could be observed after 6 months at a depth of around 60 µm below the Bowman membrane. No endothelial change was reported.[38] This demarcation line was more superficial than reported after epi-off CXL (300 µm). Caporossi et al investigated corneal microstructural modifications in keratoconic patients who underwent transepithelial CXL using 0.1% riboflavin and 15% dextran solution supplemented with EDTA. Tomography and confocal analysis were performed. Apoptosis of keratocytes was detected up to 140 µm from the epithelium. This corresponds to a depth of one-third of the depth achieved in epi-off CXL. Keratocyte apoptosis which indicates the occurrence of CXL was uneven and superficial.[39] Apoptosis of keratocyte has been demonstrated following epithelium on CXL but to a lesser extent.[40] Al-Aqaba demonstrated localized swellings of stromal nerves with loss of axonal continuity in the treated area following epi-off CXL, but not in corneas treated by the epi-on CXL in which the nerves were spared.[41]

Koppen evaluated the effect of transepithelial CXL using riboflavin and in 53 eyes of 38 patients suffering from progressive keratoconus. Proparacaine drops 0.5% preserved with BAC 0.005% were instilled every 5 minutes for 30 minutes. Stability was documented at 18 months follow-up in sphere, cylinder, K max and simulated K as measured using the placido-disk topography. BCVA improved significantly at 6 and 12 months, however, K max and the thinnest point as measured by Scheimpflug device showed statistically significant progression. The authors concluded that transepithelial CXL using the mentioned riboflavin formula was less effective than standard CXL in stabilizing progressive keratoconus.[42] This article poses a question regarding the parameters of keratoconus progression. One of the important parameters for progression is the increase in keratometry values and decrease in corrected VA. In this report both were stable at 18 months hence no progression was diagnosed, however, the Scheimpflug device measurements of K_{max} and pachymetry at the thinnest point demonstrated a statistically significant progression. On which parameter do we have to rely? This remains an open question.

Magli et al compared between epithelium-off CXL in 23 eyes and transepithelial CXL in 14 eyes in pediatric patients with progressive keratoconus. Epi-on CXL was less painful and had similar efficacy and safety profile to epi-off CXL. At 12 months: K_{max}, K_{min}, mean K, surface asymmetry index, inferior-superior symmetry index, index of height asymmetry and anterior elevation at the thinnest point showed improvement.[43] Kilic and Roberts reported on transepithelial CXL in 32 eyes of 22 patients using riboflavin 0.1% with dextran, pilocarpine 2% as a chemical enhancer and proparacaine. The authors applied alcohol 20% for 25 seconds to weaken the epithelium. There was improvement in UCVA and BCVA. No statistically significant change in sphere, cylinder or keratometry. The ocular response analyzer (ORA; Reichert Ltd, USA) assesses the biomechanical properties of the cornea and the 'true' intraocular pressure regardless of the corneal viscoelastic properties. Corneal hysteresis (CH), corneal resistance factor (CRF), intraocular pressure cornea compensated (IOPcc) did not demonstrate any change, however, the peak one signal amplitude increased after transepithelial CXL in the examined 14 patients which indicates increased corneal stiffness.[32]

Buzzonetti et al treated 13 eyes of pediatric keratoconus patients with follow-up of 18 months. The authors used riboflavin 0.1% with EDTA and trometamol as enhancers and dextran 15% (Ricrolin TE, Sooft Italia SPA). Corrected distance visual acuity improved significantly, the sphere

and cylinder did not demonstrate any change, while the K readings and high order aberrations showed significant worsening. The demarcation line mean depth was at 105 µm. Consequently, the authors concluded that transepithelial CXL did not stop the progression of keratoconus in this pediatric group.[44] The same Ricrolin TE was used by Touboul et al in transepithelial CXL as part of a prospective study comparing three different methods of CXL. (1) The standard Dresden protocol, (2) accelerated epi-off CXL using UVA at an irradiance of 30 mW/cm^2 and (3) transepithelial CXL. Confocal microscopy was performed following CXL. Results demonstrated obliteration of the subbasal nerve plexus in the first two groups but not in the transepithelial CXL group. Keratocyte density and reflectivity were altered in the first two groups but again was not affected in the transepithelial group.[45]

An interesting approach was used by Stojanovic et al; they used chemical enhancers; pilocarpine 2%, proparacaine 0.5% and gentamycin 0.3%. All eye drops contained BAC.[43] 'A round Merocel sponge (Medtronic, Inc., Minneapolis, MN) of 5 mm in diameter was inserted into the conjunctival sac to provide a depot of riboflavin and to produce microabrasions of the superficial epithelial layers caused by friction upon patient's blinking.'[43] Riboflavin 0.5% was used every 5 minutes until the achievement of stromal saturation verified by the observation of yellow flare in the anterior chamber.[43] 'Irrigation with isotonic balanced salt solution (BSS) was performed before the UVA irradiation in order to avoid the shielding effect of riboflavin covering the epithelium.'[46] UCVA and BCVA improved significantly at 12 months. There was a documented reduction in the spherical equivalent, astigmatism, K max, irregularity index and higher order aberrations.[43] The mean demarcation line depth was at 316.92 ± 49.16 m (range: 260-367) which is close to the demarcation line observed following epi-off CXL.[43] The authors used all available modifications to enhance riboflavin penetration to the cornea; chemicals to disrupt the epithelium tight junctions, and mechanical disruption of the epithelium, increasing riboflavin concentration. Riboflavin without dextran was used and verification of riboflavin absorption into the stroma by slit-lamp examination was meticulously performed. Irrigation with isotonic BSS was performed before the UVA irradiation in order to avoid the shielding effect of riboflavin covering the epithelium.

We believe that the diverse results reported in the literature are in part due to insufficient riboflavin saturation in the stroma. Most articles report on half an hour instillation of riboflavin for transepithelial CXL. In the latter article the riboflavin was instilled until verification of stromal saturation by the appearance of yellow flare in the anterior chamber, a method not reported in many articles. Sheraz Daya reports instillation of riboflavin for 30 to 45 minutes (round table discussion on the future of CXL, January 2011. Available from: http://www.bmctoday.net/crstodayeurope/2011/01/article.asp?f=the-future-of-corneal-collagen-crosslinking) while Trattler reports instillation for 60 to 90 minutes till there is enough riboflavin penetration for epi-on CXL (round table discussion on the future of CXL, January 2011. Available from: http://www.bmctoday.net/crstodayeurope/2011/01/article.asp?f=the-future-of-corneal-collagen-crosslinking). We use the Medicross TE, a riboflavin (produced by Peschke Ltd, Germany) which has a concentration of >0.25% riboflavin with BAC, NaCl and no dextran. We needed 60 to 90 minutes of riboflavin instillation every 2 minutes to achieve corneal saturation and yellow flare in the anterior chamber, in most cases it took 90 minutes. Therefore, the instillation time is as important and not only the chemical formula.

NEW EMERGING TECHNIQUES

The issue of epithelium and CXL is a hot topic, in the near future we shall see new formulas of riboflavin and new ways of providing riboflavin, such as iontophoresis; electrically charged riboflavin is put into a cup on the cornea and an electric current drives the charged riboflavin into the stroma. There are no articles in the literature on this topic; however, various reports in congresses are promising for this technique in introducing riboflavin into the stroma without epithelium removal within few minutes. This may shorten stromal saturation time and spare patients the need for epithelial removal.

REFERENCES

1. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A—induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003;135(5):620-27.
2. Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: Long-term results. J Cataract Refractive Surg 2008;34(5):796-801.
3. Caporossi A, Baiocchi S, Mazzotta C, Traversi C, Caporossi T. Parasurgical therapy for keratoconus by riboflavin—ultraviolet type A rays induced cross-linking of corneal collagen: Preliminary refractive results in an Italian study. J Cataract Refractive Surg 2006;32(5):837-45.
4. Hafezi F, Mrochen M, Iseli HP, Seiler T. Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. J Cataract Refractive Surg 2009;35(4):621-24.
5. Bakke EF, Stojanovic A, Chen X, Drolsum L. Penetration of riboflavin and postoperative pain in corneal collagen

crosslinking: Excimer laser superficial versus mechanical full-thickness epithelial removal. J Cataract Refractive Surg 2009;35(8):1363-66.
6. Kymionis GD, Grentzelos MA, Karavitaki AE, Kounis GA, Kontadakis GA, Yoo S, et al. Transepithelial phototherapeutic keratectomy using a 213-nm solid-state laser system followed by corneal collagen cross-linking with riboflavin and UVA irradiation. J Ophthalmol 2010;2010:146543.
7. Kapasi M, Baath J, Mintsioulis G, Jackson WB, Baig K. Phototherapeutic keratectomy versus mechanical epithelial removal followed by corneal collagen crosslinking for keratoconus. Can J Ophthalmol 2012; 47(4):344-47.
8. Haque S, Simpson T, Jones L. Corneal and epithelial thickness in keratoconus: A comparison of ultrasonic pachymetry, Orbscan II, and optical coherence tomography. J Refract Surg 2006;22(5):486-93.
9. Reinstein DZ, Archer TJ, Gobbe M. Corneal epithelial thickness profile in the diagnosis of keratoconus. J Refract Surg 2009;25(7):604-10.
10. Kymionis G, Diakonis V, Coskunseven E, Jankov M, Yoo S, Pallikaris I. Customized pachymetric guided epithelial debridement for corneal collagen cross-linking. BMC Ophthalmol 2009;9(1):10.
11. Malhotra C, Shetty R, Kumar RS, Veluri H, Nagaraj H. No in vivo imaging of riboflavin penetration during collagen cross-linking with hand-held spectral domain coherence tomography. J Refract Surg 2012;28:776-80.
12. Seiler T, Hafezi F. Corneal cross-linking-induced stromal demarcation line. Cornea 2006;25(9):1057-59.
13. Kanellopoulos AJ. Long-term results of a prospective randomized bilateral eye comparison trial of higher fluence, shorter duration ultraviolet A radiation, and riboflavin collagen cross-linking for progressive keratoconus. Clin Ophthalmol 2012;6:97.
14. Koppen C, Vryghem JC, Gobin L, Tassignon MJ. Keratitis and corneal scarring after UVA/riboflavin cross-linking for keratoconus. J Refract Surg 2009;25(9):819.
15. Mazzotta C, Balestrazzi A, Baiocchi S, Traversi C, Caporossi A. Stromal haze after combined riboflavin-UVA corneal collagen cross-linking in keratoconus: In vivo confocal microscopic evaluation. Clin Experiment Ophthalmol 2007;35(6):580-82.
16. Eberwein P, Auw-Hädrich C, Birnbaum F, Maier PC, Reinhard T, et al. Corneal melting after cross-linking and deep lamellar keratoplasty in a keratoconus patient. Klinische Monatsblatter fur Augenheilkunde 2008;225(1):96.
17. Kanellopoulos AJ. Collagen cross-linking in early keratoconus with riboflavin in a femtosecond laser-created pocket: Initial clinical results. J Refract Surg 2009;25(11):1034-37.
18. Alió JL, Toffaha BT, Piñero DP, Klonowski P, Javaloy J, et al. Cross-linking in progressive keratoconus using an epithelial debridement or intrastromal pocket technique after previous corneal ring segment implantation. J Refract Surg 2011;27(10):737.
19. Kiliç A, Kamburoglu G, Akinci A. Riboflavin injection into the corneal channel for combined collagen crosslinking and intrastromal corneal ring segment implantation. J Cataract Refract Surg 2012 May;38(5):878-83. doi: 10.1016/j.jcrs.2011.11.041. Epub 2012 Mar 15.
20. Kolozsvári L, Nógrádi A, Hopp B, Bor Z. UV absorbance of the human cornea in the 240-to 400-nm range. Investigative Ophthalmol Visual Sci 2002;43(7):2165-68.
21. Hayes S, O'Brart DP, Lamdin LS, Doutch 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(4):657-61.
22. Samaras K, O'Brart DR, Doutch J, Hayes S, Marshall J, Meek KM. Effect of epithelial retention and removal on riboflavin absorption in porcine corneas. J Refract Surg 2009;25(9):771.
23. Baiocchi S, Mazzotta C, Cerretani D, Caporossi T, Caporossi A. Corneal crosslinking: Riboflavin concentration in corneal stroma exposed with and without epithelium. J Cataract Refract Surg 2009;35(5):893-99.
24. Bottós KM, Dreyfuss JL, Regatieri CV, Lima-Filho AA, Schor P, Nader HB, et al. Immunofluorescence confocal microscopy of porcine corneas following collagen cross-linking treatment with riboflavin and ultraviolet A. J Refract Surg 2008; 24(7):S715.
25. Wollensak G, Spoerl E, Wilsch M, Seiler T. Endothelial cell damage after riboflavin—ultraviolet-A treatment in the rabbit. J Cataract Refract Surg 2003;29(9):1786-90.
26. Spoerl E, Mrochen M, Sliney D, Trokel S, Seiler T. Safety of UVA-riboflavin cross-linking of the cornea. Cornea 2007;26(4):385-89.
27. Raiskup F, Spoerl E. Corneal cross-linking with hypo-osmolar riboflavin solution in thin keratoconic corneas. Am J Ophthalmol 2011;152(1):28-32.
28. Filippello M, Stagni E, O'Brart D. Transepithelial corneal collagen crosslinking: Bilateral study. J Cataract Refract Surg 2012 Feb;38(2):283-91.
29. Kissner A, Spoerl E, Jung R, Spekl K, Pillunat LE, Raiskup F. Pharmacological modification of the epithelial permeability by benzalkonium chloride in UVA/riboflavin corneal collagen cross-linking. Current Eye Res 2010;35(8):715-21.
30. Raiskup F, Pinelli R, Spoerl E. Riboflavin osmolar modification for transepithelial corneal cross-linking. Current Eye Res 2012;37(3):234-38.
31. Boxer Wachler BS, Pinelli R, Ertan A, Chan CCK, Baiocchi S, Mazzotta C, et al. Safety and efficacy of transepithelial crosslinking (C3-R/CXL). J Cataract Refract Surg 2010;36(1):186-89.
32. Aylin Kilic, Roberts CJ. Biomechanical and refractive results of transepithelial cross-linking treatment in keratoconic eyes. Int J Kerat Ect Cor Dis 2012;2(1):75-78.
33. Wollensak G, Iomdina E. Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. J Cataract Refract Surg 2009;35(3):540-46.
34. Samaras KE, Lake DB. Corneal collagen cross linking (CXL): A review. Int Ophthalmol Clin 2010;50(3):89-100.
35. Leccisotti A, Islam T. Transepithelial corneal collagen cross-linking in keratoconus. J Refract Surg 2010;26(12):942.
36. Cha SH, Lee JS, Oum BS, Kim CD. Corneal epithelial cellular dysfunction from benzalkonium chloride (BAC) in vitro. Clin Experiment Ophthalmol 2004;32(2):180-84.
37. Spadea L, Mencucci R. Transepithelial corneal collagen cross-linking in ultrathin keratoconic corneas. Clin Ophthalmol 2012;6:1785.
38. Filippello M, Stagni E, Buccoliero D, Bonfiglio V, Avitabile T. Transepithelial cross-linking in keratoconus patients. Confocal Anal Optometry Vision Sci 2012;89(10):e1-e7.
39. Caporossi A, Mazzotta C, Baiocchi S, Caporossi T, Paradiso AL. Transepithelial corneal collagen crosslinking for keratoconus:

Qualitative investigation by in vivo HRT II confocal analysis. Europ J Ophthalmol 2012 Jan;22 Suppl 7:S81-88.
40. Wollensak G, Iomdina E, Dittert DD, Herbst H. Wound healing in the rabbit cornea after corneal collagen cross-linking with riboflavin and UVA. Cornea 2007;26(5):600-05.
41. Al-Aqaba M, Calienno R, Fares U, Otri AM, Mastropasqua L, Nubile M, et al. The effect of standard and transepithelial ultraviolet collagen cross-linking on human corneal nerves: An ex vivo study. Am J Ophthalmol 2012;153(2):258-66.
42. Koppen C, Wouters K, Mathysen D, Rozema J, Tassignon MJ. Refractive and topographic results of benzalkonium chloride-assisted transepithelial crosslinking. J Cataract Refract Surg 2012 Jun;38(6):1000-05.
43. Magli A, Forte R, Tortori A, Capasso L, Marsico G, Piozzi E. Epithelium-off corneal collagen cross-linking versus transepithelial cross-linking for pediatric keratoconus. Cornea 2012 Nov 5 [Epub ahead of print].
44. Buzzonetti L, Gianni Petrocelli M. Transepithelial corneal cross-linking in pediatric patients: Early results. J Refract Surg 2012 Nov;28:763-67.
45. Touboul D, Efron N, Smadja D, Praud D, Florence Malet JC. Corneal confocal microscopy following conventional, transepithelial, and accelerated corneal collagen cross-linking procedure for keratoconus. J Refract Surg 2012 Nov;28: 769-75.
46. Stojanovic A, Chen X, Jin N, Zhang T, Stojanovic F, Raeder S, et al. Safety and efficacy of epithelium-on corneal collagen cross-linking using a multifactorial approach to achieve proper stromal riboflavin saturation. J Ophthalmol 2012;2012:498435. doi: 10.1155/2012/498435. Epub 2012 Jul 30.

ABOUT THE AUTHORS

Ramez Barbara

Department of Ophthalmology, Bnai Zion Medical Center; Hadassah Optimal Medical Center; The National Center for the Treatment of Keratoconus; Technion-Israel Institute of Technology, Haifa, Israel

Correspondence Address: Department of Ophthalmology, Bnai Zion Medical Center, 47 Golomb Street, Haifa-31048, Israel, Phone: +972524448687, Fax: +97249815911, e-mail: ramezborbara@gmail.com

Lamis Abdelaziz

Department of Ophthalmology, Harrogate District Hospital, Harrogate United Kingdom

Ankur Barua

Department of Ophthalmology, Manchester Royal Eye Hospital, Manchester, United Kingdom

Hanna Garzozi

Professor, Department of Ophthalmology, Bnai Zion Medical Center; Hadassah Optimal Medical Center; The National Center for the Treatment of Keratoconus; Technion-Israel Institute of Technology Haifa, Israel

Adel Barbara

Department of Ophthalmology, Hadassah Optimal Medical Center The National Center for the Treatment of Keratoconus, Haifa, Israel