Editorial

Reflections on the Controversies on Collagen Corneal Cross-linking for Arresting Keratoconus

Since the first publication on collagen corneal cross-linking (CXL) for arresting the progression of keratoconus (KC) by the Dresden group[1] several hundreds of publications were published most of them reporting positive results of CXL in arresting the progression of the disease, improvement of uncorrected visual acuity (UCVA) and best spectacle corrected visual acuity (BSCVA), reduction of astigmatism and of maximum keratometry reading (K max) In April 2016, the treatment was approved by the FDA after a controlled randomized study. The beneficial effect of the treatment was reported even after 10 years[2] corneal topography, and endothelial cell count (ECC).

Conventional Protocol (C-CXL)

As reported by the Dresden group, implies the removal of the central 8-9 mm of the epithelium(epi-off), instillation of riboflavin 0.1% with Dextran 20% every five minutes for half an hour until a strong yellow flare is seen by the slit lamp in the anterior chamber, then the cornea is irradiated by ultraviolet A (UVA) of 370 nm, 3 mW/cm^2 for half an hour at distance of 5 cm from the cornea, during the treatment BSS is instilled every 2 minutes and riboflavin every five minutes.[1] After the completion of the treatment a therapeutic contact lens (CL) is inserted and removed after full re-epithelization which takes between 3-4 days, antibiotic drops are instilled till full epithelization and lens removal, then steroids eye drops are instilled for one month. The effects of CXL is limited to the anterior 300 µm of corneal stroma, the riboflavin acts as a photosensitizer and photo protector, it reacts with oxygen species, triggering formation of of intra- and inter-fibrillary crosslinks.[3] A minimal corneal thickness of 400 um is recommended in order to avoid endothelial cells damage.[1]

Since the introduction of C-CXL it was challenged by alternative treatment protocols, which are:

Epithelium on Protocol (epi-on)

The epithelium is not removed, the aim is to avoid the pain and eye irritation after C-CXL and the complications related to epithelial removal such as keratitis, delayed epithelization, corneal infiltrates, perforation, anterior uveitis and transient corneal scaring.[4-6] The results reported in the literature regarding the effectiveness of epi-on CXL are controversial and even when effectiveness is reported the depth of CXL and the refractive changes as expressed by the reduction of astigmatism and K max are inferior to what is achieved by epi-off CXL.[7-12] The decreased effect is due to the absorption of UVA by the epithelium, the inability of the riboflavin to penetrated the tight junctions of the epithelium, although various forms of riboflavin and absorption enhancers were used. The effect in terms of stress and strain measurements after the treatment in porcine eyes was fifth of what is achieved with epi-off, 64 % increase in corneal rigidity versus a 320 % in CXL with epi-off[13] non–homogeneous uptake of riboflavin in the stroma was observed in partial grid-pattern epithelial removal.[14] Epi-on CXL is useful in cases in which epithelial removal is not desired, such as pediatric cases, uncooperative patients, and thin corneas.[15] Iontophoresis CXL (I-CXL) is used to enhance the absorption of riboflavin, the technique is efficient in halting the progression of KC, reduces the treatment time and improve the riboflavin diffusion.[16,17] Similar stiffness of corneas treated by I-CXL or C-CXL in rabbits was reported.[18]

Accelerated CXL (A-CXL)

Several new CXL devices offer high UVA irradiation intensity with different time settings exist in the market, the aim is to reduce the treatment time by reducing the exposure of the cornea to 10 minutes instead of 30 minutes and even to 5 and 3 minutes maintaing a total dose of 5.4 J/cm^2. According Bunsen-Roscoe law of reciprocity: Effect of photochemical or photo biological reaction is directly proportional to the total irradiation dose, irrespective of the time span over which the dose is administered. Does CXL work in this way on the cornea? This is true partially because the ex vivo results in porcine corneas show that the Bunsen Roscoe reciprocity law is only valid for illumination intensities up to 40 to 50 mW/cm^2 and illumination times of more than 2 min.[19] No significant difference was found between the rapid 9 minutes 10 mW/cm^2 = 5.4 J/cm^2 and standard 30 minutes at 3 mW/cm^2 = 5.4 J/cm^2 groups in the median of Young's modulus.[20] Decreased stiffening effect with increasing UV-A intensity was reported by[21] clinically

similar results to C-CXLwere reported[22], A-CXL is effective in stopping the progression of KC.[23-25] Effectiveness was reported in A-CXL even with epi-on.[26] On the other hand a decreased depth of the demarcation line (DL) after using the A-CXL protocol with 10 minutes of irradiation time and 9 mW/cm^2 was reported suggesting a reduced effectiveness.[27] The DL is an optical line that separates the treated from the untreated stroma and is supposed to reflect the depth of treatment.[28] Even with higher UV dose (6.6 J/cm) A-CXL showed a smaller topographic flattening effect than did the C-CXL.[29]

Acceleated Pulsed CXL

Oxygen is depleted early in the A-CXL, oxygen is necessary for the process of cross-linking.[30] Pulsed protocols with A-CXL delivering ultraviolet light with an on-off pattern could allow a better diffusion of the oxygen into the corneal stroma and subsequent deeper effect.[31] The DL was significantly deeper using pulsed rather than continuous light exposure[32], and deeper apoptotic effect was noted with pulsed A-CXL.[33] Advanced oxidation protein products (AOPP) levels indicative of oxygen concentration and reactive oxygen species were higher in accelerated pulsed CXL compered to C-CXL and A-CXL in rabbits eyes.[34] Lower levels of nitric oxide, indicative of oxidative stress, were in aqueous humor of rabbits eyes by pulsed accelerated CXL compared to A-CXL AND C-CXL.[35] Do these last two researches in rabbits eye indicate more efficacy and safety of accelerated pulsed CXL? can we apply this to human KC? Clinically improved postoperative functional outcomes compared with continuous light treatment was reported and deeper stromal penetration.[33] Deeper DL was reported in accelerated pulsed CXL versus A-CXL using energy of 30 mW/cm^2 and energy dose of 7.2 J/cm^2.[32]

Cretan Protocol

It is an epi off CXL, the epithelium is removed by excimer laser, 50 um of "epithelium" are removed by the laser the refractive results were reported to be superior to the Dresden protocol. The explanation to this beneficial effect of the laser ablation of the epithelium is that the epithelium is thinner at the KC apex and by this few microns of the stroma are removed, more astigmatism reduction is obtained and more improvement in visual acuity[36], another research reports no statistically significant difference at 12 and 24 months postoperatively in the Cretean protocol group compared to C-CXL, the Kmax at 6 months was flatter in the first group but this effect did not last.[37]

Costumized CXL

With up to 10 J/cm^2 centered on the maximum of the posterior float was reported and compared to C-CXL. Epithelial healing time, ΔKmax, and regularization index (RI) were significantly better in the customized CXL group the other examined parameters were similar in the two groups. Energies up to 15 J/cm^2 were used according to the topographic corneal curvature,with three levels of energy, no difference between the costumized CXL and the C-CXL was noted although the DL was deeper in the areas of the cornea radiated by higher energies.[38]

Why do we have different results in different clinical and basic science researches?

- KC is not the same in different patients and even in the same patient it is asymmetric, in shape, anterior and posterior elevation of the cone, thickness of the cornea, the distribution of the thickness in the cornea, the degree of corneal irregularity and the rate of progression.
- Is the patient an eye rubber, eye rubbing may cause or aggravate KC[39,40] certainly this will affect progression pre and post operatively.
- Different UVA energies and different beam profiles are used. Deeper CXL centrally than peripherally has been observed; this is probably due to the top-hat beam profile of UVA which does not compensate for natural corneal curvature, the UVA beam enters the cornea at a non-orthogonal angle in the corneal periphery.[41] There are no published papers that demonstrate the superiority of enhanced peripheral beam profile over the C-CXL, it is worth to mention that the change in the beam profile was accompanied with increased beam energy to 9 mW/cm^2 (IROC, Switzerland).
- Different riboflavin solutions, with and without dextran, hypertonic, isotonic and hypotonic, with different riboflavin concentrations 0.1% to 0.25% different ways of riboflavin instillation, with or without lid speculum, the patient in sitting or is in supine position, different intervals and durations of the instillation of riboflavin, is the corneal surface washed from riboflavin before starting the UVA irradiation? Riboflavin instilled during

treatment and when and how much?. In case of epi-on CXL what are the enhancers used? Benzalkolium chloride? Gentamycin? Tetracain? ecc, is alcohol added before in order to loosen the tight junctions of the epithelium?, Is some mechanical disruption of the epithelium added by sponge or by the Daya disrupter? (A metallic device for performing multiple holes in the epithelium).

- How much CXL each KC patient needs? We still do not know, In some cases a less effective treatment may be sufficient to halt the progression, and this may explain the effectiveness of epi-on CXL.
- The photochemically induced effect of CXL in the cornea cannot be evidenced directly by staining methods or microscopic techniques. However, CXL induces several changes to collagen-containing tissue, from which indirect signs of the cross-linking effect can be deduced.[42]
- Most of the published studies are not randomized controlled studies, only four were reported in the literature. In the published researches differences exist in measuring and reporting the outcomes.[43] Some researches report short term follow up such as 3 and 6 months.
- In *ex vivo* experiments we have the most of the up mentioned variables, added to them are the age of the cornea, the hydration of the cornea, the time from death to the experiment, is whole eye used or only cornea? How the cornea is cut for the stress-strain measurements before and after the treatment? What device is used to measure the stress and strain? What formulas are used to compensate for the non linear distension of the cornea during stress strain measurements?
- In the *in vivo* studies what are the animals used for the experiments? Rats, rabbits or porks? Can we apply to conclusions of these animals studies to KC eyes in humans?
- There is no consensus on the definition of KC progression, this may affect the indications for CXL and the evaluation of the post treatment results. According to Global Consensus on Keratoconus and Ectatic Diseases (2015) progression is a consistent change in at least two of the following parameters: steepening of the anterior corneal surface, steepening of the posterior corneal surface, and thinning and/or thinning or changes in the pachymetric rate of changes.[44] Here are some of the criteria considered as a sign of progression: increase in K max of 1.5 D or more and deterioration in UCVA of more than 0.2 log MAR[43], increase of 1 D in K max, patient's self-report of deteriorating of visual acuity, need for new contact lens fitting more than once in 2 years, increase of ≥1.00 D in manifest refraction's astigmatism or of of ≥0.50 D in manifest refraction spherical equivalent, Worsening of UCDVA/CDVA >0.50 Snellen lines, Increase of topographic symmetry index SAI/SI >1.00 D, Reduction of the thinnest point at corneal optical coherence tomography pachymetry ≥10 µm, or ≥5% decrease in the thinnest pachymetry in the preceding 6 months and reduction in corneal thickness (thinnest point) >10 microns lasting more than 6 months.[45]

CONCLUSION

The up mentioned tens of variables exist in every single CXL treatment and certainly affect the results of the treatment. The different parameters used to define progression and the different devices used to measure them and non interchangeability of these devices make the indication and the evaluation of CXL results extremely variable. What is the best strategy of treatment that is able to obtain the best clinical efficacy together with the maximum safety profile?, is still to be defined. There is a need for reliable and accurate techniques for measuring corneal biomechanical properties before and after treatment. Designed prospective randomized controlled trials comparing C-CXL and all the alternative procedures are required.

REFERENCES

1. Wollensak G, Spoerl E ST. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003;135:620–7.
2. Raiskup F, Theuring A, Pillunat LE, Spoerl E. Corneal collagen crosslinking with riboflavin and ultraviolet-A light in progressive keratoconus: Ten-year results. J Cart Refract Surg 2015;41:41–6.
3. Wollensak G. Crosslinking treatment of progressive keratoconus: new hope. Curr Opin Ophthalmol 2006;17(4):356–60.
4. Koller T, Mrochen M, Seiler T. Complication and failure rates after corneal crosslinking. J Cataract Refract Surg 2009;35(8):1358–62.
5. Yakov Goldich, Uri Elbaz DSR. Anterior Uveitis after Collagen Cross-linking for Keratoconus. Int J Kerat Ect Cor Dis 2015;4(3):110–4.
6. Demirci G, Ozdamar A. Case of Corneal Perforation as a Complication after Uneventful CXL without Infection Int J Keratoconus Ectatic Corneal Dis J Kerat Ect Cor Dis. 22(33).
7. Raiskup F, Hillen M. Corneal Cross-Linking can halt the Progression of Keratoconus, but what is the Best Approach. Int J Keratoconus Ectatic Corneal Dis 2015;4(2):47–51.

8. Zhang Z-Y, Zhang X-R. Efficacy and safety of transepithelial corneal collagen crosslinking. J Cataract Refract Surg [Internet] 2012 Jul [cited 2012 Oct 12];38(7):1304; author reply 1304-5.
9. 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. Eur J Ophthalmol [Internet] 2012 Jan [cited 2012 Oct 27];22 Suppl 7:S81-8.
10. Barbara R, Abdelaziz L, Barua A, Garzozi H, Barbara A. Collagen Corneal Cross-linking and the Epithelium. Int J Keratoconus Ectatic Corneal Dis Ep Int J Kerat Ect Cor Dis 2012;1(3):179-184.
11. Leccisotti A, Islam T. Transepithelial corneal collagen cross-linking in keratoconus. J Refract Surg (Thorofare, NJ 1995) 2010;26(12):942.
12. Stojanovic A, Chen X, Jin N, et al., "Safety and Efficacy of Epithelium-On Corneal Collagen Cross-Linking Using a Multifactorial Approach to Achieve Proper Stromal Riboflavin Saturation," Journal of Ophthalmology, vol. 2012, Article ID 498435, 8 pages, 2012.
13. Wollensak G, Iomdina E. Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. J Cataract Refract Surg 2009;35(3):540-6.
14. Samaras K, O'brart DP, Doutch J, Hayes S, Marshall J, Meek KM. Effect of epithelial retention and removal on riboflavin absorption in porcine corneas. J Refract Surg [Internet] 2009 Sep 1 [cited 2017 Mar 3];25(9):771–5.
15. Filippello M, Stagni E, O'Brart D. Transepithelial corneal collagen crosslinking: bilateral study. J Cataract Refract Surg 2012 Feb;38(2):283-91.
16. Vinciguerra P, Randleman JB, Romano V, Legrottaglie EF, Rosetta P, Camesasca FI, et al. Transepithelial Iontophoresis Corneal Collagen Cross-linking for Progressive Keratoconus: Initial Clinical Outcomes. J Refract Surg [Internet] 2014 Nov 1 [cited 2017 Mar 3];30(11):746–53.
17. Vinciguerra P, Romano V, Rosetta P, Legrottaglie EF, Kubrak-Kisza M, Azzolini C, et al. Iontophoresis-Assisted Corneal Collagen Cross-Linking with Epithelial Debridement: Preliminary Results. Biomed Res Int [Internet] 2016 [cited 2017 Mar 3];2016:1–5.
18. Cassagne M, Laurent C, Rodrigues M, Galinier A, Spoerl E, Galiacy SD, et al. Iontophoresis Transcorneal Delivery Technique for Transepithelial Corneal Collagen Crosslinking With Riboflavin in a Rabbit Model. Investig Opthalmology Vis Sci [Internet] 2016 Feb 17 [cited 2017 Mar 18];57(2):594.
19. Wernli J, Schumacher S, Spoerl E, Mrochen M, B. C, GR. S. The Efficacy of Corneal Cross-Linking Shows a Sudden Decrease with Very High Intensity UV Light and Short Treatment Time. Investig Opthalmology Vis Sci [Internet] 2013 Feb 12 [cited 2017 Mar 7];54(2):1176.
20. Schumacher S, Oeftiger L, Mrochen M. Equivalence of Biomechanical Changes Induced by Rapid and Standard Corneal Cross-linking, Using Riboflavin and Ultraviolet Radiation. Investig Opthalmology Vis Sci [Internet] 2011 Nov 24 [cited 2017 Mar 7];52(12):9048.
21. Hammer A, Richoz O, Mosquera SA, Tabibian D, Hoogewoud F, Hafezi F. Corneal Biomechanical Properties at Different Corneal Cross-Linking (CXL) Irradiances. Investig Opthalmology Vis Sci [Internet] 2014 May 2 [cited 2017 Mar 15];55(5):2881.
22. Tomita M, Mita M, Huseynova T. Accelerated versus conventional corneal collagen crosslinking. J Cataract Refract Surg [Internet] 2014 Jun [cited 2017 Mar 7];40(6):1013–20.
23. Alnawaiseh M, Rosentreter A, Böhm MRR, Eveslage M, Eter N, Zumhagen L. Accelerated (18 mW/cm^2) Corneal Collagen Cross-Linking for Progressive Keratoconus. Cornea 2015;34(11):1427–31.
24. Elbaz U, Shen C, Lichtinger A, Zauberman NA, Goldich Y, Chan CC, et al. Accelerated (9-mW/cm2) Corneal Collagen Crosslinking for Keratoconus—A 1-Year Follow-up. Cornea [Internet] 2014 Aug [cited 2017 Mar 15];33(8):769–73.
25. Bozkurt E, Ozgurhan EB, Akcay BIS, Kurt T, Yildirim Y, Günaydin ZK, et al. Refractive, Topographic, and Aberrometric Results at 2-Year Follow-Up for Accelerated Corneal Cross-Link for Progressive Keratoconus. J Ophthalmol [Internet] 2017 [cited 2017 Mar 18];2017:1–6.
26. Piñero DP, Artola A, Ruiz-Fortes P, Soto-Negro R, Pérez-Cambrodi RJ. Clinical Outcomes at 1 Year following Corneal Ectasia Treatment with Accelerated Transepithelial Cross-linking. Int J Keratoco Ectatic Corneal Dis 2016;(3):93–6.
27. Kymionis GD, Tsoulnaras KI, Grentzelos MA, Liakopoulos DA, Tsakalis NG, Blazaki S V., et al. Evaluation of Corneal Stromal Demarcation Line Depth Following Standard and a Modified-Accelerated Collagen Cross-linking Protocol. Am J Ophthalmol [Internet] 2014 Oct [cited 2017 Mar 3];158(4):671–675.e1.
28. Spadea L, Tonti E, Vingolo E. Corneal stromal demarcation line after collagen cross-linking in corneal ectatic diseases: a review of the literature. Clin Ophthalmol [Internet] 2016 Sep [cited 2017 Mar 3];Volume 10:1803–10.
29. Choi M, Kim J, Kim EK, Seo KY, Kim T. Comparison of the Conventional Dresden Protocol and Accelerated Protocol With Higher Ultraviolet Intensity in Corneal Collagen Cross-Linking for Keratoconus. Cornea [Internet] 2017 Feb 22 [cited 2017 Mar 18];1.
30. Richoz O, Hammer A, Tabibian D, Gatzioufas Z, Hafezi F. The Biomechanical Effect of Corneal Collagen Cross-Linking (CXL) With Riboflavin and UV-A is Oxygen Dependent. Transl Vis Sci Technol [Internet] 2013 Nov [cited 2017 Mar 4];2(7):6.
31. Santhiago MR. Accelerated Corneal Cross-linking: We Must Acquire Knowledge as Fast. J Refract Surg [Internet] 2016 Jun 1 [cited 2017 Mar 12];32(6):362–3.
32. Moramarco A, Iovieno A, Sartori A, Fontana L. Corneal stromal demarcation line after accelerated crosslinking using continuous and pulsed light. J Cataract Refract Surg [Internet] 2015 Nov [cited 2017 Mar 4];41(11):2546–51.
33. Mazzotta C, Traversi C, Caragiuli S, Rechichi M. Pulsed vs continuous light accelerated corneal collagen crosslinking: in vivo qualitative investigation by confocal microscopy and corneal OCT. Eye (Lond) 2014;28(10).

34. Turkcu UO, Yuksel N, Novruzlu S, Yalinbas D, Bilgihan A, Bilgihan K. Protein Oxidation Levels After Different Corneal Collagen Cross-Linking Methods. Cornea [Internet] 2016 Mar [cited 2017 Jul 8];35(3):388–91.
35. Yuksel N, Ozel-Turkcu U, Yalinbas D, Novruzlu S, Bilgihan A, Bilgihan K. Comparison of Aqueous Humor Nitric Oxide Levels After Different Corneal Collagen Cross-Linking Methods. Curr Eye Res [Internet] 2016 Dec 23 [cited 2017 Jul 8];41(12):1539–42.
36. Kymionis GD, Grentzelos M a, Kounis G a, Diakonis VF, Limnopoulou AN, Panagopoulou SI. Combined transepithelial phototherapeutic keratectomy and corneal collagen cross-linking for progressive keratoconus. Ophthalmology [Internet] 2012 Sep [cited 2012 Oct 27];119(9):1777–84.
37. Comparison of Combined Transepithelial Phototherapeutic Keratectomy and Mechanical Debridement During Corneal Cross-linking. J Refract Surg @BULLET [Internet] 2017 [cited 2017 May 19];33(4).
38. Mazzotta C, Moramarco A, Traversi C, Baiocchi S, Iovieno A, Fontana L. Accelerated Corneal Collagen Cross-Linking Using Topography-Guided UV-A Energy Emission: Preliminary Clinical and Morphological Outcomes. J Ophthalmol [Internet] 2016 [cited 2017 May 19];2016:1–10.
39. Gatinel D. Eye Rubbing, a Sine Qua for Keratoconus. Int J Keratoconus Ectatic Corneal Dis 2016;(Jan-April):6–12.
40. Elizabeth Hawkes MAN. Eye Rubbing and Keratoconus: A Literature Review. Cor Dis 2014;3(3):118–21.
41. Kymionis GD, Grentzelos MA, Plaka AD, Tsoulnaras KI, Diakonis VF, Liakopoulos DA, et al. Correlation of the Corneal Collagen Cross-Linking Demarcation Line Using Confocal Microscopy and Anterior Segment Optical Coherence Tomography in Keratoconic Patients. Am J Ophthalmol [Internet] 2014 Jan [cited 2017 Mar 3];157(1):110–115.e1.
42. Raiskup F, Spoerl E. Corneal Crosslinking with Riboflavin and Ultraviolet A. I. Principles. Ocul Surf [Internet] 2013 Apr [cited 2017 Jul 7];11(2):65–74.
43. Sykakis E, Karim R, Evans JR, Bunce C, Amissah-Arthur KN, Patwary S, et al. Corneal collagen cross-linking for treating keratoconus. In: Hamada S, editor. Cochrane Database of Systematic Reviews [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 2015 [cited 2017 Jul 2]. p. CD010621.
44. Gomes JA, Tan D, Rapuano CJ, Belin MW, Ambrósio R Jr, Guell JL, Malecaze F, Nishida K, Sangwan VS. Global consensus on keratoconus and ectatic diseases. Cornea 2015 Apr;34(4):359-69.
45. Galvis V, Tello A, Ortiz AI, Escaf LC. Patient selection for corneal collagen cross-linking: an updated review. Clin Ophthalmol [Internet] 2017 [cited 2017 Jul 2];11:657–68.

Adel Barbara MD, MRCOphth
Medical Director at IVISION, Refractive Surgery center, Haifa, Israel
Medical Director at the Keratoconus Treatment Center, Haifa, Israel