Corneal Cross-linking for the Treatment of Keratoconus: Laboratory Science, Clinical Effect and the Potential Impact to Eye Banking in the United States

Tyler A. Hall, M.D., Alan Blake, CEBT, Kyle Mavin, CEBT

Keratoconus is a common bilateral corneal disorder characterized by corneal thinning, weakness and cone formation. Approximately 1 in 2000 persons or roughly 160,000 people in the United States are affected.[1] As the disorder progresses, vision becomes blurred such that roughly one in five affected individuals require corneal transplantation to restore functional vision. Corneal cross-linking (CXL) is a relatively new procedure developed to treat keratoconus by strengthening the cornea with the aim of preventing progression and the need for corneal transplantation. Since the initial published findings in 2003,[2] CXL has gained worldwide acceptance as the standard of care treatment for progressive keratoconus except in the United States (U.S.) where U.S. Food and Drug Administration (FDA) approval was lacking until April 18, 2016. With FDA approval, subsequent widespread U.S. adoption of CXL is expected with the resultant impact to American eye banking unknown.

Herein the basic and clinical science of CXL will be reviewed. Additionally, the impact CXL has had on European eye banking will be reviewed with emphasis as to the potential impact CXL may have on the American eye banking industry.

FUNDAMENTAL PRINCIPLES OF CROSS-LINKING

Despite variations in CXL methods, all retain the basic concept of saturating the corneal stroma with riboflavin (vitamin B^2) followed by exposure to ultraviolet light (UVA).[2] The corneal epithelium must be removed or disrupted to allow riboflavin to penetrate into the corneal stroma. The combination of riboflavin and UVA generates free oxygen radicles which facilitate the creation of cross-links between and within collagen fibers.[3] These cross-links are thought to play the major role in the corneal strengthening effect of CXL which has been verified through laboratory and clinical research studies.

LABORATORY RESEARCH FINDINGS

The idea of inducing cross-links within the cornea to produce stiffening and halt the progression of keratoconus was developed by Theo Seiler MD, PhD in the late 1990's at the University of Dresden in Germany. Laboratory studies conducted with porcine and rabbit corneas found CXL increased collagen fiber diameter, mechanical rigidity, and resistance to hydrothermal shrinkage.[4-6]

The combination of riboflavin and UVA directly damages DNA creating a cytotoxic effect to keratocytes in the UVA exposed portions of the stroma. Safety parameters have been developed to limit both keratocyte and endothelial toxicity.[7-8] Based upon UVA penetration of the stroma, Dr. Seiler and colleagues proposed the following treatment protocol to limit full thickness keratocyte death and endothelial damage.[7-8] This treatment protocol has commonly become known as the Dresden Protocol.

Dresden Protocol

1. Removal of the central 7-8 millimeters of corneal epithelium after topical anesthesia.
2. Application of 0.1% riboflavin solution (0.1% riboflavin-5-phosphate in 20% dextran solution) to the exposed corneal stroma for 30 minutes to achieve stromal saturation before UVA exposure.

Author Affiliations: Alabama Eye Bank, University of Alabama at Birmingham, Department of Ophthalmology

3. Irradiation of the cornea for 30 minutes from a 370 nm UVA (3 mW/cm^2) light located 1 cm from the cornea for a total power of 5.4 J/cm^2.
4. Continued application of the 0.1% riboflavin solution at five minute intervals during irradiation.
5. Bandage contact lens and antibiotic until re-epithelialization.

Variations in the Dresden Protocol have been introduced to avoid epithelial removal and decrease irradiation time although it remains the standard against which variations in CXL procedures are compared.

CLINICAL RESEARCH FINDINGS

Effects

After establishing laboratory safety and efficacy, CXL was first performed in humans by Dr. Seiler and colleagues in 1998 and found to be effective in halting the progression of keratoconus and decreasing keratometry values.[2] During a follow-up period ranging from two to four years, no cross-linked eyes experienced progression, visual acuity improved in 15 of 22 eyes and a two diopter reduction in maximal keratometry (Kmax) values occurred in 16 of 22 eyes.[2] These findings have been reproduced in numerous studies of both short (< 1 year follow-up)[9-12] and long term duration (> 1 year follow-up).[13-16] Long term studies support the permanence of CXL with a slow continued corneal flattening effect. [13-16]

The in vivo structural effects of CXL have been studied by biomicroscopy and in vivo confocal microscopy. Soon after the procedure, corneal haze extending below the epithelium to roughly 300um deep in the stroma is visible and probably corresponds to areas of corneal cross-linking and collagen compaction.[17] Corneal haze typically peaks at one month and plateaus at three months with gradual resolution thereafter.[17] Extensive keratocyte loss occurs in areas of cross-linking that is filled with focal edema.[17] Diffuse edema occurs adjacent to cross-linked areas with the border between focal and diffuse edema being seen on biomicroscopy as a stromal demarcation line located approximately 300um deep in the stroma.[18] Keratocyte repopulation begins two months post-CXL treatment and continues until keratocyte density returns to baseline at roughly twelve months.[17] In vivo confocal imaging also demonstrates loss of the superficial corneal nerve fiber plexus which begins to regenerate at one month and is complete by six months along with the return of full corneal sensation.[17] Similar to electron microscopy studies, in vivo microscopy found collagen fiber diameter to increase with reorganization of fibrils into a more parallel lamellar structure similar to non-keratoconic corneas.[18]

FDA Study Results

Avedro, Inc. received FDA approval to treat progressive keratconus with its CXL system based on a new drug submission (NDA) and data from two prospective, randomized, placebo-controlled, twelve month clinical trials conducted in the United States on eyes with progressive keratoconus.[19] Study one enrolled 58 patients with progressive keratoconus and Study two enrolled 147 patients with progressive keratoconus.[19] In each study, patients had one eye designated as the study eye and were randomized to receive one of two study treatments (CXL or sham) in their study eye.[19] At three months, patients could choose to have the sham study eye and or the non-study eye treated with CXL which resulted in roughly 56% and 89% of these eyes being treated at month three and month six, respectively.[19]

Cross-linked eyes showed Kmax progressively decrease from month three through month twelve while untreated fellow eyes showed a progressive increase.[19] At month twelve, cross-linked eyes averaged Kmax reduction of 1.4 diopters in Study one and 1.7 diopters in Study two, while the untreated fellow eyes averaged an increase of 0.5 D in Study one and 0.6 D in Study two.[19] The difference in mean change from baseline Kmax in cross-linked versus non cross-linked eyes with 95% confidence intervals was -1.9 diopters (-3.4, -0.3) in Study one and -2.3 diopters (-3.5, -1.0) D in Study two.[19]

LIMITS AND SAFETY OF CORNEAL CROSS-LINKING

Treatment Failure

Failure of CXL to stabilize keratoconus is typically defined as Kmax progression of more than one diopter occurring six months after crosslinking. Although not a complication per se, treatment failure occurs in 8 to 10% of treatment patients.[20] Risk factors for treatment failure include age less than 18 years, female gender, and Kmax of more than 58.0 D.[21-22]

Known Complications

The safety of cross-linking is composed of known and unknown complications. The vast majority of known complications result from epithelial removal. Shalchi et al. conducted an excellent systemic review of forty-five CXL

studies to report on the safety of CXL used to treat progressive keratoconus and found the reported rate of most complications to be low.[20]

TABLE 1. Complications after Corneal Crosslinking for Keratoconus[20]

Complication	*Number of Studies*	*Median Percentage*	*Range*
Microbial keratitis	7/45	0%	0-3%
Corneal scarring	5/45	0%	0-6%
Stromal edema			
6wks	6/45	17.5%	0-70%
12m	1/45	1.7%	-
Sterile infiltrates	6/45	2.5%	2-4%
Stromal haze	12/45	9.8%	0-100%
Loss of CDVA*	6/45	12.4%	0-27%

**Corrected distance visual acuity*

Stromal haze after CXL is responsive to topical steroid treatment, gradually fades with time and is rarely clinically significant. The reported effect of CXL on endothelial cell count is variable with two of fourteen studies reporting no change, nine studies reporting a reduction (median: -24 cells/mm^2; range -131 to -12 cells/mm^2) and two studies reporting an increase (median: 29.5 cells/mm^2; range 4-55 cells/mm^2).[20]

Unknown Complications

As with all new medical treatments, unknown safety issues may become apparent with time. It seems reasonable to monitor patients for limbal epithelial stem cell damage, ocular surface neoplasia, corneal scarring and progressive visually significant corneal flattening as occurred with radial keratometry.

ALTERNATIVE CROSS-LINKING PROTOCOLS

The two main categories of variation to the Dresden Protocol being investigated are avoidance of epithelial removal (epithelium-on or transepithelial CXL) and shortening of the irradiation time (accelerated CXL) in the hopes of making CXL safer, faster, and more comfortable to the patient. To avoid epithelial removal, epithelium-on CXL methods attempt to disrupt the integrity of the corneal epithelial layers either chemically with benzalkonium chloride, ethylenediaminetetraacetic acid or tetracaine eye drops or by iontophoresis to facilitate penetration of riboflavin into the corneal stroma. Compared to the standard Dresden Protocol, epithelium-on techniques have an improved safety and comfort profile primarily due to preservation of the epithelium. Unfortunately, none of the epithelium-on CXL techniques have shown the same clinical efficacy as the Dresden Protocol although improvements are being made.[23]

Accelerated CXL involves varying either the light intensity and/or the treatment time with the goal of keeping the total power to 5.4 J/cm^2. Many combinations of these parameters are being investigated with clinical efficacy similar to the standard Dresden Protocol.[23]

OTHER CLINICAL USES OF CROSS-LINKING

Infectious Keratitis

The antimicrobial effect of CXL and its ability to increase corneal resistance to enzymatic digestion make CXL a therapeutic option in treating infectious keratitis.[18] The combination of riboflavin and UVA directly damages DNA creating an antimicrobial effect that is primarily effective against bacteria.[18] Cross-linking exhibits some clinical effectiveness against fungal keratitis with mixed results against *Acanthamoeba*.[23] A history of herpetic keratitis is a contraindication to CXL as it may activate the *Herpes simplex* virus.[23] The effect of cross-linking is primarily limited to the anterior 300um of stroma making its effectiveness against deeper infections unlikely.[18]

Corneal Edema

The compaction of collagen and regularization of lamellae has been explored as a treatment for corneal edema. A decrease in stromal edema in both Fuchs' dystrophy and pseudophakic corneal edema has been demonstrated after CXL; however, this effect fades by 3 months.[18]

EUROPEAN EYE BANKING EXPERIENCE

A primary aim of CXL is to reduce the need for keratoplasty. As such, studying the penetrance of CXL into the European market where it was first introduced and its effect on the number of keratoplasty surgeries is of considerable interest to American eye banking. By all accounts, CXL has been a great success. After publication of the first clinical study of CXL in 2003, CXL received CE Mark European Union approval in 2006, the equivalent of FDA approval.[24] Soon after, CXL became the standard of care treatment for progressive keratoconus in Europe and worldwide with an estimated 200,000 corneas having already been treated.[25] The definitive effect of this widespread adoption on the number of keratoplasties performed for keratoconus remains unknown; although, most studies indicate a decrease in the number of keratoplasties following the introduction of CXL into their respective markets.[26-29] To date, only one published study has specifically examined this issue and found a 53% reduction in keratoplasty for keratoconus occurred during the same time period that CXL was introduced (2005 to 2014).[26] Whether the reduc-

tion in keratoplasty was due solely to CXL or other factors such as improved contact lens design or other procedures is unknown. The possible effect CXL has had on the number of keratoplasty surgeries might also be gleaned from the incidence of keratoplasty for keratoconus as a whole. Robert et al. reported in the province of Quebec, Canada, the percent of the total keratoplasty surgeries (penetrating and anterior lamellar keratoplasty) performed for keratoconus dropped from 14% to 11% during the 2000 to 2008 and 2009 to 2011 time periods, respectively.[27] Annually, the Eye Bank Association of America (EBAA) publishes statistics of reporting international eye banks on the incidence of keratoplasty. From 2011 to 2015, EBAA reports show a 34% drop in the number of keratoplasties (penetrating keratoplasty and anterior lamellar keratoplasty) performed internationally for keratoconus.[30] Moreover, a recently published Italian study found the total number of keratoplasties (penetrating and anterior lamellar keratoplasty) for keratoconus decreased by 27% from 2002 to 2008.[28] During a similar time period in the United States (2006 to 2015) where CXL was not available but other keratoconus treatment methods such as contact lens and intracorneal ring segments were available, the incidence of keratoplasty for keratoconus increased by 38% (n = 4849 in 2006, n = 6679 in 2015).[30]

POTENTIAL IMPACT TO AMERICAN EYE BANKING

Given the worldwide success of CXL, it seems reasonable to expect widespread adoption by U.S. cornea trained ophthalmologists. Utilization by general ophthalmologists is more difficult to predict but may occur as the procedure becomes more commonplace. Given the European experience, the number of keratoplasty procedures for keratoconus will probably begin decreasing in the year 2019, or roughly three years after the introduction of CXL into the U.S. market. Based upon the decrease in keratoconus keratoplasty elsewhere in the world (27% to 53%), a 30% decrease in the United States seems reasonable which represents roughly 1670 fewer corneas being utilized for keratoconus keratoplasty. This decrease is speculative but probable. Alternatively, it is conceivable that no change in keratoplasty rates will be observed because while patients may obtain treatment earlier in their disease process, whether those patients might have ultimately needed transplantation is unknown. Moreover, some patients with the most advanced disease have inadequate response to CXL or they cannot be treated secondary to the concern for endothelial damage related to increased UVA penetrance of their thin corneas.

Cross-linking is not currently a covered benefit of Medicare, Medicaid, or private medical insurance with the expectation that coverage might begin in 2019 or roughly three years after FDA approval (personal communication with Avedro). Until then, CXL will remain an out-of-pocket expense for patients and the ability and willingness of patients to pay for this procedure is unknown.

Cross-linked corneas may start appearing in the donor pool as early as one year after FDA approval. The current EBAA donor risk assessment interview and medical record review is robust enough to identify cross-linked patients and does not need to be adjusted. Beginning January 1, 2017, eye banks may want to begin having technicians check for a stromal demarcation line located roughly 300um deep in the stroma during slit lamp evaluation. If slit lamp evaluation proves equivocal, optical coherence tomography may be of benefit in evaluating for this finding. Corneas identified as having been cross-linked should be excluded from the potential donor pool for anterior and penetrating keratoplasty intended for optical purposes as the primary indications for CXL are anterior stromal diseases that may become recurrent in the recipient despite previous CXL. As cross-linked corneal tissue should be stronger than non-crosslinked corneas, utilization for tectonic and therapeutic keratoplasty seems reasonable. Recommendations regarding endothelial keratoplasty are more difficult to propose. If specular microscopy indicates adequate endothelial health, there is no current reason to expect cross-linked corneas, if successfully prepared, would not perform similar to non-treated corneas when utilized for endothelial keratoplasty. Regarding Descemet stripping automated endothelial keratoplasty (DSAEK), the increased mechanical rigidity of the corneal stroma imparted by CXL might make donor preparation cuts more challenging and unpredictable. However, if specular microscopy indicates adequate endothelial cell health, DSAEK donor preparation may be attempted. Regarding Descemet membrane endothelial keratoplasty (DMEK), cross-linked corneas should be expected to behave as non-treated corneas during eye bank processing and need not be excluded for endothelial keratoplasty.

CONCLUSIONS

Corneal cross-linking represents an exciting new treatment for keratoconus. Although newly approved in the United States, over ten years of experience by ophthalmologists outside the U.S. indicate CXL is safe, effective, and permanent. Widespread adoption of CXL by U.S. corneal surgeons is expected with a likely decrease in keratoplasty for keratoconus beginning in 2019.

REFERENCES

1. National Eye Institute. https://nei.nih.gov/health/cornealdisease/#12. Accessed June 28, 2016.
2. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol. 2003. 620-627.
3. Mastropasqua Leonardo. Collagen cross-linking: when and how? A review of the state of the art of the technique and new perspectives. Eye and Vision. 2015. 2:19.
4. Wollensak G, Wilsch M, Spoerl E, Seiler T. Collagen fiber diameter in the rabbit cornea after collagen crosslinking by riboflavin/UVA. Cornea. 2004. 503-7.
5. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced cross-linking. J Cataract Refract Surg. 2003. 1780-5.
6. Spoerl E, Wollensak G, Dittert DD, Seiler T. Thermomechanical behavior of collagen-cross-linked porcine cornea. Ophthalmologica. 2004. 136-40.
7. Wollensak G, Sporl E, Reber F, Pillunat L, Funk R. Corneal endothelial cytotoxicity of riboflavin/UVA treatment in vitro. Ophthalmic Res. 2003. 324-8.
8. Wollensak G, Spoerl E, Wilsch M, Seiler T. Keratocyte apoptosis after corneal collagen cross-linking using riboflavin/UVA treatment. Cornea. 2004. 43-9.
9. Hersh PS, Greenstein SA, Fry KL. Corneal collagen crosslinking for keratoconus and corneal ectasia: One-year results. J Cataract Refract Surg. 2011. 149-60.
10. Kymonis GD, Portaliou DM, Diakonis VF, Kounis GA, Panagopoulou SI, Grentzelos MA. Corneal collagen cross-linking with riboflavin and ultraviolet-A irradiation in patients with thin corneas. Am J Ophthalmol. 2012. 24-8.
11. Asri D, Touboul D, Fournie P, Malet F, Garra C, Gallois A, et al. Corneal collagen crosslinking in progressive keratoconus: multicenter results from the French National Reference Center for Keratoconus. J Cataract Refract Surg. 2011. 2137-43.
12. Kranitz K, Kovacs I, Mihaltz K, Sandor GL, Knorz MC, ,Nemeth J, et al. Corneal changes in progressive keratoconus after cross-linking assessed by Scheimpglug camera. J Refrt Surg. 2012. 645-9.
13. Vinciguerra R, Romano MR, Camesasca FL, Azzolini C, Trazza S, Morenghi E, et al. Corneal cross-linking as a treatment for keratoconus: four-year morphologic and clinical outcomes with respect to patient age. Ophthalmology. 2013. 908-16.
14. Hashemi H, Seyedian MA, Miraftab M, Fotouhi A, Asagari S. Corneal collagen cross-linking with riboflavin and ultraviolet a irradiation for keratoconus: long-term results. Ophthalmology. 2013. 1515-20.
15. Kymonis GD, <Grentzelos MA, Liakopoulous DA, Parakevopoulos TA, Klados NE, Tsoulnaras KL, et al. Long-term follow-up of corneal collagen cross-linking for keratoconus-the Cretan study. Cornea. 2014. 1071-9.
16. Lang SJ Messmer EM, Geerling G, Mackert MJ, Brunner T, Dollak S, et al. Prospective, randomized, double-blind trial to investigate the efficacy and safety of corneal cross-linking to halt the progression of keratoconus. BMC Ophthalmol. 2015. 78.
17. Mazzotta C, Hafezi F, Kymionis F, Caragiuli S, Jacob S, Traversi C, Barabino S, Randleman B. In Vivo Confocal Microscopy after Corneal Collagen Cross-Linking. The Ocular Surface. 2015.
18. Sorkin N, Varssano D. Corneal collagen crosslinking: a systemic review. Ophthalmologica. 214. 10-27.
19. Avedro, Inc. http://avedro.com/press-releases/avedro-receives-fda-approval/. Accessed July 3, 2016.
20. Shalchi Z, Wang X, Nanavaty MA. Safety and efficacy of epithelium removal and transepithelial corneal collagen crosslinking for keratoconus. Eye. 2015. 15-29.
21. Koller T, Mrochen M, Seiler T. Complications and failure rates after corneal crosslinking. J Cataract Refract Surg. 2009. 1358-62.
22. McAnena L, O'Keefe M. Corneal collagen crosslinking in children with keratoconus. J AAPOS. 2015. 228-32.
23. Randleman JB, Khandelwal SS, Hafezi F. Corneal cross-linking. Survey of Ophthalmology. 2015. 509-23.
24. Kent, C. Cross-linking: Asking the tough questions. Four surgeons discuss the risks and address the unanswered questions still associated with this procedure. http://www.reviewofophthalmology.com/content/i/1777/c/32329/. Accessed June 21, 2016.
25. Trattler W. Corneal cross-linking (CXL): For keratconus treatment and LASIK-related corneal ectasia. http://www.allaboutvision.com/conditions/corneal-crosslinking.htm. Accessed June 21, 2016.
26. Sandvik GF, Thorsrud A, Raen M, Ostern A, Soethre M, Drolsum L. Does corneal collagen cross-linking reduce the need for keratoplasties in patients with keratoconus? Cornea. 2015. 991-995.
27. Robert MC, Choronzey ME, Lapointe J, Meunier LPG, Harissi-Dagher M, Germain M, Mabon M, Brunette I. Evolution of corneal transplantation in the province of Quebec from 2000 to 2011. Cornea. 2015. 880-887.
28. Frigo AC et al. Corneal transplantation activity over 7 years: changing trends for indications, patient demographics and surgical techniques from the corneal transplant epidemiological study (CORTES). Transplantation Proceedings. 2015. 528-535.
29. Lang SJ, Bischoff M, Bohringer D, Seitz B, Reinhard T. Analysis of the changes in keratoplasty indications and preferred techniques. PLoS ONE. E112696.
30. Eye Banking Statistical Reports. Eye Bank Association of America.