Published in final edited form as:

Exp Eye Res. 2014 August ; 125: 114–117. doi:10.1016/j.exer.2014.06.001.

BAC-EDTA transepithelial riboflavin-UVA crosslinking has greater biomechanical stiffening effect than standard epithelium-off in rabbit corneas

Andre A. M. Torricelli, MD^{1,3,*}, Matthew R. Ford, BS^{1,*}, Vivek Singh, PhD⁵, Marcony R. Santiago, MD, PhD^{3,4}, William J. Dupps Jr., MD, PhD^{1,2}, and Steven E. Wilson, MD¹

¹Cole Eye Institute, Cleveland Clinic, Cleveland, Ohio

²Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Ohio

³University of Sao Paulo, Sao Paulo, Brazil

⁴Federal University of Rio de Janeiro, Brazil

⁵LV Prasad Eye Institute, HERF, Hyderabad, AP, India

Abstract

Studies suggest that standard corneal collagen crosslinking (CXL) is a safe and effective treatment to stiffen the cornea for keratoconus and other ectatic corneal disorders. The purpose of the present study was to compare the biomechanical effects of transepithelial benzalkonium chloride-EDTA (BAC-EDTA) riboflavin-UVA crosslinking to standard epithelium-off riboflavin-UVA crosslinking in a rabbit model. Corneal stiffness was quantified using optical coherence elastography at two months after treatment. The mean lateral-to-axial displacement ratio for the BAC-EDTA transepithelial CXL group was lower (greater stiffness) [0.062 ± 0.042 , mean $\pm$ SD] than epithelium-off CXL (mean $\pm$ SD: 0.065 ± 0.045) or untreated control eyes (0.069 ± 0.044). Using ANOVA with Tukey correction, a statistically significant difference was found between the BAC-EDTA transepithelial CXL group and standard epithelium-off CXL ($p=0.0019$) or the untreated control ($p<0.0001$) groups. A graph of the probability density functions for biomechanical stiffness also showed a greater shift in stiffening in the BAC-EDTA transepithelial CXL group than the standard epithelium-off CXL or untreated control group. These results demonstrated that the biomechanical stiffening effect produced by BAC-EDTA transepithelial CXL was greater than that produced by standard epithelium-off CXL in a rabbit model.

© 2014 Elsevier Ltd. All rights reserved.

Corresponding Author: Steven E. Wilson, Cole Eye institute, I-32, Cleveland Clinic, 9500 Euclid Ave, Cleveland, Oh 44195. Tel: 216.444.5887; wilsons4@ccf.org.

*These authors contributed equally to this research and manuscript

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Proprietary interest statement: The other authors have no commercial or proprietary interest in the materials presented herein.

Keywords

cornea; collagen crosslinking; biomechanical; ectasia; riboflavin; transepithelial treatment

Corneal collagen crosslinking (CXL) that uses riboflavin and ultraviolet-A (UVA) was introduced by Wollensak, Spoerl and Seiler in 2003 for the treatment of progressive keratoconus (Wollensak et al., 2003a). This treatment aims to increase biomechanical stability of the cornea in eyes with ectatic corneal disorders and previous studies have demonstrated that standard epithelium-off CXL technique is effective in halting keratoconus progression (Caporossi et al., 2010; Vinciguerra et al., 2009; Wittig-Silva et al., 2008).

Epithelial debridement during standard epithelium-off CXL is accompanied by patient discomfort, microbial corneal infections, and other complications (Kymionis et al., 2007; Mazzotta et al., 2007; Rama et al., 2009). Several investigators developed transepithelial CXL treatments to stiffen the cornea without removal of epithelium to reduce the incidence of complications (Filippello et al., 2012; Koppen et al., 2012). Importantly, these methods utilize chemicals such as benzalkonium chloride (BAC) and ethylenediaminetetraacetic acid (EDTA) to increase epithelial permeability to riboflavin. The use of combinations of permeability enhancers may have an additive effect on epithelial riboflavin permeability and thereby increase riboflavin penetration into the corneal stroma (Majumdar et al., 2008; Rathore and Majumdar, 2006).

Few studies have compared the biomechanical results of the alternative techniques for riboflavin-UVA corneal crosslinking (Armstrong et al., 2013; Wollensak and Iomdina, 2009a). BAC-EDTA transepithelial CXL was found to have greater endothelial safety than standard epithelium-off CXL in a recent study in rabbits that compared several transepithelial CXL methods (Armstrong et al., 2013). That study also suggested that the BAC-EDTA transepithelial CXL method produced greater biomechanical stiffening in the cornea than standard epithelium-off CXL but the results did not achieve statistical significance—likely due to the small sample size. The purpose of the current study was to compare the biomechanical effects of BAC-EDTA transepithelial CXL and standard epithelium-off CXL in larger groups of rabbits to determine if the biomechanical effects of these treatments were significantly different in the animal model.

Rabbits were selected for this study because their well-characterized corneal wound healing response and previous studies of transepithelial and standard epithelium-off riboflavin-UVA crosslinking (Armstrong et al., 2013; Salomao et al., 2011; Wollensak and Iomdina, 2009a). The Institutional Animal Care and Use Committee at the Cleveland Clinic approved this study. Animals were treated in accordance with the Tenets of the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

One eye of twenty-four 10 to 12 week-old female New Zealand white rabbits weighing 2.5 to 3.0 Kg each were enrolled into this study. Rabbits were divided in two treatment groups with twelve animals in each group. One animal in each group died during the two-month period after treatment due to causes unrelated to crosslinking and were excluded from the

study. Biomechanical measurements were performed at two months after treatment because biomechanical effects tend to increase over the first two months after treatment and treatment effects have been measured at this time point in prior studies (Armstrong et al., 2013; Wollensak and Iomdina, 2009b; Wollensak et al., 2003a). The untreated eyes of eleven rabbits served as untreated control eyes.

Epithelium-off and BAC-EDTA transepithelial crosslinking techniques were performed under general anesthesia induced by intramuscular injection of ketamine hydrochloride (30mg/Kg) and xylazine hydrochloride (5 mg/Kg).

Rabbits in the standard epithelium-off riboflavin-UVA crosslinking group underwent treatment by the method described by Wollensak, Spoerl, and Seiler (Wollensak et al., 2003a) using riboflavin photosensitizer solution produced by Seros Medical (Melon Park, California) containing 0.1% riboflavin-5-phosphate and 20% dextran T-500. The riboflavin solution was applied for 10 minutes prior to beginning UVA irradiation, instead of 5 minutes in the original protocol, otherwise the treatment was the same as that used in our prior study (Armstrong et al., 2013). Briefly, one percent proparacaine hydrochloride (Alcon Laboratories, Inc., Ft Worth, Texas) was applied to each eye just before treatment. Central epithelial debridement to within 1 mm of the limbus was performed with a #64 blade (Beaver, Becton-Dickson, Franklin Lake, NJ) under an operating microscope. The riboflavin solution was dropped onto the exposed stromal surface every three minutes starting ten minutes before the irradiation and every five minutes during the UV light treatment. Balanced salt solution was dropped onto the cornea between riboflavin applications to keep the cornea moist. UVA irradiation (370 nm) was applied continuously using a double UVA diode (Roithner Lasertechnik, Vienna, Austria) with an irradiance of 3 mW/cm² for a total of 30 minutes at a fixed 1 cm distance from the cornea. The radiance was controlled for this group, and for BAC-EDTA-riboflavin transepithelial CXL group, using a UVA light meter (handheld UVA meter, HHUVA1; Omega Engineering Inc., Stamford, Connecticut) before and after every treatment.

Rabbits in the BAC-EDTA riboflavin transepithelial CXL group underwent crosslinking with a commonly used formulation: 0.1% riboflavin-5-phosphate, 0.02% BAC, 0.01% EDTA, and 0.5% carboxymethylcellulose in hypotonic 100 mM NaCl, 50 mM tris buffer, pH 7.2. This formulation was compounded by Leiter's Pharmacy (San Jose, CA). One drop of proparacaine hydrochloride 1% and one drop of BAC-EDTA-riboflavin solution were applied, one minute apart, every 5 minutes for 15 minutes prior to irradiation and every five minutes during irradiation. UVA irradiation (370 nm, 3mW/cm²) was applied for a total of 30 minutes—identical to the epithelium-off crosslinking group.

Each animal in each group received two drops of ciprofloxacin ophthalmic 0.3% solution instilled into the operative eye immediately after surgery. Euthanasia was performed at two months with an intravenous injection of 100 mg/Kg pentobarbital while animal was under ketamine/xylazine general anesthesia.

Treated and untreated eyes of all rabbits from both groups were placed in Optisol preservation medium (Bausch and Lomb, Rochester, NY). Optical Coherence Elastography

(OCE) for biomechanical analysis was performed within 20 minutes of enucleation. The experimental setup of the OCE apparatus and the method used to measure corneal stiffness has been previously described (Ford et al., 2011). Briefly, the technique is based on high-resolution optical coherence tomography (OCT) imaging of the displacement of intracorneal optical features tracked with a two-dimensional cross-correlation algorithm that allows precise non-destructive estimation of local and directional corneal material properties. Intraocular pressure of the enucleated globe was maintained at a physiologic level of 15 mmHg with intravitreal saline infusion using an in-line pressure transducer while the cornea was vertically displaced with a gonioscopy lens in 20 μm intervals for a total of 140 μm of displacement. The displacement was controlled with an electronic computer-controlled microstage (Zaber Technologies Incorporated, TLA-28 Linear Actuator, Vancouver, Canada). The OCT scan parameters were set to oversample the lateral spot size by a factor of 10 to maximize capture of the speckle pattern in the image. The OCT data stream was then processed to extract the two-dimensional displacement of the cornea across the 4 mm scan width and the entire corneal thickness. Color-encoded maps of local axial and lateral displacement were obtained with corresponding maps of cross-correlation strength, an indicator of the fidelity of the displacement tracking. Only data points meeting a previously defined correlation threshold of 0.6 were analyzed because at this level the displacement error was found in less than 5% of all pixels (Ford et al., 2011). The slope of the lateral displacement divided by the imposed axial displacement was calculated – a unitless measure of corneal resistance to lateral strain – with a smaller ratio indicating greater corneal stiffness. Six regions of interest (3 anterior and 3 posterior stromal) were defined and analyzed in aggregate for each cornea.

Statistical comparisons of biomechanical effects of the different methods of crosslinking were performed using methods of repeated measures ANOVA with Tukey corrections for multiple comparisons. A $p < 0.05$ was considered statistically significant.

Corneal wound healing proceeded normally in all animals of both groups with no signs of infection being noted after epithelium-off or BAC-EDTA transepithelial crosslinking. Transient mild haze (corneal opacity) was noted in the stroma of all treated eyes.

Analysis of OCT elastography data at two months after treatment showed that the average lateral-to-axial displacement ratio for BAC-EDTA transepithelial CXL group was lower (mean $\pm$ SD: 0.062 ± 0.042) than epithelium-off CXL (mean $\pm$ SD: 0.065 ± 0.045) or untreated controls (0.069 ± 0.044). A lower lateral-to-axial displacement ratio denotes a higher corneal stiffness. By ANOVA with Tukey corrections, a statistically significant difference was found between the BAC-EDTA transepithelial CXL group and epithelium-off CXL ($p=0.0019$) or untreated control ($p<0.0001$). Interestingly, no statistically significant difference was reached between the epithelium-off CXL group and control eyes. This finding appears to relate to the high variability of mechanical stiffness in the untreated control group, which has also been noted in normal human eyes (____Andre add a couple of references that show this____). The different anterior-to-posterior stiffness patterns created by the transepithelial CXL and epithelial-off CXL compared to the control corneas are shown in Figure 1.

The probability density functions of the biomechanical properties measured for BACEDTA transepithelial CLX, epithelium-off CLX and untreated control group at two months after treatment showed a trend toward stiffening (shift to the left) for epithelium-off CLX and BACEDTA transepithelial CLX compared to untreated control eyes (Figure 2). Each curve represents the probability density function for a given treatment group calculated using the treatment or control group's measured mean and variance. The normality density of the distribution was verified prior to this calculation. It is important to note that the plots in Figure 2 were not based on a single displacement ratio for each eye, but on measurements from several regions on each eye that were incorporated into these results using the applied statistical methods.

A prior study in rabbits showed a trend towards greater corneal stiffening in BAC-EDTA transepithelial CXL compared to standard epithelium-off CXL at two months after treatment measured by OCT elastography (Armstrong et al., 2013), but the biomechanical difference did not reach statistical significance—likely due to the small number of eyes in each group (three rabbits per group). In the current study, a significant difference in lateral resistance to deformation was noted between the BAC-EDTA transepithelial treatment group and the other groups. Leftward shifts in the probability distributions (toward greater stiffening effect) are clearly visible at the distribution peaks and at the extremes of the distributions in Figure 2, and these offsets demonstrate a trend toward greater stiffness in the transepithelial group, with the lowest stiffness noted in the control group. Most likely the difference between the standard epithelium-off and untreated control groups did not reach statistical significance due to eye-to-eye variability in lateral-to-axial displacement ratios in each of these groups that is characteristic of measurements in normal eyes (Armstrong, et al., 2013). OCT elastography is an established method for providing directionally resolved displacement measurements in response to an applied stress (Ford et al., 2011; Roberts and Dupps, 2014; Schmitt, 1998) and has been shown to provide micron-level displacement resolution in corneal tissue (Schmitt, 1998). Moreover, a recent study has also demonstrated OCT elastography is capable of measuring distinctive biomechanical behaviors (lateral corneal resistance) in corneas after CXL and corneas with different stromal hydration states (Ford et al., 2014). Finally, the lack of significant between the untreated control group and the epithelium-off CXL does not conflict with the success of epithelium-off CXL reported in the clinical literature since corneal elastography behavior was not determined in those prior studies (Caporossi et al., 2010; Vinciguerra et al., 2009). Power calculations revealed that a much larger number of rabbit eyes in each group (more than 30) would be necessary to show a statistically significant difference between epithelium-off CXL and control groups.

Several previous studies have demonstrated the efficacy and safety of transepithelial CXL methods in the treatment of ectatic corneal disorders in human clinical studies (Filippello et al., 2012; Leccisotti and Islam, 2010). Few experimental studies, however, have addressed the biomechanical effects of epithelium-off or transepithelial CXL methods. Wollensak et al (Wollensak et al., 2003b) found an increase in corneal rigidity after standard epithelium-off CXL of 71.9% in porcine corneas and of 328.9% in human corneas related to a rise in stress-strain measurements. The stronger increase in the biomechanical rigidity of the human corneas compared to the porcine corneas was hypothesized to occur because of the relatively larger percentage of stroma crosslinked in the thinner human corneas. Wollensak and

Iomdina (Wollensak and Iomdina, 2009a) tested the biomechanical efficiency of CXL in rabbit eyes and demonstrated that the transepithelial CXL method they used produced only one-fifth of the increase in corneal stiffness compared to standard epithelium-off CXL. These authors suggested the reduced efficacy of the transepithelial treatment was attributable to the restricted and inhomogeneous stromal distribution of riboflavin. Importantly, however, Wollensak and Iomdina (Wollensak and Iomdina, 2009a) did not include chemicals that would break down epithelial barrier function but performed their study with 0.1% riboflavin-5-phosphate and 20% dextran T-500 dissolved in physiologic saline solution. The tight junctions of the epithelium are considered most important barriers to epithelial permeability in the cornea (Baiocchi et al., 2009). The efficacy the transepithelial CXL method used in the current study is likely attributable to the use of BAC and EDTA to break down the epithelial barrier function and thereby facilitate riboflavin penetration into the corneal stroma.

Other investigators have also demonstrated the efficacy of BAC-EDTA transepithelial CXL methods. For example, Kissner and coworkers (Kissner et al., 2010) showed in a rabbit model that CXL treatment with a hypo-osmolar solution of riboflavin containing 0.02% BAC induced sufficient epithelial permeability to riboflavin and resulted in increased corneal stiffening compared to the standard epithelium-off protocol. However, in that study the corneas were collected for analysis immediately after the CXL procedure. In another rabbit study, Raiskup et al (Raiskup et al., 2012) found that transepithelial riboflavin solutions performed better if they did not contain dextran—a component in the standard epithelium-off solution—because dextran inhibited riboflavin penetration through the epithelium. Other investigators showed that combinations of permeability enhancers, such as BAC and EDTA, had additive effects on breaking down epithelial barrier function in transepithelial CXL (Schumacher et al., 2012).

A major question raised by this study is why the transepithelial CXL treatment would produce a greater overall stiffening effect than the standard epithelium-off CXL method? Keratocyte death after BAC-EDTA transepithelial crosslinking only penetrates to approximately 1/3 depth into the stroma (Armstrong et al., 2013). However, keratocyte cell death extends to a much greater stromal depth in the standard epithelium-off CXL—even to the endothelium in some rabbit corneas that are approximately 400 μm thick (Armstrong et al., 2013). We hypothesize that the extensive keratocyte death produced by the standard epithelium-off CXL method has a deleterious long-term effect on corneal rigidity by altering normal keratocyte maintenance of collagen and other extracellular matrix materials in the stroma. Moreover, since the anterior 40% of the stroma has significantly higher cohesive strength than the posterior 60% of the stroma (Randleman et al., 2008), it is likely there is a greater contribution to the overall crosslinking treatment stiffening effect in the anterior stroma regardless of which method is used. Thus, the greater stromal penetration of riboflavin in the standard epithelium-off CXL method may not be needed to increase corneal rigidity but may coincidentally contribute to increased toxicity and may, in fact, have a detrimental effect on overall corneal stiffness because of its profound effect on the very cells responsible for maintaining the corneal stromal extracellular matrix.

Further investigation of transepithelial CXL methods in humans with keratoconus and other ectatic disorders is needed to evaluate the efficacy and safety of these treatments. This study suggests, however, that methods that use chemicals that break down epithelial barrier function have significant biomechanical effects and should be further studied in humans considering the demonstrated lower risk of corneal endothelial damage.

Acknowledgments

Supported in part by US Public Health Service grants EY023381 (WJD) and EY015638 from the National Eye Institute, Challenge and Unrestricted Grants from Research to Prevent Blindness, New York, New York and an RPB Career Development Award (WJD).

WJD has received honorarium funds from Zeimer related to the Galilei Dual Scheimpflug Analyzer (Ziemer, Port, Switzerland) and sponsored research funds from companies involved in riboflavin-UVA crosslinking trials [Topcon (Oakland, New Jersey) and Avedro (Waltham, Massachusetts)]. MRF and WJD are listed as inventors on an issued patent related to OCT elastography.

REFERENCES

- Armstrong BK, Lin MP, Ford MR, Santhiago MR, Singh V, Grossman GH, Agrawal V, Roy AS, Butler RS, Dupps WJ, Wilson SE. Biological and biomechanical responses to traditional epithelium-off and transepithelial riboflavin-UVA CXL techniques in rabbits. *J Refract Surg.* 2013; 29:332–341. [PubMed: 23659231]
- 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; 35:893–899. [PubMed: 19393890]
- Caporossi A, Mazzotta C, Baiocchi S, Caporossi T. Long-term results of riboflavin ultraviolet a corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. *Am J Ophthalmol.* 2010; 149:585–593. [PubMed: 20138607]
- Filippello M, Stagni E, O'Brart D. Transepithelial corneal collagen crosslinking: bilateral study. *J Cataract Refract Surg.* 2012; 38:283–291. [PubMed: 22104644]
- Ford MR, Dupps WJ Jr, Rollins AM, Roy AS, Hu Z. Method for optical coherence elastography of the cornea. *J Biomed Opt.* 2011; 16:016005. [PubMed: 21280911]
- Ford MR, Roy AS, Rollins AM, Dupps WJ Jr. Serial biomechanical comparison of edematous, normal, and collagen crosslinked human donor corneas using optical coherence elastography. *J Cataract Refract Surg.* 2014
- 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. *Curr Eye Res.* 2010; 35:715–721. [PubMed: 20673048]
- 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; 38:1000–1005. [PubMed: 22624899]
- Kymionis GD, Portaliou DM, Bouzoukis DI, Suh LH, Pallikaris AI, Markomanolakis M, Yoo SH. Herpetic keratitis with iritis after corneal crosslinking with riboflavin and ultraviolet A for keratoconus. *J Cataract Refract Surg.* 2007; 33:1982–1984. [PubMed: 17964410]
- Leccisotti A, Islam T. Transepithelial corneal collagen cross-linking in keratoconus. *J Refract Surg.* 2010; 26:942–948. [PubMed: 20166621]
- Majumdar S, Hippalgaonkar K, Repka MA. Effect of chitosan, benzalkonium chloride and ethylenediaminetetraacetic acid on permeation of acyclovir across isolated rabbit cornea. *Int J Pharm.* 2008; 348:175–178. [PubMed: 17897799]
- 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; 35:580–582. [PubMed: 17760642]

- Raiskup F, Pinelli R, Spoerl E. Riboflavin osmolar modification for transepithelial corneal cross-linking. *Curr Eye Res.* 2012; 37:234–238. [PubMed: 22335811]
- Rama P, Di Matteo F, Matuska S, Paganoni G, Spinelli A. Acanthamoeba keratitis with perforation after corneal crosslinking and bandage contact lens use. *J Cataract Refract Surg.* 2009; 35:788–791. [PubMed: 19304108]
- Randleman JB, Dawson DG, Grossniklaus HE, McCarey BE, Edelhauser HF. Depth-dependent cohesive tensile strength in human donor corneas: implications for refractive surgery. *J Refract Surg.* 2008; 24:S85–S89. [PubMed: 18269156]
- Rathore MS, Majumdar DK. Effect of formulation factors on in vitro transcorneal permeation of gatifloxacin from aqueous drops. *AAPS PharmSciTech.* 2006; 7:57. [PubMed: 17025238]
- Roberts CJ, Dupps WJ Jr. Biomechanics of corneal ectasia and biomechanical treatments. *J Cataract Refract Surg.* 2014
- Salomao MQ, Chaurasia SS, Sinha-Roy A, Ambrosio R Jr, Esposito A, Sepulveda R, Agrawal V, Wilson SE. Corneal wound healing after ultraviolet-A/riboflavin collagen cross-linking: a rabbit study. *J Refract Surg.* 2011; 27:401–407. [PubMed: 21162471]
- Schmitt J. OCT elastography: imaging microscopic deformation and strain of tissue. *Opt Express.* 1998; 3:199–211. [PubMed: 19384362]
- Schumacher S, Mrochen M, Wernli J, Bueeler M, Seiler T. Optimization model for UV-riboflavin corneal cross-linking. *Invest Ophthalmol Vis Sci.* 2012; 53:762–769. [PubMed: 22222507]
- Vinciguerra P, Albe E, Trazza S, Rosetta P, Vinciguerra R, Seiler T, Epstein D. Refractive, topographic, tomographic, and aberrometric analysis of keratoconic eyes undergoing corneal cross-linking. *Ophthalmology.* 2009; 116:369–378. [PubMed: 19167087]
- Wittig-Silva C, Whiting M, Lamoureux E, Lindsay RG, Sullivan LJ, Snibson GR. A randomized controlled trial of corneal collagen cross-linking in progressive keratoconus: preliminary results. *J Refract Surg.* 2008; 24:S720–S725. [PubMed: 18811118]
- Wollensak G, Iomdina E. Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. *J Cataract Refract Surg.* 2009a; 35:540–546. [PubMed: 19251149]
- Wollensak G, Iomdina E. Long-term biomechanical properties of rabbit cornea after photodynamic collagen crosslinking. *Acta Ophthalmol.* 2009b; 87:48–51. [PubMed: 18547280]
- Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol.* 2003a; 135:620–627. [PubMed: 12719068]
- 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.* 2003b; 29:1780–1785. [PubMed: 14522301]

Highlights

- Transepithelial crosslinking (CXL) produces more corneal stiffening than epithelium-off CXL
- Agents that break down epithelial barrier function are critical for transepithelial CXL
- The extent of keratocyte cell death may have a role in the crosslinking stiffening effect

Figure 1.

OCT images of A) a non-crosslinked control cornea and corneas treated with C) transepithelial CXL and E) epithelium-off CXL. Frames B, D and F show the corresponding lateral displacement maps derived from optical coherence elastography for the same three corneas. Displacements are displayed in μm , with lower displacements indicating higher elastic stiffness (deeper blue) and higher displacements indicating lower stiffness (yellow and red). Note the lower stiffness of the epithelium in all 3 states and the greater overall stiffening effects of the transepithelial approach with EDTA and BAC as enhancers.

Figure 2.

Graph of probability distribution versus lateral/axial displacement ratio obtained with OCT elastography at two months after treatment for untreated control group, BAC-EDTA transepithelial CXL, and standard epithelium-off CXL. Note that there is a shift towards left (stiffness) in the epithelium-off CXL and BAC-EDTA transepithelial CXL groups compared with the untreated control group.