Femtosecond-assisted intrastromal corneal cross-linking for early and moderate keratoconus

M. Balidis,[1,2] V.E. Konidaris,[2] G. Ioannidis,[1,3] A.J. Kanellopoulos[4,5]

Abstract

Purpose: To evaluate the effect of Femtosecond-assisted intrastromal corneal cross-linking to stabilize early and moderate keratoconus.

Methods: Twelve eyes of 9 consecutive patients (6 male), with early keratoconus (K > 48.00 D, Skewed Steepest Radial > 22°, superior–inferior difference on the 5 mm circle > 2.5 D, inferior– superior difference > 1.5 D), minimum corneal thickness > 380 μm, age < 50 years included in the study studied.

Results: Stabilization of keratoconus during the 1 year follow-up period, with Kmax remaining unchanged and Kmax-Kmin difference reduced after the first postoperative month ($p < 0.05$). There was statistically significant difference in the preoperative and 1 year postoperative value of eccentricity (Topolyser, Oculus Instruments), thinnest corneal point and irregularity in 3mm (Orbscan imaging) ($p < 0.05$). Corrected distant visual acuity, initially decreased ($p = 0.157$), followed by improvement in 3 and 12 months ($p = 0.042$).

Conclusions: Riboflavin injected intrastromal in a precisely designed corneal pocket is a painless procedure. This surgical approach provokes topographic stability of the ectatic disease and improvement of CDVA even after 12 months. Our study demonstrates the safety and efficacy of the proposed method.

Key words: keratoconus, femtosecond, cross-linking.

1. Thessaloniki Eye Foundation Private Eye clinic, Thessaloniki, Greece.
2. 1st Ophthalmology Clinic, AHEPA University Hospital,
3. Eye Clinic, Ippocration General Hospital, Thessaloniki, Greece
4. New York University Medical College and Manhattan Eye, Ear and Throat Hospital, New York NY
5. Laservision.gr Institute, Athens Greece.

Corresponding author: Miltiadis Balidis
e-mail: balidis@perfectvision.gr

Introduction

Keratoconus is a bilateral, non symmetric and noninflammatory progressive corneal degeneration. It is characterized by progressive thinning and steepening of the central cornea, resulting in increasing myopia, irregular astigmatism, and eventual loss of spectacle-corrected visual acuity. Its incidence has been estimated to be 1 in 2000 in the general population, but the increased number of eyes undergoing screening for laser refractive surgery suggests that prevalence may be higher.[1] Rigid contact lenses can be used to improve visual acuity in many patients,[2] but keratoconus frequently progresses to the point that corneal transplantation is required to restore useful vision.[3] Until recently, there was no effective way to stop progressive keratoconus so that eventually about 21% of keratoconus patients required corneal transplantation. Also keratoconus may recur following corneal transplantation and require further transplant surgery.[4]

More than ten years ago, corneal collagen cross-linking by means of Ultraviolet A light and riboflavin was proposed as a therapeutic approach to improve biomechanical and biochemical properties of the cornea. Since then the management of keratoconus with collagen cross linking (CXL) has been studied at length both in the laboratory as well as clinically and received CE marking in December 2006 for clinical use in the European Union countries.[5]

The proposed treatment counteracts progressive thinning and ectasia by photosensitized oxidation, increasing intra and interfibrillar covalent bonds, while minimizing exposure to the surrounding structures of the eye.[6] UVA CXL assisted by the photo sensitizer riboflavin, leads to a significant in-

crease in corneal collagen diameter in rabbit eyes. This morphologic alteration is leading to an increase in biomechanical stability. Cross linking effect is strongest in the anterior half of the stroma due to rapid decrease in UVA irradiance as a result of riboflavin-enhanced absorption.[7]

The standard technique involves application of a photosensitizing agent containing riboflavin to the corneal surface at regular time intervals for a total time of 30 minutes followed by continuous application of a broad ultraviolet (UV-A) beam for another 30 minutes.[1,8,9] Before riboflavin is applied, the epithelium is typically removed from the central 5- to 7-mm-diameter zone of the cornea to facilitate penetration of riboflavin into the stroma.[1,8,10] During epithelial healing patients experience significant discomfort and pain. Sterile corneal infiltrates and melting after CXL for keratoconus have been reported after epithelium-off procedures.[11] An alternative technique was described by Wachler et al.[12] Epithelium is not removed offering patients a less invasive, faster and painless CXL, while retaining the efficacy of the standard technique. This technique applies a riboflavin solution containing benzalkonium chloride (BAK) directly onto intact epithelium. Riboflavin is hydrophilic and epithelium is hydrophobic. However, underneath the epithelium, stroma is highly hydrophilic. Thus epithelium with its tight junctions is considered to be the most important barrier for permeability of riboflavin. So far riboflavin solutions used (Sooft, EDTA and Benzalkonium chloride) are thought to loosen and open up tight junction of epithelial barrier. BAK, an effective tensioactive substance, alters surface tension value, and hence facilitates penetration of other substances through biological membranes. Therefore, BAK promote riboflavin penetration into corneal stroma, without the need of de- epitheliazation. As an alternative to the above approaches, we developed a Femtosecond assisted intrastromal pocket to introduce riboflavin, and evaluate its safety and efficacy.

Patients and Methods

Twelve eyes of 9 consecutive patients, 6 male and 3 female, with early progressive keratoconus were included in the study. Early and moderate keratoconic corneas were selected with the following criteria: topographic evidence of keratoconus (K-readings > 48 D, Skewed Steepest Radial Axis (SRAX) >22º, superior–inferior difference (S-I) on the 5 mm circle > 2.5 D, inferior–superior difference (I-S) >1.5 D.), minimum corneal thickness > 380 μm, and patient age younger than 50 years.

Progression was confirmed by documenting K – reading increase of 1 or more diopters in two consecutive corneal topographies, or minimum thickness decrease of 5 μm or more in two consecutive Orbscan topographies. The research followed the tenets of the Declaration of Helsinki and written informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. The mean cohort age was 29.75 years (Std deviation ± 9.3). The preoperative CDVA for each patient was better than 0.15 Log MAR. The mean preoperative CDVA was 0.1 ± 0.09, with mean sphere -4.1 ± 2.9 diopters and cylinder -3.23 ± 3.2 diopters. The mean corneal pachymetry was 445.1 ± 38.7 μm. One year after CXL with Femtosecond laser, CDVA was 0.05 ± 0.06, mean sphere was -3.6 ± 3.1 and mean cylinder was -3.1 ± 1.2. Mean corneal pachymetry was 425.3 ± 39.8 μm.

Postoperative medications included topical ciprofloxacin HCL 0.3% four times a day for 5 days and 1% prednisolone acetate four times a day for 4 weeks. Patients were evaluated at postoperative day 1, month 1 and 3, and every 6 months thereafter to last follow-up.

Preoperative and postoperative examinations included slit lamp evaluation, corrected distance visual acuity (CDVA), corneal topography [three consecutive measurements using Oculus Instruments Wetzlar (Germany), equipped with Topolyser software, Wavelight (Erlangen, Germany), comparison charts, Orbscan corneal topographer (Bausch and Lomb Orbscan II Corneal Analysis System)], and corneal thickness at the thinnest point. Specular microscopy was performed preoperatively and 3 months postoperatively (Tomey EM-3000 Specular Microscope).

The studied parameters including mean endothelial cell density (MCD) and mean cell area (MCA) of the central cornea were analyzed. Anterior Optical Coherence Tomography scans (SOCT Copernicus OPTOPOL Technology S.A.) were also performed.

It is well known from refractive surgery, that regular use of contact lenses, especially hard gas permeable, introduce a mechanical effect on corneal surface and as a consequence altered refractive status and corneal topography. Therefore all our patients refrained from contact lens use one month preoperatively and during the follow up period (up to 2 years). Patients entering the project have been informed to avoid contact lens use. Warpage after rigid contact lenses may need more than a month to regress. Thus we can assume that changes documented in a 3 monthly corneal topography examination were due to treatment effect or disease progression.

Intrastromal Pocket Creation. The Femtosecond laser used to create stromal pockets was the Technolas Femtec 520 (Technolas Perfect Vision GmbH). This Femtosecond Diode pumped solid state laser had the following technical characteristics Laser Wavelength 1040 nm; Pulse Rate 40 kHz; Laser Pulse Duration: 400 – 800 femtoseconds; Pulse energy 1-4 μJ. For this treatment the distance between spots

was 5μm. The Femtec laser works in a spiral pattern. Spiral pattern is applied when laser pulses begin centrally and expand centrifugally out to the periphery. Also we used a curved applanation docking system, which generate less intraocular pressure (IOP) increase and more physiologic interface between the eye and the laser.

Under topical 1% proparacaine anesthesia (Alcaine; Alcon Laboratories Inc, Ft Worth, Texas) a 6 mm diameter doughnut-shaped intrastromal pocket was created by the Femtosecond laser leaving a clear central optical zone of 3 mm (Fig. 1.1 upper left). The outer diameter of the pocket was 6mm and the inner diameter 3mm. Thus a doughnut shape pocket at 200μ depth and 1.5 mm width was created. Then two 0.5 x 0.5 mm entry channels were created 180 degrees apart. From the fashioned channels, one is facilitating for riboflavin infusion and the contra latera depressurization.

Following the pocket creation, a tapered Intacs spatula mall Jameson muscle hook (Storz Bausch & Lomb GmbH) was used to open, enter and bluntly dissect the pocket (Fig. 1.2).

0.1% Riboflavin solution Infusion. 0.3 mL of 0.1% riboflavin in 20% dextran solution was infused into the pocket with 2 injections one at the upper half and one at the lower, using Intacs stromal channel Irrigation cannula (Storz Bausch & Lomb GmbH). Infusion continued until the entire pocket was colored bright yellow from riboflavin and the solution was overflowing from the opposite incision (Fig. 1.3 lower left). When corneal stroma colored yellow we start preparing the UVA radiation device. Time interval between the two stages was 5 minutes, during that period riboflavin was allowed to diffuse into stroma (Fig. 1.3)

Collagen Cross-linking. We use the UVX-PESCHKE (Meditrate GmbH Hunenberg, Switzerland) device. Cornea was irradiated with UVA 365 -375nm light at an irradiance of 3 mW/ cm2 for 30 minutes. During irradiation corneal epithelium was moistened twice (one drop at 10 and one at 20 minutes after the beginning of irradiation) with riboflavin 0.1% solution. The total fluency at the 6 mm diameter corneal plane was 5.4 J/cm^2. During UVA emission we applied one drop of riboflavin every 10 minutes (total 2 drops) to moist and protec 156 t epithelium. Riboflavin solution used could not cross the epithelial barrier.

Statistical methods: Statistical Package for the Social Sciences 17 (SPSS, inc., Chicago, IL) was used for the statistical analysis of the data collected. Due to the small number of patients, Shapiro Wilk test (appropriate for small sample sizes) was performed in order to test normality of distributions. Wilcoxon signed-rank test for non-parametric data was used to compare the pre- and postoperative parameters. Values of $p < 0.05$ were considered to differ statistically importantly.

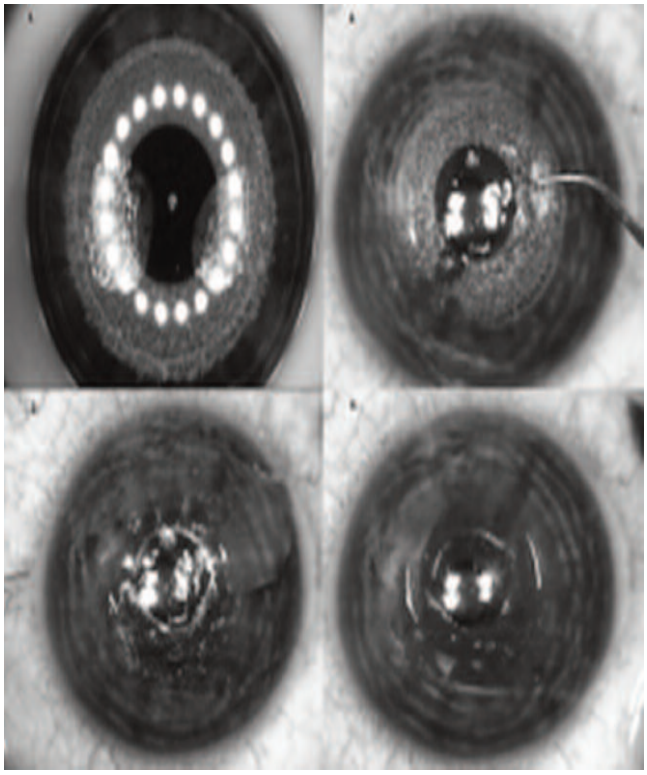

Figure 1: 1. Creation of a 6 mm diameter circular intrastromal pocket, by means of the femtosecond laser, leaving a clear optical zone of 3 mm.
2. Creation of two 0.5 mm width entry channels 180 degrees apart. Following the pocket creation, a tapered Intacs spatula mall Jameson muscle hook was used to enter and bluntly dissect the pocket.
3. Infusion of 0.3 mL of 0.1% riboflavin solution into the pocket using an Intacs stromal channel Irrigation cannula. Infusion continued until the entire pocket was colored bright yellow from the presence of the riboflavin solution.
4. A UVA irradiation source of ~ 370 nm wavelength (365 to 375 nm) was used for corneal surface irradiation.

Results

Specular microscopy was performed preoperatively and 3 months postoperatively. Mean endothelial Cell Density MCD preoperatively was 2543.83 ± 241 cells / mm^2 and MCA was 501.0 ± 137.4 μm^2. 3 months postoperatively MCD was 2495.1 ± 227.4 cells / mm^2 and MCA was 527.0 ± 114 μm^2. There was no statistically significant difference in MCD and MCA. According to Shapiro Wilk test, the studied parameters demonstrate non parametric distribution. Corrected distance visual acuity, although decreased in 1st

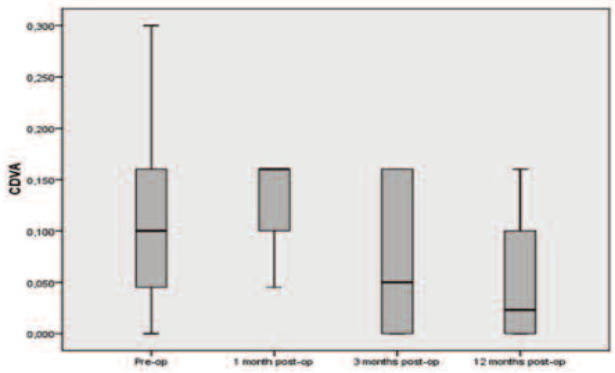

Figure 2: Change in Corrected Distance Visual Acuity.

month (p = 0.157), increased in 3rd and 12th months postoperatively. There was a statistically significant difference in CDVA between the preoperative and one year postoperative examination (p = 0.042) (Fig. 2). Additionally statistically significant difference confirmed in the preoperative and 1 year postoperative value of astigmatic power (p = 0.016), eccentricity (Topolyser, Oculus Instruments) (p= 0.044), and thinnest point of corneal thickness (p = 0.043) (Orbscan imaging). The effect observed in difference maps was a central flattening and circular midperipheral steepening at the area of the intrastromal treatment. We did not notice any induction of astigmatism as a result of these negligible incisions.

Moreover, keratoconus remained unchanged even after 12 months postoperatively, Kmax remaining unchanged and Kmin increased after the first postoperative month (p = 0.034). The values of CDVA, Kmax, Kmin, eccentricity, corneal pachymetry, thinnest point and irregularity in 3 mm as well as the statistically significant differences are shown on table 1.

	Pre-op	1 month post-op	3 months post-op	1 year post-op
CDVA	¥ 0.1 ± 0.09	0.13 ± 0.05	0.07 ± 0.08	0.05 ± 0.06
Kmax	49.7 ± 2.86	48.6 ± 2.24	49 ± 3.11	50 ± 2.57
Kmin	¥ 45.6 ±2.36	45.7 ± 2.46	46.2 ± 2.59	46.4 ± 2.28
Ecc	¥ 0.9 ± 0.3	* 0.8 ± 0.2	♯ 0.9 ± 0.3	‡ 1 ± 0.2
C.Pachymetry	¥ 445.08 ± 38.7	425.5 ± 85.02	460.67 ± 39.63	425.27 ± 39.8
Thinn	¥ 417 ±31.4	317 ± 23.3	357 ± 18.7	357 ± 30.8
Irreg	4.7 ± 2.2	5.6 ± 0.2	4.6 ± 3.2	4.4 ± 2.7

Table 1: Preoperative, 1, 3 and 12 postoperative month follow-up mean values and standard deviation for Corrected Distant Visual Acuity (CDVA) (logMAR), Kmax, Kmin and eccentricity (Ecc) (Topolyser, Oculus Instruments), corneal pachymetry (in μm) (Tomey), and thinnest corneal point (in μm) (thinn) and irregularity in 3mm (Irreg) (Orbscan imaging).
Statistically important differences are marked as:
¥: Preoperative vs 1 year (p<0.05)
**: 1 month vs 1 year (p<0.05)*
‡: 3 months vs 1 year (p<0.05)
♯: 3 months vs 1 month (p<0.05)to 375 nm) was used for corneal surface irradiation.

Comparison charts demonstrate the postoperative effect of the procedure in anterior corneal curvature. Comparing pre and 1 184 year postoperative topographies we identify the highest curvature difference in the periphery and the center of the topographic map. Safety and efficacy of the procedure is displayed on the Figure 3. There was no CDVA line loss in our study group. Slit-lamp biomicroscopy revealed minimal diffuse light scattering at the level of midstroma, delineating the annular pocket. Haze formation resolved within 3 months. Haze after cross linking differs in appearance compare to sub-Bowman scaring after PRK. In the 2 year follow up, slit lamp examination haze disappeared in almost all cases.

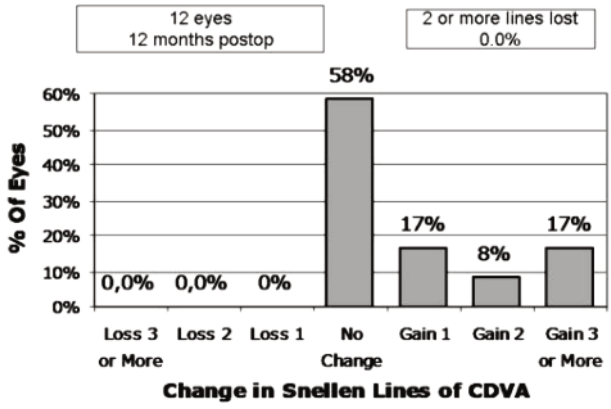

Figure 3: Safety and efficacy of the procedure.

Discussion

Keratoconus is the second most frequent indication for corneal transplantation, accounting for about 15% of the corneal transplants performed in the United States.[13] Corneal transplantation has inherent risks that could result in permanent loss of vision and significantly impact the patient's quality of life during the surgical recovery phase.[14] Any modality, such as CXL, that can delay or prevent corneal transplantation in patients with these conditions is of great benefit. CXL using UVA light with riboflavin photo sensi-

tizer to strengthen corneal tissue has shown promising results internationally, in stabilizing corneal curvature and slowing or even arresting progression of keratoconus and post-refractive ectasia.[1] Experimental studies in rabbit and porcine eyes have shown an approximate increase in corneal rigidity by 70% after CXL.[15] A clinical study of 22 cases demonstrated stabilization of keratoconus with 4-year follow-up, with mean keratometric regression of 2 D in 70% of cases, and manifest spherical equivalent refractive error regression of 1.[14] D. Corneal and lens transparency, as well as endothelial cell density and intraocular pressure, remained unchanged, whereas visual acuity improved slightly in 65% of the eyes.[8,15-17] Corneal stabilization, followed by full visual rehabilitation, leads us to believe that this combined approach may have wider applications and become a temporizing alternative to corneal transplantation.

In the standard technique, involving central epithelial removal to achieve intrastromal penetration, extended healing time causes significant discomfort and pain.[18] Angunawela et al[11] present a case of sterile corneal infiltrates and melting after CXL for keratoconus. Staphylococcal antigens, deposited at high concentrations in static tear pools beneath the bandage contact lens, triggered an enhanced cell-mediated immune reaction, as proposed by the authors. Regarding post-CXL haze, Raiskup et al.[19] reported in their retrospective survey that 8.6% of the KC eyes that underwent CXL treatment developed clinically significant permanent stromal haze. Disadvantage of standard epithelium off CXL include prolonged surgical time, increased incidence of herpetic activation and haze development, corneal edema, postoperative pain and discomfort, and reduced visual acuity (until epithelialization is complete and corneal edema is resolved).[12]

We have been motivated to develop a alternative technique in which the epithelium is not removed. Riboflavin placed intrastromally in a pre-formed pocket will absorb and activate UVA light and achieve CXL in a more controlled method. Kanellopoulos presented a novel epithelium-sparing, rapid soak-and-treat method of intrastromal riboflavin instillation, creating a femtosecond assisted pocket and utilizing higher fluence UVA light for CXL.[5]

The effect of UVA light is at the area of maximal absorbance and its close vicinity. Using hand-held spectral domain optical coherence tomography, Malhotra et al. measured riboflavin penetration during collagen cross-linking (CXL) in vivo.[20] In the epithelium-off cases the hyperreflectivity band of riboflavin was measured 54.2 +/- 5.2 μm (mean) at the end of 30 minute drop administration period. At the end of the procedure (total 60 minutes of riboflavin penetration) mean band thickness was 72.4 +/- 7.1 μm. So the greatest concentration of Riboflavin is expected to be 55 μm from the area of injection, which in our method is 200μm deep. Treatment is aimed in order to achieve greatest absorbed irradiation in mid- corneal stoma. Concerns regarding safety and UV absorption through intact epithelium were addressed in this study. Corneal stroma, due to its thickness (10fold thicker than that of the other layers) absorbs UVA light more than the other corneal structures.[21,22] UV filtering ability of the epithelium and Bowman layer may be due to their special molecular composition and higher dry mass content of the Bowman layer, resulting in a higher absorption coefficient. Concentration of riboflavin with this technique was expected to be activated by stray UVA light at the mid stromal level.[23] As confirmation of the safety of the procedure pre- and one year postoperative endothelial cell density and morphology show no significant difference in our cases. Concerns about biomechanical instability from the femto ring, the 6 mm diameter doughnut-shaped intrastromal femtosecond ring, with a clear central optical zone of 3 mm, have been countered by Kanellopoulos in his paper on the same subject.[5]

In our prospective study, there was a statistically important improvement in CDVA. Moreover, stabilization of keratoconus was established for 12 months. Kmax remained unchanged and Kmin regressed after the first postoperative month. Limitation of our study is the small cohort. The follow up period is long enough to disclose failure of stabilization.

This surgical approach merits further exploration. Riboflavin injected intrastromally in a precisely designed pocket is a painless procedure, lacking epithelialization period. A faster and longer saturation period and possibly more effective diffusion of riboflavin (as the large molecule, in regard to the Bowman's layer barrier is directly injected at depth via the intrastromal pocket), provide greater shielding near the endothelium. In cases of central or paracentral cones the cross linking effect treatment is augmented. Flattening of the cone, surrounded by mid peripheral steepening has been shown in topographic comparison maps. Introducing cross linking in the mid peripheral annulus rather than central cornea may increase the biomechanical effect further.

The effect is augmented in central topographic cones. It is possible to further customize the procedure, by fitting the femto - created ring pocket to encircle the cone. Riboflavin concentrated at the ring will maximize the cross linking effect at the mid - stromal around the protrusion, probably flattening the cone. This novel technique may become an alternative for the prevention of cornea transplantation in corneal ecstasies. Further studies and longer follow-up are needed to validate these data.

References

1. Wollensak G. Crosslinking treatment of progressive keratoconus: New hope. Curr Opin Ophthalmol 2006; 17:356-360.

2. Barnett M, Mannis MJ. Contact lenses in the management of keratoconus. Cornea 2011; 30:1510-1516.

3. Jhanji V, Sharma N, Vajpayee RB. Management of keratoconus: Current scenario. Br J Ophthalmol 2011; 95:1044-1050.

4. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998; 42:297-319.

5. Kanellopoulos AJ. Collagen cross-linking in early keratoconus with riboflavin in a femtosecond laser-created pocket: Initial clinical results. J Refract Surg 2009; 25:1034-1037.

6. Kohlhaas M, Spoerl E, Schilde T, Unger G, Wittig C, Pillunat LE. Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet a light. J Cataract Refract Surg 2006; 32:279-283.

7. Wollensak G, Wilsch M, Spoerl E, Seiler T. Collagen fiber diameter in the rabbit cornea after collagen crosslinking by riboflavin/UVA. Cornea 2004; 23:503-507.

8. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003; 135:620-627.

29. Tu KL, Aslanides IM. Orbscan ii anterior elevation changes following corneal collagen cross-linking treatment for keratoconus. J Refract Surg 2009; 25:715-722.

10. 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.

11. Angunawela RI, Arnalich-Montiel F, Allan BD. Peripheral sterile corneal infiltrates and melting after collagen crosslinking for keratoconus. J Cataract Refract Surg 2009; 35:606-607.

12. Boxer Wachler BS, Pinelli R, Ertan A, Chan CC. Safety and efficacy of transepithelial crosslinking (c3-r/cxl). J Cataract Refract Surg 2010; 36:186-188; author reply 188-189.

13. Binder PS, Lindstrom RL, Stulting RD, Donnenfeld E, Wu H, McDonnell P, et al. Keratoconus and corneal ectasia after lasik. J Refract Surg 2005; 21:749-752.

14. Price FW, Price MO. Adult keratoplasty. Ha 290 s the prognosis improved in the last 25 years? Int Ophthalmol 2008; 28:141-146.

15. 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; 29:1780-1785.

16. Sporl E, Schreiber J, Hellmund K, Seiler T, Knuschke P. Studies on the stabilization of the cornea in rabbits]. Ophthalmologe 2000; 97:203-206.

17. Wollensak G, Spoerl E, Wilsch M, Seiler T. Keratocyte apoptosis after corneal collagen cross-linking using riboflavin/uva treatment. Cornea 2004; 23:43-49.

18. Vicente LL, Boxer Wachler BS. Factors that correlate with improvement in vision after combined intacs and transepithelial corneal crosslinking. Br J Ophthalmol 2010; 94:1597-1601.

19. Raiskup F, Hoyer A, Spoerl E. Permanent corneal haze after riboflavin-uva-induced cross linking in keratoconus. J Refract Surg 2009; 25:824-828.

20. Malhotra C, Shetty R, Kumar R, Veluri H, Nagaraj H, Shetty KB. In Vivo Imaging of Riboflavin Penetration During Collagen Cross-linking With Hand-held Spectral Domain Optical Coherence Tomography. J Refract Surg 2012; 28:776-780.

21. Schnitzler E, Sporl E, Seiler T. [Irradiation of cornea with ultraviolet light and riboflavin administration as a new treatment for erosive corneal processes, preliminary results in four patients]. Klin Monbl Augenheilkd 2000; 217:190-193.

22. Wollensak G, Spoerl E, Reber F, Seiler T. Keratocyte cytotoxicity of riboflavin/uva-treatment in vitro. Eye (Lond) 2004; 18:718-722.

23. Kolozsvari L, Nogradi A, Hopp B, Bor Z. UV absorbance of the human cornea in the 240- to 400-nm range. Invest Ophthalmol Vis Sci 2002; 43:2165-2168.