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Collagen Cross-Linking. Indications, Applications, results, complications and evolving technology

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Course Description:

Synopsis: Didactic approach to the management of progressive cornea ectasia associated with keratoconus and refractive surgery. Several surgical treatment modalities utilized internationally will be presented, including: collagen cross-linking with ultraviolet radiation A in order to halt ectasia, combined in some cases with a customised excimer laser ablation to facilitate visual rehabilitation (as presented in previous ESCRS meetings by the author), these alternatives to Intracornea ring segment implantation, lamellar grafts as well as penetrating graft techniques will be analyzed. Surgical and medical treatment technique, indications, potential complications and their management as well as clinical experience pearls will be presented

Objective: The participants will share our vast experience in managing progressive keratoconus and post-LASIK ectasia in order to visually rehabilitate these patients. Pearls on indications, patient selection, surgical technique and complication management for safe and effective results will be presented and discussed with the participants.

Outline:
1-Keratoconus surgical management, Literature review:
- Thermokeratoplasty
- Lamellar grafts
- INTACS
- Penetrating keratoplasty
- Cornea Collagen Cross-linking

2-Post-LASIK ectasia-surgical management, literature review:
- How to avoid-risk factors
- Thermokeratoplasty
- Lamellar grafts
- INTACS
- Penetrating keratoplasty
- Cornea Collagen Cross-linking

3-patient selection
a) Indications
b) medical contraindications
c) pre-operative evaluation and refractive error

4-Stabilization of ectasia:
- INTACS
- Collagen cross-linking
- lamellar tissue support

5-Collagen cross-linking:
- energy source, luminance and duration
- protective riboflavin A
- cornea pachymetry issues
- topographic and elevation changes
- stabilization of ectasia
- When is it best to intervene?
- FDA issues

6-Customised enhancement techniques
a) wavefront-guided
b) topography-guided
c) Asphericity adjustment

7- Microkeratome-assisted lamellar keratoplasty technique
a) basic principles
b) pre-operative evaluation parameters
c) Surgical technique
d) Possible complications and their management
c) Clinical data and review of the literature

8- Penetrating keratoplasty considerations:
 a) basic principles
 b) pre-operative evaluation parameters
 c) Surgical technique
d) Possible complications and their management
c) Clinical data and review of the literature

9- Refractive surgery enhancements following these procedures

10-surgery in action
Step-by-step action on several procedures on tape, question-answer session and coverage of basic problem-shooting with the panelists
Evaluation of Visual Acuity, Pachymetry and Anterior-Surface Irregularity in Keratoconus and Crosslinking Intervention Follow-up in 737 Cases

Anastasios John Kanellopoulos, Vasiliki Moustou, George Asimellis

ABSTRACT
Purpose: To investigate visual acuity, corneal pachymetry, and anterior-surface irregularity indices correlation with keratoconus severity in a very large pool of clinically-diagnosed untreated keratoconic eyes, and in keratoconic eyes subjected to cross-linking intervention.

Materials and methods: Total of 737 keratoconic (KCN) cases were evaluated. Group A was formed from 362 untreated keratoconic eyes, and group B from 375 keratoconic eyes subjected to partial normalization via topography-guided excimer laser ablation and high-fluence collagen crosslinking. A control group C of 145 healthy eyes was employed for comparison. We investigated distance visual acuity, uncorrected (UDVA), best-spectacle corrected (CDVA), and Scheimpflug-derived keratometry, pachymetry (central corneal thickness, CCT and thinnest, TCT), and two anterior-surface irregularity indices, the index of surface variance (ISV) and the index of height decentration (IHD). The correlations between these parameters vs topographic keratoconus classification (TKC) were investigated.

Results: Keratometry for group A was K1 (flat) 46.67 ± 3.80 D and K2 (steep) 50.76 ± 5.02 D; for group B K1 44.03 ± 3.64 D and K2 46.87 ± 4.61 D; for group C, K1 42.89 ± 1.45 D and K2 44.18 ± 1.88 D. Visual acuity for group A was UDVA 0.12 ± 0.18 and CDVA 0.59 ± 0.25 (decimal), for group B, 0.51 ± 0.28 and 0.77 ± 0.22, and for group C, 0.81 ± 0.31 and 0.87 ± 0.12.

Correlation between ISV and TKC (r²) was for group A 0.853, and for group B 0.886. Correlation between IHD and TKC was for group A r² = 0.731, and for group B 0.701. The ROC analysis for visual acuity “area under the curve” was for CDVA 0.550, TCT 0.596, ISV 0.876 and IHD 0.857.

Conclusion: Our study indicates that the traditionally employed metrics of visual acuity and corneal thickness may not be the robust indicators nor provide accurate assessment on either keratoconus severity or postoperative evaluation. Two anterior surface irregularity indices, derived by Scheimpflug-imaging, ISV and IHD, may be more sensitive and specific tools.

Précis: Visual acuity, Scheimpflug-derived pachymetry and anterior-surface irregularity correlation to keratoconus severity in untreated cases (A), treated with crosslinking (B), and in a control group (C) reveals that visual acuity and pachymetry do not correlate well with keratoconus severity.

Keywords: Athens Protocol, Combined topography guided PRK and higher fluence CXL, Visual rehabilitation in keratoconus, Severity criteria, Keratoconus progression, Keratoconus classification, Pentacam, Keratoconic Scheimpflug tomometric indices, Visual acuity, Keratoconus, Grading anterior surface Pentacam indices, Keratoconus.Amsler and Krumeich grading, Corneal pachymetry, Receiver operating characteristic ROC analysis.

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Conflict of Interest: None declared

INTRODUCTION
Keratoconus (KCN), derived from the Greek words κρατοσ (cornea; κονον; cone, meaning cone-shaped protrusion, is a corneal disorder, defined as a noninflammatory degenerative axial thinning of an ectatic cornea.1 Vision is affected by increased myopia due to the cone protrusion, and irregular astigmatism due to substantial corneal asymmetry.1-4

Our long clinical experience with keratoconic screening and rehabilitation5-12 indicate that neither corneal pachymetry nor visual acuity (uncorrected distance visual acuity, UDVA, and best-spectacle corrected distance visual acuity, CDVA) can be reliable indicators of ectasia and/or keratoconus progression assessment.5 One may expect that the presence of large amounts of corneal irregularities might hamper sufficient spectacle-correction of visual acuity. However, at least in our experience, often enough keratoconic patients present with surprisingly high CDVA, even near 20/20, despite severe topographic irregularity and/or pachymetric thinning present. This makes keratoconus diagnosis a difficult and potentially dangerous process, as most early, many advanced and even some severe cases can be missed with traditional screening methods. We have also encountered cases with progressive keratoconus with no clinically significant reduction in visual acuity.

To the best of our knowledge, the subject of quantitative correlation of visual acuity with keratoconus grading5,11 has been reported only in very few post-review publications.

This study aims to investigate the possible correlations of visual acuity (UDVA and CDVA), corneal pachymetry, and specific Scheimpflug-imaging derived anterior-surface topographic irregularity indices with keratoconus severity, in a large pool of clinically-diagnosed keratoconic eyes, and in a group of keratoconic eyes subjected to cross-linking and anterior-surface normalization intervention, and examine the applicability of these indicators in keratoconus screening.
Cross-linking Biomechanical Effect in Human Corneas by Same Energy, Different UV-A Fluence: An Enzymatic Digestion Comparative Evaluation

Anastasios J. Kanellopoulos, MD,*† Yannis L. Loukas, PhD,‡ and George Asimellis, PhD*

Purpose: To evaluate ex vivo the possible difference in corneal cross-linking (CXL) biomechanical effect of different ultraviolet-A (UV-A) irradiances.

Methods: The study involved 25 human donor corneas, randomly allocated to 5 groups (n = 5 each). CXL was applied with UV-A irradiances of 3, 9, 18, 30, and 45 mW/cm², maintaining equal cumulative energy dose of 5.4 J/cm². UV-A was delivered on half of the cornea. The nonirradiated halves served as controls. Specimens were subjected to collagenase-A enzymatic digestion. The time to complete dissolution in each specimen was recorded.

Results: Time to dissolution in group-A (3 mW/cm² for 30 minutes) was 321 ± 13.4 minutes (range: 300–330) compared with 171 ± 8.2 minutes (range: 165–180) for their control. In group-B (9 mW/cm² for 10 minutes), it was 282 ± 19.6 minutes (range: 270–315) compared with 177 ± 6.7 minutes (range: 165–180) for their control. In group-C (18 mW/cm² for 5 minutes), it was 267 ± 19.6 minutes (range: 240–285) compared with 177 ± 7.7 minutes (range: 165–180) for their control. In group-D (30 mW/cm² for 3 minutes), it was 252 ± 12.5 minutes (range: 240–270) compared with 180 ± 10.6 minutes (range: 165–195) for their control. In group-E (45 mW/cm² for 2 minutes), it was 204 ± 17.1 minutes (range: 180–225) compared with 186 ± 8.2 minutes (range: 180–195) for their control.

Conclusions: The data in this ex vivo human corneal study indicate that the biomechanical effect of CXL studied by resistance to enzymatic digestion in human corneas is comparable between irradiances of 9, 18 and 30 mW/cm² and seems to be reduced at a fluence of 45 mW/cm².

Key Words: corneal cross-linking, keratoconus, high-irradiance CXL, corneal biomechanics, high-energy CXL, reciprocity law, Bunsen–Roscoe law, collagenase-A, enzymatic digestion, accelerated CXL, rapid CXL

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Corneal cross-linking (CXL) has been clinically used for stabilizing progressive keratoclastic for more than a decade. This photochemical reactive process is induced by peak 370-nm ultraviolet-A (UV-A) radiation absorbed by riboflavin, a photosensitive vitamin B2 molecule, with an absorption maximum at 365 nm. The procedure is broadly accepted to result in corneal biomechanical strengthening not only in advanced keratoconus but also in early-stage and iatrogenic keratoclastic.

Collagenase has been known to contribute to breakdown of collagen in the corneal stroma. This collagenase-related breakdown is a vigorous biochemical process that has been used as an indirect metric of corneal biomechanical properties. The stabilizing biochemical effect of CXL may be thus reflected by an increased amount of resistance to collagenase digestion. CXL-treated porcine corneas have demonstrated nearly double the dissolution time after pepsin, trypsin, and collagenase digestion.

The original (standard) Dresden CXL protocol introduced epithelial removal and 30-minute corneal soaking with a dextran-based 0.1% riboflavin solution. UV-A illumination settings were 30 minutes with an irradiance of 3 mW/cm², corresponding to a dissipated energy of 5.4 J/cm².

We have subsequently introduced higher fluence, same-energy protocols, and many other investigators have subsequently introduced a multitude of CXL protocols currently in use internationally. The rationale of these protocols has been justified by the Bunsen–Roscoe reciprocity law, which states a certain biological effect is directly proportional to the total radiant exposure (energy dose), irrespective of application time. The reported limitations of the reciprocity law may indicate that there exists a range of applicability of how much the clinical UV-A radiation may be increased (and correspondingly, the application time shortened), which may need to be further investigated.

Despite their widespread clinical practice, a thorough clinical comparative validation of these approaches has not yet been published. The quantitative CXL effect between several of these protocols still remains elusive. The enzymatic...
Statistical analysis was performed by SPSS software version 21.0 (IBM Corporation, New York, NY). \( P \)-values less than 0.05 were indicative of statistically significant results. Results are reported in the form average \( \pm \) SD (range: minimum to maximum).

**RESULTS**

The average time to complete dissolution in the CXL-half specimens was 265 \( \pm \) 42 minutes, whereas in the non-CXL-half specimens (controls), it was 179 \( \pm \) 8 minutes. Descriptive statistics of time to dissolution (minutes) per group are presented in Table 1. Results are illustrated in Figure 1.

Time to dissolution results showed that as UV-A irradiance increased (from group-A to group-E), the time to dissolution decreased, indicating that the standard-protocol UV-A irradiance of 3 mW/cm\(^2\) achieved the strongest enzymatic digestion set of data. There was a statistically significant global difference between groups according to the analysis of variance (1-way ANOVA) (\( P = 0.0005 \)). The following 2-tailed \( P \)-values were recorded when comparing the 5 investigative groups: group-A with group-B 0.0079, group-B with group-C 0.259, group-C with group-D 0.1878, and group-D with group-E 0.00146. In other words, the standard-protocol UV-A irradiance of 3 mW/cm\(^2\) achieved statistically significant difference in time to dissolution in comparison with the “accelerated” groups (9, 18, and 30 mW/cm\(^2\)). The latter 3 groups displayed nonsignificant differences among them. Finally, group-E (45 mW/cm\(^2\)) seemed to have less time to dissolution by a statistically significant difference (\( P = 0.00146 \)) compared with the previous group-D. In addition, except group-E (45 mW/cm\(^2\)), in the remaining groups there was a statistically significant difference between CXL-half specimens and non-CXL-half specimens (controls). The \( P \)-values for the each CXL group compared with its control indicated significant differences between all groups except group-E (45 mW/cm\(^2\)), which had a nonstatistically significant difference with a 2-tailed Mann–Whitney \( U \) test \( P \)-value of 0.116 (Table 1). The 2-tailed \( P \)-values with records of comparison of the 5 cross-linked groups among them are presented in Table 2, which shows the statistically significant difference between the standard-treatment group-A and the “accelerated” groups (B to E).

**DISCUSSION**

Higher fluence (irradiance) variations have been shown to offer comparable corneal biomechanical stiffening compared with standard irradiance (3 mW/cm\(^2\)). Clinical results suggesting comparable effectiveness with the conventional protocol in stabilizing keratoconus progression have been reported. Equivalence in clinical parameters (including visual rehabilitation and refraction) and Scheimpflug imaging and anterior-segment optical coherence tomography imaging–derived parameters have also been demonstrated. Some reports suggest that accelerated CXL seems to be effective in preventing keratectasia progression in advanced keratoconus cases but is not as effective in less progressed stages.

Adding to this skepticism, some limitations of the reciprocity law have been reported in regard to CXL.

![Figure 1](image-url)  
**FIGURE 1.** Box plot illustrating time to dissolution (in minutes) results from the 5 groups of study.

![Graph](graph-url)

**TABLE 2.** Records of Comparison of the 5 Cross-linked Groups Among Them, Indicating 2-Tailed \( P \)-Values

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Group-A: 3 mW/cm\(^2\) for 30 minutes; group-B: 9 mW/cm\(^2\) for 10 minutes; group-C: 18 mW/cm\(^2\) for 5 minutes; group-D: 30 mW/cm\(^2\) for 3 minutes; group-E: 45 mW/cm\(^2\) for 2 minutes.
High-irradiance CXL combined with myopic LASIK: flap and residual stroma biomechanical properties studied ex-vivo

Anastasios John Kanellopoulos,1,2 George Asimellis,1 Joseph B Ciolino,3 Borja Salvador-Culla,3 James Chodosh3

ABSTRACT

Background/aims To evaluate ex vivo biomechanical and enzymatic digestion resistance differences between standard myopic laser in-situ keratomileusis (LASIK) compared with LASIK+CXL, in which high-irradiance cross-linking (CXL) is added.

Methods Eight human donor corneas were subjected to femtosecond-assisted myopic LASIK. Group A (n=4) served as a control group (no CXL). The corneas in LASIK+CXL group B were subjected to concurrent prophylactic high-irradiance CXL (n=4). Saline-diluted (0.10%) riboflavin was instilled on the stroma, subsequently irradiated with UV-A through the repositioned flap. The cornea stroma and flap specimens were separately subjected to transverse biaxial resistance measurements; biomechanical differences were assessed via stress and Young’s shear modulus. Subsequently, the specimens were subjected to enzymatic degradation.

Results For the corneal stroma specimen, stress at 10% strain was 128±11 kPa for control group A versus 293±20 kPa for the LASIK+CXL group B (relative difference Δ =+129%, p<0.05). The stress in group B was also increased at 20% strain by +68% (p<0.05). Shear modulus in group B was increased at 10% strain by +79%, and at 20% strain by +48% (both statistically significant, p<0.05). The enzymatic degradation time to dissolution was 157.5±15.0 min in group A versus 186.25±7.5 min in group B (Δt =+18%, p=0.014). For the flaps, both biomechanical, as well as enzymatic degradation tests showed no significant differences.

Conclusions LASIK+CXL appears to provide significant increase in underlying corneal stromal rigidity, up to +130%. Additionally, there is significant relevant enzymatic digestion resistance confirmatory to the above. LASIK flaps appear unaffected biomechanically by the LASIK+CXL procedure, suggesting effective CXL just under the flap.

INTRODUCTION

Corneal collagen cross-linking (CXL) has been clinically employed for stabilising progressive keratectasia.1–3 This photochemical reactive process is induced by peak 365 nm ultraviolet (UV-A) radiation absorbed by riboflavin, a photosensitive vitamin B2 molecule. The procedure is broadly known as corneal cross-linking—despite the fact that there are some reports suggesting that the mechanism responsible for biomechanical strengthening4 within the stroma is related not to interlamellar cohesion increase, but to inter-fibrillar and intra-fibrillar cohesion.4 In addition, increased collagen resistance against enzymatic degradation has been associated with CXL.6–8

We have introduced an alternative CXL application, adjuvant to myopic laser in-situ keratomileusis (LASIK+CXL). The application aims to improve long-term keratometric stability9 and to reduce regression likelihood following moderate and high myopic LASIK10 by proactively restoring corneal biomechanical strength.11 Riboflavin solution is briefly applied on the exposed stromal bed at the completion of the excimer ablation; the flap is then repositioned, followed by superficial UV-A irradiation.12,13

To the best of our knowledge, the biomechanical and/or enzymatic degradation resistance modulations achieved via CXL application concurrent with LASIK have not been studied in human corneas. The purpose of this study is to evaluate ex-vivo biomechanical and enzymatic degradation resistance differences in such application.

MATERIALS AND METHODS

Eight human donor corneas were involved, obtained by the Eye Bank for Sight Restoration Inc (New York, USA), an accredited member of the Eye-Bank Association of America. The corneas were donated by eight different donors (four men, four women) of average age 62.0±9.5 (43–72) years, stored in 4°C OptiSol solution (Bausch +Lomb, Rochester, New York, USA).

Surgical technique

All corneas were subjected to femtosecond-laser assisted myopic treatment. The corneas were mounted on an artificial anterior chamber (Baron, Katena Products, Inc, Denville, New Jersey, USA). Flaps (120 μm thick, 8.5 mm diameter) were created with the WaveLight FS200 femtosecond laser (Alcon Surgical, Ft Worth, Texas, USA), observing standard docking, applanation and vacuum-suction procedures (figure 1A). After flap lifting (figure 1B), the WaveLight EX5300 excimer laser (Alcon) was employed to create a −8.00 D myopic ablation over a 6.5 mm diameter optical zone (figure 1C). During the procedure, interferometric pachymetry embedded in the EX500 provided corneal thickness data.

Isotonic saline 0.1% riboflavin solution (Vibex Rapid, Avedro Inc, Waltham, Massachusetts, USA) was instilled on the exposed stromal bed afforded by the lifted flap (figure 1C). Soaking time was 1 min (figure 1D); then excess riboflavin was wiped from the cornea surface. Special care was taken to minimise potential riboflavin soaking to the folded LASIK flap.
The corneas were then randomly formed into two groups, four in each. Control group A received no further treatment. Group B (LASIK+CXL) was subjected to cross-linking: with the flap repositioned, the cornea was UV-A irradiated at 30 mW/cm² for 80 s (total fluence 2.4 J/cm²), employing the KXL device (Avedro) (figure 1F).

The flaps were then amputated from all corneas; stroma and flap specimens were stored back to OptiSol (4°C) until testing.

Biomechanical strength testing
The stroma specimens were prepared by razor-blade manual dissection to approximately 12×12 mm. The amputated flaps were tested without additional preparation. Transverse biaxial load-cell resistance measurements were accomplished by tangential shear-force employing the Biotester 5000 (CellScale Biomaterials Testing, Waterloo, Canada). The device records the simultaneous x-axis and y-axis displacement, applied force, and time. An integrated camera captures still, 1280×960-pixel images, which provide precise x-displacement and y-displacement measurements, analysed by custom software (LabJoy V9.05). The specimens were fixed (via random orientation) on a 4×5-tine rake arrangement clamped on their centre 3.5×3.5 mm section. The tines, of 250 μm diameter, were spaced by 0.7 mm (figure 2). The specimens were then submerged into an isotonic saline bath, temperature controlled at 37°C, for 5 min before (for temperature stabilisation) as well as during testing (to eliminate temperature-related variability). Shear rate was fixed to 4.16 μm/s. Time (s), x and y displacement (μm), and x and y force (mN) were recorded every second.

Enzymatic degradation
The stroma specimens were trephined into 8.5 mm diameter round buttons; no further processing on the amputated flaps. A 0.3% collagenase-A solution (active agent: Clostridium histolyticum) (Sigma-Aldrich, St Louis, Missouri, USA) was prepared via dilution in Dulbecco’s Phosphate Buffer Saline (DPBS, Sigma-Aldrich). Stromal and flap specimens were incubated within 1.5 mL of collagenase-A solution; the test tube racks were placed at 37°C on a plate shaker at 175 rotations per min.

Figure 1 Schematic of the surgical procedure LASIK combined with prophylactic high-irradiance corneal cross-linking (LASIK+CXL).

Figure 2 Fitting of the corneal specimens (top) and the flap (bottom) on the BioTester device.
Editorial
Cross-Linking and Corneal Imaging Advances

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1. Introduction

Corneal cross-linking, a technique employing UV-A illumination and a photomediator to induce corneal rigidity, is a widely recognized procedure for the stabilization or even possibly reversal of corneal ectasia progression in patients with keratoconus and post-LASIK ectasia. A rapidly growing number of clinical reports suggest a consistent stabilizing effect of cross-linking along with a variable improvement in corneal shape and visual function in some patients. In the past ten years there has been a continuous effort into understanding, ensuring safety and efficacy, and further expanding its applications, as well as exploring modifications aiming to optimize the technique. Research in the field of CXL is highly dynamic; techniques, concepts, and indications are constantly evolving. Recent advances in corneal cross-linking include applications for infections treatment that do not respond to topical medications; accelerated, high-fluence applications; prophylactic application in refractive surgery; modified beam profiles for selective treatments; fully customized induction of refractive changes in nonectatic eyes. We welcome in this special issue several papers on this subject covering topics such as the issue of epithelial removal with hypotonic riboflavin solution, as well as a contralateral study on this subject; study investigating rate of corneal collagen cross-linking redo, investigating risk factors and safety, including a study investigating the profile of microbial keratitis following CXL; long-term investigation of safety and visual outcome of Visian toric ICL implantation after CXL in keratoconus; long-term investigation of accelerated CXL in paediatric patients; biomechanical effects investigation of the correlation between tomographic and biomechanical severity of keratoconic corneas; and a novel application of intraoperative optical coherence tomography in CXL.

Keratoconus is considered an unpredictably progressive eye disease that “softens” the cornea. The progressive thinning and “bulging” of the cornea may distort or even significantly reduce vision. In advanced cases, one or more corneal transplant procedures and possibly additional eye surgeries may be required for visual rehabilitation. As it mainly affects younger people, it has severe consequences in their quality of life and their ability to contribute to the active workforce during their most productive years. In our experience within our ophthalmology center in Greece, through extensive studies conducted the last 10 years, we have found that in unpublished data possibly more than 1 out of 35 patients display some form of keratoconus in modern cornea diagnostics, compared to 1 out of 1,000–2,000 reported in Northern Europe and the United States. In addition, we have noted a higher degree of familial correlation of keratoconus reaching 90% topographic or tomographic suspicion in one of the two parents of a known young adult with keratoconus, a marked difference compared to the 10% genetic correlation that has been previously reported.

Over the last decade a new treatment, collagen cross-linking (CXL), has been introduced. In this treatment, vitamin B2 and ultraviolet light (UV-A) are applied to the cornea in a short procedure that “stiffens” the cornea and stops disease progression.
2. Current Treatment Options for Keratoconus Management

Keratoconus progression was traditionally observed. Visual rehabilitation was managed with spectacle correction and/or soft contact lenses, until irregular astigmatism necessitated application of rigid gas permeable (RGB) contact lenses.

In cases when this was not possible or there was RGP intolerance (estimated up to 21% of cases [1]), traditionally, a penetrating keratoplasty (PK) in which the patient’s cornea is discarded and replaced with a fresh donor cornea was employed. This procedure is associated with significant morbidity [2], as usually it takes about a week for the patient to return to normal everyday life and months, if not years, before that eye can be adequately visually rehabilitated. It is noted that, despite the use of this drastic procedure, visual rehabilitation may still necessitate additional repair and/or refractive procedures in order to reduce the very common irregular astigmatism and high postoperative anisometropia associated with penetrating keratoplasty.

Even in cases where PK generally achieved acceptable visual outcomes, long-term graft survival in keratoconic eyes declined rapidly after the second decade because the endothelial cells of the donor cornea tend to be slowly rejected by the host. Primary graft survival rates have been reported to 50% at 20 years [3], falling even further with repeat grafts. An alternative to PK is deep anterior lamellar keratoplasty (DALK) which does not have the disadvantage of short lifespan and associated complications. In DALK this risk is possibly lower as the endothelial cell layer of the host is preserved: a median graft survival of 49 years for DALK versus 17 years for PK has been reported. It is noted, however, that DALK techniques are technically challenging.

Other introduced treatment options for keratoconus are the insertion of intracorneal ring segments (ICRS). These inserts appear to significantly shift the shape of the cornea and may provide significant visual rehabilitation. Although, in clinical use for several years, there is no unison assessment of their stability and safety, we have reported along with other clinicians a number of significant short- and long-term complications associated with the ICRS.

Collagen cross-linking, on the other hand, has proven that it can effectively arrest the progression of keratoconus and corneal ectasia. The standard, epithelium-off Dresden protocol has been proven to be effective in arresting keratoconus progression.

Despite substantiated safety, we have reported, along with other clinicians, a range of complications associated with CXL. In addition to the standard CXL, other protocol variations introduced include alternative levels and amounts of energy, pulsing, oxygen supplementation, riboflavin solution concentrations, and route of administration within the cornea of the riboflavin solution. The underlying premise of these alternatives is that delivering a similar effect over a shorter period of time will not compromise safety in comparison with the standard protocol.

Our team has contributed many of the evolutionary steps of the initially introduced CXL technique:

(1) higher fluence,
(2) use of dextran-free riboflavin solution,
(3) combination of CXL with topography-guided excimer normalization of ectatic corneas (the Athens Protocol),
(4) prophylactic CXL in routine myopic and hyperopic LASIK,
(5) in situ CXL through a femtosecond laser created corneal pocket,
(6) photorefractive CXL.

Specifically, we have introduced the concept of accelerated, high-fluence collagen cross-linking (CXL) in post-LASIK ectasia, as well as the utilization of prophylactic CXL in routine LASIK, and in situ, femtosecond laser-assisted treatment of corneal ectasia, in attempting corneal deturgescence in bullous keratopathy, and as a prophylactic intervention adjuvant to Boston keratoprosthesis surgery.

3. The Need for Comparative Evaluation of CXL Protocols

Over the last ten years CXL has evolved to be a standard treatment for the arrest of the progression of keratoconus. Since the original Dresden protocol (3 mW/cm² for 30 minutes), several treatment CXL protocol variations have been introduced, most of them by our team [4]. These, however, have not been fully compared as far as their correlating effect. These variations involve higher fluence, such as the use of 6, 10, 18, and 30 mW/cm², and correspondingly shorter UV-A exposure time, aiming to deliver the same (5.4 J/cm²) or more total amount of energy, and presumably adequate stiffening effect [5]. Besides the original CXL protocol parameters evaluated more extensively, all newer-introduced CXL protocols have not been evaluated and correlated as extensively either clinically or ex vivo. In order to correlate the efficacy of the standard to newer CXL protocols the following prospective studies must be conducted, both clinically and ex vivo with the following parameters:

(i) ectasia stabilization (topographic and anterior elevation stability and/or improvement),
(ii) safety in regard to visual acuity loss, corneal clarity, corneal inflammation, and endothelial cell loss,
(iii) biomechanical/biochemical response parameters.

To the best of our knowledge, so far no direct and thorough comparative study of these CXL protocols has been conducted. The lack of CXL-techniques comparison is a noteworthy shortcoming. In a recent example, in a smaller-scale precursory prospective randomised trial carried by our team, contralateral eyes of 21 patients with progressive keratoconus were randomised to either conventional or high-fluence CXL (7 mW/cm² for 15 min).

Several assessment modalities for the evaluation of CXL efficacy exist. They include ex vivo biomechanical (tensile strength), biochemical (enzymatic digestion) [6], and in vivo
methods, for example, via OCT imaging demarcation line [7], corneal hysteresis (CH), and corneal resistance factor (CRF). CH is considered indications of corneal viscous damping, reflecting the capacity of corneal tissue to absorb and dissipate energy; CRF is considered an indicator of the overall corneal resistance.

The latter may be evaluated by dynamic tonometry (visualization of fast deformation of the cornea), employing the Corvis ST (Oculus Optikgerate GmbH, Wetzlar, Germany) and the Ocular Response Analyzer (Reichert, Buffalo, NY). The Corvis ST is a functional in vivo corneal biomechanics analyzer employing a noncontact tonometer and enabling recording the corneal reaction to an air impulse. An incorporated high-speed Scheimpflug camera (4,330 frames/sec) records still frames of the oscillating cornea. The device enables assessment of corneal biomechanics for various applications of refractive surgery, keratoconus screening, and cross-linking assessment.

Several studies have evaluated the reduction in corneal biomechanical strength following refractive surgeries such as LASIK. However, there is inconclusive evidence in the peer-review literature on the specificity of these techniques in the evaluation of the effect of corneal cross-linking [8].

Ex vivo corneal biomechanical evaluation may be conducted with biaxial stress-strain measurements. The BioTester 5000 (Cell Scale, Waterloo, Ontario, Canada) is a specifically developed biomaterials biaxial strength analyzer applicable to ex vivo corneal rigidity (Young’s modulus) measurements within a temperature-controlled media bath. Two high-performance actuators (two per axis) are capable of \( \mu \text{m} \) positional resolution for accurate test motion, with inline overload-protected load cell on each axis. The device captures and graphically displays live time, force, and synchronized video images for results analysis and verification. Data are easily exported to standard spreadsheet programs. Future promising diagnostics may include devices that are based on phonon spectroscopy as demonstrated already in studies on the Brillouin-based investigative devices.

A. John Kanellopoulos
Ronald R. Krueger
George Asimellis

References


2004: Over the last 11 years we have introduced and treated over 3000 cases of KCN and ectasia with CXL combined with a topo-guided excimer normalization: the “Athens Protocol” now practiced globally!!!
**Kanellopoulos AJ: JRS Sept 09:** 358 cases with over 2 year follow-up: 160 cases Sequential (left) Vs 198 cases same-day Combined (right)

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Sequential CXL and after TCAT

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Combined TCAT + CXL: The Athens Protocol
Average K from 48.5 to 44
Refraction \(-2.5-4.5@155\) (20/70) to \(-1-1.5@10\) (20/20)
Corneal Refractive Power and Symmetry Changes Following Non-Neutralization of Ectasias Treated With Partial Topography-Guided PTK Combined With Higher-Fluence CXL (The Athens Protocol)

Anastasios John Kanellopoulos, MD; George Asimellis, PhD

ABSTRACT

PURPOSE: To investigate preoperative and postoperative anterior and posterior keratometry and simulated corneal astigmatism in keratoconic eyes treated with collagen cross-linking combined with anterior surface normalization by partial topography-guided excimer ablation (the Athens Protocol).

METHODS: Anterior and posterior corneal keratometry were measured by Scheimpflug imaging for 267 untreated keratoconic eyes. Following treatment, they were assessed 1 year postoperatively.

RESULTS: Before treatment, average anterior keratometric value was 47.06 ± 6.02 diopters (D) for flat and 51.24 ± 6.75 D for steep. The posterior keratometric values were -6.70 ± 0.99 D (flat) and -7.67 ± 0.80 D (steep). The statistically significant postoperative change: there was minimal keratometric values did not demonstrate statistically significant keratometric values flattened. The posterior surface correlation between posterior and anterior corneal astigmatism was not significant (r = 0.762 ± 0.225). The statistically significant postoperative change indicated a...
We previously reported overall reduced corneal epithelial thickness in keratoconic eyes that were treated with (1) excimer laser debridement of the top 50 μm of the epithelium, (2) partial topography-guided excimer ablation, and (3) immediate high-fluence ultraviolet-A radiation (10 mW/cm²) and short-duration (10 minutes) corneal collagen crosslinking (CXL) with riboflavin in a procedure known as the Athens protocol.¹⁻³ Our goal was to arrest the keratoclastic progression⁴ and provide a less irregular anterior corneal surface. In a study,⁵ which was performed using high-frequency scanning ultrasound biomicroscopy (UBM), the epithelial thickness in a group of untreated keratoconic eyes was compared with that in a group of keratoconic eyes treated using the Athens protocol.

Epithelial thickness assessment has been facilitated by the development of anterior segment optical coherence tomography (AS-OCT).⁶ Although there are studies of AS-OCT epithelium measurement in the peer-reviewed literature, until recently and to our knowledge, the methodology and instrumentation mainly used an on-screen caliper tool⁷; thus, only local point-thickness measurements were reported. The
Group B and Group C was performed during the first clinical visit.

The main analysis report produced by the AS-OCT system displayed total corneal (reported as pachymetry) and epithelial 3-D thickness maps covering the 6.0 mm diameter area. Corneal pachymetry was assessed by the central corneal thickness (CCT) and minimum corneal thickness. Epithelial thickness assessment comprised the following measurements: pupil center, superior, inferior, minimum, maximum, mean, peripheral, topographic thickness variability, and epithelial thickness range. These data were collected as follows (Figure 2): Each thickness map was divided into 17 sections (2.0 mm diameter pupil center disk of 12.56 mm² area; 8 sectors [octants] within the annulus between the 2.0 mm and 5.0 mm zones, each of 8.24 mm² areas; and 8 sectors [octants] within the annulus of the 5.0 to 6.0 mm zones, each of 4.32 mm² areas). For each of these sections, the mean epithelial thickness was displayed numerically in integer form with a minimum difference of 1 μm over the corresponding area.

In this study, the reported center epithelial thickness was taken from the integer indication over the center 2.0 mm disk. The mean epithelial thickness was computed by the mean of all segments, and the peripheral epithelial thickness was computed by the mean of the thickness corresponding to 18 equispaced points along the 5.0 mm radius (data harvested by mouse-over indication over the epithelial thickness map). The superior, inferior, minimum, maximum, and topographic epithelial thickness variability (computed by the standard deviation [SD] of the 17 thickness values) were provided in tabular form by the software of the AS-OCT device (Figure 2). The thickness range was computed as follows: minimum epithelial thickness – maximum epithelial thickness.

Descriptive statistics, linear regression analysis to look for possible correlations, paired analysis t tests, and analysis of variance were performed using Minitab software (version 16.2.3, Minitab, Ltd.) and Origin Lab software (version 9, Originlab Corp.). Paired-analysis P values less than 0.05 were considered an indication of statistically significant results.

RESULTS
Table 1 shows the CCT, minimum corneal thickness, epithelial thicknesses, topographic thickness variability, and epithelial thickness range measured by AS-OCT in the 3 groups.

Group A (Athens protocol) comprised 175 eyes, 74 of women and 101 of men. The mean patient age at the time of surgery was 26.8 years ± 7.2 (SD) (range 18 to 48 years). There were 87 right eyes and 88 left eyes. The Athens protocol treatment was uneventful in all cases.

Group B (untreated keratoconic) comprised 193 eyes, 92 of women and 101 of men. The mean patient age at the time of examination was 31.1 ± 9.9 years (range 18.0 to 51.0 years). There were 91 right eyes and 102 left eyes.

Group C (control) comprised 160 eyes, 67 of women and 93 of men. The mean patient age at the time of examination was 35.45 ± 9.55 years (range 18.0 to 52.0 years). There were 74 right eyes and 86 left eyes.

Epithelial Thickness
In Group A, the difference in the center epithelial thickness between each postoperative timepoint was statistically significant (all P<0.05). The difference in the mean center epithelial thickness (−4.31 μm; 95% confidence interval [CI], −6.31 to −2.30) between Group A 1 year after treatment and Group B at the time of examination was statistically significant (P<0.05, 2-sample t test). The difference in the mean center epithelial thickness (−4.75 μm, 95% CI, −6.59 to −2.92) between Group A 1 year after treatment and Group C at the time of examination was also statistically significant (P<0.05) (Figure 3).

In Group A, the difference in topographic thickness variability between each postoperative timepoint was statistically significant (all P<0.05). Figure 4 shows the epithelial thickness variability and range by group.

DISCUSSIONS
Until recently, high-frequency UBM had been the gold standard for in vivo corneal epithelial 3-D imaging. The recent, rapid development and current high-speed imaging capabilities of AS-OCT have made acquisition of in vivo 3-D pachymetry corneal maps reliable and fast. Software refinement also enables clinical assessment of corneal asymmetry and focal thinning parameters for keratoconus classification. In addition, the higher axial resolution, increased accuracy, and finer image-processing capabilities of the current AS-OCT imaging systems have enabled, among other things, 3-D imaging of epithelial thickness.

Epithelial thickness and irregularity indices (eg, center and mean epithelial thickness, epithelial thickness topographic irregularity, and thickness range)
Variable Fluence, topo-guided CXL

KXL II device (Avedro, Waltham, MA, USA) CE marked 2 year
The Athens Protocol evolution
topo-guided PTK+variable fluence topo-customized CXL
Comparison of prophylactic higher fluence corneal cross-linking to control, in myopic LASIK, one year results

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Costas Karabatsas1

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Purpose: To compare 1-year results: safety, efficacy, refractive and keratometric stability, of femtosecond myopic laser-assisted in situ keratomileusis (LASIK) with and without concurrent prophylactic high-fluence cross-linking (CXL) (LASIK-CXL).

Methods: We studied a total of 155 consecutive eyes planned for LASIK myopic correction. Group A represented 73 eyes that were treated additionally with concurrent prophylactic high-fluence CXL; group B included 82 eyes subjected to the stand-alone LASIK procedure. The following parameters were evaluated preoperatively and up to 1-year postoperatively: manifest refractive spherical equivalent (MRSE), refractive astigmatism, visual acuity, corneal keratometry, and endothelial cell counts. We plotted keratometry measurements pre-operatively and its change in the early, interim and later post-operative time for the two groups, as a means of keratometric stability comparison.

Results: Group A (LASIK-CXL) had an average postoperative MRSE of −0.23, −0.19, and −0.19 D for the 3-, 6-, and 12-month period, respectively, compared to −6.58±1.98 D preoperatively. Flat keratometry was 37.69, 37.66, and 37.67 D, compared to 43.94 D preoperatively, and steep keratometry was 38.35, 38.36, and 38.37 D, compared to 45.17 D preoperatively. The predictability of Manifest Refraction Spherical Equivalent (MRSE) correction showed a correlation coefficient of 0.979. Group B (stand-alone LASIK) had an average postoperative MRSE of −0.23, −0.20, and −0.27 D for the 3-, 6-, and 12-month period, respectively, compared with −5.14±2.34 D preoperatively. Flat keratometry was 37.65, 37.89, and 38.02 D, compared with 43.15 D preoperatively, and steep keratometry was 38.32, 38.57, and 38.66 D, compared with 44.07 D preoperatively. The predictability of MRSE correction showed a correlation coefficient of 0.970. The keratometric stability plots were stable for the LASIK CXL group and slightly regressing in the standard LASIK group, a novel stability evaluation metric that may escape routine acuity and refraction measurements.

Conclusion: Application of prophylactic CXL concurrently with myopic LASIK surgery appears to contribute to improved refractive and keratometric stability compared to standard LASIK. The procedure appears safe and provides a new potential for LASIK correction.

Keywords: myopic LASIK regression, femtosecond myopic LASIK, LASIK-CXL, LASIK-Xtra, high myopia, accelerated high-fluence collagen cross-linking

Introduction

Laser-assisted in situ keratomileusis (LASIK) is the most common form of refractive surgery,1,2 offering predictable and stable refractive and visual outcomes.3 Specifically, in correcting moderate to high myopia (equal or more than −6.00 D in the least-minus meridian),4,5 there have been reports in the past indicating significant long-term regression.6-8 The work by Alió et al7 reported that 20.8% of high myopia cases required
Combined laser in situ keratomileusis and prophylactic high-fluence corneal collagen crosslinking for high myopia: Two-year safety and efficacy

Anastasios John Kanellopoulos, MD, George Asimellis, PhD

PURPOSE: To evaluate the safety, efficacy, and refractive and keratometric stability of myopic femtosecond laser in situ keratomileusis (LASIK) with concurrent prophylactic high-fluence corneal collagen crosslinking (CXL) compared with the outcomes of standard femtosecond LASIK.

SETTING: Private clinical practice, Athens, Greece.

DESIGN: Consecutive randomized prospective comparative study.

METHODS: Eyes that had myopic LASIK or myopic LASIK with concurrent high-fluence CXL were evaluated preoperatively and up to 2 years postoperatively for manifest refraction spherical equivalent (MRSE), refractive astigmatism, visual acuity, corneal keratometry (K), and endothelial cell count.

RESULTS: One hundred forty consecutive eyes had myopic LASIK; 65 of the eyes were treated additionally with CXL. In the LASIK–CXL eyes, the mean postoperative MRSE was −0.18 diopter (D) ± 17.0 (SD) from −6.67 ± 2.14 D preoperatively. The postoperative flat K was 37.67 D from 43.92 D, and the steep K was 38.38 D from 45.15 D. The correlation coefficient of SE correction predictability was 0.975. In the LASIK-only eyes, the mean postoperative MRSE was −0.32 ± 0.24 D from −5.49 ± 1.99 D preoperatively. The flat K was 38.04 D from 43.15 D, and the steep K was 38.69 D from 44.03 D. The correlation coefficient of SE correction predictability was 0.968. The differences between the 2 groups at the 20/20 and 20/25 levels were statistically significant (P = .045 and P = .039, respectively).

CONCLUSION: Two-year results indicate that the application of prophylactic CXL concurrently with high-myopic LASIK appears to improve refractive and keratometric stability, presumably by affecting corneal biomechanical properties.

Financial Disclosure: Dr. Kanellopoulos is a consultant to Alcon Surgical, Inc., Wavelight Laser Technologie AG, Allergan, Inc., Avedro, Inc., and i-Optics Corp. Dr. Asimellis has no financial or proprietary interest in any material or method mentioned.

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Laser in situ keratomileusis (LASIK) is a widely accepted method to correct refractive errors and offers predictable and stable refractive and visual outcomes, specifically in the correction of moderate to high myopia (equal to or greater than −6.00 diopters [D] in the least minus meridian of both eyes). However, some studies have reported significant long-term regression. In a study by Alió et al., 1 in 5 (ie, 20.8%) cases with high myopia required retreatment because of overcorrection, undercorrection, or regression.

Our experience with LASIK correction of high myopia suggests a slight (0.5 D) trend toward long-term postoperative corneal steepening. We have therefore attempted prophylactic in situ corneal collagen crosslinking (CXL) on the stromal bed concurrent with LASIK, particularly in highly myopic eyes with thin residual stroma and young patients who...
patient with a between-eye difference in the amount of astigmatism greater than 0.5 D. In our opinion, it does not merit "leniency" with preoperative forme fruste keratoconus criteria. All cases in this study, as well as in our previous reports, were screened thoroughly for signs of tomographic cornea irregularity.

In this study, we propose and document that keratometric stability may be a more objective measure of 2-year efficacy and the data indicate that this measure was statistically significantly better in the LASIK–CXL group \( (P = .032) \). In our opinion, this correlation is compelling. Also, regarding visual acuity results, the difference in the percentage of cases achieving a UDVA of 20/20 and 20/25 in the 2 groups was statistically significant \( (P = .045 \) and \( P = .039 \), respectively), with the LASIK–CXL group performing better.

In conclusion, LASIK combined with prophylactic CXL intervention in eyes with high myopia appears to provide predictability as well as refractive and keratometric stability. The data reported in this study provide evidence of the safety and efficacy of this approach. The adjuvant CXL procedure may provide enhanced corneal biomechanical stability, especially in highly myopic and younger eyes.

**WHAT WAS KNOWN**

- There can be regression after LASIK in high myopia treatments. This may reflect altered cornea biomechanical properties.
- Comparative clinical data are lacking about the combined procedure of LASIK with prophylactic CXL application.

**WHAT THIS PAPER ADDS**

- Laser in situ keratomileusis combined with CXL in patients with higher myopia appears to be a safe and effective treatment.
- Laser in situ keratomileusis combined with CXL appears to reduce the likelihood of postoperative regression.

**REFERENCES**


**Figure 8.** Stability of corneal keratometry in both groups expressed in diopters up to 2 years postoperatively (CXL = corneal collagen crosslinking; LASIK = laser in situ keratomileusis).
HYPEROPIC LASIK+CXL

A drop of 0.1% riboflavin sodium phosphate solution, just prior to its spread over the exposed stromal bed
Comparison of Keratometric Stability compelling evidence that LASIK Xtra works and maybe a necessary adjunct in hyperopic LASIK

Corneal Collagen Cross-linking Combined With Simulation of Femtosecond Laser–Assisted Refractive Lens Extraction: An Ex Vivo Biomechanical Effect Evaluation

Anastasios J. Kanellopoulos, MD,* † Mark A. Kontos, MD,‡ Shihao Chen, MD, OD, MSc,§ and George Asimellis, PhD*

Purpose: To evaluate biomechanical changes induced by in situ corneal cross-linking (CXL) with stromal pocket delivered enhanced concentration riboflavin and high-fluence, high-energy UV-A irradiation.

Methods: Eight human donor corneas were subjected to intrastromal lamellar corneal tissue removal of anterior 140-μm deep, 80-μm thick × 5-mm diameter central stromal buttons, extracted through a 3.5-mm width tunnel, surfacing in the superior cornea periphery. Enhanced concentration riboflavin solution (0.25%) was instilled in the pocket. In study group A (CXL), superficial high-fluence UV-A irradiation was applied, whereas in control group B (no CXL), none. To comparatively assess changes in corneal rigidity, corneal specimens were subjected to transverse biaxial resistance measurements by application of a unidirectional tangential shear force. Biomechanical differences were evaluated through stress and Young shear modulus.

Results: Stress at 10% strain was 305 ± 24 kPa in study group A versus 157 ± 11 kPa in control group B (relative difference Δ = 107%, P = 0.021). Stress at 20% strain was 1284 ± 34 kPa in study group A versus 874 ± 29 kPa in control group B (Δ = 47%, P = 0.043). Average shear modulus in study group A at 10% strain was 6.98 ± 1.12 MPa versus 4.04 ± 0.85 MPa in control group B (Δ = 73%, P = 0.036). Average shear modulus in study group A at 20% strain was 11.46 ± 0.75 MPa versus 8.80 ± 0.72 MPa in group B (Δ = 30%, P = 0.047).

Conclusions: Adjunct CXL in this ex vivo simulation refractive lens extraction procedure seems to provide significant increase in corneal rigidity, up to +107%. These findings also support our previous reported work on laser in situ keratomileusis combined with CXL.

Key Words: femtosecond laser, refractive lens extraction, biomechanical simulation, in situ CXL, epithelium on, high-fluence CXL, high-energy CXL, higher riboflavin concentration CXL, corneal biomechanics, Young shear modulus, corneal stress–strain (Cornea 2015;0:1–7)

Corneal collagen cross-linking (CXL) has been clinically used for stabilizing progressive keratectasia.1,2 A photo-chemical reactive process induced by 365-nm UV-A light in the presence of riboflavin, a photosensitive molecule, results in increased intrafibrillar and interfibrillar covalent bonds3 and stromal collagen resistance against enzymatic degradation.4,5 The increased stromal biomechanical strength and lamellar compaction lead to corneal stabilization.6

The original protocol Dresden technique7 requires corneal epithelium removal (epithelium off) and anterior stromal saturation with riboflavin solution. Aiming to address the significant morbidity and postoperative pain associated with epithelial removal,7 several alternatives have been proposed. One such alternative is to keep the epithelium in place and attempt to loosen the epithelial tight junctions to facilitate riboflavin diffusion with prolonged use of topical anesthetics, in a procedure termed “epithelium-on” CXL.8 Our team has introduced alternative CXL techniques using higher fluence, shorter duration UV-A irradiation, and administration of riboflavin solution in an anterior intrastromal lamellar pocket created with a femtosecond laser.9

A subsequent CXL alternative application we also presented (Kanellopoulos AJ Prophylactic, ultraviolet a cross-linking combined at the completion of high risk myopic LASIK cases. Subspecialty Day Paper presentation, American Academy of Ophthalmology Annual Meeting, Nov 8, 2008, Atlanta, GA) aimed to proactively restore corneal biomechanical stability10 in myopic laser in situ keratomileusis (LASIK) corrections.11–13 Riboflavin is applied on the exposed stromal bed afforded by the open LASIK flap; the flap is then repositioned, followed by UV-A irradiation of the riboflavin-soaked stroma through the flap.14,15

Recently reported refractive error correction procedures involve intrastromal tissue dissection performed solely by a femtosecond laser.16,17 Although the mechanism of stromal tissue reduction in these procedures is different from LASIK, the issue of potential biomechanical weakening, particularly in attempted high myopic corrections has been modeled18 and investigated clinically.19 The combination of CXL with such
Long-Term Safety and Efficacy of High-Fluence Collagen Crosslinking of the Vehicle Cornea in Boston Keratoprosthesis Type 1

Anastasios J. Kanellopoulos, MD,*† and George Asimellis, PhD*

Purpose: The aim of this study was to evaluate the safety and efficacy of very high-fluence collagen crosslinking (CXL) as a means of achieving increased corneal rigidity and reduced enzymatic digestion in the vehicle cornea of Boston keratoprosthesis (KPro) type 1.

Methods: Eleven consecutive patients fitted with a KPro (5 with a previous repeat cornea graft failure, 4 with ocular cicatricial pemphigoid, and 2 with chemical burn) underwent donor vehicle cornea pretreatment with very high-fluence prophylactic CXL in a 2-step procedure. First, the donor cornea was crosslinked with an intrastromal riboflavin instillation through a femtosecond laser–created pocket. This was followed up with a superficial CXL treatment. On the completion of the CXL pretreatment, the cornea center was trephined with the femtosecond laser, and the KPro was fitted onto the crosslinked donor cornea. Visual acuity, corneal surface, and donor vehicle cornea stability were evaluated. Follow-up evaluations were conducted over the next 9 years with an mean of 7.5 years.

Results: Mean uncorrected visual acuity improved from light perception to 20/60. One patient required a follow-up surgery, because of significant melt in the host cornea. None of the eyes developed melts and/or infection, especially on the vehicle cornea on which the KPro was fitted.

Conclusions: Pretreatment with intrastromal and superficial very high-fluence CXL in conjunction with Boston type 1 KPro seems to be a safe and effective adjunctive treatment for achieving increased vehicle donor cornea rigidity. Additionally, there is an increased resistance to enzymatic degradation. This application may serve to enhance the biomechanical stability and external disease resistance of the donor vehicle cornea in patients with advanced external disease.

Key Words: prophylactic pretreatment with collagen crosslinking, Boston keratoprosthesis type 1, Dohlman keratoprosthesis, severe external disease, ocular cicatricial pemphigoid, chemical burn, repeat cornea transplantation failure

Extreme external disease has been successfully treated over the last few decades with allograft cornea transplantation, relatively histocompatible limbal stem-cell transplantation, or keratoprosthesis (KPro). There are several KPro variations. Currently, the most prevalent in clinical practice are the Boston KPro,† the AlphaCor, the odonto-KPro, the Fyodorov KPro, and the KeraClear inlay KPro.

Our team has introduced the concept of accelerated, high-fluence collagen crosslinking (CXL) in post–laser in situ keratomileusis (LASIK) ectasia and the use of prophylactic CXL in routine LASIK in treatment of cornea ectasia and in attempting corneal deturgescence in bulbus keratopathy.

In our 20 years of experience in using the Boston KPro, we encountered 2 significant complications: (1) melts and (2) erosion of the donor and host cornea interface. When the latter occurs, there is an increase in the risk of developing infectious keratitis, which will significantly increase the risk of potential endophthalmitis and may predispose to endophthalmitis exposure and/or infection. We hypothesized that the application of a prophylactic crosslinking treatment on the donor vehicle cornea might help reduce the susceptibility of these corneas to enzymatic digestion and cornea infection. This work presents an evaluation of a longitudinal case series to study the advantages of using CXL as a prophylactic intervention adjuvant to Boston KPro surgery.

MATERIALS AND METHODS

This work adhered to the tenets of the Declaration of Helsinki, and the study received the approval from the Ethics Committee of our Institution. All patients were provided a written informed consent before the treatment, and a comprehensive explanation of the benefits and risks of this procedure was presented to them by the operating surgeon (A.J.K.).
RESULTS

The mean age of the patients was 67 ± 14 years. Six patients were female and 5 were male. The visual acuity assessed from preoperative light perception and/or hand motion showed a 6-month postoperative improvement. The average UDVA was 20/80 (range: 20/100–20/40), and the CDVA was 20/70 (range: 20/80–20/32).

These patients are still being followed up. After the first postoperative year, each patient is evaluated at least annually. During the long follow-up time that these patients have been continuously monitored (minimum 1 year, maximum 9 years), 2 of the patients required subsequent injection of intracameral and triamcinolone and bevacizumab injection (Avastin; Genentech/Roche, San Francisco, CA) because of cystoid macular edema. Additionally, 1 patient needed yttrium aluminum garnet laser intervention for a retroprosthesis inflammatory membrane that was quite dense and had resulted in a CDVA reduction from 20/60 to 20/400. After this procedure, the patient’s vision returned to 20/50.
Is CXL a refractive procedure?
Most investigators speak of “disease reversal” when flattening occurs after CXL in ectasia
This is a simple 3mW CXL-alone case from 2005
No scar developed, Now 2013 has Flattened 12D!!!
Novel myopic refractive correction with transepithelial very high-fluence collagen cross-linking applied in a customized pattern: early clinical results of a feasibility study

Anastasios John Kanellopoulos
LaserVision.gr Institute, Athens, Greece, and New York Medical School, New York, NY, USA

Background: The purpose of this study is to report the safety and efficacy of a new application of collagen cross-linking using a novel device to achieve predictable refractive myopic changes in virgin corneas.

Methods: Four cases were treated with a novel device employing very high-fluence collagen cross-linking applied in a myopic pattern. Prior to treatment, riboflavin solution was applied to the intact epithelium. The collagen cross-linking device was then engaged for a total of 12 J/cm², to be applied transepithelially in a predetermined pattern. Cornea clarity, corneal keratometry, and corneal topography were evaluated by both Placido disc and Scheimpflug imaging, along with cornea anterior segment optical coherence tomography and endothelial cell counts.

Results: An average of 2.3 diopters was achieved in the first week in all four cases treated with the very high-fluence myopic collagen cross-linking intervention. There was a slight regression to 1.44 diopters at 1 month, which remained stable at 6-month follow-up. The mean keratometry change was from 44.90 diopters to 43.46 diopters. There was no significant change in endothelial cell counts or corneal clarity. There was some mild change in epithelial thickness distribution, with the treated area showing a slight but homogeneous reduction in mean thickness from 52 μm to 44 μm.

Conclusion: This report describes the novel application of very high-fluence collagen cross-linking with a predictable well defined myopic refractive (flattening) corneal effect. This technique has the advantages of essentially no postoperative morbidity, immediate visual rehabilitation, and the potential for tapering until the desired result is achieved.

Keywords: myopia, refractive correction, high-fluence collagen cross-linking, clinical results

Introduction
Collagen cross-linking has been used for many years as a means of stabilizing cornea ectasia.1–5 Although a multitude of treatments and techniques are available, it has been well documented that the procedure almost invariably results in some central anterior corneal flattening,1–5 which has often been interpreted as “disease regression.” As our understanding and the technology available for collagen cross-linking has progressed, it has been theorized that differential application of collagen cross-linking in specific areas of the cornea may produce predictable refractive changes. Several aspects of this theory need further investigation. Is it possible to achieve predictable refractive changes? Can this be achieved through an intact epithelium? Can the human cornea tolerate higher fluence of ultraviolet light? This paper describes the use of a novel...
Hyperopic correction: clinical validation with epithelium-on and epithelium-off protocols, using variable fluence and topographically customized collagen corneal crosslinking

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Purpose: To report novel application of topographically-customized collagen crosslinking aiming to achieve hyperopic refractive changes. Two approaches were evaluated, one based on epithelium-off and one based on epithelium-on (transepithelial).

Methods: A peripheral annular-shaped topographically customizable design was employed for high-fluence ultraviolet (UV)-A irradiation aiming to achieve hyperopic refractive changes. A total of ten eyes were involved in this study. In group-A (five eyes), a customizable ring pattern was employed to debride the epithelium by excimer laser ablation, while in group-B (also five eyes), the epithelium remained intact. In both groups, specially formulated riboflavin solutions were applied. Visual acuity, cornea clarity, keratometry, topography, and pachymetry with a multitude of modalities, as well as endothelial cell counts were evaluated.

Results: One year postoperatively, the following changes have been noted: in group-A, average uncorrected distance visual acuity changed from 20/63 to 20/40. A mean hyperopic refractive increase of +0.75 D was achieved. There was some mild reduction in the epithelial thickness. In group-B, average uncorrected distance visual acuity changed from 20/70 to 20/50. A mean hyperopic refractive increase of +0.85 D was achieved. Epithelial thickness returned to slightly reduced levels (compared to baseline) in group-A, whereas to slightly increased levels in group-B.

Conclusion: We introduce herein the novel application of a topographically-customizable collagen crosslinking to achieve a hyperopic refractive effect. This novel technique may be applied either with epithelial removal, offering a more stable result or with a non-ablative and non-incisional approach, offering a minimally invasive alternative.

Keywords: topography customizable crosslinking, high-fluence cross linking, epi-on and epi-off CXL, PiXL, KXL II, CXL hyperopic correction, CXL presbyopic correction

Introduction
Over the past decade, corneal collagen crosslinking (CXL) has become routine for stabilizing corneal ectasia,1,2 as well as in the management of corneal infections.3-5 Laboratory data suggest that the CXL application, consisting of riboflavin injection and ultraviolet (UV)-A irradiation, increases stromal collagen fibril diameter resulting in improved corneal biomechanical strength.6 Several clinical reports indicate that this corneal stiffening results not only in arresting ectasia progression,7,8 but also in reduction of corneal keratometry,9-11 perceived as central corneal flattening.12-15 We have reported on the use of prophylactically, higher fluence CXL as an agent for refractive stabilization in high-myopic and hyperopic LASIK,16-18 and have also recently reported
perform feasibility studies with the KXL II device (Avedro), the current study provides additional evidence for the potential of custom-designed, selected pattern application CXL in the deliberate treatment of refractive error. We have reported previously topographically central cornea flattening effects consistent with a correction of myopia of about 2.5 D\textsuperscript{27} with central application of customized CXL. The available interim data appear promising with regard to the potential for correcting low myopic refractive errors without tissue removal in an excimer-like fashion or other previously described thermal techniques combined with CXL.\textsuperscript{28} Such myopic and astigmatic corrections\textsuperscript{29} are novel applications that are currently under study with this technology with a procedure called Photorefractive Intrastromal Crosslinking (PiXL).

While customarily, CXL has been applied centrally, the specific annular application demonstrated herein presents a novelty. The peripheral topographically-customized application of crosslinking presented herein was designed to induce a preferential corneal stiffening along the peripheral annulus of 6–9 mm. As shown in Figures 2B and C, the aim of this stiffening was to induce a corneal steepening (positive) differential within the central 6 mm zone.

Six months postoperatively, a very specific hyperopic correction of +0.8 D (reaching up to +2.5 D at certain loci in group-A, and up to +1.5 D in group-B) was observed (Table 1 and Figures 3 and 4). These results were stable up until the 1 year examination, too. The fact that these refractive changes were achieved in cases involving relatively elderly patients, and the known “natural” increase of corneal rigidity with age,\textsuperscript{30} may be suggestive of the increased potential of this application in a younger population.

The corneal thickness epithelial remodeling maps, when compared to normal eyes,\textsuperscript{11} suggest that there was some modest change in the epithelial distribution. In group-A, the cornea was slightly thinner (average 532 μm from 535 μm preoperatively in group-A and 544 μm from 552 μm preoperatively in group-B). The epithelial thickness remodeling suggested a slightly different progression. Specifically, in group-A (PTK), the epithelium was reduced

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**Figure 3** Placido disk topography sagittal curvature maps (diameter 9 mm) depicting hyperopic refractive changes.

**Notes:** 1: 6 months postoperatively; 2: preoperative. (A) Example from group-A, PTK; (B) example from group-B, transepithelial.

**Abbreviation:** PTK, laser-debridement epithelial removal.
Toric Topographically Customized Transepithelial, Pulsed, Very High-Fluence, Higher Energy and Higher Riboflavin Concentration Collagen Cross-Linking in Keratoconus

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Key Words
Topography customizable cross-linking · High-fluence cross-linking · Transepithelial cross-linking · Toric cross-linking · Keratoconus · Photorefractive intrastromal cross-linking · KXL II

Abstract
Purpose: To report a novel application of toric topographically customized transepithelial collagen cross-linking (CXL) aiming to achieve refractive astigmatic changes in a keratoconic cornea. Methods: Specially formulated riboflavin transepithelial administration and delivery of high-fluence UVA in a topographically customized pattern was applied in an eye with progressive keratoconus. Visual acuity, cornea clarity, keratometry, topography, and pachymetry with a multitude of modalities, as well as endothelial cell counts were evaluated for >6 months. Results: Uncorrected distance visual acuity changed from preoperative 20/40 to 20/25 at 6 months. A mean astigmatic reduction of 0.8 D, and significant cornea surface normalization was achieved 6 months postoperatively. There was some mild change in the epithelial distribution, with the treated area having a slight normalization in the average epithelial thickness. Conclusions: We introduce herein the novel application of a topograph-
Fig. 4. Anterior segment OCT imaging pachymetry maps for the cornea (left) and corneal epithelium (right) covering the center of the 6-mm diameter area. Top panel: preoperatively; middle panel: 1-week postoperatively, and bottom panel: 6 months postoperatively.
Conclusions

CXL stabilizes ectasia

CXL has proven as biomechanical modulator helpful in LASIK

Can resist cornea enzymatic melt (Kpro, infections)

Can have predictable refractive effects (refractive CXL)

Epi-on CXL may work by modifying ribo bioavailability (concentration, solution, in-pocket installation, iontophoresis etc)

May treat infection

Our team has introduced and proven concept on numerous novel CXL applications

Industry leaders should pursue evaluation and standarized correlation of the multitude of CXL protocols currently used