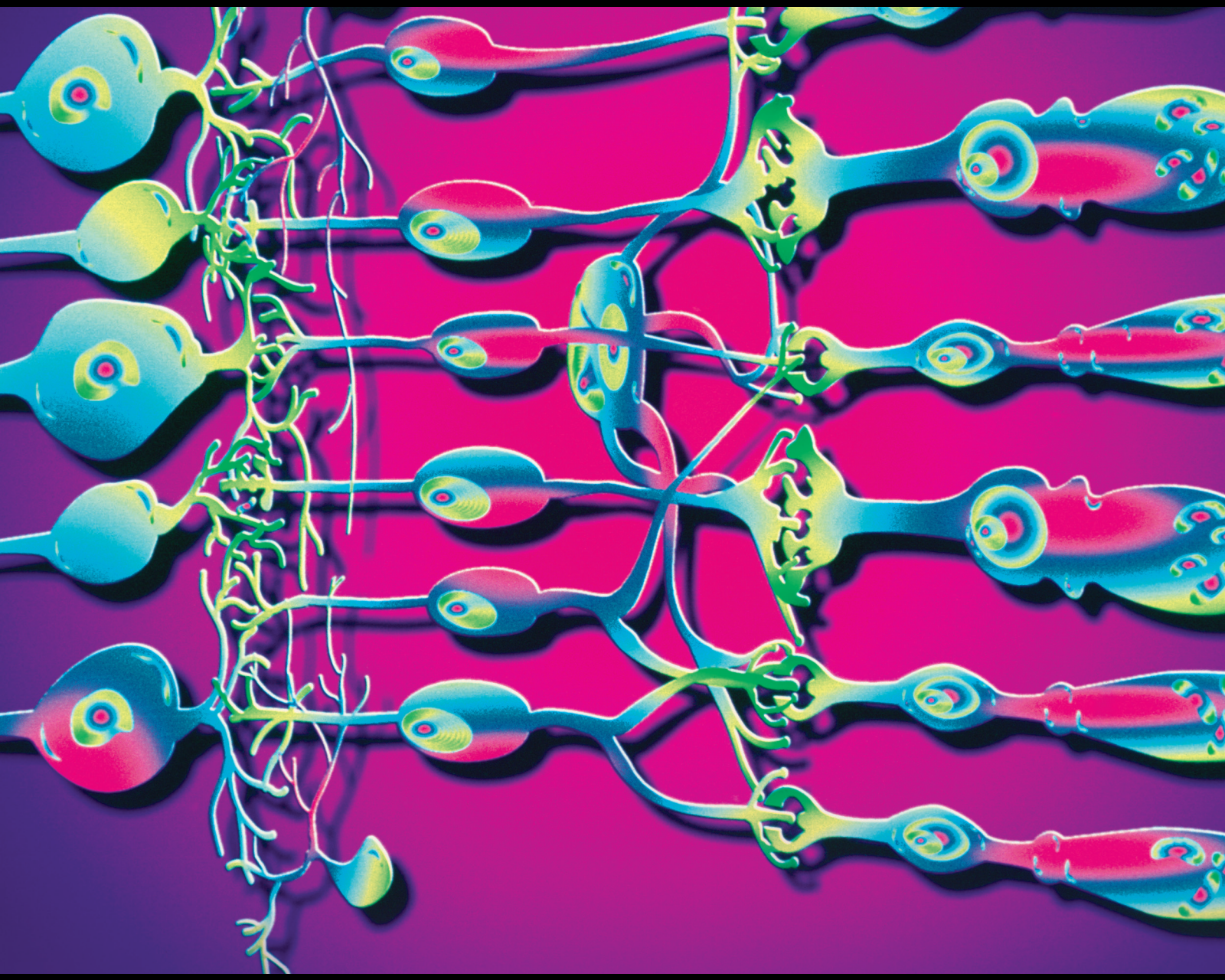
Journal of Ophthalmology

Current Concepts and Future Developments of Corneal Cross-Linking

Guest Editors: Suphi Taneri, Elias Jarade, John A. Kanellopoulos, and David Muller



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Editorial Board

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Editorial

Current Concepts and Future Developments of Corneal Cross-Linking

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The introduction of corneal cross-linking (CXL) has changed the landscape of treatment and management of keratoconus and ectasia after refractive surgery. Previously, treatment options provided only temporary visual rehabilitation without limiting disease progression. Keratoconus and ectasia resulted in significant vision-related reduction in quality of life and a substantial lifetime economic burden, with up to 20% of keratoconus cases resulting in eventual corneal transplantation [1]. CXL was introduced as the first therapeutic option for keratoconus aimed at stiffening the cornea in order to treat the underlying stromal instability. From the time that the first reports of the clinical application of cross-linking in the cornea were published in 2003 [2], CXL has rapidly been adopted as a standard therapy for treatment of progressive keratoconus in much of the world [3–5].

The potential for early intervention with corneal cross-linking *before* visual function has been compromised has resulted in a shift in the way we think about corneal biomechanics and has reawakened interest in the early diagnosis of ectasia. In this issue, J. Steinberg et al. report two new parameters to detect biomechanical changes in the keratoconic cornea after CXL using in vivo corneal visualization Scheimpflug technology. Research in the area of corneal biomechanics has the potential to enable earlier diagnosis of patients in need of CXL and better analysis of the effects of the procedure.

While many questions remain unanswered, the understanding of the photochemical mechanisms that result in the formation of cross-links in the cornea has grown exponentially in the last decade. Improved scientific understanding of the mechanisms of CXL has driven clinical research aimed at optimizing CXL for better efficiency and efficacy [6]. In this issue, A. C. da Paz et al. present a critical review of known and as yet undetermined effects of CXL on corneal structure, biomechanics, and functional aspects. J. Antoun et al. examine patient characteristics that contribute to cross-linking failure to stabilize keratometry and examine the effect of repeat treatments on eyes that have continued to progress after primary CXL.

The remaining contributions to the special issue explore modifications to the conventional protocol that incorporate new technology, such as transepithelial riboflavin formulations designed to improve patient comfort (S. Taneri et al.) and higher irradiance and pulsed UVA delivery aimed at improving procedure speed and photon efficiency (C. Mazzotta et al.). CXL combination procedures targeted at maximizing visual outcomes through the addition of simultaneous intracorneal ring implantation (P. Studeny et al.) or subsequent phakic toric implantable collamer lens insertion (R. Antonios et al.) are also discussed.

Together, the papers in this special issue describe the next generation of corneal cross-linking. The contributors explore

the potential to maximize CXL efficacy, provide equivalent or greater treatment effect in shorter total treatment times, reduce patient discomfort and speed visual recovery, and offer both stabilization and functional visual improvement through CXL combination procedures.

Suphi Taneri
Elias Jarade
John A. Kanellopoulos
David Muller

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Research Article

Safety and Visual Outcome of Visian Toric ICL Implantation after Corneal Collagen Cross-Linking in Keratoconus: Up to 2 Years of Follow-Up

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Purpose. To evaluate the long-term safety and clinical outcome of phakic Visian toric implantable collamer lens (ICL) insertion after corneal collagen cross-linking (CXL) in progressive keratoconus. *Methods*. This was a retrospective study of 30 eyes (19 patients), with progressive keratoconus, who underwent sequential CXL followed by Visian toric ICL implantation after 6 months. *Results*. At baseline, 6 eyes had stage I, 14 eyes stage II, and 10 eyes stage III keratoconus graded by Amsler-Krumeich classification. At 6 months after CXL, only K (steep) and K (max) decreased significantly from baseline, with no change in visual acuity or refraction. Flattening in keratometric readings was stable thereafter. There was significant improvement in mean uncorrected distance visual acuity (1.57 ± 0.56 to 0.17 ± 0.06 logMAR, $P < 0.001$) and mean corrected distance visual acuity (0.17 ± 0.08 to 0.11 ± 0.05 logMAR, $P < 0.001$) at 12 months after ICL implantation that was maintained at the 2-year follow-up. Mean cylinder power and mean spherical equivalent (SE) also decreased significantly after ICL implantation. A small hyperopic shift in SE (+0.25 D) was observed at 2 years that did not alter visual outcomes. *Conclusions*. Visian toric ICL implantation following CXL is an effective option for improving visual acuity in patients with keratoconus up to 2 years.

1. Introduction

Keratoconus is a progressive noninflammatory thinning disorder of the cornea leading to a decrease in visual acuity as a result of myopia and irregular astigmatism [1, 2]. Corneal collagen cross-linking (CXL) can effectively halt the progression of the disease [2], but visual acuity following CXL remains poor. In patients intolerant to rigid gas permeable contact lenses after corneal CXL, additional interventions are often necessary to improve their vision [1, 2].

Many visual rehabilitation options are available to manage keratoconus including intracorneal ring segment implantation (ICRS), phakic intraocular lenses (pIOL), and photorefractive keratectomy (PRK) and all can be combined with CXL [3–6]. In patients with poor best-corrected visual acuity ICRS implantation is performed [7–9]. The PRK is used to correct mild refraction error [3, 4], while the pIOL's are used to correct moderate to severe ametropia in patient with good best-corrected visual acuity [5, 6].

Our study group recently published the 6-month data on the safety and efficacy of CXL followed by insertion of a phakic toric implantable collamer lens (ICL) (Visian Toric V4 ICL; STAAR Surgical, Monrovia, CA) in the posterior chamber for correction of myopia and astigmatism in patients with keratoconus [9]. In this paper, we report the long-term safety and efficacy of sequential CXL, then ICL implantation, separated by 6 months, in a larger cohort of patients with moderate to severe keratoconus with moderate to severe myopia and astigmatism, and good best-corrected visual acuity.

2. Methods

2.1. Patient Selection. This was a retrospective study of patients with keratoconus who underwent sequential CXL-ICL procedure between December 2010 and March 2012 at the Beirut Eye Specialist Hospital (BESH), Beirut, Lebanon. This study was approved by the Institutional Review Board at BESH and complied with the declaration of Helsinki. All patients signed an informed consent prior to treatment and all surgical procedures were performed by one surgeon (E.J).

Patients treated according to Dr. Jarade's protocol [9] were included if they had a preoperative best-correct visual acuity better than 20/40, were hard contact lens intolerant (defined as a comfortable wearing time of less than 8 hours per day), had an endothelial count >2,200 cells/mm^2 (Noncon Robo, Konan Medical), had history of progressive keratoconus in one or both eyes (defined as an increase in maximum keratometry of 1.00 diopter (D) or more in 1 year and/or the need for new contact lens fitting more than once in the previous 2 years), and did not have any corneal surgery (including PRK and ICRS) before or after the CXL and ICL implantation. Patients were considered eligible for ICL implantation after CXL only if the keratoconus was considered stable (defined as subjective refractions [5, 9, 10] within ±0.50 D of spherical equivalent at 4 and 6 months postoperatively and was most of the time equivalent to the refraction prior to CXL).

The exclusion criteria for enrollment in this study (those who could not undergo the CXL and phakic IOL procedures consecutively) were central corneal thickness of less than 450 μm (measured by optical pachymetry (Pentacam; Oculus Optikgerate GmbH, Wetzlar, Germany)), mean K reading >56.00, endothelial cell count of less than 2,000 cells/mm^2 measured on the central part of the cornea by specular microscopy, anterior chamber depth of <2.8 mm from endothelium to anterior capsule measured by Pentacam (Oculus Optikgerate GmbH, Wetzlar, Germany), corneal opacification or scars, history of keratitis (any form), peripheral marginal degeneration, previous corneal and/or intraocular surgeries, and autoimmune and/or connective tissue disease. The central corneal thickness limit of 450 μm would account for around 400 μm of remaining stromal thickness after removal of the epithelium [11], which is considered as the safety thickness for the residual stroma to avoid endothelial cell damage during the CXL procedure [12].

The criteria for diagnosing keratoconus were based on a combination of computed slit-scanning videokeratography of the anterior and posterior corneal surfaces, keratometric readings, and corneal pachymetry [13–16]. Keratoconus was classified, according to the Amsler-Krumeich criteria, into four stages based on corneal power, thickness, transparency, and astigmatism [17].

Contact lens use was discontinued for at least 3 weeks for rigid lenses and 1 week for soft lenses prior to any ophthalmic examination, investigation, and treatment. The preoperative and postoperative screening consisted of a complete ophthalmic examination. It included uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), manifest and cycloplegic refractions, anterior and posterior segments evaluation with dilated fundus examination, and keratometric evaluation. Since the autorefractometer results of refraction are not always accurate in keratoconus and after both CXL procedures, all refractions were based on refined refraction using trial lenses, and the axis of astigmatism was chosen according to the best visual acuity obtained while rotating the astigmatism trial axis [9].

Follow-up examinations were scheduled at baseline and at 1, 3, 6, and 12 months and every 6 months thereafter.

2.2. Cross-Linking Procedure. The eye to be treated was anesthetized by applying proparacaine hydrochloride 0.5% drops on three occasions at 5-minute intervals. After positioning the patient under the operating microscope, an eyelid speculum was inserted and the central 9 mm corneal epithelium was removed with a blunt spatula. A mixed riboflavin 0.1%–20% dextran solution was instilled every 5 minutes until the riboflavin penetrated the cornea (i.e., approximately 30 minutes). The ultraviolet lamp (UV-X illumination system, version 1000; IROC AG, Zurich, Switzerland) was then focused on the apex of the cornea at a distance of 5 cm for a total of 30 minutes, providing a radiant energy of 3.0 ± 0.3 mW/cm^2. The required irradiance of 3.0 mW/cm^2 was calibrated prior to each treatment using an ultraviolet A meter (LaserMate-Q; LASER 2000, Wessling, Germany). During ultraviolet A administration, riboflavin drops were applied to the cornea every 5 minutes. The cross-linking procedure adopted in our study is in accordance with the standard "Dresden" protocol, which has been shown to result in absorption limited to the anterior two-thirds (200–400 μm) of the stroma as demonstrated by stress-strain measurements, thermomechanical measurements, and swelling studies [12].

Thinnest and central corneal thickness were continuously monitored (Sonogage Pachymeter; Sonogage, Inc., Cleveland, OH) to ensure that neither of the two parameters dropped below 400 μm. After treatment, the eye surface was washed with balanced salt solution and two drops of gatifloxacin 0.3% were instilled, followed by placement of a bandage soft contact lens. Postoperatively, patients received acetaminophen 500 mg twice daily for 3 days, one drop of gatifloxacin 0.3% six times daily for 7 days with one drop of tobramycin-dexamethasone 0.1% four times daily for 10 days, and one drop of loteprednol 0.5% five times daily, slowly tapered over 5 weeks. The bandage soft contact lens was removed on postoperative day 4 and the eye examined by slit-lamp microscopy to confirm complete corneal epithelialization. Complete assessment was performed 1 and 6 months postoperatively and included UDVA, CDVA, refraction, and anterior/posterior topography. No further progression of keratoconus was noted in any eyes throughout the 6 months of follow-up period.

2.3. ICL Insertion Procedure. The implantation of the toric ICL was performed at least 6 months after CXL. ICL power was calculated using the software provided by the manufacturer. Emmetropia was selected as the target refraction. The appropriate ICL size was determined based on the horizontal white-to-white distance measured manually with a caliper,

and the anterior chamber depth was measured with the Pentacam. A minor clinical adjustment of anterior chamber depth was performed by subtracting no more than 0.2 mm whenever corneal anterior bulging was advanced. Regarding the inaccuracy of the autorefractometer in predicting the K-reading in many keratoconus cases and to obtain accurate ICL choice using the online ICL calculator software, adjustment of extreme values of K readings obtained by autorefractometer was performed by attenuating the K-reading values to reflect the magnitude of astigmatism obtained by manifest refraction and the chosen axis of astigmatism was always the axis obtained by manifest refraction.

Laser iridotomy was performed 1 week preoperatively. The pupil was dilated with cyclopentolate and phenylephrine drops, instilled 30 minutes prior to surgery, and the horizontal axis was marked by the surgeon with the patient upright to control for cyclotorsion. General anesthesia was administered to all patients. A 3.2 mm clear corneal tunnel incision was performed in the horizontal temporal meridian (regardless of the astigmatism axis). The anterior chamber was filled with sodium hyaluronate 1%. The ICL was inserted in the posterior chamber through the incision using the injector cartridge supplied by the manufacturer. After the ICL was gently positioned in the sulcus with the axis properly aligned, the remaining viscoelastic material was completely washed out of the anterior chamber with balanced salt solution and a miotic agent was instilled. No intraoperative complications were encountered. Tobramycin-dexamethasone 0.1% eye drops were used four times a day for 10 days and then slowly tapered over 3 weeks.

2.4. Statistical Analysis. SPSS version 20.0 was used for data management and analyses. Descriptive statistics were reported as mean and standard deviation for continuous variables. Repeated-measures analysis with the Bonferroni test for post hoc analysis and the Wilcoxon Signed Rank test were computed. P value < 0.05 was considered to be statistically significant.

3. Results

The study included 30 eyes of 19 patients, among those 13 males and 6 females. Mean age was 30.44 ± 8.14 years (range: 20 to 45 years). Mean follow-up was 16 ± 5.75 months; all patients (100%) had complete follow-up from baseline up to 12 months after ICL implantation; only 10 (33%) eyes of 10 patients had 24 months of follow-up. The Visian toric ICL was implanted in all eyes; 11 patients underwent bilateral implantation while the remaining 8 patients had unilateral ICL implantation. Preoperative mean spherical power was −8.37 ± 3.89 D (range: −20.5 to −4 D) and mean cylindrical power 2.95 ± 1.40 D (range: 1 to 5.25 D). According to the Amsler-Krumeich classification, 6 eyes had stage I, 14 eyes had stage II, and 10 eyes had stage III keratoconus at baseline. Among the eyes that completed the 24 months of follow-up after ICL implantation, 6/10 had stage I, 2/10 had stage II, and 3/10 had stage III keratoconus at baseline. All eyes had an endothelial cell count greater than 2,200 cells/mm$_2$. The mean central corneal thickness was 479 ± 24 μm.

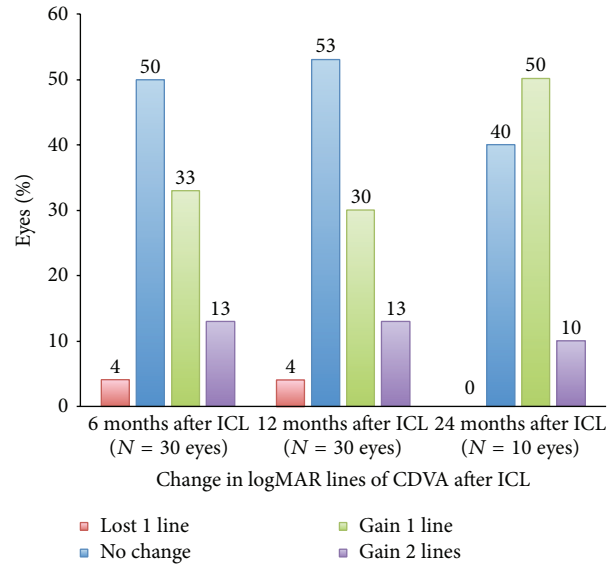

Figure 1: Change in corrected distance visual acuity (CDVA) following toric implantable collamer lens implantation (ICL).

3.1. Refractive Outcome. The preoperative values were compared to values starting 6 months after CXL, because visual acuity and corneal keratometry vary significantly in the first few months after CXL.

According to Table 1, both UDVA and CDVA values at 6 months after CXL did not differ from baseline ($P = 1.000$ and 0.231, resp.). At 6 months after ICL implantation, there was significant improvement in mean UDVA from 1.57 logMAR to 0.17 logMAR ($P < 0.001$) and mild improvement in CDVA from 0.17 logMAR to 0.11 logMAR ($P < 0.001$). Both CDVA and UDVA remained stable thereafter up to 24 months (Tables 1 and 2). No eye lost 2 or more lines in CDVA in the study (Figure 1). At 12 months, 43% (13 of 30) of eyes gained ≥1 line in CDVA, and in the smaller subset of eyes with 24 months follow-up 60% of eyes gained ≥1 line in CDVA. Overall, 60% (18 of 30) and 50% (5 of 10) of eyes had UDVA of 20/30 or better 12 months and 24 months after ICL implantation, respectively.

At 6 months after CXL, the small changes in SE and the spherical component of refraction were not significant from baseline ($P = 0.611$ and 1.000, resp.), unlike the mean change of 0.21 D in cylindrical component ($P = 0.012$) (Table 1). However, the changes in SE, sphere power and cylindrical power at 6 months after ICL implantation were all clinically and statistically significant from baseline and their values remained relatively stable up to 12 months (Table 1). However, in the smaller subset of 10 eyes with 24 months of follow-up (Table 2), small hyperopic shifts of 0.25 D in SE ($P = 0.012$) and 0.20 D in spherical power ($P = 0.005$) were noted after 6 months after ICL visit. Overall, 63.3% and 40% of eyes were within ±1.0 D SE at 12 and 24 months after ICL implantation, respectively (Figure 2).

All keratometric values showed a gradual decrease after CXL, up to the 24 months of follow-up. According to Table 1, the decrease in mean K (flat) from baseline became

Table 1: Refractive data at baseline, 6 months after CXL, and 1 and 6 months after Visian toric ICL implantation for keratoconus ($N = 30$ eyes).

Parameter (mean ± SD)	Preoperatively	6 M after CXL [^{a}P value*]	6 M after ICL [^{b}P value*]	12 M after ICL [^{c}P value*]
UDVA (log MAR)	1.57 ± 0.50	1.57 ± 0.56 [1.000]	0.17 ± 0.06 [<0.001]	0.17 ± 0.06 [1.000]
CDVA (log MAR)	0.17 ± 0.08	0.15 ± 0.06 [0.231]	0.11 ± 0.05 [<0.001]	0.11 ± 0.05 [1.000]
Sphere (D)	−8.37 ± 3.89	−8.18 ± 3.64 [0.611]	−1.38 ± 0.96 [<0.001]	−1.36 ± 0.94 [1.000]
Cylinder (D)	2.95 ± 1.40	2.74 ± 1.33 [0.012]	1.12 ± 0.68 [<0.001]	1.03 ± 0.60 [0.094]
SE (D)	−6.96 ± 3.68	−6.81 ± 3.48 [1.000]	−0.86 ± 0.86 [<0.001]	−0.83 ± 0.76 [1.000]
K (flat) (D)	46.52 ± 3.72	46.23 ± 3.21 [0.588]	45.95 ± 3.79 [0.007]	45.94 ± 3.79 [1.000]
K (steep) (D)	50.49 ± 4.42	49.55 ± 4.18 [<0.001]	49.03 ± 4.61 [<0.001]	48.98 ± 4.65 [0.587]
K (max) (D)	53.08 ± 5.17	52.01 ± 4.87 [<0.001]	51.55 ± 4.78 [<0.001]	51.55 ± 4.75 [1.000]

CXL: corneal collagen cross-linking; ICL: implantable collamer lens; SD: standard deviation; UDVA: uncorrected distance visual acuity; CDVA: corrected distance visual acuity; D: diopters; K: keratometry values.
$^{*}P$ value derived from post hoc analysis.
[a]Comparing 6 months after CXL to preoperative value.
[b]Comparing 6 months after ICL to preoperative value.
[c]Comparing 12 months after ICL to 6 months after ICL value.

Table 2: Complete case analysis of 10 eyes with 24 months of follow-up ($N = 10$ eyes).

Parameter (mean ± SD)	Preoperatively	6 M after CXL	6 M after ICL	12 M after ICL [^{a}P value$'$]	24 M after ICL [^{b}P value$'$]
UDVA (log MAR)	1.75 ± 0.56	1.84 ± 0.69	0.17 ± 0.07	0.17 ± 0.06 [0.317]	0.17 ± 0.07 [0.317]
CDVA (log MAR)	0.17 ± 0.07	0.15 ± 0.05	0.12 ± 0.05	0.12 ± 0.05 [1.000]	0.12 ± 0.05 [1.000]
Sphere (D)	−9.60 ±4.69	−9.55 ± 4.67	−1.83 ± 0.93	−1.75 ± 0.98 [0.317]	−1.63 ± 0.95 [0.005]
Cylinder (D)	2.55 ± 1.35	2.38 ± 1.29	1.15 ± 0.64	1.00 ± 0.59 [0.034]	1.05 ± 0.55 [0.102]
SE (D)	−8.32 ± 4.33	−8.36 ± 4.31	−1.34 ± 0.86	−1.17 ± 0.78 [0.027]	−1.09 ± 0.75 [0.012]
K (flat) (D)	45.57 ± 4.13	45.59 ± 3.38	44.97 ± 3.74	44.99 ± 3.76 [0.610]	44.93 ± 3.77 [0.131]
K (steep) (D)	49.17 ± 4.37	48.07 ± 4.42	47.31 ± 4.29	47.25 ± 4.35 [0.256]	47.29 ± 4.37 [0.581]
K (max) (D)	51.16 ± 4.57	50.30 ± 4.55	49.82 ± 4.36	49.80 ± 4.34 [0.715]	49.75 ± 4.35 [0.019]

CXL: corneal collagen cross-linking; ICL: implantable collamer lens; SD: standard deviation; UDVA: uncorrected distance visual acuity; CDVA: corrected distance visual acuity; D: diopters; K: keratometry values.
$'P$ value derived from Wilcoxon Signed Rank test.
[a]Comparing 12 months after ICL to 6 months after ICL.
[b]Comparing 24 months after ICL to 6 months after ICL.

statistically significant 6 months after ICL implantation, while the decreases in mean K (steep) and mean K (max) from baseline were statistically significant starting 6 months after CXL.

Overall, the safety index = [mean postoperative CDVA (logMAR)/mean preoperative CDVA (logMAR)] at 12 months and 24 months after ICL implantation was 0.73 ± 0.29 and 0.72 ± 0.25, respectively. The efficacy

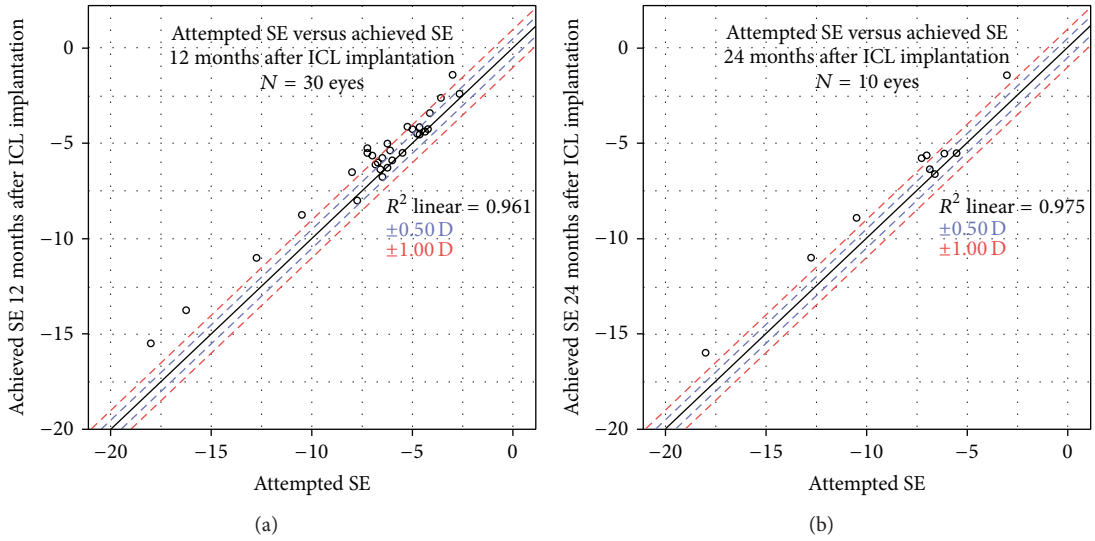

Figure 2: Attempted and achieved spherical equivalent (SE) correction at (a) 12 months and (b) 24 months after implantable collamer lens (ICL) implantation, respectively.

index = [mean postoperative UDVA (logMAR)/mean preoperative CDVA (logMAR)] at 12 months and 24 months after ICL implantation was 1.03 ± 0.26 and 1.04 ± 0.26, respectively (Figure 3).

3.2. Complications. All epithelial defects healed within 4 days after CXL. In this study, none of the patients had infectious keratitis, lens rotation, vaulting problem, cataract formation, pigment dispersion, or pupillary block. Also, none had development of clinically significant haze at any of the follow-up periods. There was, however, a transient increase of intraocular pressure that was observed in most patients during the first week after ICL implantation that was controlled with topical drops.

4. Discussion

Providing optimal refractive and vision results to patients with progressive keratoconus remains challenging to the refractive surgeon. While corneal collagen cross-linking (CXL) can halt progressive disease [2], patients with high refractive error and poor vision at baseline would remain so, after CXL, even without keratoconus progression [10, 18, 19]. Therefore, CXL is used to set the stage for other interventions to be performed. Management after CXL is tailored according to the patient's best-corrected visual acuity and refractive status. In patients with good-best corrected visual acuity and high residual refractive error after CXL, pIOL implantation provides adequate correction of ametropia [9]. Several types of toric pIOL were reported to be effective and safe in eyes with keratoconus, but only a handful of studies have evaluated their use following a CXL procedure [6, 9, 10, 18, 20–24].

The Visian toric ICL has demonstrated good efficacy and safety profiles for the correction of high ametropia in patients without keratoconus [25–32]. In our previous study [9], toric ICL implantation 6 months after CXL was proven to be an effective and safe method of improving visual acuity and refraction in selected eyes with moderate to severe keratoconus. In this paper, we assess the long-term (up to 24 months) safety and efficacy of that same procedure in 30 eyes with mild-to-moderate progressive keratoconus.

Stability of keratoconus following CXL in preparation for ICL implantation has been previously defined using stability of refraction data [5, 9, 10]. As such, ICL implantation was performed 6 months after CXL, since most patients had a stable visual acuity and manifest refraction by 4 months. The *K*-reading values however showed gradual flattening after CXL throughout the study. This flattening was not significant enough to alter the mean SE manifest refraction at the time of ICL implantation, or the outcome of the ICL procedure at 12 months. In the small subset of 10 eyes with 24 months of follow-up, the small hyperopic shift in SE might have resulted from the continuous flattening in *K* readings; however, the change did not affect vision. The continuous flattening in *K* readings and its effect on SE is most likely due to the effect of CXL [33]. It is unlikely that the 3.2 mm clear corneal incision at the time of ICL implantation (surgically induced astigmatism) would have contributed to the change in SE; the incisions were placed at the temporal site according to the surgeon's preference, regardless of the axis of manifest astigmatism. Another possible yet unlikely culprit of the change in SE is the rotation of the toric Visian ICL with loss in the refractive corrective effect [5]. Although possible, the effect of a rotation on refraction and visual acuity would have been uncovered earlier, and all our patients were happy with the end-result. After stabilization of keratoconus with CXL and ICL implantation, 60% (18 of 30) and 50% (5 of 10) of eyes had UDVA of 20/30 or better at 12 months and 24 months, respectively. Results of our study compare favorably to other reports in terms of gain in UDVA and CDVA [9, 10, 21], as reflected by the safety and efficacy indices. In our study, the slight myopic SE refraction at post-ICL implantation was

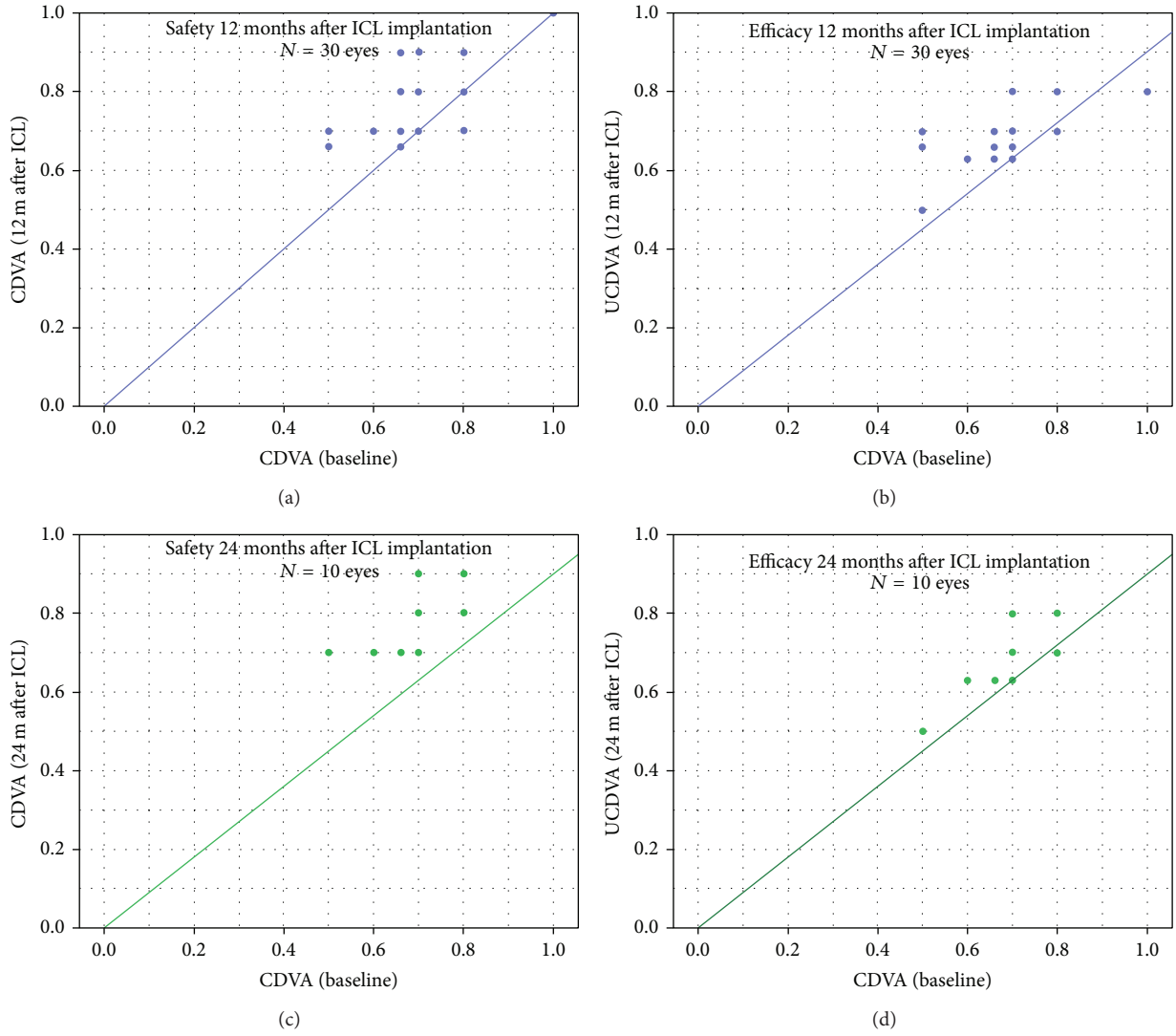

Figure 3: Safety and efficacy graphs comparing preoperative corrected distance visual acuity (CDVA) and uncorrected distance visual acuity (UDVA) 12 months (a, b) and 24 months (c, d) after ICL.

related to 2 factors. First, there is no way to customize the ICL to exactly fit the patient's refraction, and in most cases we had to use what was available (undershoot the target refraction of plano). Second, one patient had a high refractive power that exceeds the capacity of the ICL (which is limited to −18.0 D of manifest refraction at the eyeglasses plane). However, all our patients were satisfied with the resulting vision.

Only 2 other studies have evaluated the safety and efficacy of Visian toric ICL following CXL [6, 10]. Both Kymionis et al. and Shafik Shaheen et al. evaluated the outcomes of Visian toric ICL implantation 12 months following CXL. Kymionis et al. [6] in a case report published encouraging results of this procedure; at 3 months, UDVA improved from counting fingers to 20/40 and CDVA improved from 20/100 to 20/30. Shafik Shaheen et al. [10], in a case series of 16 eyes with early-stage (undefined) keratoconus, showed a favorable outcome in terms of visual acuity and SE at 3 years of follow-up; mean CDVA improved from 20/35 to 20/22, mean UDVA improving to 20/23 and mean SE improving from −8.5 D to −0.25 D. In our previous study on mild to severe keratoconus [9], the 6-month results revealed that mean CDVA improved from 0.15 logMAR to 0.12 logMAR, mean UDVA decreased from 1.67 logMAR to 0.15 logMAR, and mean SE decreased to −0.89 D with no complication.

Other types of pIOLs implanted after CXL have also been evaluated. Izquierdo Jr et al. [18] employed the iris-fixated Artiflex phakic IOL (Ophtec, USA) in 11 eyes with progressive keratoconus. Results were favorable in terms of visual acuity, sphere, and cylinder at 12 months. Güell et al. [5] employed the toric Artiflex/Artisan phakic IOL in 17 keratoconic eyes; at 24 months, 14 eyes were within ±0.50 D of the attempted SE correction and 13 eyes were within ±1.00 D of the attempted cylinder correction.

ICL implantation after CXL depends on the stability of keratoconus (both refraction and keratometry) since progression would lead to refractive changes and drop of

visual acuity [34]. A continuous flattening in K readings after CXL occurred in our study with no significant effect on SE, UDVA, nor CDVA at 1 year; in the smaller subset of 10 eyes a statistically but nonclinically significant change in SE was observed following CXL at the 2-year follow-up, but both UDVA and CDVA were not affected. Although we do believe that a longer time interval would possibly show a greater change in keratometry, we are still uncertain whether an equivalent amount of change in refractive error would accompany this flattening, possibly related to the altered biomechanics of cross-linked corneas. As demonstrated in our results, the change in keratometry did not significantly alter the SE and more importantly did not alter the UDVA and CDVA. The small hyperopic shift observed in our study deserves further investigation with long-term studies to assess its long-term impact on vision, but it does not warrant delaying ICL implantation. The continuous flattening effect of CXL with the accompanying risk for a hyperopic shift can last more than 2 years [35, 36]; therefore, targeting mild undercorrection rather than delaying the ICL implantation for 12 months would improve predictability and may be a solution. Moreover, implanting an ICL at 6 months as opposed to 12 months offers the patient the benefit of earlier functional visual recovery.

In conclusion, the results of toric ICL implantation 6 months after CXL at 1 year and at 2 years compare to the outcomes at 6 months in a previous study; it is an effective and safe method of improving visual acuity and refraction in keratoconus eyes with high myopia and astigmatism and good best corrected visual acuity.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Rate of Corneal Collagen Crosslinking Redo in Private Practice: Risk Factors and Safety

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Objective. To report the rate of progression of keratectasia after primary crosslinking (CXL) and evaluate the safety and efficiency of CXL redo. *Materials and Methods*. We conducted a retrospective analysis of the patients who underwent CXL between 2010 and 2013 at the Beirut Eye Specialist Hospital, Lebanon. Progression of keratectasia was based on the presence of an increase in maximum keratometry of 1.00 D, a change in the map difference between two consecutive topographies of 1.00 D, a deterioration of visual acuity, or any change in the refraction. Primary and redo CXL were done using the same protocol. *Results*. Among the 221 eyes of 130 patients who underwent CXL, 7 eyes (3.17%) of five patients met the criteria of progression. All patients reported a history of allergic conjunctivitis and eye rubbing and progressed within 9 to 48 months. No complications were noted and all patients were stable 1 year after CXL redo. *Conclusion*. Allergic conjunctivitis and eye rubbing were the only risk factors associated with keratoconus progression after CXL. A close followup is thus mandatory, even years after the procedure. CXL redo seems to be a safe and efficient technique to halt the progression after a primary CXL.

1. Introduction

Keratoconus (KC) is a noninflammatory corneal disease characterized by corneal deformation and thinning caused by structural changes in the corneal collagen, inducing irregular astigmatism, myopia, and protrusion, which leads to mild to marked impairment in vision quality [1]. Corneal ectasia is one of the most devastating complications after laser-assisted in situ keratomileusis (LASIK). The disease is characterized by a progressive thinning and steepening of the central and inferior portions of the cornea, inducing a loss of uncorrected visual acuity, best-corrected visual acuity, and topographic evidence of asymmetric inferior corneal steepening [2–5].

Corneal collagen crosslinking (CXL) was introduced in 2003 by Wollensak et al. to halt the progression of keratectasia [6–8]. During CXL, riboflavin interacts with ultraviolet-A (UV-A) light to cause crosslinking of protein fibrils followed by formation of interchain disulfide bonds, thus arresting the progression of keratoconus by increasing the biomechanical stability of the cornea [7, 8]. In the meantime, CXL has become an increasingly well-accepted low invasive intervention with high success and low complication rates [9–11]. Long-term stabilization and improvement after CXL have been reported in many prospective studies [11–13]. However, failure and progression of keratectasia after CXL have been reported. Recently, Kymionis et al. [14] reported that topographic keratoconus progression might occur several years after CXL, despite stability for a long-term period. While Greenstein and Hersh showed that no preoperative characteristics were predictive of CXL failure for keratectasia [15], other studies attributed progression after primary CXL to specific risk factors and patient characteristics. Koller et al. reported an 8% CXL failure rate one year after CXL for keratoconus, with preoperative maximum K value of more than 58.0 D as a risk factor for progression [16]. In addition, due to the few cases of progression after CXL, there is no

consensus about the definition of progression of keratectasia after CXL. The number of cases of keratoconus progression after original CXL procedure is expected to increase with time with no clear consensus about the best treatment modality of those cases.

To our knowledge, no previous study evaluated the safety and efficiency of CXL redo. We hence report the rate of progression and the risk factors after CXL in our private practice and evaluate the technique, safety, and efficiency of CXL redo after primary CXL.

2. Materials and Methods

2.1. Setting. We conducted a retrospective analysis of the patients with progressive keratectasia who underwent CXL between March 2010 and March 2013 at the Beirut Eye Specialist Hospital Beirut, Lebanon. Diagnosis of keratoconus was based on a combination of computed slit-scanning videokeratography of the anterior and posterior corneal surfaces, keratometric readings, and corneal pachymetry. Keratoconus was classified into four stages based on corneal power, astigmatism, corneal transparency, and corneal thickness, according to the classification of Amsler-Krumeich [17].

2.2. Participants. All patients included in this study had a history of progressive keratectasia either from keratoconus or from corneal ectasia after LASIK in one or both eyes and underwent a primary CXL in order to stabilize the disease.

All patients included in this study had a central corneal thickness >400 μm. Central corneal thickness (CCT) and thinnest corneal location were measured using Pentacam topography with epithelium on prior to the procedure. Because of the potential deswelling effect in the corneal stroma of the dextran in riboflavin solution [18], the CCT and thinnest corneal location were measured using ultrasound pachymeter all through the period of riboflavin application during the entire period of CXL procedure. Therefore, after removing the epithelium, CCT and thinnest corneal location were measured although the period of riboflavin application and hypoosmolar riboflavin was additionally instilled every 20 seconds for 5 minutes and repeated up to 2 times until adequate minimal corneal thickness of more than 400 μm was reached.

Exclusion criteria for primary and redo CXL were preoperative corneal opacities, ocular pathology other than keratectasia, especially the cornea guttata or other endothelial irregularities, age younger than 18 years, actual or intended pregnancy, not available for follow-up examinations for 1 year, and connective tissue disease.

2.3. Data Collection. Progressive keratectasia was suspected by an increase in maximum K readings in several consecutive recordings in the last 6 months with or without progressive corneal thinning as well as deterioration in visual acuity and manifest refraction. We evaluated the progression of KC after CXL based on the presence of 2 or more of the following criteria: increase (≥1 D) in K value ($k1$, $K2$, or K max), a change in the map difference between two consecutive topographies of ≥1 D, a deterioration of VA defined as a drop of one or more lines, or any change in the refraction as a change of 0.5 D.

Based on our observation, the cornea may endure major topographic changes in the first 6 months after CXL with significant changes in manifest refraction and visual acuity. We set a baseline corneal topography 6 month after CXL treatment and we noticed no further clinically significant changes are happening then after apart from a nonsignificant minor flattening of k-reading over time with no further steepening happening 6 months after CXL treatment in stable keratoconus. Any steepening in k-reading that happens 6 months after primary CXL is considered as sign of keratoconus progression, mainly when it was associated with one of the aforementioned criteria of keratoconus progression. Therefore, the effect of CXL is considered only 6 months after primary CXL and corneal stability is judged after that.

All patients undergoing CXL were followed up closely in the postoperative period at day one, one week, one month, 3 months, sixth months, and one year and then half yearly after. Corneal topography was repeated at each visit starting 3 months after CXL and then 6 months after CXL corneal topography is considered as the baseline topography after CXL based on which the progression of KC is considered. Thus, all patients are evaluated at 6 months after CXL by a complete ophthalmic workup including assessment of uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), manifest and cycloplegic refractions, and anterior and posterior segment evaluation with dilated fundus examination, as well as an anterior/posterior topography. Corneal topography (Pentacam 70700, Oculus, Germany) was conducted with undilated pupils under scotopic conditions by a single experienced technician. Baseline strategy for the treatment of recurrent allergic episodes was based on topical mild steroids (Fluorometholone 0.1%) to be used four times a day for 10 to 14 days as needed. In case of severe exacerbations, topical antihistamine and topical cyclosporine 0.1% were added to the regimen.

2.4. Surgical Procedure. Primary and redo corneal collagen crosslinking (CXL) were done using the same protocol. All surgeries were performed by the same surgeon (EJ). The eye to be treated was anesthetized by applying proparacaine hydrochloride 0.5% drops on three occasions spaced by five minutes. After positioning the patient under the operating microscope, a lid speculum was inserted and the central 9 mm corneal epithelium was removed with a blunt spatula. A mixed riboflavin 0.1% dextran solution (Collagex, isotonic 0.1%, Lightmed USA Inc.) was instilled every 2 minutes until the riboflavin penetrated the cornea, that is, after approximately 30 minutes. The ultraviolet (UV) lamp (UV-X illumination system, version 1000; IROC AG, Zurich, Switzerland) was then focused on the apex of the cornea at a distance of 5 cm for a total of 30 minutes, providing a radiant energy of $3.0 \pm 0.3\,\text{mW/cm}^2$. The required irradiance of $3.0\,\text{mW/cm}^2$ was calibrated prior to each treatment using a UVA meter (LaserMate-Q; LASER 2000, Wessling, Germany). During UVA administration, riboflavin drops were applied to

the cornea every 2 minutes. Thinnest and central pachymetry were continuously monitored to ensure that none of the two parameters dropped below 400 μm. After treatment, the eye surface was washed with balanced salt solution and two drops of gatifloxacin 0.3% were instilled, followed by the placement of a bandage soft contact lens. Postoperatively, patients received acetaminophen 500 mg twice daily for 3 days, one drop of gatifloxacin 0.3% six times daily for 7 days along with one drop of tobramycin-dexamethasone 0.1% four times daily for 10 days, followed by one drop of Loteprednol 0.5% 5 times daily, slowly tapered over 5 weeks. The bandage soft contact lens was removed on postoperative day 4, and the eye examined by slit-lamp microscopy to confirm complete corneal epithelialization.

Complications after CXL redo such as significant stromal haze, sterile corneal infiltrates, recurrent erosion syndrome, corneal edema, Desçemets membrane folds, corneal melting and perforation were noted if present. Stability after CXL redo was also assessed.

3. Results

3.1. Primary CXL. Two hundred twenty-one eyes of 130 patients underwent a corneal collagen CXL for progressive keratoconus or post-LASIK ectasia in our department between March 2010 and March 2013. The demographic and topographic data of the initial 221 eyes are mentioned in Table 1.

Table 1: Baseline patient characteristics of 221 eyes of 130 patients who underwent CXL between March 2010 and March 2013 at our private clinic. PRK: photorefractive keratectomy; ICRS: intrastromal corneal ring segments; *n*%: number (percentage).

Characteristics	Value
Gender	
Male (*n*%)	68 (52%)
Female (*n*%)	62 (48%)
Age, years	
16–30	95 (73.1%)
30–50	35 (26.9%)
Stage of KC (Amsler-Krumeich)	
Stage 1 (*n*%)	96 (43%)
Stage 2 (*n*%)	100 (45%)
Stage 3 (*n*%)	25 (12%)
Keratometry, diopters	
Flattest meridian	
40–44	132 (60%)
44–46	89 (40%)
Steepest meridian	
45–58	122 (55%)
58–68	99 (45%)
Maximal keratometry	
48–58	103 (47%)
58–68	118 (53%)
Pachymetry, microns	
400–450	156 (70%)
450–580	65 (30%)
Associated surgeries *n*%	70 (32%)
ICRS *n*%	50 (23%)
PRK *n*%	20 (9%)

3.2. CXL Redo. Although the majority of the eyes remained stable after primary CXL (according to the aforementioned criteria), seven eyes (3.17%) of five patients met the criteria of progression and necessitated a CXL redo. Patients characteristics are summarized in Table 2. Mean age was 26 (one patient was 19 years old and 4 patients were aged between 26 and 30 years), with male/female ratio of 3/2. All patients who progressed reported a history of allergic conjunctivitis and eye rubbing. Their preoperative maximum *K* value was > 58.0 D in 3 eyes and <58 D in 4 eyes (mean of 58.6 D). Two eyes (of one patient) had CXL alone, 4 eyes had CXL subsequent to ICRS implantation, and one eye had simultaneous CXL with PRK. Four eyes had a stage 2 keratoconus, 2 eyes had a stage 4 keratoconus, and one eye had a post-LASIK ectasia.

Progression of KC was noticed more than one year (14 to 48 months) after the original CXL in 6 eyes of four patients (2 males and 2 females, one patient was 19 years old, and 3 were 26–28 years old) and one eye (30-year-old male) was diagnosed with KC progression 9 months after the original CXL. The mean time of KC progression after original CXL was 29.14 months. Progression was noted by all the patients after a decrease in CDVA and was evidenced by progression in corneal topography (Figure 1). Progression was simultaneously noted in both eyes in all patients who had bilateral disease evolution.

No major complications after CXL do and redo such as significant stromal haze, sterile corneal infiltrates, recurrent erosion syndrome, corneal edema, Desçemets membrane folds, corneal melting and perforation were noted in any patient. At one year after CXL redo, all patients remained stable by either UDVA, CDVA, or topographic readings. The characteristics of the 5 patients are summarized in Tables 2, 3, and 4.

4. Discussion

Despite the proven effect of CXL in halting the progression of KC and corneal ectasia with stabilization in the majority of cases [7, 19, 20], KC progression still can happen after primary CXL treatment [14, 16]. In most of the studies, the reported failure rate varied from 0% [6, 21] to 16.5% [22]. The time of progression after CXL was reported to be as early as few months [16] to 5 years after CXL [14]. The most adopted definition of KC progression after CXL in most of the reports in the literature was an increase in the maximum keratometry readings of >1.00 D over the 6 months after CXL value [14, 16]. In our study, 2 eyes (eye 1 and eye 4) presented with evidence of progression based on worsening of their visual acuity despite a progression of their *k* readings of less

Table 2: Patient characteristics: all eyes had allergic conjunctivitis. ∗: missing data. CCT: central corneal thickness; PRK: photorefractive keratectomy; ICRS: intrastromal corneal ring segments; LASIK: laser-assisted in situ keratomileusis.

Eye	Age	Gender	Diagnosis	Stage of Kc.	Baseline CCT	**Allergic conjunctivitis**	Associated surgeries	Time (months) since first CXL	Slit lamp evaluation (first visit)
Eye 1	27	F	Keratoconus	Stage 2	489	Yes	PRK	14	No haze papillae
Eye 2	19	F	Keratoconus	Stage 2	523	Yes	ICRS	38	No haze papillae
Eye 3	30	M	Post-LASIK ectasia		420	Yes	ICRS	9	No haze papillae
Eye 4	26	M	Keratoconus	Stage 4	414	Yes	None	23	Striae papillae
Eye 5	26	M	Keratoconus	Stage 4	418	Yes	None	24	Striae + haze papillae
Eye 6	28	M	Keratoconus	Stage 2	∗	Yes	ICRS	48	No haze papillae
Eye 7	28	M	Keratoconus	Stage 2	∗	Yes	ICRS	48	No haze papillae

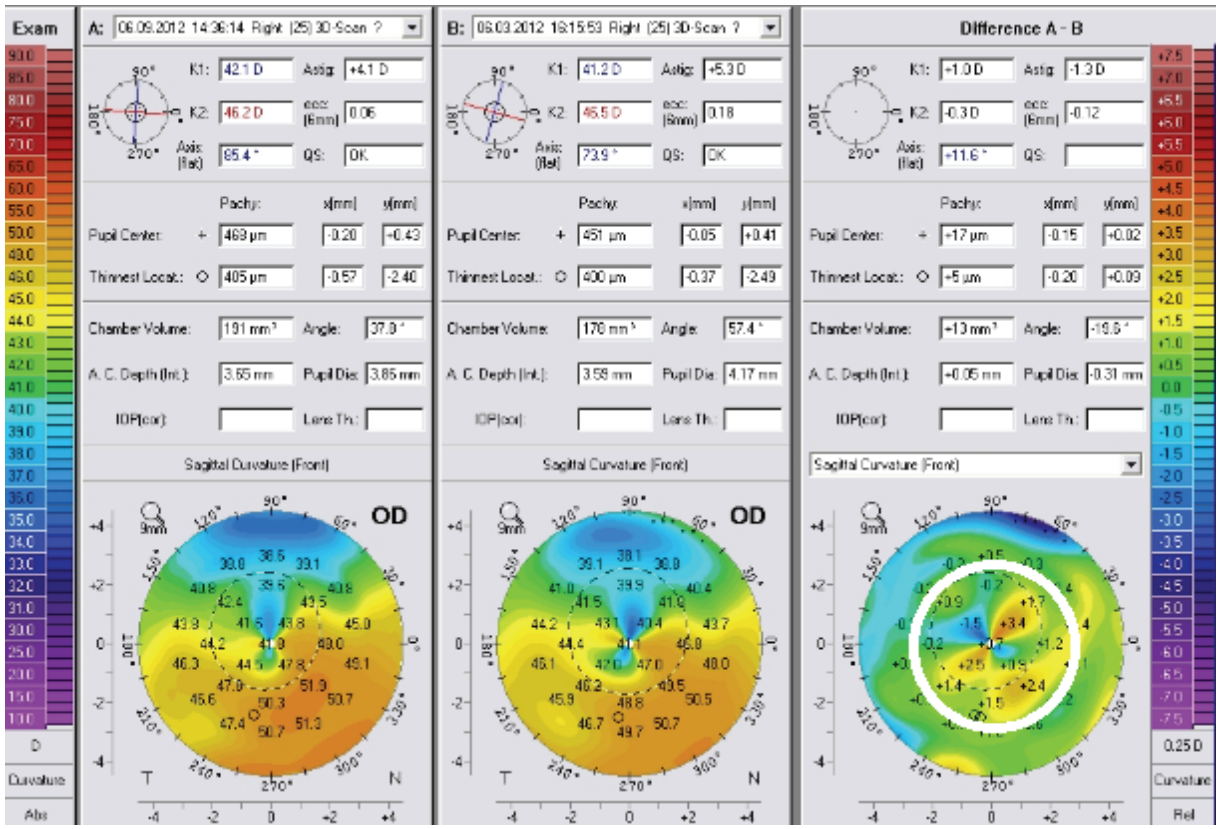

Figure 1: A map difference showing progression after primary corneal collagen crosslinking. B represents corneal topography 6 months after primary CXL, and A shows corneal topography 12 months after primary CXL. The map difference (difference A-B) shows the progression after initial CXL with +2.5 and +3.4 D of difference between successive topographies (white circle).

than 1.00 D (0.9 in eye 1 and 0.68 in eye 2). Thus, a change in the map difference between two consecutive topographies of 1.00 D (or maybe less), a deterioration of visual acuity (excluding other possible non-cornea-related reasons for deterioration), or any change in the refraction must be taken into account when evaluating the stability after CXL. Any of these indicators are considered as progression and necessitate a redo of CXL. The failure of CXL as a continued progression of keratoconus during the first year postoperatively has been reported in several studies [16, 23, 24]. The failure rate has been reported to be around 7 to 9%. Koller et al. reported a failure rate of 7.6% during the first postoperative year [16]. Hersh et al. and Sloot et al. [23, 24] reported a failure rate of 9.8% and 9%, respectively, while Baenninger et al. reported a failure rate of 16.5% in patients aged <35 years [22]. In our study, we found that the failure rate is 3.17% which

Table 3: Progression of keratometric readings. K readings remained stable one year after CXL redo. ∗: missing data. #: difference.

Eye	Preoperatively			6 months after CXL			At diagnosis of progression			**Sign of progression**	One year after CXL redo		
	$K1$	$K2$	$k$max	$K'1$	$K'2$	K'max	$K''1$	$K''2$	K''max		$K\hat{}1$	$K\hat{}2$	$K\hat{}3$
Eye 1	40.6	50.2	53.9	41.2	45.5	50.7	42.1	46.2	51.3	**#k1: 0.9 D, #Kmax 0.6 D ↓CDVA**	42.00	45.8	51.00
Eye 2	45.2	48.9	51.3	42.93	45.45	∗	43.15	46.67	∗	**#k2 1.22 D**	43.00	46.87	∗
Eye 3	53.53	60.25	67.5	40.84	41.16	45.22	40.5	43.38	49.97	**#k2: 1.84 D #kmax 4.75 D**	40.00	43.25	49.80
Eye 4	51.6	55.1	57.1	52.7	54.38	∗	53.2	55.06	∗	**#k1 0.5 D #kmax 0.68 D ↓CDVA**	52.98	54.9	∗
Eye 5	54.5	56.8	63.2	48.3	51.4	∗	57.05	59.33	∗	**#k1 8.75 D #k2 7.92 D**	56.8	59.2	∗
Eye 6	∗	∗	∗	44.07	47.45	59.4	44.49	47.5	62.11	**#kmax 2.71 D**	44.3	46.9	61.89
Eye 7	∗	∗	∗	47.69	50.05	59.51	47.74	49.71	61.7	**#kmax 2.19 D**	47.8	49.59	61.2

is significantly less than the rates in the previous reports. Although our CXL technique is the same technique described in the aforementioned reports, the lower failure rate at our practice can be attributed to any of the following factors. First, this chart review was performed in our private clinic, and the lower failure rate might be due mostly to the fact that unhappy progressive keratoconus patients might be lost to followup. Second, many of the patients at our private clinic had CXL associated refractive surgeries (32%), such as PRK (9%) or ICRS (23%). Few reports imply not only the safety of the latter procedures, but also their possibility to add up to CXL's collagen stabilization [25–28]. Such procedures might have reduced our failure rate. Third, we considered 6 months after CXL as baseline data and KC progression was judged based on the corneal topography performed at 6 months after initial CXL. Therefore, we might have reduced the selection error due to the keratometric fluctuation during the first 6 months after CXL, which may have contributed to the lower rate of KC progression in our study. In fact, some studies reported that the initial fluctuation and maybe worsening of keratometric readings are observed in the first months following CXL [16]. This change may be due to transient haze, corneal edema, and remodeling [29, 30]. Accordingly, we evaluated significant changes in keratometric values for assessment of CXL efficacy only 6 months after CXL.

Risk factors associated with progression after primary CXL remain unclear. In our practice, a history of allergic conjunctivitis with eye rubbing was found to be a common risk factor to all patients in the progression group. However, because of the small number of patients with keratoconus progression, we could not conduct a multifactorial analysis to determine other risk factors. Further prospective studies with multifactorial analysis are thus necessary to determine other risk factors associated with progression of keratoconus after a primary CXL. Similarly, Raiskup-Wolf et al. reported progression in 2 patients with neurodermatitis, a condition in which constant skin and ocular rubbing is present [13]. In fact, the relationship between eye rubbing and keratoconus has been studied in previous reports [30, 31]. Eye rubbing leads to biomechanical and biochemical alterations [32]. It injures the epithelium and leads to cytokine and metalloproteins release [32]. Stromal thinning occurs and this contributes to the keratoconus disease progression [32]. In our case, we think that the eye rubbing and the mechanical trauma it caused played an important role in the recurrence of the disease [31–33]. Other postulated factors for progression such as female sex and elevated maximal keratometry were not predominant factors in our study. In the study of Koller et al. there were differences between the failure subgroup with the total group in sex, where females had significantly more failure rates than males (females 62.5% versus 38.8% in males; $P = 0.048$), and preoperative maximum K reading of less than 58.00 D was found to reduce the failure rate to 3% [34]. In our cases, the gender was not a risk factor for progression (3 males, 2 females), nor the K max (K max >58 D in 2 eyes and <58 D in 3 eyes). Finally, one out of the five patients in our study with CXL failure in our study had a post-LASIK ectasia. Post-LASIK ectasia might have a higher rate of failure. Hersh et al. reported a reduced effect of CXL in cases of post-LASIK ectasia compared with keratoconus [23]. It was postulated that the reduced effect could be due to the influence of the flap, which may impede the diffusion of riboflavin or change the behavior of the anterior stroma to the crosslinking process [23]. Finally, the small group of failure makes multivariate analysis nonconclusive. In our study, 5 out of 7 eyes had associated surgeries, 4 eyes had CXL subsequent to ICRS implantation, and one eye had simultaneous CXL with PRK. These two types of associated surgeries were not found to increase failure rate in literature reviews and are considered safe in combination with CXL in keratectasia [11, 35, 36].

The majority of the studies report the failure of CXL during the first year postoperatively [16, 22, 24]. In a recent paper published by Kymionis et al., a topographic examination revealed an increase in the keratometric values indicating keratoconus progression 4 and 5 years after CXL, despite stability for a long-term period. In our series, four patients presented with a progression time after crosslinking ranging from 14 months to 48 months. To our knowledge, this is

Table 4: Change in error of refraction (EOR), uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA) from baseline to one year after CXL redo. UDVA and CDVA remained stable one year after CXL redo. ∗: missing data; S: sphere; C: cylinder; A: axis.

Eye	EOR									UDVA/**CDVA**							
	Baseline			6 mo CXL + associated surgeries			Refraction at progression			Baseline		6 months after		Time of progression		1 year after CXL redo	
	S	C	A	S′	C′	A′	S″	C″	A″								
Eye 1	**−1.5**	**+4.25**	**170**	**−0.5**	**+1.00**	**20**	**−2.75**	**+2.75**	**175**	*20/100*	**20/30**	*20/25*	**20/20**	*20/100*	**20/40**	*20/100*	**20/40**
Eye 2	**−7.00**	**+3.50**	**150**	**−3.00**	**+1.75**	**170**	**−2.75**	**+2.75**	**10**	*20/200*	**20/30**	*20/70*	**20/40**	*20/80*	**20/40**	*20/100*	**20/40**
Eye 3	**−9.00**	**+3.50**	**20**	**−0.5**	**+0.75**	**170**	**−0.50**	**+3.00**	**135**	*20/400*	**20/200**	*20/25*	**20/20**	*20/50*	**20/30**	*20/50*	**20/25**
Eye 4	**−13.00**	**+2.00**	**75**	**−13.00**	**+1.75**	**70**	**−16.00**	**+1.5**	**70**	*CF*	**20/50**	*CF*	**20/30**	*CF*	**20/30**	*CF*	**20/30**
Eye 5	**−14.50**	**+0.75**	**90**	**−13.50**	**+1.75**	**70**	**−18.00**	**+2.5**	**55**	*CF*	**20/50**	*CF*	**20/30**	*CF*	**20/30**	*CF*	**20/40**
Eye 6	∗	∗	∗	**−4.00**	**+2.75**	**105**	**−5.00**	**+3.25**	**105**	∗	∗	*20/50*	**20/25**	*20/70*	**20/25**	*20/60*	**20/30**
Eye 7	∗	∗	∗	**−1.75**	**+2.25**	**170**	**−3.50**	**+4.00**	**170**	∗	∗	*20/40*	**20/30**	*20/100*	**20/30**	*20/100*	**20/25**

the second case series reported in the literature, in which patients with stability after CXL for a long-term period showed topographic recrudescence. The exact pathophysiology of keratoconus progression after years of stability following CXL is not known but could be related to the new collagen laydown. Richoz et al. evoked the role of corneal stromal regeneration and rejuvenation as a possible explanation in the recurrence of the disease [8]. Also, in our study we found that the patient's age is not predictive of failure; the younger patient in our series (19 years old) had CXL failure at 38 months postop, while patients around 30 years old had failure at different times 9 to 48 months.

In the literature, the safety, efficiency, and the technique of CXL redo were not previously evaluated. To our knowledge, this is the first report to assess long-term safety and efficacy of CXL redo. We performed the primary and redo corneal collagen CXL using the same classical protocol, and the 7 eyes we treated with CXL redo were stable 1 year after the second CXL. No complications after CXL redo such as significant stromal haze, sterile corneal infiltrates, recurrent erosion syndrome, corneal edema, Desçemets membrane folds, corneal melting and perforation were noted in any of the patients we treated with CXL redo. However, we did not perform an endothelial cell count preoperatively and after CXL and the effect of CXL redo on the endothelial health was not evaluated which constitutes a limitation to our study.

Recently, Kanellopoulos and Asimellis introduced a novel, noninvasive, quantitative technique utilizing anterior segment OCT images to quantitatively assess the depth and cross-sectional area of CXL in the corneal stroma. Despite the usefulness of the aforementioned method, OCT was not performed systematically in all patients who underwent CXL treatment in our study; therefore, the value of OCT in determining the depth and effectiveness of CXL treatment was not studied in our group, and this is considered as a limitation factor of our study [37].

5. Conclusion

In conclusion, according to our understanding of keratocytes turnover in the cornea, the effect of CXL may be transient and progression of KC after primary CXL may happen. Thus, a close followup is mandatory in patients after CXL, even after a stability of years after the procedure and CXL redo procedures for those cases who progressed seems to be a safe and efficient technique to halt the progression of keratoconus or post-LASIK corneal ectasia after a failed primary CXL.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Corneal Biomechanical Changes after Crosslinking for Progressive Keratoconus with the Corneal Visualization Scheimpflug Technology

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Purpose. To evaluate the effect of corneal crosslinking in progressive keratoconus by applying in vivo corneal visualization Scheimpflug technology. *Design.* Longitudinal retrospective study. *Subjects and Controls.* Seventeen eyes of patients treated with corneal crosslinking for progressive keratoconus. *Methods.* Corneal visualization Scheimpflug technology analyses (research software version 6.07r08) of subjects with progressive keratoconus before and 3 months after corneal crosslinking (CXL) were reviewed retrospectively. *t*-test (for normal distribution) and Wilcoxon matched-pairs test (if not normally distributed) were used to test for statistically significant differences between pre- and post-CXL analyses. *Results.* We demonstrated statistically significant differences for the intraocular pressure (median: +3 mmHg, $P = 0.004$), the central corneal pachymetry (pachy; mean: −35 μm, $P < 0.001$), the timespan between the air impulse release and the first applanation of the cornea (A1time; median: +0.12 ms, $P < 0.05$), and the timespan between the air impulse release and the second applanation of the cornea (A2time; median: −37 ms, $P < 0.05$). *Conclusions.* With the A1time and the A2time, we identified two parameters that demonstrated a statistically significant improvement of the biomechanical properties of the cornea after CXL. Despite the known initial decrease of the pachymetry after CXL, none of the analyzed parameters indicated a progression of the keratoconus.

1. Introduction

Keratoconus (KC) is a bilateral noninflammatory disease of the cornea characterized by progressive corneal thinning and ectasia [1]. Introduced in 2003, corneal crosslinking (CXL) was the first treatment aimed at the pathogenetic cause of KC by potentially changing the intrinsic biomechanical properties of the corneal collagen [2]. Histologically, CXL causes an increase of the fiber diameter and chemical bonding between corneal microstructural components, leading to a higher mechanical stiffness of the cornea [3, 4]. A reduced susceptibility to enzymatic degradations has also been described [5, 6].

Over the past few years, long-term studies concentrating on in vivo topography and tomographic analyses have been published to demonstrate that CXL is an effective treatment for stopping the natural course of progressive KC [7–9].

The next step in extending our knowledge of CXL, and thereby in optimizing treatment, is to gain a better understanding of not only the morphological but also the in vivo biomechanical effects of CXL. We could then make a better evaluation of the different CXL strategies, which may possibly enable individualized therapy strategies.

The aim of the current study was to analyze biomechanical changes of the cornea after CXL for progressive KC by applying in vivo corneal visualization Scheimpflug

technology (CST) combined with new research software (version 6.07r08).

2. Materials and Methods

2.1. Setting. This retrospective study was performed in cooperation between the Department of Ophthalmology, University Medical Center Hamburg-Eppendorf, and the Care Vision Eye Clinic Hamburg.

2.2. Participants. Patients received corneal crosslinking (CXL) for progressive keratoconus.

Inclusion criteria for CXL were

(a) diagnosis of a progressive keratoconus that is pentacam topography documented progression of maximum K-values > 0.5 D within 6 months. The diagnosis of KC was confirmed by asymmetry of the corneal surface (KISA%) of >100 [11]. Further, all patients were classified as stage 1 or 2 according to the Amsler-Krumeich classification [12];

(b) best corrected visual accuracy of at least 0.4 logMAR;

(c) absence of corneal scars;

(d) signed consent form including the information of the off-label status of the therapy.

Exclusion criteria for CXL were

(a) central corneal thickness < 400 microns (even after intraoperative corneal swelling with a hypotonic solution);

(b) pregnancy;

(c) severe dry eye syndrome;

(d) inflammation of the ocular surface, the anterior chamber or the eyelids.

None of the included eyes received surgical procedure before the CXL.

2.3. Data Collection. Biomechanical analyses were performed with the Corvis ST (CST; Oculus Inc., Dudenhofen, Germany). After using the automated export function of the CST, the data were recalculated by applying the CST research software version 6.07r08 developed by Oculus. This recalculation process adds additional parameters to the analyses. Moreover, it provides extra quality scores that help in further assessing the value of the data. The CST and the analyzed parameters of the original software have been explained elsewhere [13].

The new research software adds the following parameters.

(i) Deflection amplitude [mm] (Figure 1): displacement of the corneal apex in reference to the overlaid cornea in initial state (blue line). The movement of the corneal apex is compensated by the whole eye movement. Only the movement of the cornea is described by this parameter. The red line in Figure 1 displays the deformation amplitude (DA). The DA is determined by the deformation of the corneal apex in vertical direction. It is the sum of deflection amplitude and whole eye movement (green line).

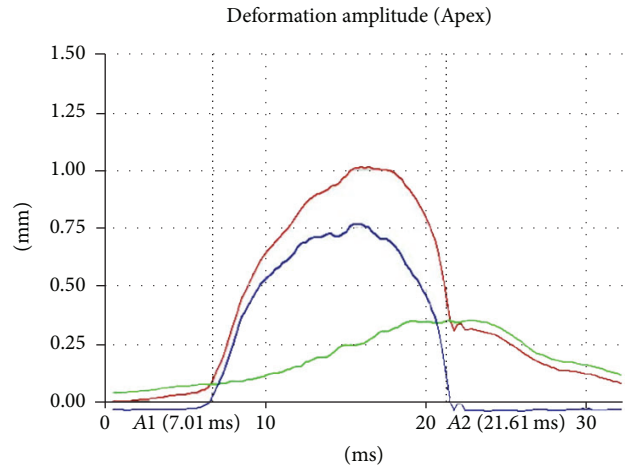

Figure 1: Deformation/deflection/eye movement diagram. Blue line: deflection; red line: deformation; green line: whole eye movement.

(ii) Deflection length [mm] (Figure 2): length of a line (blue line, Figure 2) that describes the deflected part of the cornea compared to the undeformed cornea in the initial state (red dotted line). The two end points (blue circles) are fitted to the positions where the shape of the outskirts of the cornea does not differ from the cornea in the initial state. This allows more robust information to be obtained on the applanated part of the cornea at the time of the first and second applanation compared to just the applanation length.

(iii) Radius (3P) [mm]: radius of curvature at maximum deformation, 3-point-fit.

(iv) A1 deflection length [mm]: deflection length at the time of the first applanation.

(v) HC deflection length [mm]: deflection length at the time of the highest concavity.

(vi) A2 deflection length [mm]: deflection length at the time of the second applanation.

(vii) HC deflection amp. [mm]: deflection amplitude of the highest concavity.

(viii) A1 deflection amp. [mm]: deflection amplitude of the first applanation.

(ix) A2 deflection amp. [mm]: deflection amplitude of the second applanation.

(x) Deflection amp. max. [mm]: maximum deflection amplitude.

(xi) Deflection amp. max. [ms]: time of the maximum deflection amplitude.

The CST analyses were performed with a median of 0 days before and 84 days (3 month) after CXL. The exact time intervals for every patient are displayed in Table 1.

Table 1: Descriptives.

Patient	Sex	Age	Days before CXL	Days after CXL	BAD_D	Pachy min (μm)	Astig. (D)	*K*max (D)
1	Male	21	0	98	7.18	453	4.20	51.66
2	Female	28	0	84	7.06	489	2.50	54.79
3	Female	47	−21	56	4.42	464	3.00	49.74
4	Male	27	0	91	7.62	462	4.70	50.59
5	Male	20	0	70	7.84	454	7.80	58.00
6	Male	39	0	77	14.07	520	2.00	60.05
7	Male	24	0	84	9.30	480	5.90	59.70
8	Male	29	−23	70	10.76	450	6.10	59.25
9	Male	20	0	91	5.56	508	4.70	54.28
10	Male	37	0	91	8.22	501	2.20	59.99
11	Male	21	−16	91	6.19	522	4.20	47.48
12	Male	43	0	84	0.82	545	6.30	49.46
13	Male	27	0	91	9.27	462	5.70	57.00
14	Male	29	0	77	12.11	454	6.90	62.57
15	Male	47	0	91	8.19	451	6.40	54.09
16	Male	46	0	70	0.67	571	6.80	49.53
17	Male	25	−44	84	3.30	475	2.20	46.75

Days before CXL and days after CXL: time interval between Corvis ST analysis and corneal crosslinking; BAD_D: Belin-Ambrosio display-enhanced ectasia total deviation value. [10]; pachy min: corneal thickness at the thinnest point; Astig: topometric astigmatism front surface; *K*max_: steepest keratometry of the front surface.

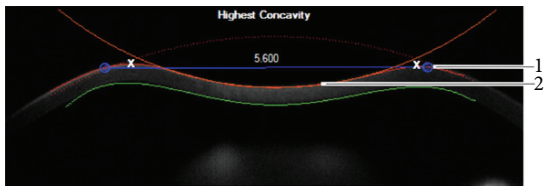

Figure 2: Display of the deflection length and the highest concavity. (1) Deflection length; (2) highest concavity.

The CST displays a quality specification grade (QS) based on the patient's alignment and the integrity of the data record for every analysis. Only CST analyses with a status "OK" for all available QS were included in the statistical analyses. If the patient had used contact lenses, a minimum of 14 days (hard lenses) or 4 days (soft lenses) of contact lens abstinence was maintained. To avoid a potential bias attributed to diurnal variations of the corneal thickness and the anterior and posterior corneal surface, both the pre-CXL and the 3-month post-CXL analyses were performed between 8 and 10 a.m. [14]. Only one eye of every subject was included in the statistical analyses.

2.4. Surgical Technique. Standard corneal CXL was conducted using Dresden protocol as previously reported [2]. According to that, CXL was conducted under sterile conditions in the operating room. Oxybuprocaine 0.4% eye drops were applied for preoperative local anesthesia. After inserting the eyelid speculum, the central 9 mm of the corneal epithelium was cautiously removed using 20% alcohol solution (20 seconds) and a blunt knife.

After de-epithelialization, an ultrasound-pachymetry was performed to ensure a central corneal thickness >400 μm. In case of a central corneal thickness >400 μm, a photosensitizer, riboflavin 0.1% solution (10 mg riboflavin-5-phosphate in 10 mL dextran-T-500 20% solution) was applied every 2 minutes for 30 minutes. If the pachymetry was <400 μm, we applied 0.1% hypo-osmolar riboflavin solution. After 30 minutes, the application of riboflavin was followed by another pachymetry to confirm a central corneal thickness of at least 400 μm. If the corneal thickness was thinner then 400 μm, we continued the application of the hypotonic solution until the pachymetry exceeded 400 μm and continued with the hypotonic solution throughout the irradiation. If the central pachymetry was >400 μm in both measurements, we applied the riboflavin 0.1% solution as described above. The application of riboflavin was continued every 5 minutes during the following irradiation.

The UVA irradiation was started using UV diodes (370 nm; Peschke Lasertechnik, Waldshut-Tiengen, Germany) with the UVA light focusing on the cornea while protecting the limbus. Before each treatment, the desired irradiance of 3 mW/cm^2 was controlled with a UVA meter (LaserMate-Q; LASER 2000, Wessling, Germany) and, if necessary, regulated with the potentiometer. The patient's cornea was irradiated with the UVA-light diodes for 30 minutes. Treated eyes were dressed with a soft contact lens bandage for 4 days and were medicated with antibiotics (ofloxacin drops 4 times/day), nonsteroidal anti-inflammatory drugs (diclofenac drops 4 times/day), and lubricants (phospholipidic microemulsion drops tapered 8 times/day). All eye drops were free of preservatives. After CL removal on 4th postoperative day the therapeutic regimen was changed to

fluorometholone 0.2% drops (4 times/day) and lubricants (phospholipidic microemulsion drops, 8 times/day). During the 5th and 10th week after CXL, fluorometholone was reduced by 1 eye drop every two weeks.

Our study adhered to the tenets of the Declaration of Helsinki. Informed consent for retrospective data analysis and approval of the Institutional Review Board (IRB)/Ethics Committee for the study were obtained.

2.5. Statistical Analysis. Before statistical analyses, all CST parameters were automatically exported into a spreadsheet program (Microsoft Office Excel) using the original software of the device. The data were recalculated using the new research software of the CST developed by Oculus (v.6.07r08). For statistical analyses, the general purpose statistical software (STATA version 11.0; StataCorp) was applied. For normal distributions, a t-test was used to test for statistically significant differences between pre- and postoperative analyses. If a parameter was not normally distributed, a Wilcoxon matched-pairs test was used. A P value less than 0.05 was considered statistically significant.

3. Results

Our database contained records of 22 patients with progressive KC who received CST analyses before and after CXL. Of these, 17 eyes of 17 patients (2 females, 15 males; mean age 27 years; min/max: 20/47 years) displayed CST analyses with high quality results before and median 3 months after CXL. Descriptives are displayed in Table 1.

More than 75% of the examinations were conducted only minutes before the treatment and mean followup was 3 months. High values of the Belin Ambroso Index (BAD_D), the topographic astigmatism and/or the maximum keratometry of the corneal surface (Kmax) could be demonstrated for every patient before the treatment.

Table 2 displays the changes of the exported CST parameters before and 3 months after CXL.

Of the 24 automatically exported parameters of the CST, 4 parameters displayed statistically significant differences between pre- and postoperative examination: the intraocular pressure (IOP; median: +3 mmHg), the central corneal pachymetry (pachy; mean: −35 μm), the timespan between the air impulse release and the first applanation of the cornea (A1time; median: +0.12 ms), and the timespan between the air impulse release and the second applanation of the cornea, which occurs when the cornea passed the point of maximum impression and is on its way back to the initial state (A2time; median: −37 ms) (see also Table 3).

All these statistically significant changes indicate an increase of the corneal stiffness after the treatment. Even considering the parameters without statistically significant changes, none of the analyzed parameters demonstrated an alteration, which might suggest a worsening of the corneal biomechanical properties after CXL. The differences of the parameters with statistically significant changes are outlined in Table 3.

We calculated the sample sizes for the new deflection parameters based on our data (Table 4).

Based on the distribution and the number of subjects included (see also N in Table 2) the required sample sizes vary between 21 and >10,000. It should be born in mind that the calculated sample size also strongly depends on the follow-up period. An extended followup might reduce the calculated sample size distinctively.

4. Discussion

We identified statistically significant differences between four parameters obtained before and 3 months after CXL: IOP, central corneal pachymetry, "A1time," and "A2time."

Over the past few years, several methods for measuring the geometric structure of the cornea have been used for analyzing corneal geometrics and to describe corneal pathologies. These devices have improved our understanding of corneal pathologies and helped us to identify pathologies like KC in its early stages. Besides these improvements in structural analyses, in vivo analyses of biomechanical changes have been developed. The first device allowing in vivo analyses of the cornea was the Ocular Response Analyzer (ORA) (Reichert Technologies) [15, 16]. Only a few study groups have analyzed in vivo biomechanical changes in progressive KC after CXL [17–20]. Unfortunately, ORA analyses of post-CXL changes have not yielded consistent findings [17, 18, 20, 21].

Recently, Oculus introduced a new device for in vivo analyses of corneal biomechanics called "Corneal Visualization Scheimpflug Technology" (CST). Like the ORA, it uses a precise collimated air pulse to cause the cornea to move inwards. However, in contrast to the ORA, it uses high-speed Scheimpflug technology to follow the movement of the cornea throughout the whole dynamic process of inward and outwards motion. In this way a range of parameters are generated which enable complex analyses of the viscoelastic properties of the cornea. Until now, only a few studies comparing biomechanical properties of KC and normal eyes have been published [13, 22].

Tian et al., using the CST software version 1.00r30, found the deflection amplitude (DA) to be the best parameter for differentiating between KC and normal eyes (NE) by demonstrating a higher DA in KC (their "DA" is equivalent with our "defampmax (mm)") [13]. They also demonstrated a lower concavity curvature and faster corneal applanation velocity in KC compared to NE. Ali et al., comparing data of 45 KC eyes and 103 NE, also found the DA to be the potentially strongest parameter for differentiating between KC and NE, with DA being higher in KC (KC: 1.37 ± 0.21 mm; NE: 1.05 ± 0.11 mm, $P < 0.001$). However, because of the only minor ROC (receiver operating characteristic) areas with no ideal cut-off values, they concluded that the DA may be a useful adjunct in KC assessment and monitoring but cannot solely discriminate between normal and keratoconic corneas.

Table 2: CST parameter before and 3months after CXL.

Parameter (unit)	Before CXL				3 months after CXL				P^*
	N	Min/Max	Median (Q25/Q75)	Mean (±SD)	N	Min/Max	Median (Q25/Q75)	Mean (±SD)	
IOP (mmHg)	17	7.50/17.00	11.00 (9.00/12.50)	11.26 (2.76)	17	8.00/45.50	14.00 (12.00/16.00)	15.12 (8.31)	**0.004**†
pachy (μm)	17	415/562	487 (473/539)	498 (42.99)	17	365 /548	452 (438 /502)	463 (45.33)	**<0.001**
defampmax (mm)	17	0.94/1.41	1.18 (1.08/1.26)	1.16 (0.13)	17	0.44/1.51	1.09 (1.04/1.21)	1.10 (0.22)	0.155†
A1time (ms)	17	6.49/7.50	6.93 (6.78/7.13)	6.96 (0.28)	17	6.61/10.68	7.12 (6.87/7.35)	7.29 (0.92)	**0.024**†
A1length (mm)	17	1.33/1.89	1.70 (1.53/1.79)	1.65 (0.18)	17	1.22/1.86	1.69 (1.60/1.76)	1.64 (0.18)	0.891
A1velocity (ms)	17	0.09/0.22	0.14 (0.13/0.17)	0.15 (0.04)	17	0.03/0.22	0.15 (0.13/0.17)	0.15 (0.05)	0.902
A2time (ms)	17	21.41/22.86	22.25 (21.84/22.46)	22.16 (0.46)	16	19.07/22.93	21.65 (21.54/21.95)	21.66 (0.83)	**0.039**†
A2length (mm)	17	0.80/2.41	1.27 (1.09/1.87)	1.43 (0.47)	17	0.78/2.12	1.55 (1.08/1.82)	1.49 (0.44)	0.702
A2velocity (ms)	17	−0.67/−0.26	−0.46 (−0.52/−0.39)	−0.45 (0.11)	16	−0.57/−0.24	−0.44 (−0.49/−0.34)	−0.42 (0.10)	0.348
hctime (ms)	17	16.17/18.25	17.09 (17.09/17.56)	17.27 (0.50)	17	15.25/18.71	17.09 (16.63/17.56)	17.04 (0.77)	0.292
peakdist (mm)	17	2.14/5.62	4.81 (4.68/5.10)	4.46 (1.07)	17	2.01/5.92	4.58 (2.49/5.08)	3.97 (1.36)	0.165
radius (mm)	17	4.27/9.48	5.80 (5.25/6.29)	6.04 (1.27)	17	4.52/9.96	6.04 (5.25/6.56)	6.21 (1.41)	0.488
radius3p (mm)	17	4.27/9.48	5.96 (5.30/6.35)	6.12 (1.28)	17	4.52/9.96	6.04 (5.25/6.56)	6.23 (1.43)	0.683
A1deformationamp (mm)	11	0.09/0.32	0.12 (0.10/0.15)	0.14 (0.06)	8	0.07/0.22	0.13 (0.11/0.18)	0.14 (0.05)	0.544
hcdeformationamp (mm)	11	0.99/1.55	1.14 (1.05/1.33)	1.19 (0.18)	8	0.95/1.41	1.14 (1.07/1.25)	1.16 (0.15)	0.838
A2deformationamp (mm)	11	0.18/0.53	0.38 (0.33/0.42)	0.37 (0.09)	7	0.21/0.44	0.38 (0.28/0.43)	0.36 (0.08)	0.270†
A1deflectionlength (mm)	11	1.85/3.15	2.30 (2.02/2.46)	2.32 (0.36)	7	1.55/2.89	2.33 (2.06/2.79)	2.38 (0.48)	0.612†
hcdeflectionlength (mm)	11	5.50/6.12	5.80 (5.75/6.03)	5.85 (0.19)	8	5.70/6.16	5.90 (5.78/5.96)	5.89 (0.15)	0.699
A2deflectionlength (mm)	10	1.90/3.63	3.00 (2.51/3.09)	2.85 (0.48)	5	2.05/3.50	3.12 (3.04/3.31)	3.00 (0.56)	0.095†
hcdeflectionamp (mm)	11	0.81/1.36	0.97 (0.89/1.09)	1.01 (0.17)	11	0.75/1.36	0.97 (0.91/1.09)	1.00 (0.17)	0.592†
A1deflectionamp (mm)	11	0.07/0.31	0.10 (0.07/0.11)	0.11 (0.07)	11	0.04/0.47	0.10 (0.07/0.18)	0.14 (0.12)	0.188
A2deflectionamp (mm)	11	0.03/0.33	0.13 (0.06/0.19)	0.14 (0.09)	11	0.00/0.69	0.15 (0.09/0.19)	0.20 (0.20)	0.247
deflectionampmax (mm)	11	0.81/1.40	0.98 (0.92/1.09)	1.02 (0.17)	8	0.76/1.26	1.00 (0.93/1.13)	1.02 (0.16)	0.885†
deflectionampmax (ms)	11	15.99/16.83	16.40 (16.17/16.74)	16.44 (0.31)	8	14.35/17.20	15.99 (15.89/16.38)	16.01 (0.81)	0.254

P values reaching statistical significance are in bold font.

IOP: intraocular pressure; pachy: central corneal pachymetry; defampmax: maximum corneal deformation amplitude; A1time: time of the first applanation; A1length: Length of the first applanation; A1velocity: velocity of the corneal apex at the first applanation; A2time: time of the second applanation; A2length: length of the second applanation; A2velocity: velocity of the corneal apex at the second applanation; hctime: highest concavity, time of the maximum deformation; peakdist: distance between both non-deformed peaks; radius: radius of curvature at maximum deformation, calculated with "parabolic fit"; radius3p: radius of curvature at maximum deformation, 3-point-fit; A1deformationamp: deformation amplitude at the time of the first applanation; hcdeformationamp: deformation amplitude at the time of the highest concavity; A2deformationamp: deformation amplitude at the time of the second applanation; A1deflectionlength: deflection length at the time of the first applanation; hcdeflectionlength: deflection length at the time of the highest concavity; A2deflectionlength: deflection length at the time of the second applanation; hcdeflectionamp: deflection amplitude of the highest concavity; A1deflectionamp: deflection amplitude of the first applanation; A2deflectionamp: deflection amplitude of the second applanation; deflectionampmax (mm): maximum deflection amplitude; deflectionampmax (ms): time of the maximum deflection amplitude.

*Paired *t*-test.

†Differences are not normally distributed. Therefore, Wilcoxon matched-pairs test was used.

Table 3: Differences for the parameters with statistically significant changes after CXL.

Parameter (unit)	Before CXL		3 months after CXL		Differences	
	Median (Q25/Q75)	Mean (±SD)	Median (Q25/Q75)	Mean (±SD)	Median (Q25/Q75)	Mean (±SD)
IOP (mmHg)†	11.00 (9.00/12.50)	11.26 (2.76)	14.00 (12.00/16.00)	15.12 (8.31)	**+3.00 (+4.50/0.00)**	+3.85 (7.33)
pachy (μm)*	487 (473 /539)	498 (42.99)	452 (438 /502)	463 (45.33)	−41 (−14 /−50)	**−35.00 (26.05)**
A1time (ms)†	6.93 (6.78/7.13)	6.96 (0.28)	7.12 (6.87/7.35)	7.29 (0.92)	**+0.12 (+0.32/0.00)**	+0.33 (0.80)
A2time (ms)†	22.25 (21.84/22.46)	22.16 (0.46)	21.65 (21.54/21.95)	21.66 (0.83)	**−0.37 (+0.10/−0.79)**	−0.47 (0.78)

*Normally distributed.
†Not normally distributed.
The relevant differences are in bold font.

Table 4: Sample size calculation for the deflection-based parameters calculated using the new CST research software.

Parameter	Delta (Mean/SD)	Required sample size*
A1deflectionlength (mm)	−0.08 (0.53)	347
hcdeflectionlength (mm)	−0.03 (0.20)	350
A2deflectionlength (mm)	−0.26 (0.40)	21
hcdeflectionamp (mm)	0.003 (0.11)	10555
A1deflectionamp (mm)	−0.026 (0.06)	44
A2deflectionamp (mm)	−0.06 (0.16)	58
deflectionampmax (mm)	−0.018 (0.123)	368
deflectionampmax (ms)	0.43 (0.98)	43

*Based on our data ($n = 17$, 3-month followup), this sample size is required to prove the differences with the t-test at the significance level of 0.05 and a test power of 80%.

Tomita et al. used the CST for analyzing biomechanical changes of the cornea after CXL for progressive KC [19]. They compared the results of accelerated versus conventional CXL in progressive KC by applying ORA and CST with a follow-up of 1 year. Similar to our study, they included subjects with KC classified as first or second stage according to the Amlser-Krumeich classification [12]. Unfortunately, they did not give information about the criteria of KC progression leading to the indication for CXL. As in previous studies, they could not demonstrate statistically significant changes after conventional CXL using the ORA for in vivo biomechanical analyses. However, using CST analyses, they found changes in three parameters: the deformation amplitude (DA; −0.02 mm), the distance between corneal bending points ("peak distance"; +0.42 mm) and the radius of the curvature at the time of highest concavity of the cornea (+0.1 mm). Although these changes indicate a higher stiffness of the cornea, the changes in these parameters were not statistically significant at 1 year after conventional CXL.

We demonstrated statistically significant changes in the A1time (median: +0.12 ms) and the A2time (median: −0.37 ms). In addition, the IOP (median: +3 mmHg) and the central corneal pachymetry (pachy; mean: −35 μm) demonstrated statistically significant changes. In line with previously published data, the DA decreased slightly, but not on a statistically significant level (−0.9 mm, $P = 0.155$). Also the peak distance and the radius at the time of the highest concavity changed with the same direction as that demonstrated by Tomami et al., but again did not reach a statically significant level (peak distance: −0.49 mm, $P = 0.165$; radius: +0.17 mm; $P = 0.488$).

The changes of "A1time" and "A2time" at 3 month after CXL indicate an increase in the corneal resistance after the treatment. Because of the increased "stiffness" of the corneal tissue, the time until the cornea reaches the status of the first applanation (A1time) increased and the time of the second applanation (after the cornea passed the point of maximum deformation [A2time]) decreased. Correspondingly, Tian et al. demonstrated a statistically significant longer "A1time" and a shorter "A2time" in NE than in KC on analyzing the biomechanical properties of the cornea in KC and NE with the CST [13].

Huseynova et al. showed that the biomechanical analyses measured with the CST are strongly influenced by the IOP and the pachymetry [23]. This is of curial importance, especially in cross-sectional-analyses. In longitudinal studies, the IOP or the pachymetry should not matched or accounted before statistical analyses to avoid generating confounding factors. An increase in pachymetry leads to an increase in the measured IOP values since more pressure is needed to applanate the cornea regardless of the real IOP [24, 25]. We demonstrated a statistically significant increase in the IOP despite a decrease of the pachymetry. This relationship can be seen as a further biomechanical indication of an increase of corneal stiffness. Accordingly, Tian et al., analyzing KC and NE, demonstrated a statistically significantly lower IOP in their KC group [13]. The initial decrease of corneal pachymetry after CXL has been described in several studies [26–28]. These studies also demonstrated an increase in the pachymetry over the months following treatment. Because pre- and post-CXL measurements were performed between 8 and 10 a.m., the diurnal change of IOP should be negligible [29, 30]. Several study groups have already demonstrated a high repeatability and accuracy of IOP and pachymetry measurement with the CST [23, 31, 32].

We used the new research software version 6.07r08 for our analyses. This software additionally provides new deflection parameters. Focusing on deformation-related parameters, as in the mentioned above, the sum of the deflection (real corneal apex movement) and the whole eye movement are analyzed. As shown in the methods section, the deflection describes the displacement of the corneal apex in reference to the overlaid cornea in its initial state. Therefore, the movement of the corneal apex is compensated by the whole

eye movement and only the "real" movement of the cornea is described.

Unfortunately, despite applying the new test software, we could not demonstrate further statistically significant differences in the deflection-based parameters. This is probably related to the small sample size and the short follow-up of our study. Different study groups have demonstrated ongoing changes of the cornea even years after the CXL [7–9].

The strength of our study is the methodological standardization: We only implemented CST analyses with objectively measured high-quality standards (included QS "OK" or "Model deviation"; excluding all analyses with an "Alignment" or "Lost images" warning). Excluding confounding factors such as wrong alignment is crucial because the CST measures corneal changes caused by a precise collimated air pulse. If the alignment is off-center, the analysis is severely compromised. In addition, 76% of the included pre-CXL analyses were performed only minutes before the CXL, reducing the probability of potential changes between the last examination before the CXL and the time of the treatment. Further, all analyses were performed between 8 and 10 a.m. to reduce diurnal variations. We were able to apply the latest CST research software. As already mentioned, besides an improved feedback regarding the quality of the analyses (additional QS scores), this version provides (deflection-based) parameters that are not compromised by back and forth movements of the eye that occur as a reaction to the air impulse during the measurement.

We acknowledge the fact that our study has certain limitations. We only included 17 eyes in our retrospective analyses. Additionally, 24% of the pre-CXL analyses were not performed immediately before CXL but within 6 weeks before the treatment (range 16–44 days before CXL). This might affect the results due to potentially unknown biomechanical changes between the pre-CXL measurement and the treatment. Therefore, a prospective study design with requested CST on the same day as CXL would be helpful to minimize the time interval between analysis and treatment. Alike, the time range of the post-CXL examinations might bias the results. As displayed in Table 1, 89% of the examinations post-CXL were conducted 70–90 days after the treatment. One patient was examined 56, and another patient 98 days after CXL. These outliers potentially affect the results by either not detecting subtle changes due to a too short timespan after CXL, or overrating changes in the context of our median 3 month follow up. Unfortunately, we could not find other prospective or retrospective studies analyzing changes after CXL for progressive KC which report the time range of their follow up intervals pre- and post CXL. Therefore, further comparisons are impossible [7, 33–35]. Nevertheless, because of a high percentage of examinations conducted only few minutes before the treatment (76% of the included examinations) and almost 90% of the examinations performed within a time range of 20 days at the 3 month follow up, we think that our results contribute to the understanding of biomechanical changes after CXL for progressive KC.

5. Conclusions

We identified two parameters, A1time' and A2time, that indicate an improvement of the corneal biomechanical properties after corneal CXL for progressive KC. None of the other CST parameters revealed statistical significant changes, demonstrating at least a stabilizing effect of CXL on corneal biomechanics. However, studies with a longer followup and larger sample sizes are warranted.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Johannes Steinberg and Toam Katz contributed equally to this work.

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Clinical Study

Clinical Outcomes after Complete Intracorneal Ring Implantation and Corneal Collagen Cross-Linking in an Intrastromal Pocket in One Session for Keratoconus

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Purpose. The aim of this work was to evaluate the results after combined surgery implantation of full rings and CXL in one session in a group of patients with keratoconus during a 12-month follow-up. *Material and Methods.* The study included 22 eyes of 20 keratoconic patients, mean age of 28.41 (from 18 to 50) years. A full ring was inserted and afterwards 0.1% riboflavin solution was injected into the corneal pocket through the incision tunnel. The cornea was irradiated with UV-A light for 30 minutes. Postoperative visits were scheduled for the first week and months 1, 3, 6, 12, and 24 after surgery. Minimal follow-up time was 12 months. *Results.* The mean UDVA improved by 6 lines from before the operation to 1 year after the operation, the mean CDVA improved by approximately 2.5 lines, and the mean K improved by 3.94 D. Statistically significant reductions of sphere ($P < 0.001$), cylinder ($P = 0.004$), and spherical ($P < 0.001$) equivalents were found 1 month after surgery. *Conclusion.* The combined surgery MyoRing implantation and CXL seems to be a safe method in the treatment of keratoconus. We noticed an improvement of the refractive error in all of our patients.

1. Introduction

Keratoconus is an ectatic corneal disorder with progressive steepening and corneal thinning, especially in the inferior part of the cornea. Ultraviolet-A irradiation of the cornea after the application of riboflavin induces cross-links between the collagen elements with subsequent stiffening of the tissue [1]. Although this treatment may stop the progression of keratoconus and stabilise the cornea, the ability to achieve visual rehabilitation for improved visual outcome is limited [2, 3].

Corneal remodelling by inserting intrastromal implants can improve the visual acuity, changing the curvature of the ectatic cornea [4]. Incomplete rings available in the market for many years are Intacs (Addition Technology, Inc.), Ferrara ring (Ferrara Ophthalmics Ltd.), and Keraring (Mediphacos Ltd.). Implanting of a complete intrastromal ring, MyoRing (Dioptex GmbH, Austria), is an alternative technique, which has been proven to be safe and effective in previous studies in the treatment of keratoconus [5–9]. The ring is implanted into an intrastromal pocket created with either a specified microkeratome PocketMaker (Dioptex GmbH, Austria) [5] or a femtosecond laser [8, 10]. The depth of the corneal pocket has been proposed to be 300 or 250 μm in the previous studies [5, 11]. The main advantages of a full ring are easy implantation, excellent centration, and the postoperative possibility of adjusting the position of the ring, if necessary [6]. The corneal pocket can also be used for the direct application of the riboflavin into the cornea. Bypassing the epithelium by injecting riboflavin directly into an intracorneal pocket seems to be a safe and effective method, preserving the epithelium and avoiding pain and discomfort seen after epithelial removal [12]. The combination of full ring implantation and corneal cross-linking (CXL) not only can lead to an improvement but also can lead to a long-term stability of visual acuity in patients with keratoconus [12].

The aim of this work is to evaluate the results after combined surgery implantation of full rings and CXL in one session in a group of patients with keratoconus during a 12-month follow-up.

Table 1: Visual and refractive outcomes over time.

Variable	Mean ± SD					P Value
	Preoperative	Postoperative				
		1 Months	6 Months	1 Year	2 Years	
UDVA (logMAR)	0.89 ± 0.38	0.61 ± 0.34	0.42 ± 0.26	0.33 ± 0.23	0.29 ± 0.26	=0.012
CDVA (logMAR)	0.44 ± 0.20	0.36 ± 0.22	0.22 ± 0.12	0.19 ± 0.09	0.16 ± 0.10	<0.001
Sphere (D)	−4.01 ± 3.21	−1.54 ± 2.57	−0.94 ± 2.07	−1.25 ± 0.94	−1.8 ± 0.58	<0.001
Cylinder (D)	−2.98 ± 2.67	−1.15 ± 1.78	−1.53 ± 2.10	−1.37 ± 1.43	−0.95 ± 1.54	=0.025
Mean K (D)	51.05 ± 4.51	47.27 ± 5.27	46.8 ± 4.64	47.11 ± 4.57	47.34 ± 5.85	<0.001
Corneal astigmatism (D)	4.62 ± 3.23	3.12 ± 1.92	1.66 ± 0.9	2.96 ± 1.65	2.35 ± 1.14	=0.048

CDVA = corrected distance visual acuity; K = keratometry; UDVA = uncorrected distance visual acuity.
P value—change from preoperatively to 1 year postoperatively (paired Student t test).

2. Materials and Methods

This was a retrospective, consecutive, nonrandomised, interventional case series including a total of 22 eyes of 20 keratoconic patients with ages ranging from 18 to 50 years. Informed consent was obtained from all patients. Institutional ethical review board approval was obtained for the procedures and the tenets of the Helsinki Declaration were followed.

Keratoconus diagnosis was based on corneal topography and slit-lamp observation: asymmetric bow tie pattern, the presence of stromal thinning, conical protrusion of the cornea at the apex, Fleischer ring, and Vogt striae. Patients were classified according to the Amsler-Krumeich classification [13]. Inclusion criteria were keratoconic eyes with no corneal scar, minimum corneal thickness 350 μm, and uncorrected distance visual acuity (UDVA) worse than 0.25 logMAR. Exclusion criteria were active ocular diseases, history of herpes keratitis, hyperopic spherical equivalent (SE), previous intraocular or corneal surgery, systemic connective tissue disease, and pregnancy.

All surgical procedures were performed by 1 surgeon (P.S.). After topical anesthesia a closed intracorneal pocket was created via a small incision tunnel by means of the PocketMaker microkeratome. The diameter of the pocket was 9.0 mm and the depth was 300 μm. The incision tunnel was approximately 4.0 mm wide and 2.0 mm long. A detailed description of the creation of the corneal pocket using a microkeratome was described by Daxer [14]. The MyoRing was then inserted into the pocket. The diameters of the rings used in this study were 5 or 6 mm with a thickness of 240, 280, or 320 μm, according to the nomogram recommended by the manufacturer. A sterile standard dose of riboflavin without dextran (0.1% riboflavin, Mediocross-sine, Medio-HAUS Medizinprodukte GmbH, Germany) was continuously injected over 1 minute into the corneal pocket via a standard cannula of 0.3 mm diameter through the incision tunnel. The instillation of the dye resulted in a yellowish colour of the anterior and posterior stroma, visible in the slit-lamp microscopy. The cornea was irradiated with UV-A light of 365 nm (Peschke Meditrade GmbH, Switzerland) and UV intensity of 3 mW/cm^2 for 30 minutes. The intracorneal tunnel is self-sealing, and the procedure requires no suturing.

Preoperatively and at all postoperative visits, patients had a complete ocular examination. The examination included UDVA, corrected distance visual acuity (CDVA), manifest refraction, slit lamp microscopy, and Pentacam imaging (Oculus GmbH, Germany). The primary outcome measures were the safety of the procedure, defined as the number and percentage of eyes losing more than 2 lines of Snellen UDVA, the safety index, defined as mean postoperative CDVA/mean preoperative CDVA [15], the UDVA and CDVA, manifest refractions, and keratometry. Keratometry and corneal thickness were measured using the Pentacam Scheimpflug imaging system. The UDVA and CDVA were obtained in decimal scaling and transformed into logMAR for statistical analysis.

Postoperative visits were scheduled for the first week and months 1, 3, 6, 12, and 24 after surgery. The minimal follow-up time was 12 months and 11 eyes had a follow-up time of 24 months.

Preoperative data versus postoperative data were analysed using the paired t-test. Statistical measures are the mean ± standard deviation and significant P values are less than 0.05. Statistical analysis was performed using SPSS statistic software, version 15.0, for Windows (SPSS, Inc., IL, USA).

3. Results

A total of 22 eyes of 20 patients with a mean age of 28.41 (±8.94) years were included; 14 patients were male (70%) and 6 were female (30%). According to the Amsler-Krumeich grading system 4 eyes had a keratoconus grade I (18.18%), 7 eyes had a keratoconus grade II (31.82%), and 11 eyes had a keratoconus grade III (50%). No intraoperative complications occurred. No MyoRing was explanted after surgery. Only five eyes had a temporary slight haziness of the cornea, which completely disappeared within one week. We have not noticed any serious postoperative complications. One patient recorded deterioration in UDVA from 0.2 to 0.05 1 month after surgery and 6 months after surgery UDVA returned to the original value of 0.2. The safety index was 1.7 at 1 year.

Table 1 summarises the visual and refractive outcomes over time. A significant improvement in UDVA was observed 1 month after surgery ($P = 0.014$). We noticed further improvement in subsequent periods (Figure 1). The difference between the first month and the sixth month was statistically significant ($P = 0.011$) and the difference

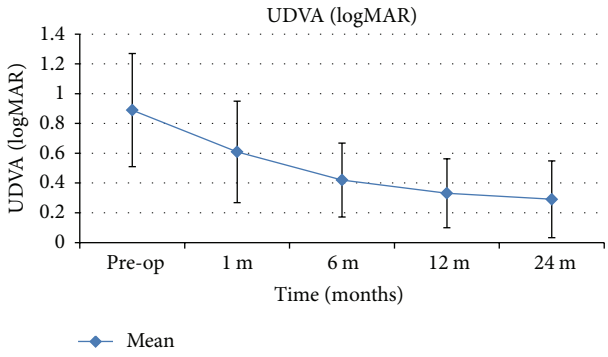

Figure 1: Mean UDVA over time. The error bars represent the SD in logMAR (UDVA: uncorrected distance visual acuity).

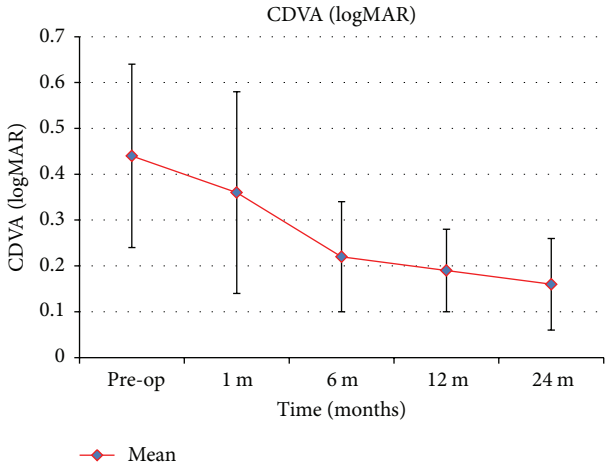

Figure 2: Mean CDVA over time. The error bars represent the SD in logMAR (CDVA: corrected distance visual acuity).

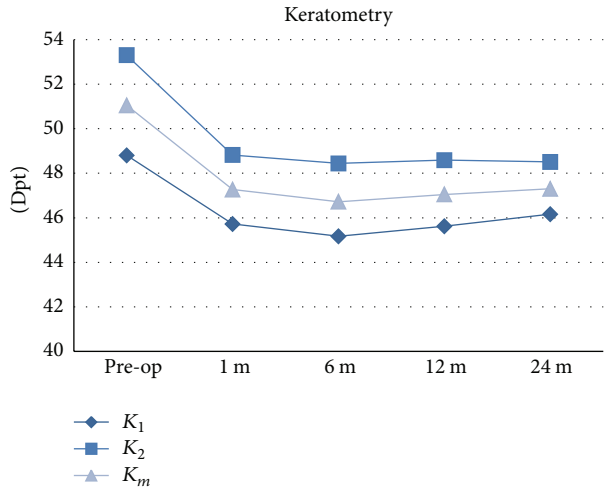

Figure 3: Keratometric changes after MyoRing implantation and CXL. D: diopters; K_1: corneal dioptric power in the flattest meridian for the 3 mm central zone; K_2: corneal dioptric power in the steepest meridian for the 3 mm central zone; K_m: mean corneal power in the 3 mm central zone.

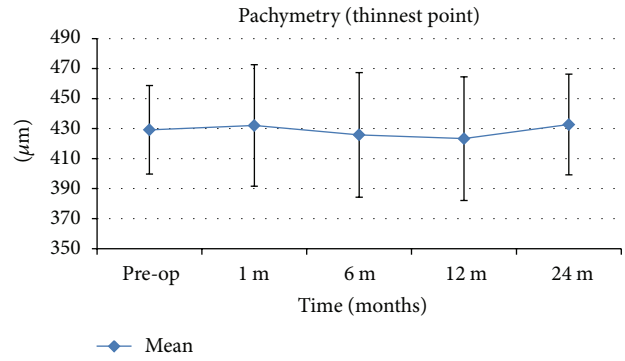

Figure 4: Pachymetric changes after MyoRing implantation and CXL. μm: micrometer. The error bars represent the SD in μm.

between the sixth month and the twelfth month was not statistically significant ($P = 0.227$). Statistically significant reductions of sphere ($P < 0.001$), cylinder ($P = 0.004$), and spherical ($P < 0.001$) equivalents were found 1 month after surgery. No significant changes in manifest refraction were detected during the remaining follow-up. The improvement in CDVA 1 month after surgery was not statistically significant ($P = 0.243$) but we noticed further increasing in subsequent periods (Figure 2). The difference between the first month and the sixth month was statistically significant ($P = 0.001$) and the difference between the sixth month and the twelfth month was not significant ($P = 0.209$).

Regarding corneal topographic outcomes (Table 2 and Figure 3) there was significant central corneal flattening (mean keratometry) 1 month after surgery ($P < 0.001$). However further improvement was no longer statistically significant (between 1 month and 6 months, $P = 0.191$, and between 6 months and 1 year, $P = 0.502$). Also, the mean value of corneal astigmatism (keratometry in flat meridian-keratometry in steep meridian) decreased significantly only in the first month after operation ($P = 0.031$).

In the postoperative period, we did not notice any thinning of the cornea and the preoperative and postoperative differences in the mean thinnest corneal point were not statistically significant. Preoperatively, the pachymetry was $429.18 \pm 29.47\,\mu$m and 1 month postoperatively it was $432.09 \pm 40.79\,\mu$m ($P = 0.576$) and 1 year postoperatively it was $423.29 \pm 41.23\,\mu$m ($P = 0.210$) (Table 2 and Figure 4).

4. Discussion

Many clinical studies have demonstrated the effectiveness of CXL to stop the progression of keratoconus [2, 16, 17]. The CXL causes photopolymerisation of collagen fibrils in the corneal stroma and it subsequently modifies the biomechanical properties of the cornea, especially the resistance to stretching [18, 19]. The main disadvantage of standard CXL with removal of epithelium is a greater risk of infection and pain. The method of CXL without removing the epithelium was therefore proposed. In some studies, however, the effect of transepithelial CXL has been proven as limited in terms of biomechanical and functional efficacies [20, 21]. In 2009,

Table 2: Keratometry (D) and pachymetry (μm) over time.

Variable	Mean ± SD					P Value
	Preoperative	Postoperative				
		1 Months	6 Months	1 Year	2 Years	
K_1	48.80 ± 4.68	45.72 ± 5.37	45.17 ± 4.72	45.62 ± 4.55	46.16 ± 5.78	<0.001
K_2	53.30 ± 4.95	48.82 ± 5.35	48.44 ± 4.74	48.59 ± 4.75	48.51 ± 5.98	<0.001
K_m	51.05 ± 4.52	47.27 ± 5.28	46.72 ± 4.63	47.05 ± 4.57	47.3 ± 5.85	<0.001
Pachymetry (thinnest location)	429.18 ± 29.47	432.09 ± 40.49	425.80 ± 41.50	423.29 ± 41.23	432.73 ± 33.60	=0.210

D = diopters; μm = micrometres; K_1 = corneal dioptric power in the flattest meridian for the 3-mm central zone, K_2 = corneal dioptric power in the steepest meridian for the 3-mm central zone, K_m = mean corneal power in the 3-mm central zone.
P value—change from preoperatively to 1 year postoperatively (paired Student t test).

Kanellopoulos described the technique of CXL with the intrastromal application of riboflavin into the pocket created by femtosecond laser [22].

More recently, techniques combining intrastromal corneal ring segment and CXL with the intrastromal administration of riboflavin have been described. A theoretical advantage of this method is the combination of two effects onto the ectatic cornea. Alió et al. compared 2 techniques of CXL using an epithelial debridement or intrastromal pocket technique after previous corneal ring segment implantation in eyes with keratoconus. They report that CXL with intrastromal riboflavin injection seemed to be as effective for corneal and refractive changes as classic CXL, although with potentially less postoperative pain [23]. Also Kılıç et al., in their study of 131 eyes with keratoconus, treated by CXL with a riboflavin injection into the corneal channel, combined with intrastromal corneal ring segment implantation, concluded that this technique is effective and the intrastromal riboflavin injection into the tunnel is safe and may provide more penetration without epithelial removal [24]. But, there may be one potential risk and disadvantage. The tunnel for segment implantation and riboflavin injection is relatively narrow and is located in the middle periphery of the cornea so the saturation of the central part of the cornea with riboflavin may not be absolutely perfect. Daxer et al. described the technique of MyoRing implantation and CXL with the intrastromal application of riboflavin into the pocket in one session. Authors presented one case report with a very good result. UDVA increased by 7 lines from 0.05 to 0.25, and the average central K reading decreased by 11 diopters. They noticed corneal haze during the early postoperative period. It diminished in the first month after surgery [12].

In our work, we evaluated the annual results of combined treatment with the intrastromal CXL application of riboflavin and full corneal ring implantation in a group of 22 eyes with keratoconus. One month after surgery we noticed a statistically significant improvement in all the followed parameters. The mean UDVA increased from 0.89 logMAR to 0.61 logMAR, mean CDVA from 0.44 logMAR to 0.36 logMAR, mean K from 51.05 D to 47.27 D, mean sphere from −4.01 D to −1.54 D, and mean cylinder from −2.98 D to −1.15 D and similar improvements have also been described by Jabbarvand et al. and Alio et al. They implanted only MyoRing, without the use of CXL. Jabbarvand et al. in a group of 98 eyes, describe, one month after MyoRing implantation, an improvement of the mean UDVA from 1.17 logMAR to 0.66 logMAR, mean CDVA from 0.85 logMAR to 0.51 logMAR, mean K from 51.9 D to 45.0 D, mean sphere from −5.48 D to 0.08 D, and mean cylinder from −5.3 D to −2.21 D. Between 1 month and 12 months after implantation monitored parameters have remained unchanged, or they changed only slightly. 1 year after surgery the mean UDVA was 0.62 logMAR, mean CDVA was 0.52 logMAR, mean K was 45.0 D, mean sphere was 0.09 D, and mean cylinder was −2.23 D [10]. 1 month after MyoRing implantation in a group of 12 eyes Alio et al. described an improvement of the mean UDVA from 1.36 logMAR to 0.69 logMAR, mean cylinder from −6.75 D to −2.07 D, and mean sphere from −4.82 D to −0.5 D. CDVA remains unchanged, 0.1 logMAR. As in the previous study, the results one year after surgery compared with results one month after surgery remained almost unchanged [8].

In our work we noticed a further improvement of the results between 1 month and 1 year after surgery. One year after surgery UDVA was 0.33 logMAR, CDVA 0.19 logMAR, mean K 47.11 D, mean sphere −1.37, and mean cylinder −1.37 D. Improvement in UDVA and CDVA was statistically significant ($P = 0.008$; $P = 0.011$, resp.).

After CXL, a slight improvement in long-term follow-up period is a common finding [2]. In contrast, after implantation of the rings, the findings are stable after 1 month and do not change. It can be assumed that the slight improvement of followed parameters a year after surgery can be attributed to the effect of CXL only.

5. Conclusion

The combined surgery MyoRing implantation and CXL seems to be a safe method in the treatment of keratoconus. We noticed an improvement of the refractive error in all of our patients. The exact resolution between the effects of CXL with intrastromal submitted riboflavin and MyoRing implantation will require additional studies with a longer follow-up period.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Theoretical Basis, Laboratory Evidence, and Clinical Research of Chemical Surgery of the Cornea: Cross-Linking

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Corneal cross-linking (CXL) is increasingly performed in ophthalmology with high success rates for progressive keratoconus and other types of ectasia. Despite being an established procedure, some molecular and clinical aspects still require additional studies. This review presents a critical analysis of some established topics and others that are still controversial. In addition, this review examines new technologies and techniques (transepithelial and ultrafast CXL), uses of corneal CXL including natural products and biomolecules as CXL promoters, and evidence for *in vitro* and *in vivo* indirect effectiveness.

1. History

The concept of using collagen cross-linking photochemically induced, for increasing corneal stiffness, as a conservative method to stabilize ectasia progression was first conceived in Germany in the 1990s by Theo Seiler and collaborators [1–4]. Collagen cross-linking (CXL) opened a new horizon for conscious biomechanical manipulation of the cornea [5], which uses the concept of biomechanical customization of therapeutic and refractive corneal surgery [6]. The original "Dresden cross-linking clinical protocol" involves topical anesthesia, central corneal abrasion, and application of riboflavin 0.1% with 20% dextran T-500 until stromal saturation is observed through biomicroscopy. The traditional procedure is followed by ultraviolet A (UVA) light of 365–370 nm at an irradiance of 3 mW/cm^2, which corresponds to a dose of 5.4 J/cm^2 for 30 min [3].

The photopolymerization effect on corneal collagen results from the reaction of the photosensitizer agent riboflavin and UVA light (370 nm), which is the absorptive peak of riboflavin. This reaction generates reactive oxygen species that can react with various molecules and subsequently induce chemical covalent bonds that bridge the amino groups of collagen fibrils [7]. Hayes et al. (2013) demonstrated riboflavin/UVA-induced cross-links at the surface of the collagen fibrils and within the proteoglycan (PG) rich coating surrounding them [8]. In another study, Zhang et al. (2011) reported that riboflavin/UVA treatment causes beyond CXL among collagen molecules and among PG core proteins, as well as limited linkages between collagen and PG such as mimecan, decorin, keratocan, and lumican [9].

2. Other Approaches

2.1. Natural Cross-Linking. Human collagen undergoes progressive changes including a decrease in solubility, elasticity, and permeability, as well as an increase in thermal stability and resistance to enzymatic digestion with aging. The precise chemical changes of these transformations are unknown. However, an *in vitro* study has suggested that these physical changes involve progressive CXL among collagen molecules

[10]. A detailed study of the collagen fibrils in normal human corneas showed a small but significant age-related increase in collagen fibrils for diameter, intermolecular spacing, and elongation [11]. Expansion of the collagen intermolecular spacing suggests molecules other than collagen are deposited between the fibrils during aging, which subsequently push the collagen molecules further apart. This is consistent with a recent study that demonstrated glycation-induced expansion of intermolecular spacing and subsequent CXL of molecules with age [10]. Considering the isolated ultrastructural dimensions of collagen fibrils, one would expect a tendency toward biomechanical strengthening of the cornea during aging [12].

Hyperglycemia was shown to influence corneal biomechanical properties by inducing stromal collagen CXL through glycosylation and lysyl oxidase (LOX) enzymatic activity [13]. People with diabetes mellitus have increased central corneal thickness, corneal hysteresis, and a corneal resistance factor, possibly reflecting a greater stiffness of diabetic corneas [14].

2.2. Biomolecules and Natural Products. Several studies have demonstrated several molecules that might promote collagen cross-link. Natural products such as genipin [15, 16] and proanthocyanidins (PAs) [17] can form cross-links between collagen fibrils. Avila et al. (2012) demonstrated in an *ex vivo* study that corneal CXL with genipin was similar to the UV traditional procedure, with minimal toxicity to endothelial cells [16]. PAs are natural products with polyphenolic structures that have the potential to give rise to stable hydrogen bonded structures and generate nonbiodegradable collagen matrices. Han et al. (2003) demonstrated the feasibility of using PAs from grape seeds to cross-link collagenous materials [17].

Biomolecules, such as the leucine-rich proteoglycans (e.g., decorin, lumican, and keratocan), regulate the orderly assembly of extracellular matrices, corneal transparency, tensile strength of skin and tendons, viscoelasticity of blood vessels, and tumor cell proliferation. Experiments *in vitro* showed that SLRPs interact with collagen through specific binding sites and delay formation of collagen fibrils. To modulate cornea collagen fibrillogenesis decorin binds to collagen types I, II, III, VI, and XIV [18, 19].

3. *In Vitro* Effectiveness Evidences

3.1. Increase in Collagen Fiber Diameter. Riboflavin/UVA-induced collagen CXL increases the corneal collagen fiber diameter, which was more pronounced in the anterior portion of the stroma of the rabbit cornea as observed on transmission electron microscopy [23].

3.2. Resistance to Enzymatic Digestion. The stabilizing biochemical effect of CXL can be explained by changes in the tertiary structure of collagen fibrils induced by CXL preventing access of the proteolytic enzymes to their specific cleavage sites by steric hindrance. In porcine corneas cross-linked with riboflavin/UVA, CXL causes an impressive doubling in the time following pepsin, trypsin, and collagenase digestion, particularly in the anterior half of the cornea [22].

3.3. Modulus of Elasticity (Young's Modulus). Many published studies report an increase in cornea stiffness after collagen CXL. Wollensak et al. (2003) found a significant increase in biomechanical rigidity by a factor of 4.5 in human corneas following riboflavin/UVA-induced collagen CXL, which was indicated by an increase in Young's modulus. The increase in biomechanical stiffness in porcine eyes was also significant by a factor of 1.8 [20]. In another study, Wollensak and Iomdina (2009) found a highly significant increase in corneal stiffness after CXL treatment of rabbit corneas with an impressive durability over time, as demonstrated by a 78.4%–87.4% (by a factor of 1.6) increase in Young's modulus by and a 69.7%–106.0% increase in ultimate stress over the entire 8-month follow-up [21]. Some limitations of this method are that the strip specimens originated from a curved sample, the corneal structure is disrupted because the lamellae are cut, and several crucial constraints are ignored (e.g., real pachymetry and meridional differences) [31].

3.4. Atomic Force Microscopy. Atomic force microscopy (AFM) has a shaping probe tip that can scan the sample surface at an atomic distance. By monitoring the interaction force between the tip and the sample surface, this instrument can create topographical images of the sample surface at high resolutions [32]. When the probes approach the sample surface, tiny interaction forces, such as Van der Waals and electrostatic forces, occur between the probe and sample. The resulting cantilever is recorded by measuring the displacement of a laser beam reflected from the backside of the cantilever. AFM can be applied to identify the collagen bundles and to determine their diameters [33]. This technique provides quantitative information on the surface morphology of the collagen fibrils at a high resolution [32]. Yamamoto et al. (2002) clearly obtained surface topographic images of human corneal and scleral collagen fibrils using AFM [32]. Further AFM studies are important to examine cross-link induced modification in corneal collagen fibrils. Seifert et al. (2014) developed a method that allows for atomic force microscopy-based measurements of gradients of Young's modulus in soft tissues. In the abovementioned study, the authors demonstrated the depth-dependent distribution of the stiffening effect caused by riboflavin/UV CXL in porcine corneas [34].

3.5. X-Ray Scattering. X-ray scattering is a specialized technique that provides structural information about the constituent collagen in the corneal stroma. The wide-angle equatorial scattering pattern produced from the lateral packing of molecules within the stromal collagen fibrils can be used to determine the intermolecular spacing within the fibrils, as well as the arrangement and distribution of fibrillar collagen in the intact cornea [35, 36]. X-ray scattering is a unique method for measuring the lateral space between individual fibril-forming collagen molecules at less than a 1 mm resolution. This space is influenced by both the fibril

hydration and the extent of molecular CXL [35]. Studies of corneal collagen organization in keratoconus (KC) suggest that the mechanism of tissue thinning in this disease involves fibrillar or lamellar collagen slippage, decreased lamellar interweaving [35, 37], and distortion of the orthogonal matrix [37]. The authors of study proposed that development of interventional cross-linking strategies may limit collagen slippage and should be beneficial for delaying the progression of keratoconus [35, 37]. In another study that analyzed CXL in human corneas using X-ray scattering, Hayes et al. (2011) concluded that UVA/riboflavin induced cross-links do not have a measurable effect on the axial stagger or the tilt of collagen molecules within the fibrils when analyzed using X-ray scattering method [36].

3.6. Second Harmonic Generation Microscopy. Second harmonic generation (SHG) microscopy has been used extensively in medicine and biology to obtain images of highly ordered structures, such as collagen fibers, microtubulin, and skeletal muscle, with high resolution and contrast. This nonlinear optical microscopy results from a coherent second-order nonlinear scattering wherein a noncentrosymmetric structure emits light at half the wavelength of the incident (pump) optical field. Collagen fibers, being intrinsically noncentrosymmetric, emit SHG and thus produce high-contrast images without the need for staining [38].

Collagen fibrils are aligned uniformly in the corneal stroma and are therefore believed to be responsible for SHG from the cornea. SHG imaging has thus allowed visualization of collagen organization and can be processed to generate three-dimensional reconstructions of collagen structure [39].

In 12 of 13 human keratoconic corneal samples obtained after penetrating keratoplasty for KC, SHG could detect differences in the organizational pattern of lamellae, including a marked loss or decrease in anterior lamellae interweaving and lamellae that inserted into Bowman's layers [40].

Analysis of porcine corneas with and without riboflavin/UVA CXL treatment using SHG showed that stromal collagen fibrils in untreated corneas had a more regular, linear, and parallel orientation. However, treated corneas had wavy stromal collagen fibrils [41].

4. *In Vivo* Indirect Effective Evidences

4.1. Visual Acuity. The primary goal of CXL is to improve the biomechanical rigidity of corneal collagen to stop ectasia progression [1, 2]. In the first published clinical trial, Wollensak et al. (2003) reported stability after CXL treatment of the eyes of 19 patients with progressive KC and with a mean follow-up of 20 months (from 3 to 33 months) [3]. In this series, visual acuity (VA) slightly improved in 15 eyes (65%). The improved uncorrected visual acuity (UCVA) recorded during the follow-up is partially explained by the sphere and spherical equivalent reduction. However, these data also may be related to a progressive reduction of the mean K power. Furthermore, the increased best spectacle-corrected visual acuity (BSCVA) may be linked to a reduction in the difference between superior and inferior corneal hemimeridians (flat-test versus steeper), expressed by the improvement in corneal symmetry indexes. Moreover, an increased BSCVA may be sustained by the statistically significant early reduction in coma aberration [42].

4.2. Keratometry. In the first published clinical trial [3], there was a variable disease regression observed in 16 cases (70%) by a reduction of the maximal keratometry readings and refractive error [3]. Similar results were observed in other studies examining CXL for KC [43–50] and keratectasia [48, 51–54]. Corneal reshaping [55] appears to be a more reliable expression of CXL induced clinical and topographic changes. Mean clinical and topographic improvements were recorded from the end of the third postoperative month and continued thereafter, reaching reliable stability in 24 months [46]. In addition, Koller et al. (2009) found KMax to be an important prognostic variable, which was associated with a significant reduction in complications when excluding cases with a KMax higher than 58D [56]. A higher chance of ectasia regression, observed by flattening, was more likely if KMax was higher than 54D [57].

4.3. Biomicroscopy. A stromal demarcation line, biomicroscopically detectable as early as 2 weeks after CXL treatment, was described by Seiler and Hafezi (2006) as the first clinical evidence of a physical effect of CXL on corneal tissue [58]. The demarcation line does not refer to biomechanical properties but represents the transition between cross-linked anterior corneal stroma, with modified refractive and reflection properties, and the untreated posterior corneal stroma [58]. Caporossi et al. (2010) found stromal edema, clinically detectable by slit-lamp examination in 70% of patients, occurred in the first 30 postoperative days. Temporary haze occurred in 9.8% of cases, 14 cases in the first 3 months, and 2 cases after 6 months but disappeared progressively after topical preservative-free steroid therapy [46].

4.4. Scheimpflug Photography and Optical Coherence Tomography. The stromal demarcation line is also observed via Scheimpflug photography [59–62] and optical coherence tomography (OCT) [46, 63]. Visante OCT scans show a higher reflectivity (hyperdensity) of this line, and after 6 months, stromal reflectivity becomes more homogeneous, reducing the visibility of the line in some eyes much more than in others [46].

4.5. Pachymetry. The pachymetric map provides the thinnest point data, which is critical for ensuring the safety parameters for the endothelium [64]. The thickness map also should be important for monitoring results after CXL. Corneal thinning has been documented in the early CXL postoperative course, with a gradual return on corneal thickness toward preoperative values within the first year after CXL [45, 46, 62, 65].

4.6. Ocular Response Analyzer. Until the launching of the ocular response analyzer (ORA) (Reichert Inc., Depew, NY) in 2005 [66], corneal biomechanical studies were limited to

laboratory *in vitro* studies and virtual mathematical corneal finite element models [67, 68]. ORA is a modified noncontact tonometer (NCT) that was designed to provide a more accurate measurement of IOP through an understanding of compensation for corneal properties [66].

During an ORA measurement, a precisely metered air pulse is delivered to the eye, causing the cornea to move inward, past a first applanation (flattening), and into a slight concavity. Milliseconds after the first applanation, the air pump generating the air pulse is shut down and the pressure applied to the eye decreases in an inverse-time symmetrical fashion. As the pressure decreases, the cornea passes through a second applanated state while returning from concavity to its normal convex curvature [66].

An electrooptical collimation detector system monitors the corneal curvature in the central 3.0 mm diameter throughout the 20-millisecond measurement. A filtered (smoothed) version of the detector signal defines 2 precise applanation times corresponding to 2 well-defined peaks produced by inward and outward applanation events. Two corresponding pressures of an internal air supply plenum are determined from the applanation times derived from the detector applanation peaks [66].

The system registers the independent applanation pressures during the ingoing (P1) and outgoing (P2) phases. The difference between the 2 pressures is called corneal hysteresis (CH) [69, 70]. Corneal resistance factor (CRF) is also calculated from P1 and P2 with an optimized function designed to augment the correlation with thickness in a normal population [66, 70]. CH and CRF were significantly lower in keratoconus, but CH and CRF were unchanged after CXL [71–73]. Hysteresis is a viscoelastic property of the cornea that is not directly related to stiffness [74]. A new set of parameters derived from the waveform ORA signal that monitors the deformation response of the cornea during an ORA measurement has been reported [72–76]. These parameters had a better diagnostic performance for keratoconus [75, 76]and improved after CXL [74, 76].

4.7. Corvis. Corvis has an ergonomic design. The patient is comfortably positioned with proper placement of the chin and forehead and then asked to focus on a central red LED. A frontal view camera is mounted with a keratometer-type projection system for focusing and aligning the corneal apex. The examination is programmed for automatic release when alignment is achieved with the first Purkinje reflex of the cornea [77].

This equipment is a NCT system integrated with an ultrahigh speed (UHS) Scheimpflug camera that was introduced by Ambrósio Jr et al. (2013) [77]. The CorVis ST (Scheimpflug Technology) records 4,330 frames per second, with a Scheimpflug camera that covers 8 mm horizontally, to monitor the corneal response to a fixed profile air pulse with a maximal internal pump pressure of 25 kPa. The addition of an UHS Scheimpflug camera allows dynamic inspection of the actual deformation process that provides further details for biomechanical characterization of the cornea.

The recording starts with the cornea at the natural convex shape. The air puff forces the cornea inward (ingoing phase) through applanation (first or ingoing applanation) into a concavity phase until it achieves the highest concavity (HC). Thereafter, the cornea undergoes a second applanation before achieving its natural shape [77]. The parameters derived from the corneal response such as corneal speed during deformation, corneal applanation length, deformation amplitude (greatest displacement of the apex at the point of HC), and radius of curvature at HC are important measures of corneal viscoelastic properties and stiffness. Such parameters are useful for the diagnosis of ectasia [75] and assessing CXL results.

In an ancillary study conducted at the Ohio State University in an industry-sponsored FDA trial of corneal collagen CXL, subjects were evaluated biomechanically using the CorVis ST before and after the procedure. Preliminary analysis at 1-month postprocedure was performed with 11 keratoconic subjects randomly selected for treatment, compared with 8 keratoconic subjects randomly selected for the sham group. A significant difference ($P < 0.0014$) was found in the radius of curvature at HC in subjects who received treatment, which is consistent with increased stiffness. Subjects in the sham group showed no difference ($P = 0.6981$) at 1 month [77].

4.8. Confocal Microscopy. In vivo confocal analysis showed disappearance of keratocytes in the anterior midstroma to a depth of 340 μm [55] and a clear vertical transition area between the edematous hyporeflective stroma with apoptotic bodies and normoreflective deep stroma. After 6 months, the reflectivity of the anterior midstroma was inverted (hyper) compared with initial postoperative reflective previously demonstrated [55]. Changes in the stromal reflectivity after the sixth month are an important indirect (confocal) sign of corneal CXL [55]. In general, after the third month, there is new collagen synthesis meditated by repopulating keratocytes and lamellar compaction, expressed by the hyperreflectiveness of the extracellular matrix, combined with newly formed collagen fibers identified with *in vivo* confocal scans [55, 78]. In addition to this finding, nerve plexus degeneration was noted up to 6 months postoperatively following CXL [79].

Confocal microscopy demonstrated numerous hyperreflective spherical structures more abundantly in the anterior stroma, and they were visible up to a depth of 300 μm after CXL [80]. It is not clear what these structures represent; however, they may represent damaged keratocytes or nuclear and cellular fragments. The stroma had a spiculated appearance and extended to a depth at 300 μm that could be secondary to changes in stromal hydration [80].

The increase of collagen fiber diameter could partly explain the increased scattering of the collagen fibers creating a net-like formation observed at the first and third months after CXL [81]. In addition, revelation of the otherwise unseen collagen fibers in the confocal microscopy images also suggest alterations of the normal collagen fiber formation that is responsible for the transparency of the cornea in normal conditions. This may also have implications on the vision function and contrast sensitivity [81].

Table 1: *In vitro* and *in vivo* evidences of corneal cross-linking protocols.

Protocol	*In vitro*	*In vivo*
Epi-off CXL (Dresden Protocol)	Increased Young's modulus [20, 21], resistance to enzymatic degradation [22], and collagen fiber diameter [23]	Improvement in VA, K reading, refraction, and halt of ectasia progression
Epi-on CXL	Riboflavin penetration requires more time than with epi-off techniques Epithelium permeabilization can be achieved with molecules as cyclodextrins [24] and benzalkonium chloride in association with NaCl [25]	Improvement in VA and topographic findings Halt of ectasia progression There is a lot of controversy about results of this technique [26]
Ultrafast CXL	Young's modulus similar to traditional CXL [27]	Equivalent in VA, refraction and pentacam parameters [28, 29], and OCT imaging [29]
Athens protocol	No data available	Superiorly with a better BSCVA, mean K reduction, spherical equivalent, and corneal haza score [30]

5. Another Crosslinking Protocols

5.1. Transepithelial Cross-Linking (Epithelial Damage versus Amphiphilic Molecules). Analysis of the light transmission spectra of porcine corneas following riboflavin/UVA corneal CXL treatment suggests a need for completely removing the epithelium to allow adequate and homogeneous penetration of riboflavin into the stroma [82]. A grid pattern of full thickness epithelial debridement appears to allow some riboflavin stromal penetration; however, this was less significant compared with that observed after complete central epithelium removal [82]. An application of 20% alcohol in the presence of an intact epithelium is not sufficient to allow adequate riboflavin penetration into the corneal stroma [82]. A riboflavin complex with ethylenediaminetetraacetic acid (EDTA) and trometamol was used for transepithelial CXL after superficial scraping. However, the uptake was considerably less than in corneas with epithelium removed [83]. Pharmacological permeabilization of epithelium was achieved by applying cyclodextrins that enhance riboflavin solubility in water and to improve its permeability through bovine corneas [24]. Raiskup et al. (2012) showed that a riboflavin solution without dextran, but including 0.01% benzalkonium chloride and 0.44% NaCl promoted the permeability through the epithelium, resulting in a sufficient concentration of riboflavin in the stroma [25]. Recently, Bottos et al. (2013) described riboflavin nanoemulsions that could penetrate the corneal epithelium. A greater stromal concentration was detected after 240 min when compared with corneas submitted to the standard protocol [84]. Bikbova and Bikbov (2014) showed the effectiveness of the impregnation of riboflavin 0.1% in eyes of 19 patients by iontophoresis in transepithelial collagen CXL with a decrease in the average keratometry 1 year after the procedure [85].

5.2. Athens Protocol. Kanellopoulus et al. (2009) studied topography-guided PRK at least 6 months following CXL and topography PRK followed immediately by CXL in a single procedure in adults with advancing KC to stabilize ectasia and rehabilitate vision (with topography-guided PRK) [30]. The simultaneous procedure appeared to be superior to sequential treatments in rehabilitation of keratoconus with minimal haze formation, and in addition to a reduction in the patient's time away from work. Perhaps CXL will have a wider application as prophylaxis in laser refractive surgery [30]. In another study, the same author found potentially promising results with the same-day and simultaneous topography-guided PRK and collagen CXL as a therapeutic intervention in highly irregular corneas with progressive corneal ectasia after LASIK [86].

5.3. Ultrafast Cross-Linking. According to the Bunsen and Roscoe (1862) law, the effect of a photochemical or photobiological reaction is directly proportional to the total irradiation dose, irrespective of the time span over which the dose is administered [87]. Schumacher et al. (2011) found an increase in Young's modulus statistically equivalent in the group of porcine corneas treated with illumination intensity of 10 mW/cm^2 and 3 times shorter illumination time of 9 min compared with a group with an intensity of 3 mW/cm^2 that required an illumination time of 30 min [27]. High fluence and UV light used with shorter exposure appears to be safe and effective in stabilizing keratoconus, and this technique appears to be similar but more comfortable for patients [28].

In Table 1 are showed *in vitro* and *in vivo* evidences of Dresden protocol and the new approaches of CXL.

6. Conclusions

Clinical assessment of biomechanical properties represents an area of active research. Novel nondestructive methodologies have been described, including radial shearing speckle pattern interferometry [88, 89], Brillouin optical microscopy [90], and other forms of dynamic corneal imaging [91, 92]. These approaches may soon be developed into commercially available instruments.

CXL has revolutionized the treatment of ectatic diseases. However, considering the goal of the procedure is to stiffen corneal tissue, thereby stabilizing ectasia progression, characterization of the cornea should go beyond shape analysis into biomechanical assessment. Such characterization is critical

for enabling conscious optimization and further improvements in CXL techniques. Such advances should significantly affect the indication, planning, and postoperative evaluation of ectasia treatments.

Conflict of Interests

Amanda C. da Paz, Patrícia A. Bersanetti, and Marcella Q. Salomão declare that they have no conflict of interests; Renato Ambrósio Jr.: consultant for Alcon and Oculus Optikgeräte GmbH; P. Schor: patent PI1001009-2, deposited on 03/26/2010, at Brazilian National Institute of Industrial Property (INPI—http://www.inpi.gov.br).

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Clinical Study

Evaluation of Epithelial Integrity with Various Transepithelial Corneal Cross-Linking Protocols for Treatment of Keratoconus

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Purpose. Corneal collagen cross-linking (CXL) has been demonstrated to stiffen cornea and halt progression of ectasia. The original protocol requires debridement of central corneal epithelium to facilitate diffusion of a riboflavin solution to stroma. Recently, transepithelial CXL has been proposed to reduce risk of complications associated with epithelial removal. Aim of the study is to evaluate the impact of various transepithelial riboflavin delivery protocols on corneal epithelium in regard to pain and epithelial integrity in the early postoperative period. *Methods*. One hundred and sixty six eyes of 104 subjects affected by progressive keratoconus underwent transepithelial CXL using 6 different riboflavin application protocols. Postoperatively, epithelial integrity was evaluated at slit lamp and patients were queried regarding their ocular pain level. *Results*. One eye had a corneal infection associated with an epithelial defect. No other adverse event including endothelial decompensation or endothelial damage was observed, except for epithelial damages. Incidence of epithelial defects varied from 0 to 63%. Incidence of reported pain varied from 0 to 83%. *Conclusion*. Different transepithelial cross-linking protocols have varying impacts on epithelial integrity. At present, it seems impossible to have sufficient riboflavin penetration without any epithelial disruption. A compromise between efficacy and epithelial integrity has to be found.

1. Introduction

Corneal collagen cross-linking (CXL) is the only conservative therapy for keratoconus that has been demonstrated to stiffen the cornea and halt the progression of the ectasia. CXL results in an increase in tensile strength of the cornea as a result of an interaction between riboflavin photosensitizer and ultraviolet light, which results in an increase in covalent bonding within or between collagen fibers that make up the anterior stromal lamellae [1]. The conventional protocol described by Wollensak et al. requires debridement of the central 9 mm of the corneal epithelium to facilitate diffusion of a solution containing 0.1% riboflavin with 20% dextran T500 to the corneal stroma [2].

Recently, transepithelial or "epithelium-on" CXL with modified technique has been proposed to reduce the risk of complications associated with epithelial removal [3, 4]. Provided that sufficient effect is obtained, transepithelial CXL is highly desirable from both the patient's and the ophthalmologist's perspective because ideally this approach avoids the pain, risk of infection, transient visual impairment, and all other consequences and potential complications of epithelial debridement [5].

A number of modified riboflavin formulations have been introduced to facilitate diffusion through the corneal epithelium. To our knowledge, to date, there has been no comparison of transepithelial formulations to evaluate whether these goals of transepithelial CXL are met. The purpose of this short-term study is to evaluate and compare the impact of various transepithelial riboflavin delivery protocols on the corneal epithelium in regard to pain and epithelial integrity in the early postoperative period.

Table 1: Overview of the different riboflavin formulations, formulation compositions, and the UVA light source, and if iontophoresis was used in this study.

Riboflavin formulation	Formulation composition	UVA delivery device	Iontophoresis
Ricrolin TE (Sooft, Italy)	0.1% riboflavin-5-phosphate, 15% dextran T500, sodium edetate, trometamol, and NaCl	UV-X 1000, IROC Innocross, Switzerland	N/A
Medio-Cross TE (Peschke Meditrade GmbH, Germany)	0.25% riboflavin-5-phosphate hydroxypropyl methylcellulose, benzalkonium chloride, NaCl	UV-X 1000, IROC Innocross, Switzerland	N/A
ParaCel (Avedro Inc., USA)	0.25% riboflavin-5-phosphate, hydroxypropyl methylcellulose, sodium edetate, trometamol, benzalkonium chloride, NaCL	KXL, Avedro Inc., USA	N/A
Ricrolin+ (Sooft, Italy)	0.1% riboflavin-5-phosphate, sodium edetate, trometamol, sodium dihydrogen phosphate dihydrate, and sodium phosphate dibasic dehydrate	KXL, Avedro, Inc., USA	I-ON CXL generator (Sooft, Italy)
VibeX Xtra (Avedro Inc., USA)	0.25% riboflavin-5-phosphate and NaCl	KXL, Avedro, Inc., USA	N/A

Table 2: Overview of the 6 different treatment protocols used in this study.

Group	Riboflavin formulation	Soak time (minutes)	UVA irradiance (mW/cm^2)	UVA time	Total energy (J/cm^2)
1	Ricrolin TE	30	3	30 minutes	5.4
2	Medio-Cross TE	30	3	30 minutes	5.4
3	ParaCel	15	45	2 minutes 40 seconds	7.2
4	Ricrolin+ (with Iontophoresis)	5	10	9 minutes	5.4
5	ParaCel and VibeX Xtra (2 stage application)	3 + 7	45	2 minutes 40 seconds, continuous irradiation	7.2
6	ParaCel and VibeX Xtra (2 stage application)	3 + 7	45	5 minutes 20 seconds, pulsed irradiation (1 s on, 1 s off)	7.2

2. Patients and Methods

One hundred and sixty-six eyes of 104 subjects affected by progressive keratoconus underwent transepithelial CXL between 05/2011 and 12/2013 at the Center for Refractive Surgery, St. Francis Hospital, Münster, Germany. Inclusion criteria included keratoconus I–III according to the Amsler-Krumeich classification with documented progression in the previous 12 months, defined as an increase in maximum keratometry (K Max) or subjective cylinder of 1.00 diopter (D) or more or subjective deterioration of visual acuity. Exclusion criteria included endothelial decompensation, central corneal opacities, history of herpetic keratitis, active corneal infection, aphakia, concomitant ocular or systemic autoimmune disease, pregnancy, and breastfeeding. Informed consent was obtained from all patients.

2.1. Patient Examinations. All eyes were evaluated by slit lamp examination to assess the presence or absence of any epithelial defects on each postoperative day until the eye was quiet and the epithelium was unremarkable. Visibly loose epithelium was considered as defective. On the first postoperative day, all patients were queried if they had experienced ocular pain of any level since transepithelial CXL. At every following visit the patients were again asked if they had experienced any ocular pain since the last visit. Optical coherence tomography (OCT) was used to qualitatively assess riboflavin diffusion postoperatively in some patients.

2.2. Surgical Procedure. Riboflavin application procedure was determined by a stepwise optimization protocol using one of 6 treatment regimens. In all cases, riboflavin application and subsequent UVA irradiation were performed according to manufacturer recommendations for the use of the riboflavin formulation and recommended parameters for UVA irradiation. The riboflavin formulations used are presented in Table 1, with the corresponding UVA delivery device used for the study treatments.

2.3. Surgical Technique and Procedure. In all treatments, the subject was placed in a supine position. Preservative free anesthetic eye drops were administered preoperatively and a lid speculum was applied. The corneal epithelium was left intact, and riboflavin application and UVA treatment were performed according to one of six regimens described below and summarized in Table 2.

Postoperative care included the use of a soft bandage contact lens in all of the eyes in Groups 4–6. No bandage contact lens was used in Group 1 and no bandage contact lens was used in the first 5 of eyes of Groups 2 and 3, respectively. The use of BSCL was introduced after observing epithelial defects in the first 5 eyes of Groups 2 and 3 in order to minimize stress on the epithelium by lid movements.

In Group 1, Ricrolin TE (Sooft, Italy) was applied at a rate of 1 drop every 2 minutes for approximately 30 minutes. Riboflavin was not rinsed from the cornea, and 3 mW/cm^2 of

irradiance was applied to the cornea for 30 minutes, for a total energy dose of 5.4 J/cm^2. During illumination the cornea was kept moist by further application of Ricrolin TE at a rate of 1 drop every 2 minutes.

In Group 2, Medio-Cross TE (Peschke Meditrade GmbH, Germany) was applied at a rate of 1 drop every 2 minutes for approximately 30 minutes. Riboflavin was not rinsed from the cornea, and 3 mW/cm^2 of irradiance was applied to the cornea for 30 minutes, for a total energy dose of 5.4 J/cm^2. During illumination the cornea was kept moist by further application of Medio-Cross TE at a rate of 1 drop every 2 minutes.

In Group 3, ParaCel (Avedro Inc., USA) was applied at a rate of 1 drop every 60 seconds for approximately 15 minutes. Riboflavin was rinsed from the cornea using BSS, and 45 mW/cm^2 of irradiance was applied to the cornea for 2 minutes and 40 seconds, for a total energy dose of 7.2 J/cm^2. No further ParaCel was applied during illumination.

In Group 4, Ricrolin+ (Sooft, Italy) was administered after applying preservative-free anesthetic eye drops 10 minutes, 5 minutes, and immediately before, while only one application of anesthetic eye drops was used in all other groups as recommended by the respective manufacturers. An iontophoresis technique was utilized with a constant current and two electrodes. A circular reservoir with a surrounding annular suction ring was affixed to the cornea during the procedure. A stainless steel grid inside this reservoir served as the cathode at a minimal distance from the cornea, and an anode was affixed to the subjects' forehead. The reservoir was filled with Ricrolin+ solution. The generator was used to apply a constant current of 1 mA for a period of 5 min. After the 5-minute impregnation period, 10 mW/cm^2 of irradiance was applied to the cornea for 9 minutes for a total energy dose of 5.4 J/cm^2.

In Group 5, a two-stage application procedure for ParaCel and VibeX Xtra (Avedro Inc., USA) was used. ParaCel was applied at a rate of 1 drop every 90 seconds for 3 minutes. The cornea was then rinsed with VibeX Xtra completely coating the cornea. Additional VibeX Xtra was applied at a rate of 1 drop every 60 seconds for 7 minutes. A total riboflavin soak time of 10 minutes was achieved. Forty five mW/cm^2 of irradiance was continuously applied to the cornea for 2 minutes and 40 seconds, for a total energy dose of 7.2 J/cm^2.

In Group 6, the same two-stage application procedure for ParaCel and VibeX Xtra was used as in Group 5. However, the irradiance was applied in a pulsed mode in which the UV light was alternately turned on for one second and turned off for one second. The total energy dose was 7.2 J/cm^2.

3. Results

One hundred sixty-six eyes were treated with transepithelial CXL according to 6 treatment regimens, with 110 eyes in Group 1, 8 eyes in Group 2, 12 eyes in Group 3, 10 eyes in Group 4, 13 eyes in Group 5, and 13 eyes in Group 6. Minimum corneal thickness was 335 μm in Group 1, 396 μm in Group 2, 367 μm in Group 3, 442 μm in Group 4, 377 μm in Group 5, and 460 μm in Group 6, respectively.

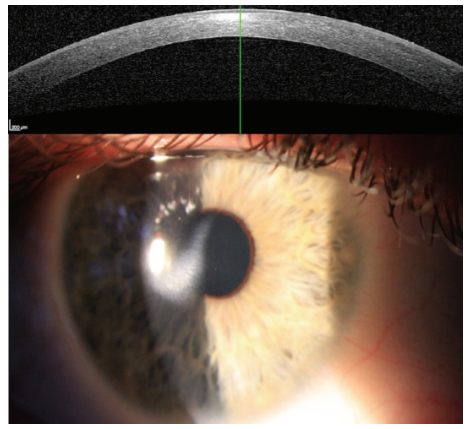

Figure 1: Paracentral subepithelial opacification after infection following Medio-Cross TE CXL.

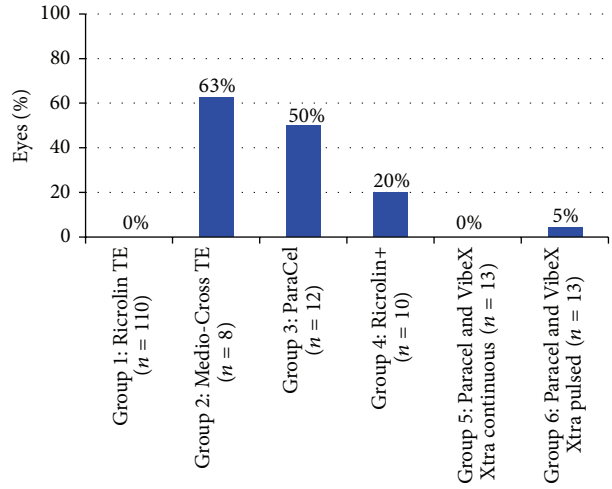

Figure 2: Percentage of eyes presenting with epithelial defect following transepithelial CXL.

There was no serious complication except for one eye in treatment protocol 2 that had a corneal infection associated with an epithelial defect.

Visual acuity was decreased to hand motion in the acute phase. After 18 months, central visual acuity was fully restored; however, a paracentral subepithelial opacification was still visible (Figure 1).

No other adverse event including endothelial decompensation or endothelial damage was observed in any eye, except for epithelial damages. The incidence of postoperative epithelial defects according to treatment protocol is presented in Figure 2.

Postoperative epithelial defects were most commonly observed on the first postoperative day. Often the complete illuminated epithelium was affected leading to a detachment as an intact sheet similar to a LASEK flap (Figure 3).

In some eyes, the epithelium was closed during the follow-up period. However, parts of it were loose and mobile over the corneal stroma leading to pain perception.

The incidence of reported postoperative pain is shown in Figure 4. In all groups, reported pain was the greatest in

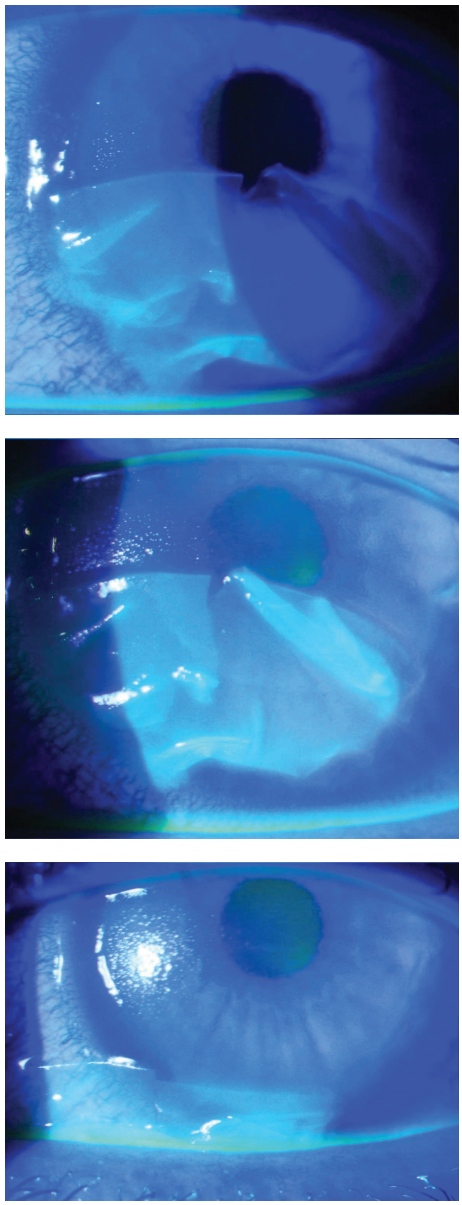

Figure 3: Epithelial sloughing after bandage contact lens removal, one day post-op transepithelial CXL with Medio-Cross TE.

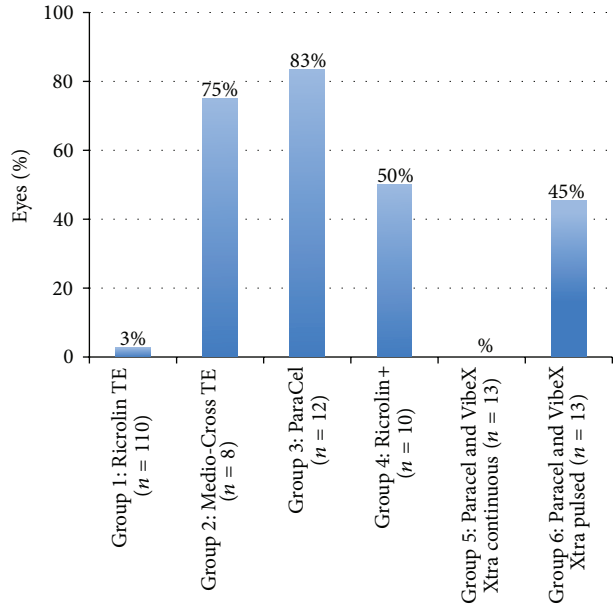

Figure 4: Percentage of eyes with postoperative pain following transepithelial CXL.

the 24 hours following the procedure, resolved by complete epithelial healing after 1–4 days.

OCT revealed limited or superficial hyperreflectivity in eyes treated according to the protocol for Group 1. OCT evaluation was comparable between the remaining groups, with deeper reaching hyperreflectivity observed in the corneal stroma in the postoperative period in Groups 2–6.

4. Discussion

Standard riboflavin formulations containing 0.1% riboflavin and 20% dextran show minimal penetration through intact or partially disrupted epithelium [6, 7]. The optimal approach for transepithelial CXL must minimize the impact on the corneal epithelium while permitting a sufficient amount of riboflavin to diffuse into the stromal tissue where cross-linking occurs. Epithelial disruption without full debridement leaves the cornea vulnerable to early postoperative infection and delays the return to gas permeable contact lens wear and visual recovery.

The results of this study reveal variability in postoperative recovery following transepithelial CXL with different treatment regimens. The use of Ricrolin TE resulted in the least disruption of the corneal epithelium, with no epithelial defects reported in any case and minimal postoperative discomfort. However, some epithelial disruption is necessary to allow diffusion of riboflavin to the corneal stroma. Reports assessing the diffusion of Ricrolin TE revealed a shallow penetration of the riboflavin which may be insufficient for cross-linking [5, 8, 9]. This finding prompted the exploration of further treatment protocols.

Qualitative evaluation of the depth of the riboflavin penetration with OCT revealed deeper penetration to the stroma following the remaining protocols in this study. However, variability was observed in the frequency of epithelial defects. Eyes treated with Ricrolin+ and Iontophoresis showed epithelial defects in 20% of eyes and pain in 50% of eyes. Based on our observation of eyes with apparently loose epithelium that leads to pain perception in the absence of an epithelial defect, we hypothesize that eyes experienced pain more often than they had epithelial defects because of subtle epithelial disruptions which were not detectable at slit lamp exam. Fifty percent of eyes in the ParaCel (alone) group and greater than 50% of eyes in the Medio-Cross TE group presented with epithelial defects in the first postoperative day.

Both the ParaCel and Medio-Cross TE formulations contain benzalkonium chloride, which acts as an epithelial permeability enhancer. The disruptive effects of BAC are both duration and concentration dependent [10], and therefore it is logical that reduction of the duration of exposure to BAC might reduce the incidence of epithelial defects. This was the rationale for the development of the two-stage riboflavin application method employing sequential application of 0.25% riboflavin with BAC (ParaCel) and 0.25% riboflavin without BAC (VibeX Xtra). According to a theoretical model proposed by Avedro, Inc., the initial soak with the riboflavin and BAC solution is sufficient to open the epithelial junctions and to provide the initial dose of riboflavin. Once the junctions have been sufficiently loosened, further exposure to BAC is not thought to provide any additional benefit, and it is flushed away. The remainder of the presoak time is completed using a BAC-free, dextran-free riboflavin solution [11].

The two-stage application appeared to be a near optimal protocol with respect to epithelial integrity, resulting in zero incidences of postoperative epithelial defects in Group 5 and a reduction in the percentage of eyes experiencing postoperative pain (0%) as compared to the use of ParaCel alone (83%). However, when pulsed, illumination was introduced to the treatment protocol of Group 5; that is, in Group 6, greater pain perception was observed. We may speculate that the prolonged treatment time may lead to desiccation of the ocular surface adding to the epithelial trauma.

While OCT evaluation of the depth of riboflavin penetration provides evidence of the efficacy of the two-stage application protocols, a clinical means of quantifying the concentration of riboflavin in the stroma as a function of depth would have added to this study. To our knowledge, no such technology currently exists. Therefore, longer term follow-up is necessary to evaluate the relative efficacy of these cross-linking protocols in regard to stabilization of the progression of keratoconus.

In conclusion, the findings of this study suggest that different transepithelial cross-linking protocols have varying impacts on epithelial integrity. At present, it seems impossible to have sufficient riboflavin penetration without any epithelial disruption. A compromise between efficacy and epithelial integrity has to be found. In children, it may be desirable to minimize discomfort and accept a less than maximum efficacy as the procedure may be repeated later on. In contrast, in very thin corneas, it may be an option to use an "aggressive" protocol to maximize efficacy even if the epithelium sloughs off postoperatively in order to have the epithelium as a protective spacer to the endothelium. Longer term outcomes of these various treatment protocols will follow and will provide insight into the selection of an appropriate treatment protocol for each of these patient scenarios.

Conflict of Interests

The authors Suphi Taneri, Saskia Oehler, and H. Burkhard Dick have no financial interests. Mrs. Grace Lytle is an employee of Avedro Inc.

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Clinical Study

Pulsed Light Accelerated Crosslinking versus Continuous Light Accelerated Crosslinking: One-Year Results

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Purpose. To compare functional results in two cohorts of patients undergoing epithelium-off pulsed (pl-ACXL) and continuous light accelerated corneal collagen crosslinking (cl-ACXL) with dextran-free riboflavin solution and high-fluence ultraviolet A irradiation. *Design*. It is a prospective, comparative, and interventional clinical study. *Methods*. 20 patients affected by progressive keratoconus were enrolled in the study. 10 eyes of 10 patients underwent an epithelium-off pl-ACXL by the KXL UV-A source (Avedro Inc., Waltham, MS, USA) with 8 minutes (1 sec. on/1 sec. off) of UV-A exposure at 30 mW/cm^2 and energy dose of 7.2 J/cm^2; 10 eyes of 10 patients underwent an epithelium-off cl-ACXL at 30 mW/cm^2 for 4 minutes. Riboflavin 0.1% dextran-free solution was used for a 10-minutes corneal soaking. Patients underwent clinical examination of uncorrected distance visual acuity and corrected distance visual acuity (UDVA and CDVA), corneal topography and aberrometry (CSO EyeTop, Florence, Italy), corneal OCT optical pachymetry (Cirrus OCT, Zeiss Meditec, Jena, Germany), endothelial cells count (I-Conan Non Co Robot), and in vivo scanning laser confocal microscopy (Heidelberg, Germany) at 1, 3, 6, and 12 months of follow-up. *Results*. Functional results one year after cl-ACXL and pl-ACXL demonstrated keratoconus stability in both groups. Functional outcomes were found to be better in epithelium-off pulsed light accelerated treatment together with showing a deeper stromal penetration. No endothelial damage was recorded during the follow-up in both groups. *Conclusions*. The study confirmed that oxygen represents the main driver of collagen crosslinking reaction. Pulsed light treatment optimized intraoperative oxygen availability improving postoperative functional outcomes compared with continuous light treatment.

1. Introduction

Riboflavin UV-A induced corneal collagen crosslinking (CXL) represents a relatively new procedure available for the conservative treatment of progressive keratoconus [1, 2] and secondary corneal ectasia [3] due to its capacity in increasing biomechanical corneal resistance [4, 5] and intrinsic anticollagenase activity [6]. The physiochemical basis of crosslinking lies in the photodynamic types I-II reactions [7] induced by the interaction between 0.1% riboflavin molecules absorbed in corneal tissue and UV-A rays delivered at 3 mW/cm^2 for 30 minutes (5.4 J/cm^2 energy dose) releasing reactive oxygen species (ROS) that mediated crosslinks formation between and within collagen fibers [8, 9].

Conventional epithelium-off crosslinking (CXL) demonstrated its safety and long-term efficacy in stabilizing progressive keratoconus and secondary ectasias in different clinical trials [10–15]. On the other hand the procedure is time consuming lasting about 1 hour [16]. The Bunsen-Roscoe law of reciprocity [17–19] theoretically demonstrated that the photochemical process behind crosslinking depends on the absorbed UV-A energy and its biological effect is proportional to the total energy dose delivered in the tissue [17–19]. According to this concept it is theoretically possible to deliver the same energy dose ensuring a proportional biological effect by setting different UV-A powers and exposure times in order to accelerate and shorten the crosslinking procedure in accelerated crosslinking (A-CXL)

modality [18–20]. According to photochemical crosslinking studies based on kinetics model [7] the UV-A illumination caused a rapid depletion of oxygen in a riboflavin soaked cornea and turning the UV light off led to replenishment of the oxygen to its original level within 3 to 4 minutes [7]. Krueger et al. [21] and Herekar [22] have also observed a rapid oxygen depletion during corneal crosslinking with riboflavin and concluded that the reactive oxygen species (ROS), specifically, singlet oxygen, are the predominant CXL reaction drivers. Under aerobic conditions, which are present during the first 10 to 15 seconds of UV-A exposure, sensitized photooxidation of the substrate (proteoglycan core proteins [23] and collagen in the corneal matrix) occurs mainly by its reaction with photochemically generated ROS, such as singlet molecular oxygen. This is consistent with a type II photochemical mechanism. After the first 10 to 15 seconds, oxygen becomes totally depleted and the reaction between the substrate and riboflavin becomes consistent with a predominantly type I photochemical mechanism [7]. Pulsing the UV light during crosslinking treatment theoretically restarts the photodynamic type II reaction achieving an additional oxygen concentration allowing more singlet oxygen release for crosslinking of collagen molecules. We report a comparative clinical study of continuous (cl-ACXL) and pulsed light (pl-ACXL) accelerated corneal collagen crosslinking in a series of 20 patients with progressive keratoconus investigating the functional outcomes at one-year follow-up and estimating the treatment penetration by means of in vivo confocal microscopy (IVCM).

2. Methods

After specific informed consent subscription, 20 patients affected by progressive keratoconus were enrolled in the study. They were divided into 2 treatment groups: 10 eyes of 10 patients (pulsed light treatments), with age between 13 and 26 years (mean: 21.5 years), underwent an epithelium-off pulsed light accelerated corneal collagen crosslinking (pl-ACXL) by the KXL I UV-A source (Avedro Inc., Waltham, MS, USA) with 8 minutes (1 sec. on/1 sec. off) of UV-A exposure at 30 mW/cm^2 with an energy dose of 7.2 J/cm^2; 10 eyes of 10 patients (continuous light treatments), with age between 11 and 24 years (mean: 18,5 years), underwent an epithelium-off continuous light accelerated corneal collagen crosslinking (cl-ACXL) with the same instrument, UV-A power setting at 30 mW/cm^2 for 4 minutes of continuous UV-A light exposure, and energy dose of 7.2 J/cm^2. The riboflavin solution used in both treatment groups was composed of dextran-free riboflavin 0.1% with hydroxyl, propyl, methyl, and cellulose (VibeX Rapid, Avedro Inc., Waltham, MS, USA), with 10 minutes of corneal soaking. Treated eyes were dressed by a soft contact lens bandage for 3 days and medicated with ciprofloxacin eye drops, diclofenac, and sodium hyaluronate eye drops 4 times/day.

2.1. Inclusion Criteria. The parameters we considered to establish keratoconus progression and inclusion criteria for each group were worsening of UCVA/BSCVA > 0.50 Snellen lines, increase of SPH/CYL > 0.50 D, increase of topographic symmetry index SAI/SI >1 D, increase of mean K reading > 1 D, reduction of the thinnest point at corneal OCT pachymetry ≥10 μm, and clear cornea at biomicroscopic examination. We considered "significant" for the inclusion in the study the variation of at least 3 of the parameters listed above (one clinical plus two instrumental).

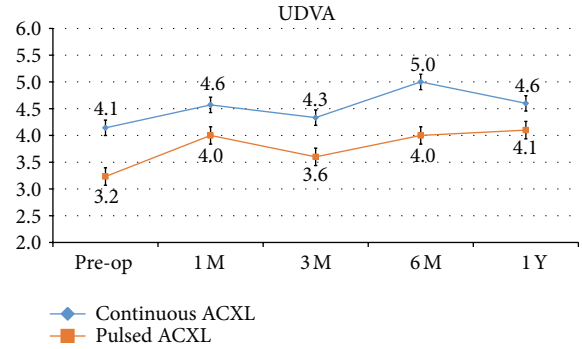

Figure 1: Uncorrected distance visual acuity (UDVA) after continuous light (blue line) and pulsed light (orange line) accelerated crosslinking gained +0.5 and +0.9 decimal equivalents, respectively, at one-year follow-up.

2.2. Assessment Criteria. Pre- and postoperative examination included uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), corneal topography simulated K average readings (K ave.), apical curvature (AK), and surface aberrometry (coma aberration) by CSO EyeTop Topographer (Costruzione Strumenti Oftalmici, Florence, Italy). In vivo scanning laser confocal microscopy was performed by the HRT II (Rostock Cornea Module, Heidelberg, Germany) and anterior segment OCT analysis by the Cirrus OCT instrument (Zeiss Meditec, Jena, Germany) in order to assess treatment penetration. Statistical analysis was performed using Wilcoxon test. All analyses were done using SPSS v16.0. A P value ≤ 0.05 was considered to be statistically significant.

3. Results

UDVA showed a statistically not significant improvement of +0.5, SD ± 1.2 (P = 0.65) and +0.9, SD ± 1.1 (P = 0.10) decimal equivalents in cl-ACXL and pl-ACXL, respectively, at one-year follow-up; see Figure 1.

CDVA showed an improvement, even not statistically significant, in both groups by a mean value of +1.6 SD ± 1.0 (P 0.56) and 1.8 SD ± 1.3 (P = 0.55) decimal equivalents, respectively, one year after treatment; see Figure 2.

Topographic simulated K average value demonstrated a not statistically significant decrease one year after cl-ACXL by a mean value −0.13 Diopters, SD ± 0.13 (P = 0.088), while after cl-ACXL the reduction of K average was found to be statistically significant by a mean value of −1.2 Diopters, SD ± 0.4 (P = 0.049); see Figure 3.

Table 1: Summary of the results.

	ΔUDVA (de)	ΔCDVA (de)	Δ*K* ave. (D)	ΔAK (D)	ΔComa (*μ*m)
Continuous light ACXL (cl-ACXL)	+0.5	+1.6	−0.13	+0.15	+0.44
Pulsed light ACXL (pl-ACXL)	+0.9	+1.8	−1.2	−1.39	+0.02

de: decimal equivalents; UDVA: uncorrected distance visual acuity; CDVA: corrected distance visual acuity; Δ*K* ave.: delta simulated *K* average reading; ΔAK (D): delta apical curvature; Δcoma: delta coma aberration.

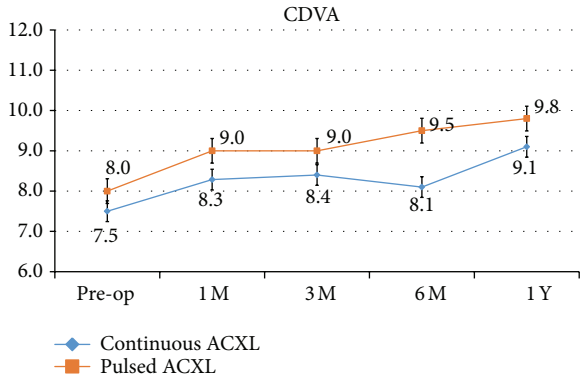

Figure 2: Corrected distance visual acuity (CDVA) after continuous light (blue line) and pulsed light (orange line) accelerated crosslinking gained +1.6 and 1.8 decimal equivalents on average, respectively, one year after treatment.

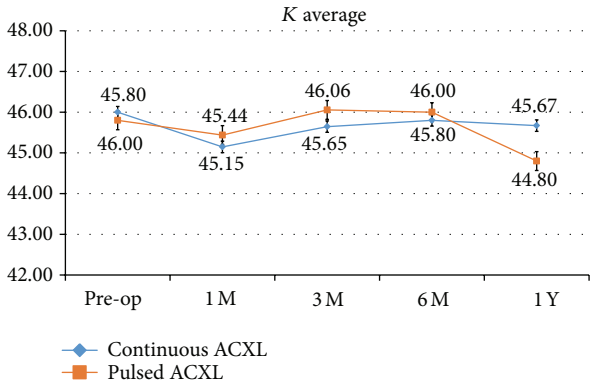

Figure 3: Topographic simulated *K* average (*K* ave.) value after continuous light (blue line) and pulsed light (orange line) accelerated crosslinking demonstrated a not statistically significant decrease by a mean value −0.13 Diopters; the reduction of *K* average was found to be statistically significant by a mean value of −1.2 Diopters after pulsed light accelerated CXL.

Apical curvature value (AK) provided by the topographer showed a slight not statistically significant increase in cl-ACXL and a statistically significant decrease in pl-ACXL by a mean value of +0.15 Diopters, SD ± 0.8 ($P = 0.077$), and −1.39 Diopters, SD ± 0.38 ($P = 0.05$), respectively, at one-year follow-up; see Figure 4.

Coma aberration values showed a statistically not significant difference one year after treatment by a mean value of +0.44 *μ*m, SD ± 0,41 (*P* 0.58), in cl-ACXL and +0.02 *μ*m, SD ± 0,02 (0.068), in pl-ACXL; see Figure 5.

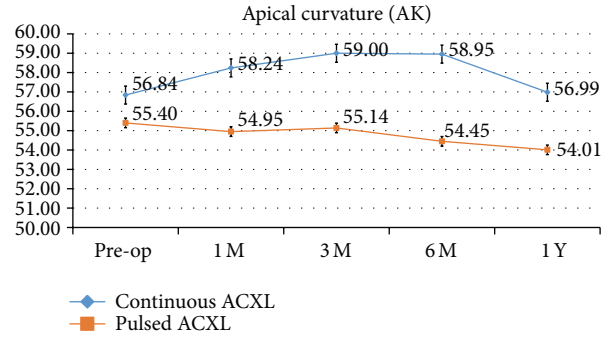

Figure 4: Topographic derived apical curvature value (AK) after continuous light (blue line) and pulsed light (orange line) accelerated crosslinking showed a statistically significant decrease in pulsed light accelerated CXL by a mean value −1.39 Diopters at one-year follow-up; no statistically significant differences were recorded in AK value after continuous light accelerated CXL.

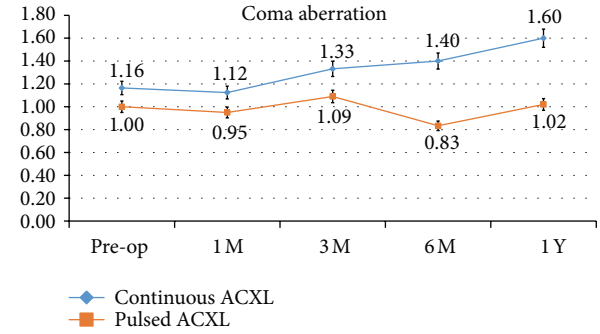

Figure 5: Coma values showed a statistically not significant difference one year after treatment. Continuous light accelerated crosslinking (blue line) was associated with a slight statistically not significant change of the coma by a mean value of +0.44 *μ*m, while pulsed light treatment (orange line) showed a stable value during the follow-up.

In vivo confocal microscopy (IVCM) after cl-ACXL showed an uneven demarcation line at mean depth of 160 *μ*m (range: 150–180 *μ*m) that was well visible one month after treatment. A deeper demarcation line was recorded after pl-ACXL at a mean depth of 200 *μ*m (range: 190–215 *μ*m) measured from the epithelial surface. A demarcation line was detectable at slit lamp examination; see Figures 6 and 7 and Table 1.

Preoperative mean endothelial cell density was 2450 cells/mm^2 (range: 2082 to 3026 cells/mm^2) in the cl-ACXL group and 2672 cells/mm^2 (range: 2459–3016 cells/mm^2) in

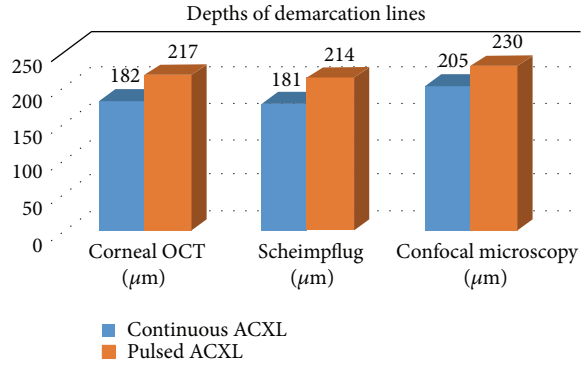

Figure 6: Depths of average demarcation lines recorded one month after continuous light (blue bar) and pulsed light (orange bar) accelerated crosslinking evaluated by corneal OCT (left), Scheimpflug camera (middle), and in vivo confocal microscopy (right) showing a deeper penetration of pulsed light treatment (orange bars) by a mean value of 215 μm (±20 μm) versus a lower penetration of continuous light treatment (blue bars) by a mean value of 160 μm (±20 μm).

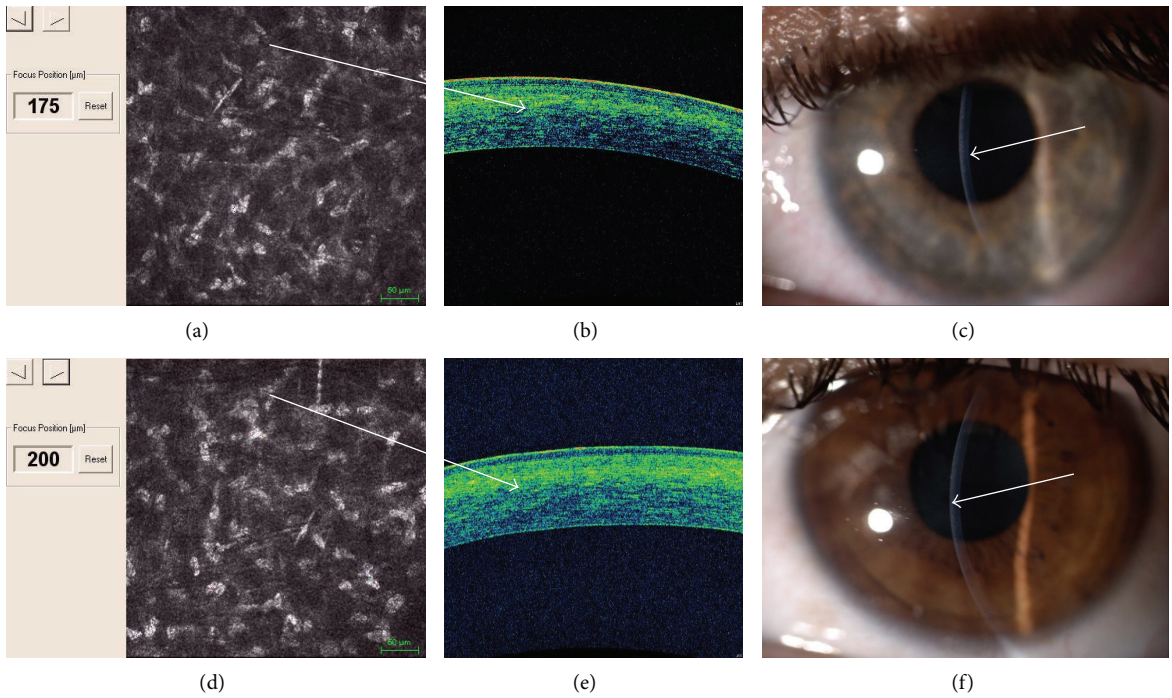

Figure 7: In vivo confocal microscopy (IVCM) after continuous light accelerated crosslinking showed keratocytes apoptosis until 175 μm (up left (a)), an uneven demarcation line is well detectable with spectral domain corneal OCT showing hyperreflective corneal tissue (up, white arrow (b)); the demarcation line was also visible at slit lamp one month after treatment (up right, white arrow (c)). In vivo confocal microscopy (IVCM) after pulsed light accelerated crosslinking showed keratocytes apoptosis until 200 μm (down left (a)); a deeper demarcation line is well detectable with spectral domain corneal OCT showing hyperreflective corneal tissue (down, white arrow (b)); the demarcation line was also visible at slit lamp one month after treatment (down right, white arrow (c)).

the pl-ACXL group. Postoperative endothelial cells count at 12 months was 2355 cells/mm^2 on average (range: 2172–2950 cells/mm^2) in the cl-ACXL group and 2495 cells/mm^2 (range: 2400–3125 cells/mm^2) in pl-ACXL group.

No adverse events were recorded in both treatment groups during the follow-up.

4. Discussion

This comparative analysis, even if in a small case series, demonstrated the efficacy of continuous and pulsed light accelerated crosslinking in stabilizing keratoconus progression after one year of follow-up. Pulsed light treatment showed a slightly better functional outcome both in

uncorrected and in corrected distance visual acuity even if there is no statistically significant difference between the two treatment modalities. UCVA was found to be slightly better in pl-ACXL patients and it may correlate with the statistically significant improvement of mean K values and reduction of apical curvature recorded in this cohort of patients. Conversely, there is no statistically significant difference in CDVA that improved in both groups at one-year follow-up. This slight difference could be attributed to the small number of the eyes included in the study. No adverse events were recorded in both treatment groups.

Pulsed light treatment showed a deeper apoptotic effect, meanly at 215 μm of stromal depth (range: 190–235 μm), while continuous light accelerated treatment revealed a penetration of 160 μm on average (range: 150–180 μm), both at confocal and at corneal OCT analysis as shown in Figures 6 and 7.

These findings were found to be slightly better than those recently reported in the literature [24] probably due to the higher energy dose used in our treatments (7.2 J/cm^2 instead of 5.4 J/cm^2) and pulsed light modality. Indeed, pulsing the UV-A light inducing an intraoperative oxygen reuptake while prolonging treatment time at 8 minutes may influence a deeper penetration of oxidative damage [25].

Accelerated corneal collagen crosslinking with pulsed and continuous UV-A light illumination reached the anterior part of the corneal stroma until 200 μm of depth. This aspect assumes a physicochemical relevance because, as reported in the literature [26], the most important biomechanical effect related to crosslinking is concentrated in the anterior mid-stroma. Anyway the penetration of accelerated crosslinking remains under the value of conventional procedure (300 μm) at 3 mW/cm^2 for 30 minutes of UV-A exposure. Actually we do not know if this factor may negatively influence the biomechanical stability of keratoconus in a long-term follow-up. Conventional epithelium-off CXL procedure (riboflavin 0.1% plus dextran 20%, UV-A 3 mW/cm^2 = 5.4 J/cm^2 for 30 minutes) remains the gold standard in the conservative treatment of early-stage progressive keratoconus particularly in pediatric patients, even if, in our preliminary experience, the accelerated crosslinking with epithelium removal demonstrated its safety for endothelium both in pulsed and in continuous light treatment modality, shortening the CXL procedure time under 20 minutes, being well tolerated by patients. Pulsed light treatment seems slightly more capable to penetrate deeper in the corneal stroma compared to continuous light treatment giving better functional outcome even if in a limited case series. The functional improvement of accelerated CXL with pulsed energy could be traced back in an optimization of oxygen availability thanks to the on/off cycle of oxygen delivery. Anyway both treatments were found to have a similar efficacy in stabilizing keratoconus during the follow-up period. Pulsed and continuous light accelerated crosslinking represents safe evolving crosslinking procedures in order to achieve keratoconus stabilization in a short treatment time. The efficacy of these techniques still needs to be investigated in the mid-long-term follow-up and in a large cohort of patients.

Conflict of Interests

The authors declare that they have no financial interests in the paper.

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