Individualized Corneal Cross-linking With Riboflavin and UV-A in Ultrathin Corneas: The Sub400 Protocol

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• PURPOSE: To determine whether corneal cross-linking (CXL) with individualized fluence ("sub400 protocol") is able to stop keratoconus (KC) progression in ultrathin corneas with 12-month follow-up.

• DESIGN: Retrospective, interventional case series.

• METHODS: Thirty-nine eyes with progressive KC and corneal stromal thicknesses from 214 to 398 μm at the time of ultraviolet irradiation were enrolled. After epithelium removal, ultraviolet irradiation was performed at 3 mW/cm^2 with irradiation times individually adapted to stromal thickness. Pre- and postoperative examinations included corrected distance visual acuity (CDVA), refraction, Scheimpflug, and anterior segment optical coherence tomography imaging up to 12 months after CXL. Outcome measures were arrest of KC progression at 12 months postoperatively and stromal demarcation line (DL) depth.

• RESULTS: Thirty-five eyes (90%) showed tomographical stability at 12 months after surgery. No eyes showed signs of endothelial decompensation. A significant correlation was found between DL depth and irradiation time ($r = +0.448$, $P = .004$) but not between DL depth and change in K_{max} ($r = -0.215$, $P = .189$). On average, there was a significant change ($P < .05$) in thinnest stromal thickness (-14.5 ± 21.7 μm), K_{max} (-2.06 ± 3.66 D) and densitometry ($+2.00 \pm 2.07$ GSU). No significant changes were found in CDVA ($P = .611$), sphere ($P = .077$), or cylinder ($P = .915$).

• CONCLUSIONS: The "sub400" individualized fluence CXL protocol standardizes the treatment in ultrathin corneas and halted KC progression with a success rate of 90% at 12 months. The sub400 protocol allows for the treatment of corneas as thin as 214 μm of corneal stroma, markedly extending the treatment range. The DL depth did not predict treatment outcome. Hence, the depth is unlikely related to the extent of CXL-induced corneal stiffening but rather to the extent of CXL-induced microstructural changes and wound healing. (Am J Ophthalmol 2021;224:133–142. © 2020 Elsevier Inc. All rights reserved.)

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Keratoconus (KC) is a chronic progressive ophthalmic disease that leads to bulging and thinning of the cornea, resulting in increasing irregular astigmatism and, ultimately, poor vision. Because of a study published in 1986 showing a prevalence of 0.05% in a single state of the US population, KC has been classified as a rare disease.[1] However, recent studies including modern screening methods have demonstrated a prevalence 5 to 10 times higher in Western Europe and up to almost 100 times higher in certain regions of the Middle East compared with the historic 1986 study.[2,3]

First introduced in 2003, corneal cross-linking (CXL) is a treatment that can prevent KC progression.[4,5] CXL has radically changed the visual prognosis of patients with KC and other ectasias, namely, postoperative ectasia and pellucid marginal degeneration.[4,5] CXL acts to halt KC progression by increasing the biomechanical stiffness of the cornea.[6] In the original standard CXL ("Dresden") protocol, the corneal stiffening was achieved first by debriding the corneal epithelial cells, saturating the corneal stroma with riboflavin, and then irradiating the corneal stroma with ultraviolet (UV)-A light at 365 nm.[6] The riboflavin in the stroma absorbs the UV energy, resulting in a photochemical reaction that generates reactive oxygen species (ROS). The ROS induce covalent bonds between the collagen fibers and the proteoglycans of the extracellular matrix (EM).[7,8] At the same time, the riboflavin acts to

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shield deeper corneal layers (particularly the corneal endothelium) from UV-induced damage and cell death.[7,8]

Before CXL could be used clinically, the concern for endothelial cell safety had to be addressed. The Dresden protocol specified that a minimal corneal thickness of 400 μm after epithelial debridement needs to be present for CXL to be performed. This measurement was made based on riboflavin diffusion calculations and the total amount of UV energy that would be delivered to the cornea, especially at the endothelial level. As a result, this 400 μm corneal thickness limitation has been excluding many corneas with ectasias like KC that may have benefit from CXL-induced strengthening from receiving that treatment.

Since 2009, various modifications of the epithelium-off Dresden protocol to overcome the 400 μm limit have been developed.[9–11] These techniques aimed at modifying stromal thickness to allow for a safe and effective CXL treatment. Two examples include hypo-osmolaric riboflavin used to swell a thin cornea to a thickness of more than 400 μm, and in contact lens-assisted CXL (CACXL), the stroma is artificially "thickened" by placing a contact lens over the cornea. A third approach has been to leave islands of epithelium over the thinnest areas of the corneal stroma. Although all 3 were promising, each of these techniques has major limitations and was not standardized. For these reasons, our group developed and published an algorithm that rather adapted the overall fluence in the CXL procedure based on the patient's individual stromal thickness (sub400 protocol) to cross-link the stroma, still protecting the corneal endothelium from damaging amounts of UV-A irradiation.[12]

In this study, the algorithm[12] was used to individualize irradiation settings based on each patient's minimal corneal thickness at the end of riboflavin soaking but immediately before administering UV-A irradiation. We then investigated whether CXL with individualized fluence was able to stop KC progression in ultrathin corneas at 1 year after treatment.

PATIENT AND METHODS

THE STUDY WAS PERFORMED IN PATIENTS WHO PRESENTED with progressive KC and corneal stromal thicknesses of <400 μm. Surgeries were performed between May 2016 and December 2018 at the ELZA Institute in Dietikon/Zurich, Switzerland, and the data were collected retrospectively. Approval from Cantonal ethics committee of the Canton of Zurich was granted for retrospective data collection (BASEC number 2018-02369), data were collected through a search in the patient database system, and written consent was received from all patients. This study was conducted in accordance with the Declaration of Helsinki, the principles of Good Clinical Practice, the Human Research Act, the Human Research Ordinance, and local regulations.

TABLE 1. Technical Specifications Used in the Sub400 Protocol

CXL Technical Settings	
Parameter	Individualized CXL
Treatment target	Keratoconus
Fluence (total) (mJ/cm^2)	Variable
Soak time (min) and interval	20 (q2)
Intensity (mW/cm^2)	3
Treatment time (min)	Variable
Epithelium status	Off
Chromophore	0.1% riboflavin
Light source	CXL-365
Irradiation mode	Continuous

CXL = corneal cross-linking.

Male and female patients with progressive corneal ectasia and a corneal stromal thickness <400 μm were enrolled in the study. Progressive ectasias were considered to be KC eyes with an increase in anterior keratometry ≥1 D within the last 12 months and/or primary ectasia in patients aged 9-19 years,[13,14] who were, based on age at presentation, also considered to be progressive and, therefore, were treated at the initial presentation. The maximum increase in the anterior sagittal keratometry was evaluated through the differential maps via comparison of the Scheimpflug tomography or Placido examinations. Comprehensively, differential maps of the anterior sagittal curvature were electronically generated and evaluated. In cases in which the differential maps were not available due to software incompatibility or because the patient had a previous examination stored in a nonelectronic format, maximum keratometry was used to compare the 2 examinations. In all cases, progression of ectasia was characterized by an increase of at least 1 D in the anterior sagittal curvature preoperatively.

Exclusion criteria included a history of >10 pack-years of tobacco smoking,[15–17] pregnancy or lactating women, preexisting ocular trauma, previous ocular surgery, inability to understand the nature of the study and/or give consent, and patients under guardianship.

Clinical data, including corrected distance visual acuity (CDVA), refraction, and biomicroscopy, were recorded before surgery and postoperatively at 1 month and 12 months after CXL. To assess the depth of the demarcation line (DL) after CXL, anterior segment optical coherence tomography (OCT) was performed during the 1-month postoperative consultation using spectral-domain OCT technology (Spectralis HRA version 1.10.0.0; Heidelberg Engineering, Heidelberg, Germany). The distances from the DL to the anterior stroma and from the DL to the endothelium were recorded. In all patients, such

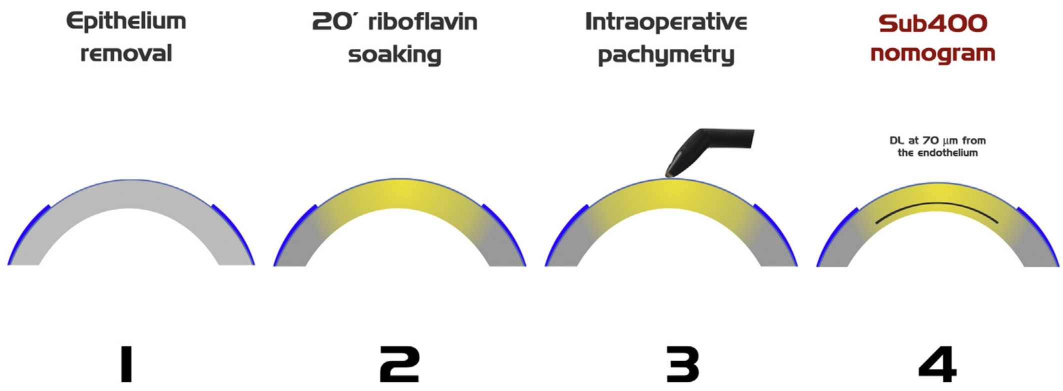

FIGURE 1. Schematic drawing of the principle of the sub400 protocol. DL = demarcation line.

measurements were performed by the same examiner and distances were measured at the thinnest point of the cornea.

All subjects had corneal evaluations performed using a rotational Scheimpflug system (Pentacam HR; Oculus, Wetzlar, Germany) by the same trained individual. The standard resolution setting was used to capture images (25 images per scan), and the following parameters were recorded: thinnest corneal stromal thickness, anterior radius of curvature in the 3.0 mm zone centered on the thinnest location of the cornea (ARC3 mm), maximum anterior keratometry (K_{max}), total anterior densitometry (AntDens), total central densitometry (CenDens), total posterior densitometry (PostDens), and total average densitometry (TotalDens). According to the Scheimpflug system's standard parameters, AntDens corresponds to the 120 μm most superficial corneal layers and PostDens corresponds to the 60 μm closest to the endothelium.

• **THE SUB400 PROTOCOL:** Table 1 and Figure 1 summarize the technical specifications and CXL surgical principles. CXL was performed by mechanically removing the epithelium over 9 mm of the central cornea. After de-epithelialization, the cornea was soaked with sodium edetate and trometamol-enriched riboflavin phosphate 0.1% hypotonic solution (Ricrolin+; Sooft, Montegiorgio, Italy) for 20 minutes. Ultrasound pachymetry was performed every 5 minutes during soaking to monitor eventual changes in corneal stromal thickness (SP-1000; Tomey, Nagoya, Japan). Guided by the preoperative Scheimpflug images, the intraoperative pachymetry measurements were performed in the thinnest area of the cornea. As a routine, 10 measurements were taken in the thinnest area and the lowest value was considered. At the end of the soaking period, corneas were rinsed with balanced salt solution to rinse off any surplus of riboflavin, and ultrasound pachymetry was performed to determine minimal stromal thickness. This intraoperative pachymetry measurement was performed at the end of the riboflavin instillation, as this corneal thickness value was required to determine the patient's individualized fluence requirement—per our published nomogram—aiming to obtain a DL 70 μm above the corneal endothelium.[12]

To facilitate the clinical application of individualized fluence, irradiation intensity/irradiance was kept fixed at 3 mW/cm^2, whereas treatment time was modified. A table was created depicting the individual fluence applicable to thin corneas in increments of 10 μm (Table 2). Then, CXL was performed at 365 nm using a commercially available CXL device (CXL-365; Schwind Eye-Tech-Solutions, Kleinostheim, Germany) at an intensity of 3 mW/cm^2.

Postoperatively, a bandage contact lens was placed, and antibiotic and corticosteroid drops were administered. Patients were re-examined at the slit lamp on postoperative day 1, and daily until the epithelium was closed, as well as after 1 month and 12 months after the procedure. The contact lens was removed on day 4. Postoperative drops included fourth-generation fluoroquinolone antibiotics twice a day for 7 days, followed by 0.1% fluorometholone drops twice a day for 12 weeks, and preservative-free artificial tears as needed.

• **STATISTICAL ANALYSIS:** Statistical analysis was performed using IBM SPSS Statistics (version 25; SPSS, Inc; FML Liquifilm;. Allergan, Inc., Irvine, CA). The Shapiro-Wilk test was used to test all variables for normality. In situations in which both variables were normally distributed, the *t* test (2-tailed) was used. In cases where at least one of variables was not normally distributed, the related-samples Wilcoxon-signed rank nonparametric test was used for further analysis. In cases of normally distributed variables, the results were reported as mean ± standard deviation, whereas in variables abnormally distributed, they were reported as medians and interquartile ranges. Statistical analysis was performed with a confidence interval of 95%. Pearson or Spearman correlation tests were performed for normally and abnormally distributed variables, respectively. Correlations were considered significant at the 0.05 level (2-tailed).

TABLE 2. Table Describing the Individual Fluence in Increments of 10 μm

Individualized CXL		
Sub400 Protocol		
Minimum Stromal Required Thickness (μm)	UV Irradiation Duration (min)	Demarcation Line Depth (μm)
200	1	130
210	01:20	140
220	01:40	150
230	2	160
240	02:30	170
250	3	180
260	03:30	190
270	4	200
280	5	210
290	6	220
300	7	230
310	9	250
320	10	255
330	12	265
340	14	275
350	16	283
360	18	290
370	20	300
380	23	310
390	26	320
400	29	330

CXL = corneal cross-linking; UV = ultraviolet.

RESULTS

THIRTY-NINE EYES FROM 32 PATIENTS WITH CORNEAL STROmal thickness <400 μm were enrolled in the study. The mean age was 29.1 ± 10.1 years (range: 13-55 years): 8 eyes (21%) were from patients between 13 and 20 years old, 14 eyes (36%) from patients between 21 and 30 years old, 11 eyes (28%) from patients between 31 and 40 years old, and 6 eyes (15%) were from patients over 40 years of age. Thirty-four eyes (87%) had KC with an increase in anterior keratometry ≥1 D within the last 12 months—mean 2.5 ± 2.0 D (range: 1.0-9.9 D)—and 5 (13%) had primary ectasia aged 13-19 years.

The CDVA at baseline and 12 months postoperatively were, respectively, 0.39 ± 0.22 and 0.41 ± 0.25 logMAR (logarithm of the minimum angle of resolution). Spherical power varied between −4.90 ± 5.44 and −4.07 ± 6.02 D, and cylindric power varied between −4.36 ± 2.82 and −4.00 ± 4.18, respectively, at presentation and 1 year after. No significant changes were found in CDVA (P = .611), sphere (P = .077), and cylinder (P = .915) from baseline to 12 months postoperatively. No eyes showed signs of endothelial decompensation.

• **DEMARCATION LINE:** Figure 2 display different treated eyes, with DL markings. The distance from the DL to the anterior stroma was 275 ± 61 μm (range: 126-385 μm) and from the DL to the endothelium 93 ± 47 μm (range: 0-250 μm). A significant correlation was found between DL depth and irradiation time (r = +0.448, P = .004). Notably, there was a considerable standard deviation in DL across the entire set of patients (Figure 3). In most cases, the distance from the DL to the epithelium was larger than anticipated (Figure 4).

• **CORNEAL THICKNESS AND KERATOMETRY:** Intraoperative minimum ultrasound pachymetry after riboflavin soaking was 343 ± 46 μm (range: 214-398 μm), of which 1 eye (2.6%) had between 214 and 250 μm, 3 eyes (7.7%) between 251 and 300 μm, 16 eyes (41%) between 301 and 350 μm, and 19 eyes (48.7%) between 351 and 400 μm. Plots showing pre- and intraoperative thinnest corneal thickness vs K_{max} for each case are available as Supplemental Material.

Scheimpflug data showed that, on average, there was a significant change from baseline at 12 months in thinnest thickness (−14.5 ± 21.7 μm, P < .05) and in K_{max} (−2.06 ± 3.66 D, P = .001), but no difference in ARC3 mm (0.05 ± 0.32 mm, P = .089). ARC3 mm values were 6.50 ± 1.03 mm at baseline and 6.52 ± 0.77 mm at 1 year after (P = .089). Mean K_{max} values were 58.5 ± 7.6 D at baseline and 56.4 ± 7.8 D at 1 year after (P = .001).

In the individual eyes, K_{max} remained stable (less than 1 D change) or improved at 1 year after CXL in 35 of 39 eyes, demonstrating that CXL successfully halted progression in 90% of the eyes from this series. Eight eyes had a K_{max} flattening of up to 1.0 D (20%), 14 eyes between 1.1 and 2.0 D (36%), 4 eyes between 2.1 and 3.0 D (10%), 3 eyes between 4.1 and 8.0 D (8%), and 3 eyes had a K_{max} flattening above 8.1 D (8%). Three eyes (8%) showed an increase in K_{max} below 1.0 D (range: 0-0.4 D).

Four eyes (10%) from 3 patients showed an increase of ≥1 D in K_{max} (range: 1.3-2.8 D), consistent with treatment failure and continued progression. All 4 failed treatment eyes were highly progressive before CXL, 2 of which had progressed up to 7.4 and 9.9 D within 6 months preoperatively. Curiously, those 2 mentioned very high preoperatively progressive eyes were from the same 42-year female patient, who had hypothyroidism, a factor that can influence corneal biomechanics.[18,19] In these 4 treatment failure eyes, the minimum intraoperative pachymetry averaged 330 μm (range: 320-343 μm) and preoperative K_{max} averaged 59.2 D (range: 50.3-64.7 D).

There was no correlation between the preoperative K_{max} and postoperative change in K_{max} treatment (r = −0.240, P = .141). Also, preoperative K_{max} values were not significantly higher in patients with treatment failure vs patients without treatment failure (59.2 ± 6.2 vs 63.3 ± 9.6 D, P = .914). There was no correlation between preoperative

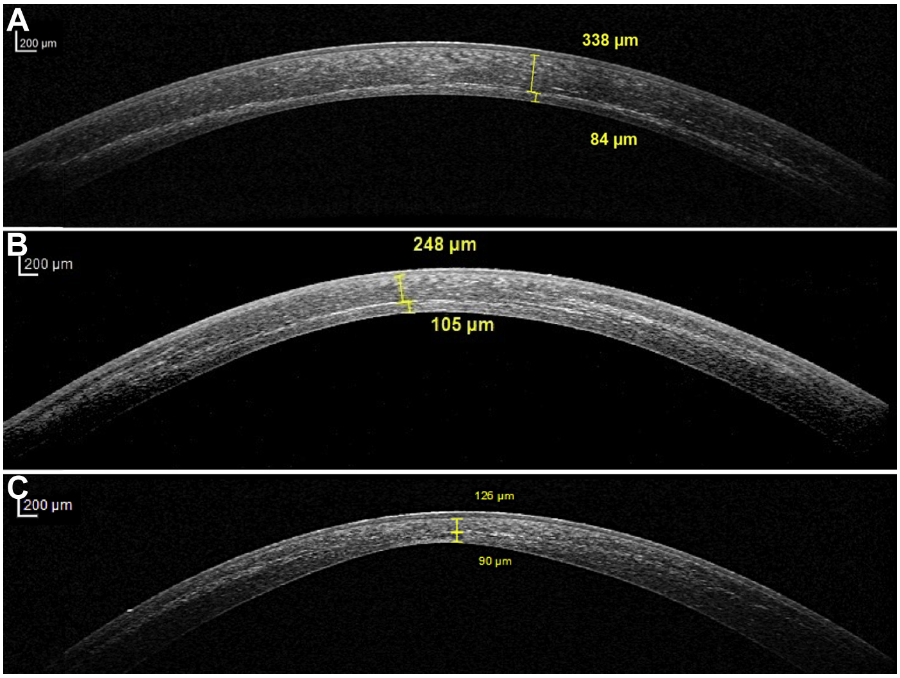

FIGURE 2. Anterior segment optical coherence tomography of a demarcation line in an ultrathin cornea after 1 month of individualized corneal cross-linking using the sub400 protocol. Thickness measurements were made at the thinnest point of the cornea according to pachymetry mapping and, as desired, show different demarcation line depths. Respectively in images A, B, and C, the corneas present stromal thicknesses of 421, 353, and 216 μm, and distances between the demarcation line and the endothelium of 84, 105, and 90 μm.

corneal thickness and the postoperative K_{max} flattening ($r = 0.061$, $P = .713$).

• **DENSITOMETRY:** There was a significant increase from baseline at 12 months in AntDens (+3.12 ± 3.36 GSU, $P < .05$), CenDens (+1.97 ± 2.09 GSU, $P < .05$), PostDens (+0.90 ± 1.47 GSU, $P < .05$), and TotalDens (+2.00 ± 2.07 GSU, $P < .05$). Although a significant increase in densitometry was observed as expected, all patients remained within the typical clinical pattern of mild opacity after CXL. No patient had "deep stromal haze"[20] or scarring in the evaluated period, as seen in Figure 5, showing representative OCT images of corneas with the highest degrees of flattening observed in our study.

DISCUSSION

THE RESULTS OF THIS STUDY DEMONSTRATE THAT INDIVIDualized CXL with the sub400 protocol was able to successfully prevent KC progression of ultrathin keratoconic corneas in 90% of cases after 1 year of follow-up. This study introduces for the first time the concept of individualizing total energy during CXL, according to intraoperative pachymetry. The individualized CXL with the sub400 protocol is based on a model taking into account the diffusion of oxygen and also correlations between CXL density and experimentally determined amount of corneal stiffening,[12] so that each cornea can receive an individual amount of total energy.

Currently, many corneas with advanced KC that would likely benefit from undergoing CXL cannot be treated with the Dresden protocol because of a stromal thickness of less than 400 μm. This initiated the development of modified techniques aiming to increase corneal stromal thickness artificially. However, such alternatives have limitations that create variable outcomes and often reduce efficacy.

The first approach, first published in 2009 by Hafezi and associates, was preoperative swelling of the cornea with hypo-osmolaric riboflavin.[9,21,22] The authors reported on 20 eyes treated with this technique. There were no cases of endothelial cell loss, and keratectasia was stable at the 6-month postoperative follow-up.[9] Another approach was the CA-CXL proposed by Jacob and associates,[10] where a contact lens soaked in iso-osmolaric riboflavin is used to "increase" the effective thickness of the cornea. A third approach was a customized epithelial debridement approach called "epithelial island cross-linking" proposed by Mazzotta and colleagues.[11] This approach spared epithelial cells around the thinnest point of the cornea; the riboflavin-soaked island attenuates the UV-A energy. As

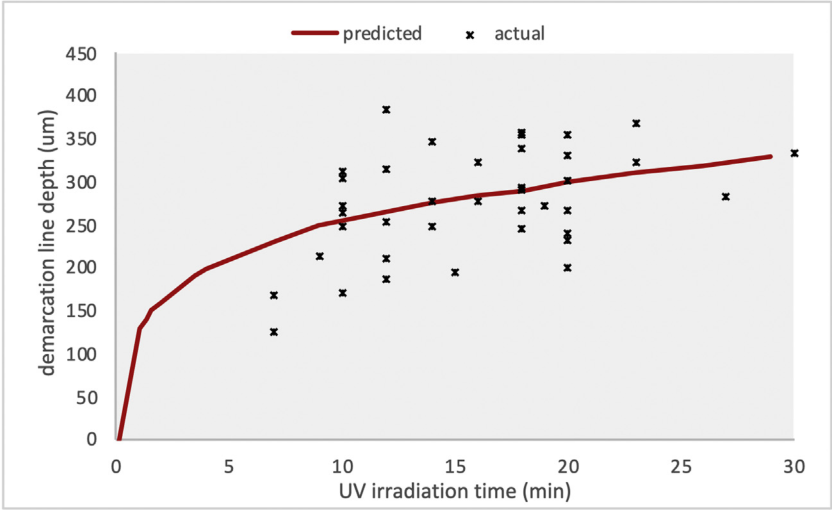

FIGURE 3. Nonlinear relation between the ultraviolet (UV) irradiation time and predicted demarcation line. The measured data points did follow the prediction, but presented a rather high standard deviation.

a potential consequence, the edge of the epithelial island would refract the UV-A energy into the intermediate stroma, potentially increasing the cross-linking effect in an undesired manner.[11]

In essence, each of these approaches has its limitations, because the modifications introduced to "increase" corneal thickness interfere with some of the fundamental key factors involved in the cross-linking reaction. The swelling approach with hypo-osmolaric riboflavin leads to a stiffening effect similar to CXL using iso-osmolaric riboflavin in a 400 μm cornea. However, the swelling effect of hypo-osmolaric riboflavin is variable: some corneas swell massively, whereas other corneas have little reaction.[9,23] This variability makes this swelling approach highly unpredictable. The second approach is CACXL. The greatest stiffening effect of cross-linking is observed in the anterior cornea.[24] Biomechanical stress-strain measurements, thermal shrinkage tests,[25] and Brillouin microscopy[24] have shown that CACXL results in a 30% reduction of the stiffening effect compared with the epi-off Dresden protocol, most probably due to a reduction in available oxygen.[25,26] Custom-shaped small-incision lenticule extraction lenticules have also been used in a similar capacity to the contact lens.[27] Finally, the "epithelial island" approach exhibits an unequal DL between epithelialized and de-epithelialized areas,[11] where areas of the intact epithelium cause not only UV attenuation but also oxygen restriction and further biomechanical loss.[28,29] In addition, it appears that the cross-linking effect is shallower in areas under the epi-on region (150 μm) than in the epi-off regions (250 μm).[30]

All current CXL techniques for thin corneas aim to increase stromal thickness. In theory, other measures should also be considered like controlling the depth of the cross-linking reaction: besides modification of stromal thickness, modification of riboflavin concentration would allow us to control the amount of chromophore present in the anterior layers of the stroma. The more riboflavin reacts with the photons provided by the UV-A light in the anterior cornea, the less energy is available in the deeper layers of the stroma, making the CXL reaction shallower. In practice, such an approach would require a multitude of riboflavin solutions with different concentrations, which is not feasible in daily clinical practice.

Another consideration is rather than modifying stromal thickness or riboflavin concentration, the total irradiation (fluence) could be adapted to the corneal thickness of the individual patient. This approach seems to be the most logical because it would require just a single type of riboflavin solution. Practically speaking, the treating surgeon would reduce irradiation time to meet the fluence required in the individual cornea.

As logical as this "sub400" approach might seem, it was impossible to implement it back in 2009 because too little was known about the CXL reaction. Specifically, riboflavin diffusion and kinetics was unknown at that time, and oxygen as a central element of the CXL process had not even been identified.[26,31] The "sub400" protocol is based on a published algorithm that accounts for stromal riboflavin, oxygen, and UV availability during the cross-linking procedure.[26,31] It is based on estimating diffusion of riboflavin and oxygen by Fick's law of diffusion and UV energy by the Lambert-Beer law of light absorption. The presence of these 3 factors determines the speed and amount of the induced photochemical reactions (types I and II). It is assumed that the amount of singlet oxygen (S_{oxy}) produced during the treatment interacts with the available EM and thereby forms the relevant cross-links. The concentration

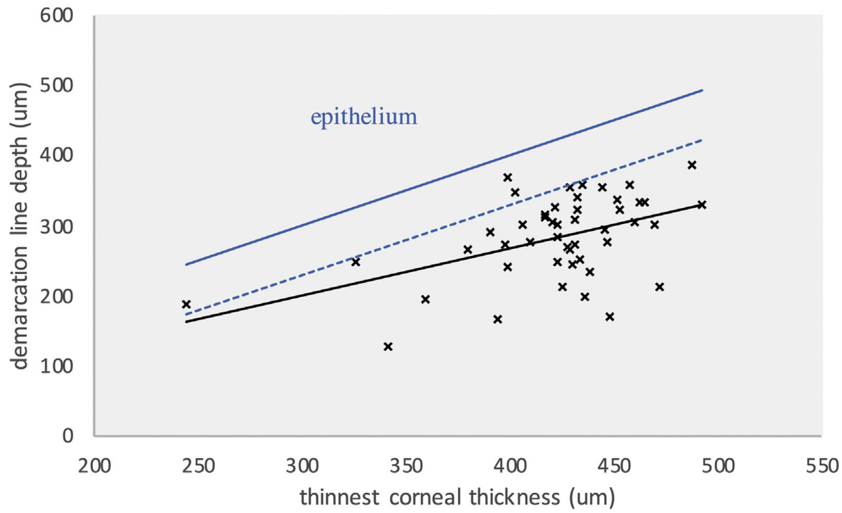

FIGURE 4. Demarcation line depth vs thinnest corneal thickness. The black continuous line is the trend line of the measured data points, the blue continuous line indicates the location of the epithelium, and the blue dashed line the 70 μm distance margin.

of those additionally induced cross-links [CXL] can then be estimated from.

$$[\mathrm{CXL}] = [\mathrm{CXL}_0] + [\mathrm{S}_{\mathrm{oxy}}] \cdot \left\{ 1 - e^{-\frac{k_{\mathrm{RFH2ox}}}{k_{\mathrm{EMox}}} \cdot [\mathrm{EM}] - \left(1 - e^{k_{\mathrm{EMox}} - \Delta t}\right)} \right\},$$

where [.] denotes concentration of riboflavin, Δt indicates the calculation time step, and k_{RFH2ox} and k_{EMox} are the reaction rate constants for RFH_2 oxidation and EM oxidation, respectively. $[\mathrm{CXL}_0]$ represents the CXL concentration at the previous time step. This model permits the prediction of not only the amount of biomechanical stiffening achieved after CXL but also the duration of UV irradiation required to achieve a CXL concentration that corresponds to the threshold of keratocyte apoptosis (when applied to clinical protocols) and as such to predict the penetration depth of a modified CXL treatment. The accuracy of the theoretical model has been verified previously in preclinical experiments, where the predicted CXL concentration did strongly correlate ($R^2 = 0.95$) with the biomechanical stiffening in porcine, murine, and lapin corneas.[12] These experimental data suggest that sub400 can be used to individualize UV-A irradiation duration with standard CXL lamp settings.

Based on this algorithm,[12] the present study introduces a standardized epithelium-off CXL method for the treatment of corneas with a stromal thickness of less than 400 μm: rather than artificially modifying corneal thickness, the sub400 protocol adapts the total UV-A energy to the patient's individual stromal thickness. The present study verified and confirmed that CXL with individualized fluence was able to stop KC progression of corneas as thin as 214 μm with 90% of success at 1 year after CXL.

It is important to emphasize that the pachymetry considered to calculate the irradiation dose of the sub400 protocol is based on intraoperative pachymetry, after the riboflavin soaking. Despite this, we have also assessed the preoperative Scheimpflug data of all eyes. Whereas the cornea with the thinnest stromal thickness was 214 μm (intraoperatively measured with an ultrasound pachymeter, without the epithelium), the thinnest cornea included showed a total thickness of 325 μm (preoperatively measured with Scheimpflug tomography, with the epithelium). Therefore, clinically, a cornea that presents with a Scheimpflug assessment of 325 μm total thickness (with the epithelium) or more can be treated with the sub400 protocol.

This study has some limitations. Although the vast majority of eyes in this study had preoperative progression documented by differential corneal imaging maps, a fraction (15%) of the eyes were from patients over 40 years and thus would be less likely to progress naturally. Therefore, one would think that they would not progress despite CXL: interestingly, all of these eyes had confirmed preoperative progression, and despite CXL 2 of these eyes showed postoperative progression and were considered to be failures. In other cases, the extreme corneal shape in far-progressed ectasias made Placido-based topography and Scheimpflug imaging less reproducible. Also, primary ectasias in children or adolescents were primarily treated.[13] A further limitation, not inherent specifically to this study, are the metrics used to assess the CXL effects. Currently, there is no clinical consensus on ideal metrics.[32] Therefore, besides using $K_{\max}$, like the majority of studies, we have also evaluated the anterior radius of curvature in a 3.0 mm zone. Using these 2 indices, we were able to demonstrate that CXL was able to at least halt KC progression. Interestingly, the reduction found in the point of maximum keratometry

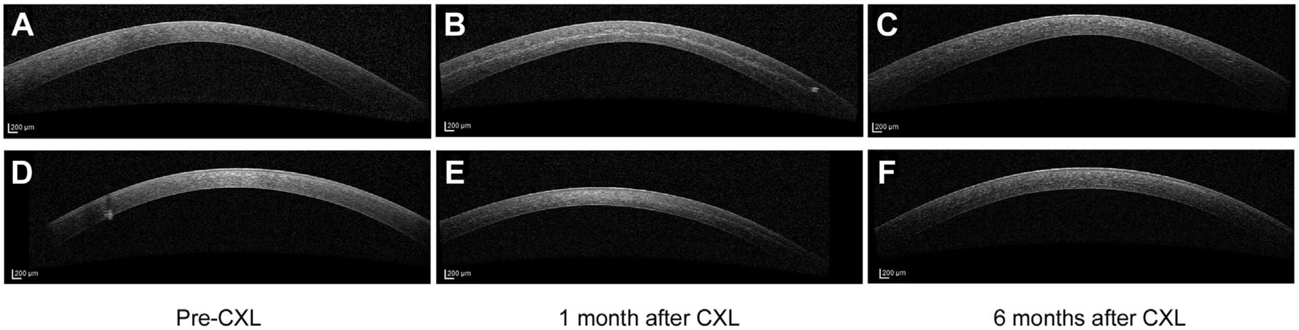

FIGURE 5. Representative optical coherence tomography (OCT) images of corneas of 2 patients (A-C and B-F) with the highest degrees of flattening observed in our study, before (A, D), at 1 month (B, E), and at 6 months (C, F) after corneal cross-linking (CXL). None of the eyes treated in this study showed "deep stromal haze" or stromal scarring.

but not in a 3 mm zone could suggest improvement of corneal regularity after CXL. A further limitation is that we were unable to evaluate endothelial cell density. However, no cornea showed clinical signs of endothelial decompensation: the total fluence in our study never exceeded the 5.4 J/cm^2 used in the original Dresden protocol, and other published studies used fluences up to 14 J/cm^2, without observing endothelial damage.[33,34]

Furthermore, experimental evidence suggests that the actual threshold of endothelial damage might be traditionally overestimated.[34] It is important to note that, despite (1) the aforementioned current indirect evidence of endothelial security, and (2) that we have not observed any signs of clinical decompensation throughout the present study, decompensation would be just an end stage sign of endothelial compromise; thence, in light of the lack of endothelial count, subtle endothelial changes could not be verified. Finally, another limitation was that DL depths demonstrated considerable variability preventing the identification of a systematic deviation from the predictions with the limited number of patients included in this study. However, it seems likely that the algorithm somewhat underestimated the demarcation depth, which could be overcome in the future by applying higher irradiances, or prolonging the irradiation time.

The ocular structures are particularly sensitive to light-induced damage.[35] The main reason why the Dresden protocol imposed a stromal thickness of more than 400 μm was to protect the corneal endothelium. So, aiming to protect sensitive structures such as the corneal endothelium, rough estimates of riboflavin concentration were calculated before the introduction of CXL in 2003.[34,36,37] From such estimates, the "400μm rule" was created and globally disseminated as the minimal required stromal thickness for epithelium-off CXL. Excess exposure of the corneal endothelium to UV irradiation (above a threshold of 0.35 mW/cm^2) would lead to cell death by apoptosis,[38] putting cornea homeostasis and transparency at stake.

The fluence of 5.4 J/cm^2 at a stromal thickness of 400 μm that was originally established in the Dresden protocol represents the baseline fluence used in our "sub400" protocol and is then reduced in thinner corneas following our published algorithm. Interestingly, recent assessments using 2-photon imaging tomography indicate that there is a discrepancy of a factor of 1.7 between the concentration of corneal riboflavin using the new 2-photon imaging technology and the old theoretical estimates.[38] This discrepancy might allow for substantially higher baseline fluences in the near future.[39]

Another technique for the treatment of thin corneas, called the "M" protocol, was recently proposed by Mazzotta and colleagues.[40] This "M" protocol gathers all published and validated clinical data on the penetration depth of various epi-off and epi-on CXL techniques that had been published over the years. The "M" protocol matched the in vivo scanning laser confocal microscopy and OCT morphological data[5] with the mathematical assessment of the cross-link concentration threshold according to the measured DL, assuming the Dresden protocol as benchmark. It demonstrates that the maximum interaction between UV-A, riboflavin, oxygen, and collagen-proteoglycans complex would be in the first 200 μm—where the 70% of riboflavin-UV-A interactions occur, whereas the remaining 30% of CXL photo-oxidative reaction would be dissipated in the deep stroma between 200 and 300 μm.[41]

The CXL techniques include using continuous or pulsed light, with and without iontophoresis, and a range of different intensities, ranging from 3 to 30 mW/cm^2. In contrast, the "sub400" protocol introduced here uses 1 single intensity in an epi-off setting, based on our published algorithm. Although both the "M" and the "sub400" protocol may achieve similar results, the "sub400" protocol requires less sophisticated technology.

The "sub400" individualized fluence CXL protocol standardizes the treatment in ultrathin corneas and is able to halt KC progression with a success rate of 90% at

12 months, allowing treatment of corneas as thin as 214 μm of corneal stroma. This finding extends the clinical range of cases that can be safely cross-linked to far progressed KC stages. Furthermore, a significant correlation was found between DL depth and irradiation but not between DL depth and change in K_{max}. In other words, the DL depth did not predict treatment outcome. Hence, the DL depth is not likely to relate to the extent of CXL-induced corneal stiffening but rather to induced wound healing. In particular, it is also not a measure of how much ROS are created, given that there was a considerable variation across patients of similar irradiation times. Still, DL depth might be a clinically relevant parameter for retrospective patient-specific assessment of the susceptibility to CXL-induced damage and its penetration.

Finally, the principles behind the sub400 protocol apply to all corneas thickness and not just for corneas with a thickness under 400 μm. The irradiance of 3 mW/cm^2 was chosen to allow for every UV illumination device on the market—even the oldest—to be used with this nomogram. The next step is to investigate how this algorithm could be adapted to use baseline fluences substantially higher than 5.4 J/cm^2; it has been previously shown in topography-guided high-irradiance pulsed-light CXL that fluences of 10 J/cm^2 and even 15 J/cm^2 can achieve higher penetration of the cornea with a reduced exposure time compared with the Dresden protocol.[42,43] Even though the algorithm relies on many constants that could be modified, we believe that future development will not be based on changing the curve of irradiation calculations, but rather adjusting overall fluence, using higher values consistent with the latest published studies. Therefore, it is possible that extending our algorithm to other CXL protocols could be used to perform more effective CXL in the future, rendering corneas stronger and more resistant to corneal ectasia progression.

The introduction of CXL has changed the natural course of corneal ectatic disease, and as a result, reduced the need for corneal transplants.[44] Our new “sub400 protocol” approach in ultrathin corneas will broaden the range of indications of CXL and further decrease the need for corneal transplantations. However, stabilizing extremely progressed corneal ectasias will only be beneficial if visual rehabilitation can be achieved. The recent advances in contact lens designs, particularly the rise of scleral contact lenses, allow for a satisfactory CDVA even in stromal thicknesses of less than 250 μm.

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