Int Ophthalmol (2019) 39:765–775
https://doi.org/10.1007/s10792-018-0876-4

ORIGINAL PAPER

Corneal densitometry after accelerated corneal collagen cross-linking in progressive keratoconus

Myriam Böhm · Mehdi Shajari · Matthias Remy · Thomas Kohnen

Received: 3 November 2017 / Accepted: 21 February 2018 / Published online: 26 March 2018

Abstract

Purpose To analyze changes in corneal densitometry 3 months after accelerated corneal collagen cross-linking (CXL) measured with Scheimpflug tomography.

Methods In this study we reviewed charts and anterior segment data of patients who had undergone accelerated pulsed epithelium-off CXL (30 mW/cm^2 for 4 min, 8 min total radiation time) for treatment of progressive keratoconus in the Department of Ophthalmology, Goethe University, Frankfurt, Germany. Visual, topographic, pachymetric and densitometric data were extracted before surgery and at the 3-month follow-up. Corneal densitometry measurements from different corneal layers and zones obtained using Scheimpflug tomography (Pentacam HR, Oculus).

Results The study investigated 12 eyes of 12 patients. The anterior (120 µm) stromal layer within the 0.0 to 2.0 mm and 2.0 to 6.0 mm concentric zones showed a significant elevation of mean densitometry 3 months post-surgery ($P = 0.045$; $P = 0.015$) compared to baseline. A mean stromal demarcation line was apparent at a depth of 203.00 µm ± 13.53 (SD). After accelerated CXL, no change in mean corrected distance visual acuity (LogMAR) was observed but a thinning of the cornea measured by a significant reduction in central pachymetry (µm).

Conclusion Accelerated CXL results in an increase in corneal densitometry, particularly in the anterior stromal layer within the two central concentric zones (0.0 to 2.0 mm and 2.0 to 6.0 mm) of the cornea at 3 months postoperatively. The changes in corneal densitometry of the anterior stromal layer did not correlate with postoperative visual acuity or central pachymetry.

Keywords Corneal collagen cross-linking · Densitometry · Scheimpflug tomography · Keratoconus

Thomas Kohnen has received research grants from Hoya Surgical Optics GmbH (Frankfurt, Germany); J&J Vision (Abbott Medical Optics, Inc. [Santa Ana, CA, USA]); Novartis (Alcon Laboratories, Inc. [Fort Worth, TX, USA]); Oculentis GmbH (Berlin, Germany); Oculus Optikgerate GmbH (Wetzlar, Germany); Schwind Eye-Tech Solutions GmbH (Kleinostheim, Germany); and Carl Zeiss Meditec AG (Jena, Germany); and is a consultant to Geuder AG (Heidelberg, Germany); J&J Vision (Abbott Medical Optics, Inc. [Santa Ana, CA, USA]); Novartis (Alcon Laboratories, Inc. [Fort Worth, TX, USA]); Oculus Optikgeräte GmbH (Wetzlar, Germany); Santen GmbH, (Munich, Germany); Schwind Eye-Tech Solutions GmbH (Kleinostheim, Germany); STAAR Surgical AG (Nidau, Switzerland); TearLab Corp (San Diego, CA, USA); Thea Pharma GmbH (Berlin, Germany); Thieme Compliance GmbH (Erlangen, Germany); Ziemer Ophthalmology GmbH (Emmendingen, Germany); and Carl Zeiss Meditec AG (Jena, Germany).

M. Böhm · M. Shajari · M. Remy · T. Kohnen (✉)
Department of Ophthalmology, Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany
e-mail: kohnen@em.uni-frankfurt.de

Abbreviations

BSS	Balanced salt solution
CDVA	Corrected distance visual acuity
CXL	Corneal collagen cross-linking
D	Diopter
GSU	Grayscale unit
LogMAR	Logarithm of the minimum angle of resolution
OCT	Optical coherence tomography
UDVA	Uncorrected distance visual acuity
UV-A	Ultraviolet-A

Introduction

Keratoconus is a degenerative noninflammatory disorder of the eye, showing a progressive corneal ectasia with increasing irregular astigmatism accompanied by possible scarring or hydrops. If the disease progresses, it may ultimately end in corneal transplantation. The etiology of keratoconus seems to be multifactorial and is still to this day, not completely understood. However, it seems to have genetic and environmental components [1] and one of the most important risk factors for developing keratoconus is chronic eye rubbing which, apart from other known risk factors such as atopy or Down's syndrome, is the only modifiable factor [2].

Corneal collagen cross-linking (CXL) is a treatment option that aims to slow or halt progression of keratoconus and other ectatic corneal diseases with the application of riboflavin (vitamin B2) as a photosensitizer combined with ultraviolet-A (UV-A) light exposure. During the treatment, the epithelium of the cornea is removed and riboflavin (vitamin B2) eye drops are applied with a certain exposure time; thereafter, the eye is radiated with UV-A light. Several methods of "epi-on" (transepithelial) CXL have been proposed in order to keep the corneal epithelium intact which should be less painful and help avoid other CXL-associated adverse events. However, the evidence so far is that epi-off CXL remains the most effective method of strengthening the cornea and slowing keratoconus progression [3]. The duration of this procedure depends on the protocol and the CXL procedure applied, but is usually from a few minutes to an hour [1, 4, 5]. Classic CXL following the Dresden protocol with 3 mW/cm^2 for 30 min applying a total energy of 5.4 J/cm^2 is the most widely used and studied protocol as compared to modified accelerated CXL protocols available [6], which also provide promising, however, controversy results concerning treatment efficacy [7–10].

Clinical results after CXL showed a reduction of keratometric values, astigmatism, improved visual acuity and leads to an increased corneal rigidity with stiffening of the anterior corneal stroma, thereby halting keratoconus progression [4, 11–14]. However, CXL often leads to a preliminary loss of vision postoperatively. This reduction of visual acuity can either be attributed to changes in corneal topography or a loss of transparency of the corneal stroma [15, 16]. During the first month after CXL, an increased corneal optical density (haze) [17, 18] can often be observed. Prior studies used a confocal microscope or Scheimpflug tomography technique to measure densitometry in order to identify an increased optical density [17–20].

Pircher et al. [21] showed that CXL following the Dresden protocol induces stromal changes leading to an increased densitometry, particularly in the anterior stroma of the central (0.0 to 2.0 mm) corneal zone [21]. However, this loss of corneal transparency did not correlate with the postoperative visual acuity values [21].

The aim of the current work was to analyze changes in corneal densitometry 3 months after accelerated CXL using Scheimpflug tomography in eyes with progressive keratoconus.

Materials and methods

Patient population and study design

In this retrospective study, data analysis was conducted including all patients suffering from progressive keratoconus [22] over 18 years of age, who underwent accelerated CXL (Avedro, Inc., 230 Third Avenue, Waltham, MA 02451) from September 2014 to April 2015 in the Department of Ophthalmology, Goethe University, Frankfurt, Germany. Except for keratoconus, the eyes did not show any other ocular pathology. Patients with prior surgery or other eye disorders (corneal dystrophies, pterygium, prior corneal infections, cornea verticillata) that have an influence on corneal curvature were excluded from data analysis.

The study was performed in accordance with the Declaration of Helsinki, and ethical approval was

obtained from the local ethics commission. Visual acuity, topographic, pachymetry and densitometry data were extracted from patients' charts, Scheimpflug tomography (Pentacam HR, Oculus) and the corneal demarcation line were measured with optical coherence tomography (Visante™ OCT, Zeiss) and analyzed before surgery and at follow-up 3 months after treatment.

Surgical technique and accelerated CXL protocol

The same surgeon (T.K.) performed all procedures using the accelerated CXL protocol. Accelerated CXL was performed with Avedro's KXL® System (Avedro, Inc., 230 Third Avenue, Waltham, MA 02451). The KXL® device has a homogeneous beam intensity profile with a beam diameter of 9 mm leading to an even dose of UV-A light energy applied across the surface of the cornea. Prior to the development of the KXL® device, CXL was performed using devices with Gaussian beam profiles, which resulted in a higher irradiation intensity in the center of the cornea [23].

The "epithelium-off" protocol for accelerated CXL was followed. First, a topical anesthetic (Proparacaine-POS® 0.5% eye drops, active substance: proxymetacainhydrochlorid) was instilled on the cornea and a speculum was inserted. Under standard hygienic precautions, debridement of the epithelium was performed and Riboflavin (VibeX Rapid™: 0.1% riboflavin, hydroxypropyl methylcellulose) was applied to completely saturate and cover the exposed corneal stroma reapplying riboflavin at least once every two minutes for a total up to 10 min saturation time. Prior to starting irradiation, the eye was rinsed with a balanced salt solution, BSS® (BSS® balanced salt solution, Alcon) and then given pulsed (1 s "on", 1 s "off") UV-A treatment at 30 mW/cm^2 for 4 min with 8 min total UV-A time where a dose of 7.2 J/cm^2 (365 nm) was initiated. During UV-A treatment, the cornea was saturated with BSS® as needed. After treatment antibiotic and corticosteroid drops were administered and a bandage soft contact lens was placed on the eye and removed after epithelialization.

Follow-up

Patients received standard care with local antibiotic (Floxal EDO® eye drops, active substance: ofloxacin) and corticosteroid (Dexa EDO® eye drops, active substance: dexamethasone) treatment 3 drops per day for 4 days, followed by a local corticosteroid treatment (Efflumidex® eye drops, active substance: fluorometholone) 4 drops per day for 1 week and a weekly reduction of one drop per week thereafter. Moreover, the patients received lubricant eye medication (Cellufresh® eye drops, active substance: carboxymethylcellulose sodium) 4 drops per day for 4 weeks. Postoperative follow-up examinations were performed daily from day 1 to day 4 until reepithelialization was complete. During a complete ophthalmological examination, 1 and 3 months postoperative, the uncorrected and corrected distance visual acuity (UCVA and CDVA, respectively) and corneal topography including the densitometry measurement were performed.

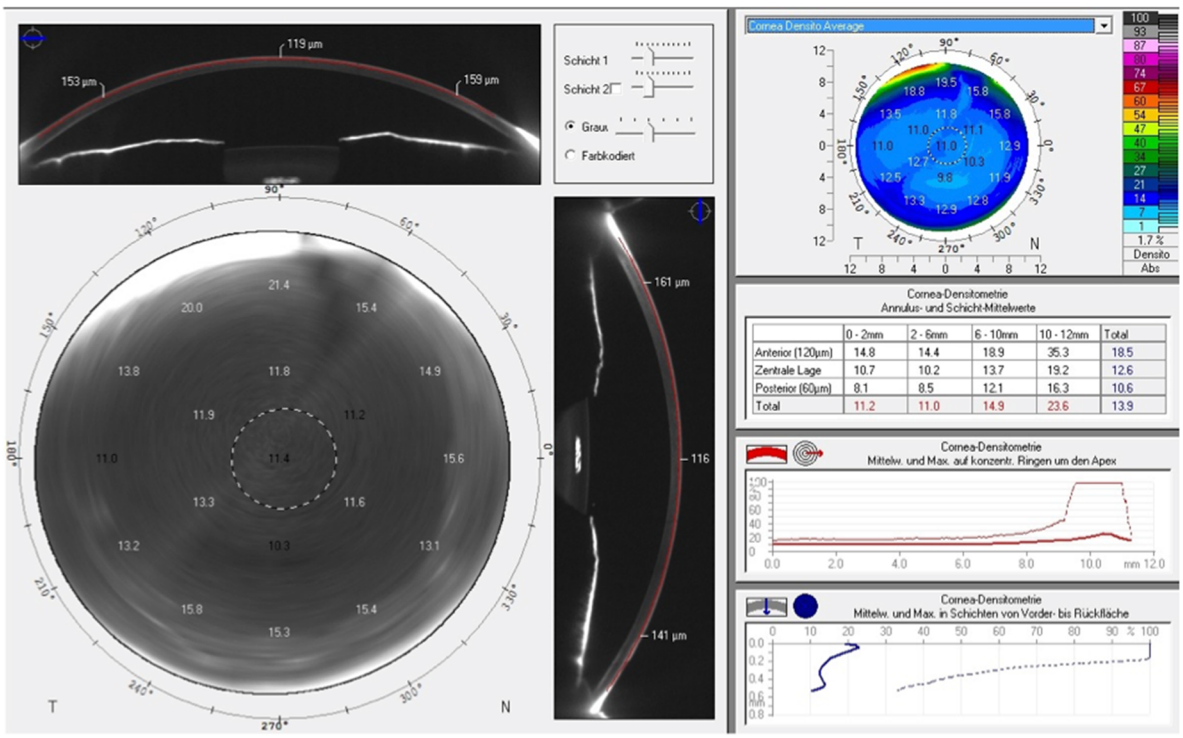
Imaging corneal densitometry

Changes in corneal transparency were measured with backward light scattering as measured by Scheimpflug tomography (Pentacam HR, Oculus Optikgeräte GmbH). Densitometry values were analyzed preoperatively and 3 months postoperatively (Fig. 1). Experienced operators performed all measurements in a dark room. Patients were always instructed to blink two times immediately before the measurement and then to open the eye wide without blinking to ensure that the eyelid did not cover the cornea. Only examinations with automatically triggered Scheimpflug scans (25 images in 2 s) that revealed the device's software quality check "OK" were used for analysis. The standardized Scheimpflug densitometry is expressed in grayscale units (GSUs), which defines backward light scatter on a scale of 0 (minimum scatter; maximum transparency) to 100 (maximum scatter; minimum transparency). Scheimpflug tomography allows the investigator to analyze the corneal densitometry at various concentric zones (0.0 to 2.0, 2.0 to 6.0, 6.0 to 10.0, 10.0 to 12.0 mm) and stromal depths of the cornea [anterior stromal layer (120 µm), posterior stromal layer (60 µm), middle stromal layer between these two layers] (Fig. 1).

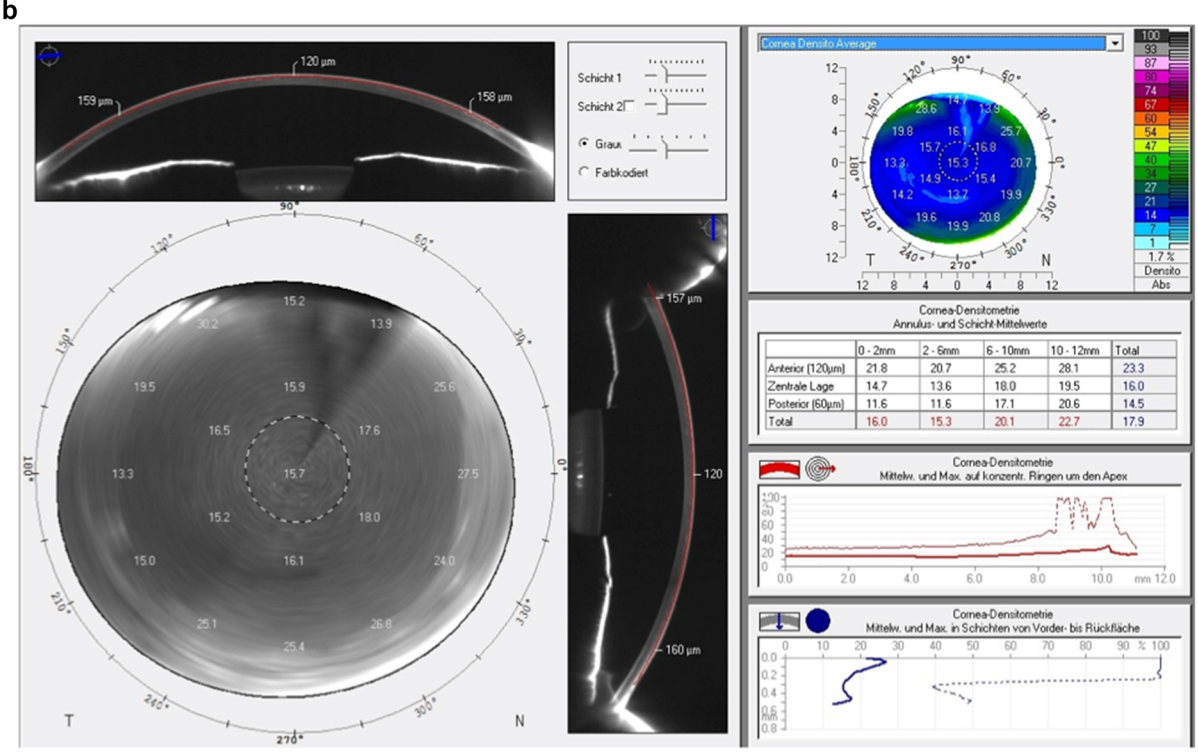
Statistical analysis

A power calculation was conducted with BiAS for Windows (Version 11.01) and determined that with $n = 12$ patients' eyes a power of at least 90% is

a


119 µm
153 µm
159 µm
Schicht 1
Schicht 2
Grau
Farbkodiert
161 µm
116
141 µm
Cornea Densito Average
Cornea-Densitometrie
Annulus- und Schicht-Mittelwerte
0 - 2mm 2 - 6mm 6 - 10mm 10 - 12mm Total
Anterior (120µm) 14.8 14.4 18.9 35.3 18.5
Zentrale Lage 10.7 10.2 13.7 19.2 12.6
Posterior (60µm) 8.1 8.5 12.1 16.3 10.6
Total 11.2 11.0 14.9 23.6 13.9
Cornea-Densitometrie
Mittelw. und Max. auf konzentr. Ringen um den Apex
Cornea-Densitometrie
Mittelw. und Max. in Schichten von Vorder- bis Rückfläche
Densito
Abs

b


120 µm
159 µm
158 µm
Schicht 1
Schicht 2
Grau
Farbkodiert
157 µm
120
160 µm
Cornea Densito Average
Cornea-Densitometrie
Annulus- und Schicht-Mittelwerte
0 - 2mm 2 - 6mm 6 - 10mm 10 - 12mm Total
Anterior (120µm) 21.8 20.7 25.2 28.1 23.3
Zentrale Lage 14.7 13.6 18.0 19.5 16.0
Posterior (60µm) 11.6 11.6 17.1 20.6 14.5
Total 16.0 15.3 20.1 22.7 17.9
Cornea-Densitometrie
Mittelw. und Max. auf konzentr. Ringen um den Apex
Cornea-Densitometrie
Mittelw. und Max. in Schichten von Vorder- bis Rückfläche
Densito
Abs

◀**Fig. 1** Example of corneal densitometry analysis by Scheimpflug tomography (Pentacam HR, Oculus Optikgeräte GmbH) before and after accelerated corneal Crosslinking in keratoconus patients. **a** Preoperative corneal densitometry measurement. **b** Corneal densitometry 3 months postoperative after accelerated corneal Crosslinking. Scheimpflug densitometry measured in gray-scale-units (GSU) at different concentric zones (0.0 to 2.0; 2.0 to 6.0; 6.0 to 10.0; 10.0 to 12.0 mm) and stromal depths of the cornea (anterior stromal layer (120 µm), posterior stromal layer (60 µm), middle stromal layer between these two layers). The standardized Scheimpflug densitometry is expressed in gray-scale-units (GSUs), which defines backward light scatter on a scale of 0 (minimum scatter; maximum transparency) to 100 (maximum scatter; minimum transparency)

achieved [21]. Data management was performed using Excel® for Mac 2011 version 14.5.9 (Microsoft) and SPSS® Statistics for Mac version 24.0 (IBM) for statistical analyses. Shapiro–Wilks analysis was performed to determine whether the sample was normally distributed. If the sample followed normal distribution changes at 3 months follow-up, it was compared with the baseline using a paired *t* test. If the data were not normally distributed, a Wilcoxon test was utilized to determine the level of significance. Due to multiple testing adjustments using Bonferroni method were conducted. All data are reported as mean ± 2 standard deviations. A two-sided probability value (*P*) less than 0.05 was considered statistically significant. Pearson correlation coefficients (*r*) were calculated to assess the correlation of UCVA, CDVA and pachymetry with corneal densitometry values of the anterior stromal layer (0.0 to 12.0 mm).

Results

In this study, 12 eyes of 12 patients suffering from progressive keratoconus who were treated with accelerated CXL in the Department of Ophthalmology, Goethe University, Frankfurt, were retrospectively analyzed. The mean age of the 9 men and 3 women was 36 ± 13 years (range 18 to 55 years). All patients attended a 3-month postoperative follow-up examination. No surgery-related complications were recorded, and epithelial closure was observed within 4 days in all patients.

There was no significant change in mean UCVA and CDVA (Table 1, Fig. 2) from preoperatively to 3 months postoperatively ($P = 0.473$; $P = 0.864$). The mean spherical equivalent ($P = 0.976$), maximum keratometry ($P = 0.363$) and simulated keratometry ($P = 0.110$) also showed no significant changes. However, the central pachymetry showed a significant decrease from 470.08 ± 32.02 µm to 453.08 ± 35.44 µm (mean ± standard deviation) ($P < 0.001$) 3 months after accelerated CXL. The mean stromal demarcation line showed at a depth of 203.67 ± 13.53 µm (mean ± standard deviation) (Table 1).

Table 2 shows the corneal densitometry results for backward light scattering as measured by Scheimpflug tomography (Pentacam HR, Oculus). Three months after accelerated CXL, the mean corneal densitometry of the three stromal layers (anterior, middle and posterior) had increased compared to baseline measurements preoperatively. The anterior stromal layer showed a significant increase in mean densitometry ($P < 0.05$) (Table 2, Fig. 3), whereas the middle and posterior layers just showed a slight but nonsignificant increase in mean densitometry. Within the anterior (120 µm) stromal layer, the two central concentric zones (0.0 to 2.0 mm and 2.0 to 6.0 mm) showed a significant elevation of mean densitometry (GSU) 3 months post-surgery ($P < 0.05$) compared to the baseline (Table 2, Fig. 3). No significant correlation ($P > 0.05$) between corneal densitometry values of the anterior stromal layer (0.0 to 12.0 mm), UCVA, CDVA and pachymetry ($r < 0.2$, $r < 0.2$, $r < 0.4$) was observed at the postoperative examination visit.

Discussion

Today CXL is the only and thus indispensable procedure to manage the treatment of progressive keratoconus or other corneal ectatic disease by halting or even stopping ectatic progression [1, 11]. However, often a reduction of visual acuity can be postoperatively detected [4, 19], which might be attributable to loss of corneal transparency.

Currently, differing propositions exist in order to explain the loss of transparency of the corneal stroma as an increase in collagen fiber diameter [24], cellular reaction by activation of keratocytes [25, 26] and a change in the refractive index [27] of the stroma after corneal CXL. The most likely reason for corneal haze after CXL seems to be a complex wound healing process comprising of a transformation of keratocytes

Table 1 Changes in uncorrected distance visual acuity (logMAR), corrected distance visual acuity (logMAR), manifest refraction spherical equivalent (D), maximum keratometry (D), simulated keratometry (D), central corneal thickness (μm), demarcation line (μm) data before and 3 months after accelerated corneal CXL

Mean ± SD							
Time point	UDVA (logMAR)	CDVA (logMAR)	MRSE (D)	Kmax (D)	SimK (D)	CCT (μm)	DM line (μm)
Preoperative	1.08 ± 0.41	0.47 ± 0.31	− 4.16 ± 4.50	59.52 ± 7.00	49.53 ± 4.77	470.08 ± 32.02	–
3 months	1.01 ± 0.43	0.48 ± 0.41	− 4.13 ± 5.30	60.10 ± 7.03	49.97 ± 4.72	453.08* ± 35.44	203.67 ± 13.53

UDVA uncorrected distance visual acuity, *CDVA* corrected distance visual acuity, *MRSE* manifest refraction spherical equivalent, *Kmax* maximum keratometry, *SimK* simulated keratometry, *CCT* central corneal thickness, *DM line* demarcation line, *D* diopter

*P value < 0.05

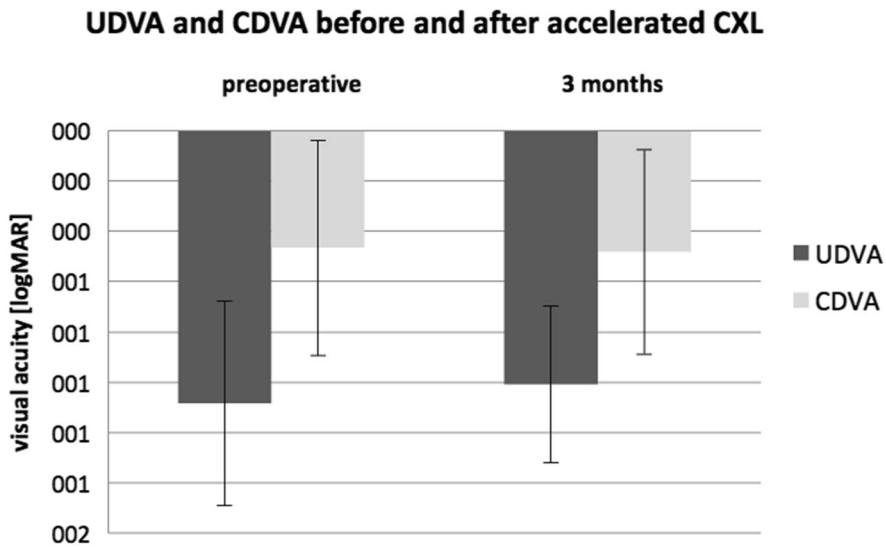


Fig. 2 Changes in uncorrected distance visual acuity (UDVA) and distance corrected visual acuity (DCVA) before and 3 months after accelerated corneal cross-linking

into myofibroblasts, which are associated with stromal remodeling [25, 26, 28].

This study investigates corneal densitometry as measured by Scheimpflug tomography and their possible effect on clinical parameters, such as visual acuity and pachymetry after accelerated CXL in progressive keratoconus patients. In the study, corneal densitometry was assessed using the Scheimpflug imagining device that provides the option to analyze corneal stromal changes in different corneal depths and concentric zones.

The results of the current study reveal an increase of corneal densitometry in all three stromal layers (anterior, middle and posterior) 3 months after accelerated CXL, however, only a significant increase in the anterior stromal layer (120 μm). The highest change in densitometry and significant backward light scattering was measured in the anterior stromal layer, particularly in the two central zones (0.0 to 2.0 mm and 2.0 to 6.0 mm). These findings are in line with the results of Pircher et al. [21] and Kim et al. [29] who also demonstrated a significant increase in densitometry after conventional CXL, especially in the anterior stroma of the central zone 3 months after CXL in eyes with progressive keratoconus [21, 29]. Their results similarly showed the greatest corneal changes in the anterior stromal layer within the 0.0 to 2.0 mm concentric zone of the cornea.

Thus, the significant increase in densitometry 3 months after accelerated CXL is restricted to the anterior stromal layer of the cornea. It might be reasonably assumed that stray light in the anterior

Table 2 Scheimpflug densitometry measurements (Pentacam HR, Oculus Optikgeräte GmbH) in stromal layers and concentric zones over time

Time point (n = 12)				
Parameter (mean ± SD)	Preoperative	3 months	Delta	P value
Stromal layer over 0–12 mm zone				
Anterior	24.07 ± 5.48	28.22 ± 5.23	4.15	0.03*
Middle	15.21 ± 3.06	16.56 ± 2.36	1.35	> 0.20
Posterior	12.72 ± 2.48	13.37 ± 1.85	0.65	> 0.20
Zone (mm)—Anterior layer (120 µm)				
0.0 to 2.0	29.69 ± 10.49	38.80 ± 10.61	9.12	0.045*
2.0 to 6.0	22.78 ± 4.89	28.82 ± 4.89	6.03	0.015*
6.0 to 10.0	19.41 ± 4.44	20.68 ± 3.06	1.54	> 0.20
10.0 to 12.0	32.90 ± 10.98	33.78 ± 13.20	0.86	> 0.20
Zone (mm)—Middle layer				
0.0 to 2.0	16.41 ± 3.56	18.82 ± 3.87	2.40	> 0.20
2.0 to 6.0	15.17 ± 1.77	13.60 ± 3.44	-1.58	> 0.20
6.0 to 10.0	13.60 ± 3.44	14.23 ± 2.64	0.63	> 0.20
10.0 to 12.0	21.62 ± 5.16	23.90 ± 8.25	2.28	> 0.20
Zone (mm)—Posterior layer (60 µm)				
0.0 to 2.0	11.46 ± 2.73	11.68 ± 2.06	0.23	> 0.20
2.0 to 6.0	11.58 ± 1.92	12.14 ± 0.94	0.57	> 0.20
6.0 to 10.0	12.25 ± 2.95	12.90 ± 2.57	0.65	> 0.20
10.0 to 12.0	18.27 ± 3.85	19.89 ± 4.11	1.63	> 0.20

*P value < 0.05 (Bonferroni adjusted P values)

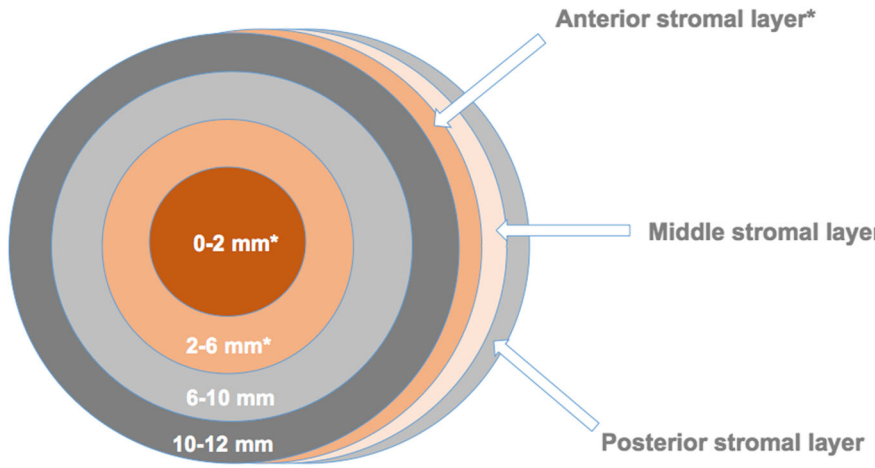


Fig. 3 Statistical significance (*P< 0.05) of changes in corneal densitometry in different stromal layers and concentric zones 3 months after accelerated CXL

stromal layer is more pronounced because the riboflavin concentration, UV-A intensity [30, 31] and oxygen concentration [32] decline toward the middle and deeper posterior stromal layers.

In contrast to prior studies the UV-A-irradiation device (KXL® System, Avedro, Inc., 230 Third Avenue, Waltham, MA 02451) used in this study has no Gaussian beam profile but a homogeneous beam intensity profile leading to an even dose of UV-A light energy applied across the surface of the cornea. CXL performed using devices with Gaussian beam profiles for example, as in the study by Pircher et al. [21] resulted in a higher irradiation intensity in the center of the cornea [23]. Nevertheless, in this study, a statistically significant increase in densitometry readings at 3 months postoperative could only be observed in the 0.0 to 2.0 mm and 2.0 to 6.0 mm corneal concentric zones but not in the 6.0 to 10.0 mm zone, despite the

UV-A irradiation being performed in a 9.0 mm diameter beam with a homogeneous beam intensity profile. Koller et al. [33] support this finding concluding that the intended depth of CXL is achieved only in the central part of the cornea, and 3 mm away from the center the CXL depth just reaches 65% of the central depth. This finding is also in line with Pircher et al. [21] who reported an increase in densitometry in the 0.0 to 2.0 mm and 2.0 to 6.0 mm corneal zone [21]. Thus, a homogenous beam profile with an even UV-A exposure does not result in different corneal densitometry distribution after accelerated CXL compared to treatment with a Gaussian beam profile after CXL following the Dresden protocol applying a total energy of 5.4 J/cm^2 compared to a total energy of 7.2 J/cm^2 following the accelerated CXL protocol in this study [6]. Future studies are needed in order to study different irradiation procedures and their effect on densitometry distribution and visual outcome to verify our finding.

The UCVA and CDVA showed no significant changes 3 months after accelerated CXL. Recent studies did not demonstrate a correlation between corneal densitometry values and visual acuity outcomes [19, 21]. Likewise in this study, UCVA and CDVA did not correlate with the changes in corneal densitometry of the anterior stromal layer (0.0 to 12.0 mm). Thus, despite the significant increase in densitometry in the anterior stromal layer 3 months postoperative, no significant effect on the visual acuity outcome was observed. The mean stromal demarcation line in this study showed at a depth of 203.67 ± 13.53 µm, which is similar to the results of Moramarco [34] showing a demarcation line at a depth of 213 ± 47.38 µm also after using pulsed UV-A light CXL with 8 min (1 s on/1 s off) of UV-A exposure at 30 mW/cm^2 and energy dose of 7.2 J/cm.

Moreover, in this study maximum keratometry and simulated keratometry showed no significant changes, which is in line with the findings of Toker et al. and Moineau et al. [35, 36] reporting that 30 mW/cm^2 accelerated CXL treatment modalities appeared to be effective in stabilizing keratoconus progression, however, less effective in achieving topographic improvement showing no changes in keratometric values compared to baseline. However, a study by Mita et al. [37] found a significant reduction in maximum keratometry 6 months after 30 mW/cm^2 accelerated cross-linking with the KXL system. In line with the results of prior studies [38–41], the present study demonstrates a significant reduction of the central pachymetry 3 months after accelerated CXL. A study conducted by Greenstein et al. [38] also demonstrated a change in corneal thickness over time. All pachymetry measurements thinned 1 and 3 months after CXL following the Dresden protocol and rethickened to almost preoperative values after 12 months [38]. The physiology of this initial corneal thinning after CXL is unclear: however, structural changes occur in corneal collagen fibrils [42, 43], such as compression of collagen fibrils, changes in corneal hydration [44] and edema [45, 46], keratocyte apoptosis [25, 47, 48] and changes in glycosaminoglycans [49] which are discussed in the literature. A study by Greenstein et al. [19] investigated the natural history of corneal haze measured by Scheimpflug imaging and slitlamp biomicroscopy after CXL following the Dresden protocol. This study illustrated that corneal haze reached its maximum 3 months after CXL and then decreased between 3 and 12 months after CXL. In contrast, Koc et al. found that corneal densitometry values increased after accelerated corneal CXL and did not return to preoperative values 12 months after CXL [50]. CXL-associated corneal haze appears as a dust-like change in the corneal stroma or a midstromal demarcation line [51]. Even though Scheimpflug densitometry allows a quantitative measurement, its specific correlation to clinical corneal haze remains to be further assessed. Greenstein et al. [19] were able to demonstrate a close approximation of their densitometry measurements to their results from slitlamp haze grading, indicating that densitometry does measure clinical corneal haze. Presumably, corneal thinning and stromal haze as measured by increased densitometry values demonstrated in our study might similarly result from complex structural and physiological wound healing changes in the cornea after CXL [52]. This means that increased densitometry values and thinning may be distinct clinical components of the basic CXL healing process. Shetty et al. [53] found that corneal densitometry measurements in different zones with the Pentacam did not affect the repeatability of the thinnest corneal thickness measurement.

Another possibility would be that the thinning of the cornea is the essential cause of the clinical stromal haze that can be seen and measured. But in the study, no correlation between a reduced central pachymetry and the corneal densitometry of the anterior stromal

layer (0.0 to 12.0 mm) after 3 months could be demonstrated.

There are three main limitations of this study, namely, a relatively short follow-up of only 3 months, a small sample size and the retrospective design. In the future, further research with a larger sample size, a control group and a longer follow-up period would be necessary to verify these findings.

In conclusion, corneal changes induced by accelerated CXL performed using the KXL® System irradiation device seemed to be greatest in the anterior stromal layer (120 µm) in two central zones (0.0 to 2.0 mm and 2.0 to 6.0 mm) of the cornea. The increase in densitometry and, thus, decrease in corneal transparency did not, however, influence visual acuity, since no correlation between the changes of corneal densitometry of the anterior stromal layer (0.0 to 12.0 mm), UCVA and CDVA was found. Moreover, the observed reduction of central pachymetry after CXL did not correlate with the changes of corneal densitometry of the anterior stromal layer (0.0 to 12.0 mm) and visual acuity.

Funding Myriam Böhm: none; Mehdi Shajari: Oculus; Matthias Remy: Avedro Inc.: Travel has been funded; Thomas Kohnen receives grant support from Hoya, J&J Vision (Abbott), Novartis (Alcon), Oculentis, Oculus, Schwind, and Zeiss; and is a consultant to Geuder, J&J Vision (Abbott), Novartis (Alcon), Oculus, Santen, Schwind, STAAR, TearLab, Thea Pharma, Thieme Compliance, Ziemer, and Zeiss.

Compliance with ethical standards

Conflict of interest Myriam Böhm and Mehdi Shajari declare that they have no conflict of interest. Matthias Remy has received a travel honorarium from Avedro (Waltham, MA 02451, USA).

Human and animal rights This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent No informed consent was obtained from patients since this was a retrospective study and data were anonymized. This is in accordance with the ethical vote obtained from the local ethics committee.

References

1. Sykakis E, Karim R, Evans JR, Bunce C, Amissah-Arthur KN, Patwary S, McDonnell PJ, Hamada S (2015) Corneal collagen cross-linking for treating keratoconus. The Cochrane database of systematic reviews (3):Cd010621. https://doi.org/10.1002/14651858.cd010621.pub2
2. Raiskup F, Lenk J, Herber R, Gatzioufas Z, Sporl E (2017) Therapeutic options in keratoconus. Klin Monbl Augenheilkd. https://doi.org/10.1055/s-0043-111797
3. Raiskup F, Velika V, Vesela M, Sporl E (2015) Cross-linking in keratoconus: "Epi-off" or "Epi-on"? Klin Monbl Augenheilkd 232(12):1392–1396. https://doi.org/10.1055/s-0035-1546155
4. Wollensak G, Spoerl E, Seiler T (2003) Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 135(5):620–627
5. Wernli J, Schumacher S, Spoerl E, Mrochen M (2013) The efficacy of corneal cross-linking shows a sudden decrease with very high intensity UV light and short treatment time. Invest Ophthalmol Vis Sci 54(2):1176–1180. https://doi.org/10.1167/iovs.12-11409
6. Zygoura V, Alio del Barrio J, Gatzioufas Z, Saw V, Raiskup F (2015) Evaluation of corneal stromal demarcation line depth following standard and a modified-accelerated collagen cross-linking protocol. Am J Ophthalmol 159(1):211–212. https://doi.org/10.1016/j.ajo.2014.10.018
7. Kymionis GD, Tsoulnaras KI, Grentzelos MA, Liakopoulos DA, Tsakalis NG, Blazaki SV, Paraskevopoulos TA, Tsilimbaris MK (2014) Evaluation of corneal stromal demarcation line depth following standard and a modified-accelerated collagen cross-linking protocol. Am J Ophthalmol 158(4):671
8. Webb JN, Su JP, Scarcelli G (2017) Mechanical outcome of accelerated corneal crosslinking evaluated by Brillouin microscopy. J Cataract Refract Surg 43(11):1458–1463. https://doi.org/10.1016/j.jcrs.2017.07.037
9. Males JJ, Viswanathan D (2018) Comparative study of long-term outcomes of accelerated and conventional collagen crosslinking for progressive keratoconus. Eye 32(1):32–38. https://doi.org/10.1038/eye.2017.296
10. Woo JH, Iyer JV, Lim L, Hla MH, Mehta JS, Chan CM, Tan DT (2017) Conventional versus accelerated collagen cross-linking for keratoconus: a comparison of visual, refractive, topographic and biomechanical outcomes. Open Ophthalmol J 11:262–272. https://doi.org/10.2174/1874364101711010262
11. Raiskup F, Theuring A, Pillunat LE, Spoerl E (2015) Corneal collagen crosslinking with riboflavin and ultraviolet-A light in progressive keratoconus: 10-year results. J Cataract Refract Surg 41(1):41–46. https://doi.org/10.1016/j.jcrs.2014.09.033
12. Kohlhaas M, Spoerl E, Schilde T, Unger G, Wittig C, Pillunat LE (2006) Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. J Cataract Refract Surg 32(2):279–283. https://doi.org/10.1016/j.jcrs.2005.12.092
13. Baumeister M, Klaproth OK, Gehmlich J, Buhren J, Kohnen T (2009) Changes in corneal first-surface wavefront aberration after corneal collagen cross-linking in keratoconus.

Klin Monbl Augenheilkd 226(9):752–756. https://doi.org/10.1055/s-0028-1109627
14. Koller T, Pajic B, Vinciguerra P, Seiler T (2011) Flattening of the cornea after collagen crosslinking for keratoconus. J Cataract Refract Surg 37(8):1488–1492. https://doi.org/10.1016/j.jcrs.2011.03.041
15. Asri D, Touboul D, Fournie P, Malet F, Garra C, Gallois A, Malecaze F, Colin J (2011) Corneal collagen crosslinking in progressive keratoconus: multicenter results from the French national reference center for keratoconus. J Cataract Refract Surg 37(12):2137–2143. https://doi.org/10.1016/j.jcrs.2011.08.026
16. Seiler TG, Schmidinger G, Fischinger I, Koller T, Seiler T (2013) Complications of corneal cross-linking. Ophthalmologe 110(7):639–644. https://doi.org/10.1007/s00347-012-2682-0
17. Mazzotta C, Balestrazzi A, Baiocchi S, Traversi C, Caporossi A (2007) Stromal haze after combined riboflavin-UVA corneal collagen cross-linking in keratoconus: in vivo confocal microscopic evaluation. Clinical Exp Ophthalmol 35(6):580–582. https://doi.org/10.1111/j.1442-9071.2007.01536.x
18. Raiskup F, Hoyer A, Spoerl E (2009) Permanent corneal haze after riboflavin-UVA-induced cross-linking in keratoconus. J Refract Surg 25(9):S824–S828. https://doi.org/10.3928/1081597x-20090813-12
19. Greenstein SA, Fry KL, Bhatt J, Hersh PS (2010) Natural history of corneal haze after collagen crosslinking for keratoconus and corneal ectasia: scheimpflug and biomicroscopic analysis. J Cataract Refract Surg 36(12):2105–2114. https://doi.org/10.1016/j.jcrs.2010.06.067
20. Lopes B, Ramos I, Ambrosio R Jr (2014) Corneal densitometry in keratoconus. Cornea 33(12):1282–1286. https://doi.org/10.1097/ico.0000000000000266
21. Pircher N, Pachala M, Prager F, Pieh S, Schmidinger G (2015) Changes in straylight and densitometry values after corneal collagen crosslinking. J Cataract Refract Surg 41(5):1038–1043. https://doi.org/10.1016/j.jcrs.2014.07.043
22. Kohnen T, Neuhann T, Knorz MC (2014) Evaluation and quality assurance of refractive surgical interventions by the German Ophthalmology Society and the German Professional Association of Ophthalmologists (status 2014). Klin Monbl Augenheilkd 231(6):642–650. https://doi.org/10.1055/s-0034-1368481
23. Accelerated cross-linking with Pulsed illumination (2013). Clinical Update & Research News Volume 3(1): 1–4
24. Wollensak G, Wilsch M, Spoerl E, Seiler T (2004) Collagen fiber diameter in the rabbit cornea after collagen crosslinking by riboflavin/UVA. Cornea 23(5):503–507
25. Wollensak G, Spoerl E, Wilsch M, Seiler T (2004) Keratocyte apoptosis after corneal collagen cross-linking using riboflavin/UVA treatment. Cornea 23(1):43–49
26. Wilson SE, Kim WJ (1998) Keratocyte apoptosis: implications on corneal wound healing, tissue organization, and disease. Invest Ophthalmol Vis Sci 39(2):220–226
27. Vinciguerra P, Albe E, Trazza S, Rosetta P, Vinciguerra R, Seiler T, Epstein D (2009) Refractive, topographic, tomographic, and aberrometric analysis of keratoconic eyes undergoing corneal cross-linking. Ophthalmology 116(3):369–378. https://doi.org/10.1016/j.ophtha.2008.09.048
28. Netto MV, Mohan RR, Ambrosio R Jr, Hutcheon AE, Zieske JD, Wilson SE (2005) Wound healing in the cornea: a review of refractive surgery complications and new prospects for therapy. Cornea 24(5):509–522
29. Kim BZ, Jordan CA, McGhee CNJ, Patel DV (2016) Natural history of corneal haze after corneal collagen crosslinking in keratoconus using Scheimpflug analysis. J Cataract Refract Surg 42(7):1053–1059. https://doi.org/10.1016/j.jcrs.2016.04.019
30. Steven P, Hovakimyan M, Guthoff RF, Huttmann G, Stachs O (2010) Imaging corneal crosslinking by autofluorescence 2-photon microscopy, second harmonic generation, and fluorescence lifetime measurements. J Cataract Refract Surg 36(12):2150–2159. https://doi.org/10.1016/j.jcrs.2010.06.068
31. Kamaev P, Friedman MD, Sherr E, Muller D (2012) Photochemical kinetics of corneal cross-linking with riboflavin. Invest Ophthalmol Vis Sci 53(4):2360–2367. https://doi.org/10.1167/iovs.11-9385
32. Marshall J HP, Muller D (2013) Corneal collagen cross-linking; past, present, future [ebook]
33. Koller T, Schumacher S, Fankhauser F 2nd, Seiler T (2013) Riboflavin/ultraviolet a crosslinking of the paracentral cornea. Cornea 32(2):165–168. https://doi.org/10.1097/ICO.0b013e318269059b
34. Moramarco A, Iovieno A, Sartori A, Fontana L (2015) Corneal stromal demarcation line after accelerated crosslinking using continuous and pulsed light. J Cataract Refract Surg 41(11):2546–2551. https://doi.org/10.1016/j.jcrs.2015.04.033
35. Moineau N, Sauvan L, Benichou J, Ho Wang Yin G, Hoffart L (2017) High-irradiance accelerated corneal collagen crosslinking for the treatment of keratoconus: a retrospective study. J Fr Ophtalmol 40(4):319–328. https://doi.org/10.1016/j.jfo.2016.11.014
36. Toker E, Cerman E, Ozcan DO, Seferoglu OB (2017) Efficacy of different accelerated corneal crosslinking protocols for progressive keratoconus. J Cataract Refract Surg 43(8):1089–1099. https://doi.org/10.1016/j.jcrs.2017.05.036
37. Mita M, Waring GOt, Tomita M (2014) High-irradiance accelerated collagen crosslinking for the treatment of keratoconus: six-month results. J Cataract Refract Surg 40(6):1032–1040. https://doi.org/10.1016/j.jcrs.2013.12.014
38. Greenstein SA, Shah VP, Fry KL, Hersh PS (2011) Corneal thickness changes after corneal collagen crosslinking for keratoconus and corneal ectasia: 1-year results. J Cataract Refract Surg 37(4):691–700. https://doi.org/10.1016/j.jcrs.2010.10.052
39. Koller T, Iseli HP, Hafezi F, Vinciguerra P, Seiler T (2009) Scheimpflug imaging of corneas after collagen cross-linking. Cornea 28(5):510–515. https://doi.org/10.1097/ICO.0b013e3181915943
40. Grewal DS, Brar GS, Jain R, Sood V, Singla M, Grewal SP (2009) Corneal collagen crosslinking using riboflavin and ultraviolet-A light for keratoconus: 1-year analysis using Scheimpflug imaging. J Cataract Refract Surg 35(3):425–432. https://doi.org/10.1016/j.jcrs.2008.11.046

41. Vinciguerra P, Albe E, Romano MR, Sabato L, Trazza S (2012) Stromal opacity after cross-linking. J Refract Surg 28(3):165. https://doi.org/10.3928/1081597x-20120301-04
42. Seiler T, Hafezi F (2006) Corneal cross-linking-induced stromal demarcation line. Cornea 25(9):1057–1059. https://doi.org/10.1097/01.ico.0000225720.38748.58
43. Bottos KM, Dreyfuss JL, Regatieri CV, Lima-Filho AA, Schor P, Nader HB, Chamon W (2008) Immunofluorescence confocal microscopy of porcine corneas following collagen cross-linking treatment with riboflavin and ultraviolet A. J Refract Surg 24(7):S715–719
44. Wollensak G, Aurich H, Pham DT, Wirbelauer C (2007) Hydration behavior of porcine cornea crosslinked with riboflavin and ultraviolet A. J Cataract Refract Surg 33(3):516–521. https://doi.org/10.1016/j.jcrs.2006.11.015
45. Dohlman CH, Hedbys BO, Mishima S (1962) The swelling pressure of the corneal stroma. Investig Ophthalmol 1:158–162
46. Wollensak G, Iomdina E, Dittert DD, Herbst H (2007) Wound healing in the rabbit cornea after corneal collagen cross-linking with riboflavin and UVA. Cornea 26(5):600–605. https://doi.org/10.1097/ICO.0b013e318041f073
47. Mazzotta C, Traversi C, Baiocchi S, Caporossi O, Bovone C, Sparano MC, Balestrazzi A, Caporossi A (2008) Corneal healing after riboflavin ultraviolet-A collagen cross-linking determined by confocal laser scanning microscopy in vivo: early and late modifications. Am J Ophthalmol 146(4):527–533. https://doi.org/10.1016/j.ajo.2008.05.042
48. Mazzotta C, Baiocchi S, Caporossi O, Buccoliero D, Casprini F, Caporossi A, Balestrazzi A (2008) Confocal microscopy identification of keratoconus associated with posterior polymorphous corneal dystrophy. J Cataract Refract Surg 34(2):318–321. https://doi.org/10.1016/j.jcrs.2007.09.028
49. Michelacci YM (2003) Collagens and proteoglycans of the corneal extracellular matrix. Braz J Med Biol Res 36(8):1037–1046
50. Koc M, Uzel MM, Tekin K, Kosekahya P, Ozulken K, Yilmazbas P (2016) Effect of preoperative factors on visual acuity, corneal flattening, and corneal haze after accelerated corneal crosslinking. J Cataract Refract Surg 42(10):1483–1489. https://doi.org/10.1016/j.jcrs.2016.08.017
51. Wollensak G, Hammer T, Herrmann CI (2008) Haze or calcific band keratopathy after crosslinking treatment? Ophthalmologe 105(9):864–865. https://doi.org/10.1007/s00347-008-1831-y
52. Corbett MC, Prydal JI, Verma S, Oliver KM, Pande M, Marshall J (1996) An in vivo investigation of the structures responsible for corneal haze after photorefractive keratectomy and their effect on visual function. Ophthalmology 103(9):1366–1380
53. Shetty R, Agrawal A, Deshmukh R, Kaweri L, Rao HL, Nagaraja H, Jayadev C (2017) Effect of post crosslinking haze on the repeatability of Scheimpflug-based and slit-scanning imaging devices. Indian J Ophthalmol 65(4):305–310. https://doi.org/10.4103/ijo.IJO_690_16