Corneal stromal demarcation line after accelerated crosslinking using continuous and pulsed light

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PURPOSE: To evaluate and compare the depth of corneal stromal demarcation line after accelerated collagen crosslinking (CXL) using continuous and pulsed light ultraviolet-A (UVA) exposure.

SETTING: Department of Ophthalmology, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.

DESIGN: Retrospective case series.

METHODS: Patients with progressive keratoconus were assigned to 1 of 2 treatment protocols using the same irradiation device for accelerated CXL. Patients assigned to Group A received accelerated CXL using continuous UVA light exposure at 30 mW/cm^2 for 4 minutes. Patients assigned to Group B received accelerated CXL using pulsed UVA light with 8 minutes (1 second on/1 second off) of UVA exposure at 30 mW/cm^2 and energy dose of 7.2 J/cm^2. One month after surgery, corneal stromal demarcation line depth was measured by 2 independent observers using anterior segment optical coherence tomography (AS-OCT).

RESULTS: A total of 60 patients were assessed. Corneal stromal demarcation line was easily identified on AS-OCT scans in all eyes by both observers. The mean depth of stromal demarcation line was 149.32 $\pm$ 36.03 μm in Group A and 213 $\pm$ 47.38 μm in Group B. The difference in stromal demarcation line depth between groups was statistically significant ($P < .001$).

CONCLUSIONS: Using accelerated CXL, the corneal stromal demarcation line was significantly deeper using pulsed rather than continuous light exposure.

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Keratoconus is a noninflammatory, progressive, bilateral, ectatic disease representing the first cause of corneal transplantation in Europe and the second in the United States.[1,2] The incidence of keratoconus varies with the diagnostic parameters used and is estimated to be between 1 in 320 and 1 in 2000 individuals.[3,4] The onset of keratoconus usually occurs during childhood or adolescence.[5–7] Prevention of keratoconus progression is of the utmost importance, as it may reduce the need for keratoplasty in young patients.[8] Corneal collagen crosslinking (CXL) is at present the only treatment option capable of slowing down or halting the progression of corneal ectasia.[9–12]

In recent years, newer crosslinking protocols with higher irradiances over shorter times have been proposed.[13]

Following conventional CXL, a corneal stromal demarcation line is usually detectable using slit lamp examination as early as 2 weeks after treatment at a depth of approximately 300 μm.[14]

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The demarcation line should indicate the transition zone between the crosslinked anterior corneal stroma and the untreated posterior corneal stroma, which results from the difference in refractive indices or reflective properties of the crosslinked versus untreated corneal stroma. For this reason, the stromal demarcation line is commonly used as a measure of the extension of CXL treatment into the stroma. In a recent study using confocal microscopy, the demarcation line was found between an anterior edematous stromal zone with low cell density and a more posterior zone with less edema and more keratocytes.[14] The demarcation line can be better visualized using anterior segment optical coherence tomography (AS-OCT) or confocal microscopy.[15]

Recent studies have shed light on the chain of chemical events occurring during the photochemical activation of riboflavin activation with ultraviolet light, highlighting the importance of corneal oxygenation during treatment.[16,17] With pulsed fractionation of ultraviolet-A (UVA) radiation, crosslinking efficiency may be improved by allowing re-diffusion of oxygen during UVA light exposure pauses.[16]

The aim of this study was to evaluate and to compare the depth of the corneal stromal demarcation line measured by AS-OCT after continuous and pulsed light CXL.

PATIENTS AND METHODS

Study Group and Protocol

A retrospective, single-center, comparative study was conducted, which included patients who underwent CXL from June 2013 through December 2013 at the Department of Ophthalmology, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy. This study was approved by the Institutional Review Board and performed in accordance with the tenets of Declaration of Helsinki.

Inclusion criteria for corneal CXL were documented keratoconus progression with central corneal pachymetry of more than 400 μm. Progression of ectasia was defined as change in corneal curvature in the cone area of at least 1.0 diopter (D) on tangential topography (Pentacam) or a thinning of more than 10 μm in minimal pachymetry observed in 2 consecutive topography maps over a period of 6 months. Exclusion criteria were central corneal thickness of less than 400 μm, concomitant or previous history of herpetic keratitis, dry eye, corneal infection, ocular or systemic autoimmune disease, central or paracentral corneal opacities, pregnancy, or lactation.[18]

Patients were examined before surgery and at 1, 3, and 6 months postoperatively. All patients underwent slitlamp examination (DC3; Topcon Corp.) and AS-OCT (3D-OCT 2000; Topcon Corp.).

Surgical Technique

Corneal CXL treatment was performed with a high-intensity UVA illuminator (KXL I, Avedro Inc.) by 4 surgeons (A.M., A.I., A.S., L.F.). Surgical procedure was performed under topical anaesthesia with application of 4% lidocaine and 0.2% oxybuprocaine hydrochloride drops. Thirty minutes before treatment, 2% pilocarpine drops were instilled to reduce the amount of ultraviolet light reaching the posterior segment.[14] The procedure was conducted under sterile operating conditions. Patients were randomly divided in 2 groups: Group A (accelerated standard crosslinking with continuous light), and Group B (accelerated corneal crosslinking with pulsed light illumination, 1 second on/1 second off). After application of a lid speculum, the corneal epithelium was debrided in the central 9-mm diameter area with a blunt metal spatula. A solution of riboflavin 0.1% and HPMC 1% (Vibex Rapid, Avedro Inc.) was instilled for 10 minutes, at 1- to 2-minute intervals. Following completion of the riboflavin soak, the solution was rinsed from the eye with balanced salt solution. Delivered UVA energy was 7.2 J/cm^2, with an irradiation of 30 mW/cm^2 for patients with standard illumination (4 minutes) or pulsed illumination (1 second on/1 second off; 8 minutes).

A soft therapeutic contact lens was applied until complete re-epithelialization. Topical netilmicin 0.3% drops (Nettacin) and 0.15% dexamethasone phosphate drops (Etacortilen) were given 4 times daily for 7 days and, 3 times daily for 1 month, and twice daily for 2 months. Sodium hyaluronate drops (Lubristill) were administred 6 times daily for 3 months.

Anterior Segment Optical Coherence Tomography

AS-OCT scans were performed under identical light conditions preoperatively and at 1, 3, and 6 months postoperatively. The stromal demarcation line was identified within an enhanced image of the cornea on the horizontal meridian and was measured using the caliper tool provided by the manufacturer. Two independent examiners

Table 1. Comparison of patients included in the study at baseline.

Characteristic	Continuous Light (Group A)	Pulsed Light (Group B)	*P*
Age, years (Min, max values)	24.8 ± 5.8 (16, 39)	24.3 ± 6.6 (17, 42)	>.05
CCT (μm) (Min, max values)	486.9 ± 34.8 (434, 562)	509.3 ± 43.2 (426, 578)	>.05
Average K value (D) (Min, max values)	46.7 ± 2.9 (41.9, 51.5)	45.6 ± 2.8 (42.4, 53.4)	>.05
Maximum K value (D) (Min, max values)	48.6 ± 3.8 (43, 56)	47 ± 6 (42.4, 55.1)	>.05

CCT = central corneal thickness; D = diopter; max = maximum; K = keratometry; Min = minimum.
Data are mean ± standard deviation, or minimum and maximum values.

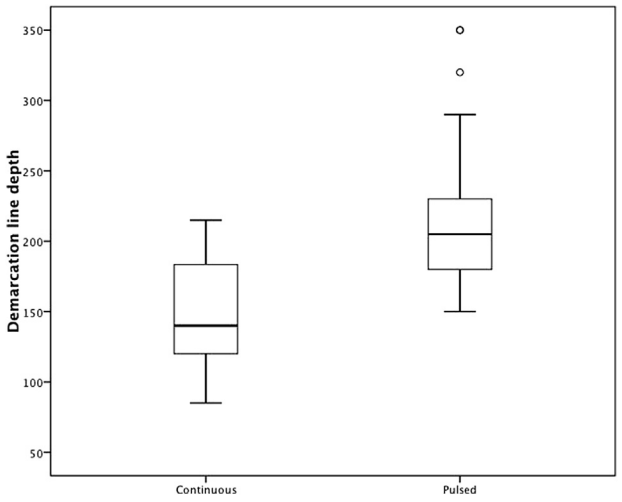

Figure 1. Corneal stromal demarcation line depth after accelerated CXL using continuous light (Group A) and pulsed light (Group B).

(A.M., A.I.) measured the depth of the demarcation line centrally. The measurement accuracy was also recorded by scoring the visibility of the demarcation line (0 = line could not be detected; 1 = line visible, but measurement not very accurate; 2 = line easily identified and reliable measurement). Only measurements with a score of 2 were included in this study.

Statistical Analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS software version 22.0 (SPSS Inc.). A *P* values of 0.05 of less were considered statistically significant.

RESULTS

The study comprised 60 patients (70 eyes), 30 (30 eyes) in Group A and 30 (40 eyes) in Group B. The 2 groups were similar in age preoperative corneal central thickness, and in steep and flat K readings (Table 1). All patients reported some degree of pain during the first 2 days after treatment. No adverse events were recorded in either treatment group during the follow-up. The corneal stromal demarcation line was identified easily (score 2) on AS-OCT in all eyes by both observers at 1 month. The mean depth of the demarcation line was 149.32 ± 36.03 μm in Group A and 213 ± 47.38 μm in Group B. The difference in demarcation line depth between groups was statistically significant ($P < 0.001$, *t* test) (Figure 1). At 3 months after CXL, the demarcation line had disappeared (score 0) in 63 of 70 measured eyes. In the remaining 7 eyes, the demarcation line was scored by both observers as visible, but not visible enough to enable an accurate measurement (score 1). At 6 months after surgery, the line was scored by both observers as invisible (score 0) for all included eyes.

DISCUSSION

The results of this study show that the stromal demarcation line detected at 1 month postoperatively was significantly deeper in pulsed light compared to continuous light accelerated CXL (Figures 2 and 3).

Accelerated CXL has been demonstrated to be equal to standard length CXL in terms of corneal photochemical reaction as well as clinical outcomes.[19] According to the photochemical law of reciprocity (Bunsen-Roscoe law), the same photochemical effect is in fact achieved with reduced illumination time and correspondingly increased irradiation intensity. A CXL energy dose of 7.2 J/cm^2 has been demonstrated by biaxial corneal extensiometry and papain digestion to be more effective compared with a dose of 5.4 J/cm^2 in laboratory studies.[20] Several new commercially available CXL devices offer high UVA irradiation intensity with reduced exposure time.

Seiler and Hafezi first reported the identification of a corneal stromal demarcation line at depth of approximately 300 μm that was visible as early as 2 weeks after conventional CXL.[14-21] The same authors concluded that the corneal stromal demaration line represents a clinical sign to directly monitor the effective depth of the CXL treatment.[14-21] Mazzotta et al. also identified the presence of a transition area between an edematous zone and a deeper zone with

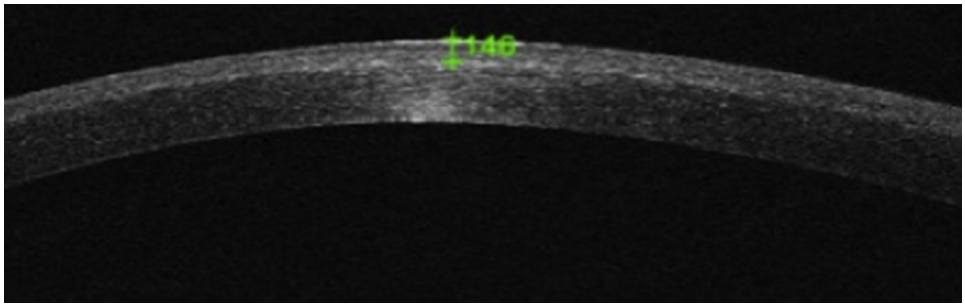

Figure 2. AS-OCT scan of the corneal stroma demarcation line 1 month after CXL in Group A (continuous light).

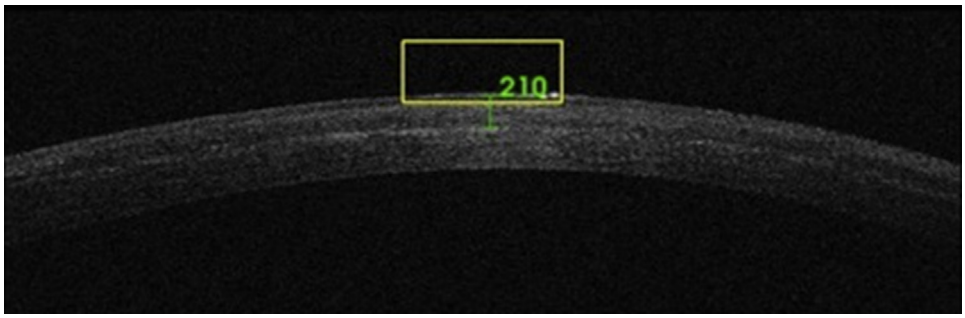

Figure 3. AS-OCT scan of the corneal stroma demarcation line 1 month after CXL in Group B (pulsed light).

less edema and regular keratocyte population; in this study, the average depth of the demarcation line was approximately 320 μm.[22] In another study by Doors et al., the corneal demarcation line after CXL was visualized using AS-OCT with the best visibility at 1 month after treatment; the average depth in their study was 313 μm.[23] Two independent studies by Kymionis et al[21] and Tomita et al[19] showed that the demarcation line depth was deeper (350 versus 290 μm) in conventional CXL treatment (30 minutes CXL performed in accordance with the Dresden protocol) compared to accelerated high-intensity 10-minute CXL (Table 2). Yam et al, in a retrospective interventional case series of 40 eyes treated with CXL, visualized the demarcation line after 6 months with AS-OCT (approximately 281 micronm).[24]

Recently, Mazzotta et al. observed with both confocal microscopy and corneal OCT analysis, a more superficial demarcation line in continuous light accelerated crosslinking (160 μm) compared to pulsed light (215 μm) treatment.[17] Our study confirms and expands previous findings of Mazzotta et al.; we did, in fact, observe a very similar difference in a much larger number of patients. In addition, we followed up our patients for a 6-month postoperative course, and found that the demarcation line disappeared in all patients within 6 months regardless of the light protocol used.

In our study, the demarcation line depth was significantly deeper in Group B than Group A. Considering that the depth of the demarcation line after CXL could be representative of CXL effectiveness, we might hypothesize that pulsed light accelerated CXL could be more effective clinically than standard continuous light accelerated CXL. Another explanation for this finding may be that the longer treatment time using pulse light irradiation might have caused a greater stromal de-swelling than the one that occurred with continuous light responsible of the deeper depth of treatment observed in patients in Group B.

In both of our groups, treatment depth was contained within 200 μm of depth in the corneal stroma. The biomechanical effect of crosslinking was therefore applied only to the anterior corneal stroma.

Demarcation line in conventional crosslinking procedures (3 mW/cm^2 for 30 minutes) has been reported to be significantly deeper on average (~300 μm).[14,22] Whether this difference in treatment depth could

Table 2. Comparison of different demarcation line depth valuated with AS-OCT or confocal microscopy.

Authors, Year	Patients (N)	Standard CXL	Accelerated CXL	Time of Demarcation Line Detection	AS-OCT	Confocal Microscopy	Demarcation Line Depth
Seiler and Hafezi, 2006[14]	16	X		2 weeks		X	300 μm
Mazzotta et al., 2008[22]	44 eyes	X		1 month		X	340 μm
Doors et al., 2009[23]	28	X		1 month	X		313 μm
Yam, 2012[24]	40 eyes	X		6 months	X		281 μm
Kanellopulos and Asimellis, 2013[15]	94 eyes	X		2 weeks	X		305 μm
Kymionis et al., 2014[21]	16	X (1)	X (2)	1 month	X		1: 350 μm 2: 288 μm
Tomita et al., 2014[19]	48 eyes	X (1)	X (2)	1 month	X		1: 350 μm 2: 294 μm
Mazzotta et al., 2014[17]	20		X pl vs cl ACXL	1 month	X	X	pl-ACXL: 215 μm cl-ACXL: 160 μm

AS-OCT = anterior segment optical coherence tomography; CXL = collagen cross-linking.

have a different impact on the biomechanical stability of keratoconic cornea in the long run it is currently not known.

At 3 months after CXL, the demarcation line had disappeared in almost all eyes. We therefore recommend performing AS-OCT no later than 1 month after CXL to obtain the most accurate measurement of demarcation line depth.

In conclusion, our study found that the corneal stromal demarcation line was deeper after pulsed light accelerated crosslinking treatment compared to standard continuous light accelerated crosslinking. The clinical significance of our finding is yet to be elucidated by comparative clinical trials assessing the long-term stability of corneal ectasia in relation to the efficacy of CXL, as measured by demarcation line depth.

WHAT WAS KNOWN

- After corneal CXL procedures, a stromal demarcation line can be observed with the corneal stroma at 1 month postoperatively.
- The depth of the demarcation line is considered an indirect measurement of CXL penetration within the stroma.

WHAT THIS PAPER ADDS

- In a large cohort of patients, the stromal demarcation line was significantly deeper in pulsed versus continuous accelerated CXL.
- In both continuous and pulsed accelerated CXL, the demarcation line was always visible at 1 month postoperatively and disappeared between 3 months and 6 months postoperatively.

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