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# Standard corneal collagen crosslinking versus transepithelial iontophoresis-assisted corneal crosslinking, 24 months follow-up: randomized control trial

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**ABSTRACT.**

***Purpose:*** **To compare the results of standard corneal crosslinking (CXL) and transepithelial iontophoresis-assisted CXL after 24 months follow-up.**

***Material and methods:*** **Corneal crosslinking (CXL) was performed in a series of 149 eyes of 119 patients with keratoconus I–II of Amsler classification. Depending on the CXL method, patients were divided into two groups: (1) 73 eyes with standard CXL and (2) 76 eyes with transepithelial iontophoresis-assisted CXL. Depending on the group, epithelium removal or administration of riboflavin solution by iontophoresis for 10 min was performed, after which standard surface UVA irradiation (370 nm, 3 mW/cm$^2$) was performed at a 5-cm distance for 30 min.**

***Results:*** **A statistically significant difference in corrected distance visual acuity (CDVA) was observed between the two groups, with a better outcome in the second group after 6 months ($p = 0.037$); however, no significant difference was found 24 months after treatment ($p = 0.829$). Stabilization and regression of keratometry values were achieved in both groups, but standard CXL was more effective. The average demarcation line depth in the standard CXL group was $292 \pm 14$ μm after 14 days and $172 \pm 16$ μm in the transepithelial iontophoresis-assisted CXL group. No demarcation line was detected after 1 month and 3 months in 45% and 100% of the eyes in the second group respectively.**

***Conclusion:*** **Transepithelial iontophoresis-assisted collagen crosslinking showed to be less effective than standard CXL after 24 months of follow-up, possibly due to a more superficial formation of corneal collagen crosslinks, however the stopping of disease progression was achieved 24 months after procedure.**

**Key words:** crosslinking – iontophoresis – keratoconus – riboflavin

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## Introduction

Corneal collagen crosslinking was successfully introduced by Wollensak et al. in 2003 and became a standard, minimally invasive, and safe treatment for progressive keratoconus (Romano et al. 2012) and secondary ectasia (Wollensak et al. 2003; Wollensak 2006; Mazzotta et al. 2008). The aim of this procedure is to slow or possibly stop disease progression to avoid the need for corneal transplantation (Rabinowitz 1998). The standard technique involves removal of the epithelium to enable riboflavin to penetrate into stromal tissue, where it causes highly reactive oxygen species to be released, triggering formation of crosslinks that consists of intra- and interfibrillary covalent bonds (Wollensak 2006).

However, epithelial removal causes significant pain and discomfort in the early postoperative period, and several variations of standard crosslinking procedure have been proposed since its introduction. These variations are aimed at avoiding epithelial debridement and increasing the patient's comfort and safety. Transepithelial corneal crosslinking (CXL) is one such variation. In this procedure, benzalkonium chloride and ethylenediaminetetraacetic acid are applied preoperatively for 3 hr (Leccisotti & Islam 2010) or tetracaine-containing anaesthetic eye drops are applied for 30 min (Chan & Wachler 2007), followed by subsequent instillation of a riboflavin solution into the eye for 30 min. Creating stromal pockets with a femtosecond laser to inject the riboflavin solution has also been proposed (Kanellopoulos 2009; Vinciguerra et al. 2009).

As a negatively charged, water-soluble molecule with a low molecular weight (376.40 g/mol), riboflavin is known to be useful for iontophoresis. Mastropasqua et al. (2014) in their experimental study confirmed that transepithelial iontophoresis imbibition provide greater and deeper riboflavin saturation than epi-on technique, however it did not reach the concentration obtained with standard epi-off technique. Several studies have described iontophoresis as a promising means of riboflavin impregnation of the corneal tissue (Bikbova & Bikbov 2014; Mastropasqua et al. 2014; Vinciguerra et al. 2014). Concerning experimental studies, there are several reports available e.g. Cassagne et al. (2014) studied transcorneal delivery by iontophoresis of riboflavin in a rabbit model using High-Performance Liquid Chromatography (HPLC), and they found that 5 min of iontophoresis allowed riboflavin diffusion with two-fold less riboflavin concentration than conventional application (936.2 ± 312.5 ng/ml and 1708 ± 908.3 ng/ml, respectively, $p < 0.05$). Novruzlu et al. (2015), using ultraperformance liquid chromatography coupled with electrospray ionization tandem mass spectrometry found that iontophoresis was increasing the riboflavin molecules transmission into the stroma without epithelial disruption. The recent study performed by Hayes et al. (2015) examined different delivery protocols for CXL procedure in *ex vivo* porcine corneas, where they found iontophoresis-assisted protocols increase stromal penetration of riboflavin, however the most effective penetration they observed in modified iontophoresis protocol (two 5-min iontophoresis-assisted deliveries of Ricrolin$^+$ with a 15-min soakage time in between) which resulted in significant stromal penetratin of riboflavin.

The effectiveness of epi-off CXL has been well described in several studies with adequate follow-up (O'Brat et al. 2013).

In the present study, we compared iontophoresis-assisted transepithelial CXL (application time – 10 min; Bikbova & Bikbov 2014) with standard CXL in patients with progressive keratoconus to clarify if the epi-on iontophoresis-assisted CXL is equally effective.

# Patients and Methods

### Study group and protocol

This randomized controlled clinical study included 149 eyes of 119 patients with progressive keratoconus who underwent CXL procedure at Ufa Eye Research Institute from January 2010 to December 2014 with follow-up for 2 years for every patient. All patients provided informed written consent. The study was approved by the ethics committee of Ufa Eye Research Institute (Ref number 467.34.8469) following the tenets of the Declaration of Helsinki and local laws regarding research on human subjects and registered at ClinicalTrials.gov (NCT02456961).

Inclusion criteria were of age over 18 years and a documented progression of disease as defined by the following changes over 1 year: an increase in the steepest keratometry value by 1.0 diopter (D) or more in manifest cylinder, or an increase of 0.5 D or more in manifest spherical equivalent refraction by repeated keratotopography ODP-scans ARK-1000 (Nidek, Aichi, Japan).

Exclusion criteria were a pachymetry value of <400 $\mu$m, history of previous ocular infection (e.g. herpes), pregnancy or breastfeeding, or corneal scarring. Patients were randomized by unrestricted randomization to either standard CXL or iontophoresis-assisted transepithelial CXL.

### Measurements and devices

Patients were examined at baseline, 1, 3, 6, 12, 24 months post CXL. At each follow-up, a standard examination was done to assess uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), refractometry, keratometry, corneal topography (ODP-scan ARK-1000), pachymetry and postoperative demarcation line depth (Visante OCT; Carl Zeiss Meditec, Jena, Germany). To control the safety of the procedure, endothelial cell density was counted in all patients, and corneas were scanned using laser scanning confocal microscope. Images of the endothelium were acquired with a confocal scanning laser ophthalmoscope (Heidelberg Retina Tomograph III/Rostock Corneal Module; Heidelberg Engineering GmbH, Heidelberg, Germany). Endothelial cell density was assessed using the software provided by the system.

During CXL pachymetry, measurements were performed with a handheld ultrasound pachymeter (SP-3000; Tomey, Nagoya, Japan).

The CXL device included two UVA diodes of 370 nm wavelength with a focusing distance of 5 cm and an irradiance of 3 mW/cm² (UFalink, Ufa Eye Research Institute, Ufa, Russia). Before each treatment, a calibration was performed to confirm the correct UVA emission level.

### Surgical technique

*Standard CXL*

The epi-off technique was performed following the standard protocol with epithelial removal (9 mm) and application of riboflavin 0.1% with dextran (T-500) for 30 min (Spoerl et al. 2011) followed by surface UVA irradiation at a 5-cm distance for 30 min. During UVA exposure, riboflavin + dextran drops were continued every 2 min.

*Transepithelial CXL*

Impregnation of the cornea with a riboflavin 0.1% hypotonic solution was performed by using an iontophoresis device (galvanizator; Potok-1, Moscow, Russia) (Bikbova & Bikbov 2014). The passive electrode (anode) was applied to the inferior part of the cervical vertebrae. The active electrode (cathode), a bath tube made of glass or plastic with a capacity 10–12 ml, was then applied to the open eye. After the tube was taped to the skin of the orbital margins, it was filled with riboflavin 0.1%. During the procedure, no pressure was applied on the eyeball, but the eye was in direct contact with the riboflavin solution. The current intensity was initially 0.2 mA for 1 min and then gradually increased to 1.0 mA at 0.2 mA for at 10-second intervals increments to determine individual tolerance and avoid patient discomfort (appearance of 'electric' sensation). The total time that the riboflavin solution was administered by iontophoresis was 10 min.

The efficiency of riboflavin penetration into the corneal stroma was checked by slit-lamp on a dark blue-cobalt filter. An intense yellow glow in the anterior chamber indicated complete impregnation with riboflavin in 10 min of iontophoresis in all patients.

Standard surface UVA irradiation (370 nm, 3 mW/cm$^2$; UFalink) was then applied at a 5-cm distance for 30 min. During UVA exposure, hypotonic riboflavin drops were continued every 2 min.

Postoperatively, the post CXL medication consisted of antibiotics for 2 weeks and topical steroids after epithelial healing for 2 weeks in the standard CXL group. In the transepithelial CXL group, corticosteroid drops were used for 2 weeks after CXL.

### Statistical analysis

Decimal visual acuity was converted to the logarithm of minimal angle of resolution (logMAR).

Statistical analysis was performed using STATSDIRECT software (StatsDirect, Ltd, Cheshire, UK). Data were recorded as mean ± standard deviation. Baseline measurements between groups were compared using independent sample *t*-test. All data samples were first checked by means of the Smirnov test. When parametric analysis was possible, the Student's *t*-test for paired data was performed for all parameter comparisons between preoperative and postoperative examinations. When parametric analysis was not possible, the Wilcoxon rank-sum test was applied to assess the significance of differences between preoperative and postoperative data, using the same level of significance ($p < 0.05$) in all cases.

# Results

This study included 149 eyes of 119 patients, including 82 men (68.9%) and 37 women (31.1%), aged 18–48 years old (average, 28.4 ± 2.5). One hundred and forty-nine eyes had progressive keratoconus of grade I–II according to the Amsler classification (without stromal scarring). Depending on the method of CXL, patients were divided into two groups: (1) 62 patients (73 eyes) who underwent standard CXL and (2) 57 patients (76 eyes) who had transepithelial iontophoresis-assisted CXL.

Table 1 shows the baseline characteristics of the patients. Both groups were comparable, and keratoconus progression was not significantly different between them.

After standard CXL, epithelial healing occurred within 3–4 days. Three patients (3.8%) showed slight stromal oedema at 1 month after CXL, which was resolved in 3–4 months. Four patients (5.2%) had impaired epithelial healing with central haze development at the 6-month follow-up. In the transepithelial CXL group, patients did not report any postoperative pain or worsening of vision associated with the classic crosslinking technique. In all eyes, the epithelium was intact and no complications were observed.

Although the great majority of eyes are stabilized after CXL, further progression of the disease was detected in one patient in the transepithelial group (1.3%).

### Visual acuity and keratometry

Table 2 shows the outcomes at all follow-up period. Corrected distance visual acuity (CDVA) was significantly different between the two groups (Fig. 1), with a better outcome in the transepithelial CXL group ($p = 0.044$). The largest observed difference between the two groups occurred after 6 months ($p = 0.037$); however, no significant difference was found at 24 months after treatment ($p = 0.829$).

Stabilization and regression of keratometry values were achieved in both groups, but standard CXL was found to be more effective (Fig. 2). The central keratometry value had a slight tendency to decrease in the standard CXL group over time, but it remained stable in the transepithelial CXL group from 6 months after procedure.

Anterior corneal astigmatism was slightly reduced 24 months after the procedure, but the change did not reach statistical significance in the transepithelial CXL group ($p \geq 0.061$).

### Pachymetry and demarcation line and confocal microscopy

Transepithelial iontophoresis did not cause corneal swelling, as was confirmed by intraoperative pachymetry. Corneal thickness measurements remained within preoperative range.

Corneal thickness at the thinnest point decreased from baseline values of 484 ± 41 μm to 462 ± 40 μm in the standard CXL group and 479 ± 44 μm to 463 ± 45 μm in the transepithelial group 12 months after CXL. Twenty-four months post CXL; it recovered to 471 ± 32 μm and 472 ± 32 μm respectively.

The average demarcation line depth in the standard CXL group was 292 ± 14 μm after 14 days and 172 ± 16 μm in the transepithelial CXL group in 68% of patients (Fig. 3). However, in the second group no demarcation line was detected 1 month and 3 months after CXL in 45% (34 eyes) and 100% (76 eyes) of the patients (Table 2). The keratometry changes, demarcation line depth and percentage of its occurrence over time are presented in Table 3.

Figure 4 showing the confocal microscopy over time in both groups. Confocal microscopy revealed that epithelium 1 month after standard CXL was thinner than that preoperatively with gaining its normal thickness after 3–6 months, in the transepithelial group 1 month postoperative, there was hyperreflectivity observed within the epithelial layer (Fig. 4A). After 3–6 months, epithelium returned to its normal structure.

Confocal microscopy showed the keratocytes loss in the anterior and intermediate corneal stroma with a 'honeycombed' appearance in both groups (Fig. 4B), and reduced number

**Table 1.** Transepithelial via iontophoresis versus epi-off corneal crosslinking (CXL) for keratoconus, baseline characteristics ($n = 149$).

| Parameter | Standard CXL | Transepithelial CXL |
|---|---|---|
| Age, year (range) | 30 (18–42) | 28 (18–44) |
| Keratometry, D (mean ± SD) | 47.61 ± 3.01 | 46.92 ± 3.28 |
| Uncorrected distance visual acuity, LogMAR (mean ± SD) | 0.81 ± 0.39 | 0.82 ± 0.24 |
| Spherical equivalent (D) | 5.41 ± 3.74 | 4.01 ± 3.54 |
| Corrected distance visual acuity, LogMAR (mean ± SD) | 0.32 ± 0.29 | 0.33 ± 0.31 |
| Pachymetry thinnest point, μm (mean ± SD) | 484 ± 41 | 479 ± 44 |
| Endothelium, cells/mm$^2$ | 2744 ± 301 | 2739 ± 312 |

**Table 2.** Summary of visual and refractive outcomes†.

| | Standard CXL | | | | | Transepithelial CXL via iontophoresis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 month | 3 month | 6 months | 12 months | 24 months | 1 month | 3 months | 6 months | 12 months | 24 months |
| UDVA, LogMar | 0.69 ± 0.39 | 0.66 ± 0.45 | 0.52 ± 0.27 | 0.66 ± 0.41 | 0.68 ± 0.56 | 0.56 ± 0.41 | 0.58 ± 0.41 | 0.57 ± 0.51 | 0.56 ± 0.439 | 0.53 ± 0.42 |
| CDVA, LogMar | 0.36 ± 0.31 | 0.35 ± 0.29 | 0.37 ± 0.35 | 0.29 ± 0.18* | 0.30 ± 0.27* | 0.31 ± 0.41 | 0.31 ± 0.39 | 0.28 ± 0.27* | 0.25 ± 0.29* | 0.26 ± 0.56* |
| Keratometry, D; K Av | 47.28 ± 2.96 | 46.82 ± 2.96 | 46.49 ± 2.21 | 45.39 ± 2.34* | 45.46 ± 2.12* | 46.68 ± 1.97 | 46.18 ± 2.54 | 45.98 ± 2.65 | 45.93 ± 2.46* | 45.95 ± 2.79* |
| Astigmatism, D | 3.68 ± 3.01 | 3.54 ± 2.91 | 2.98 ± 2.01* | 2.89 ± 2.12* | 2.91 ± 1.24* | 3.54 ± 1.39 | 3.37 ± 1.31 | 3.23 ± 1.01* | 3.16 ± 1.58 | 3.10 ± 1.87 |
| CCT, $\mu$m | 486 ± 36* | 475 ± 41* | 472 ± 41* | 462 ± 40* | 471 ± 32* | 473 ± 44 | 476 ± 40* | 464 ± 44* | 463 ± 45* | 472.28 ± 32.29 |

UDVA = uncorrected distance visual acuity, CDVA = corrected distance visual acuity, K Av = mean corneal power in the 3-mm zone, CCT = corneal thickness at thinnest location, D = diopter, CXL = corneal crosslinking.
Visual acuity and refractive outcomes are shown.
†Values are mean ± SD. *$p < 0.05$.

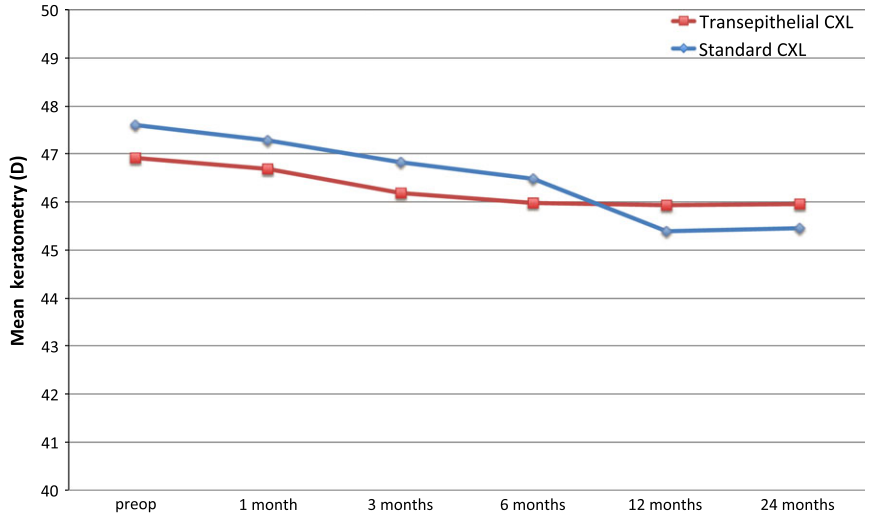


**Fig. 1.** Difference in keratometry values in standard versus transepithelial corneal crosslinking for keratoconus.

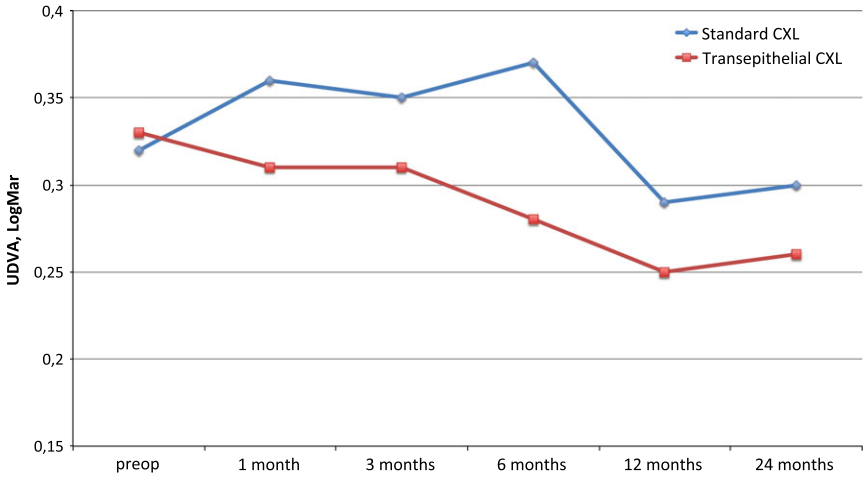


**Fig. 2.** Difference in corrected distance visual acuity (CDVA) (LogMar) in standard versus transepithelial corneal crosslinking for keratoconus.

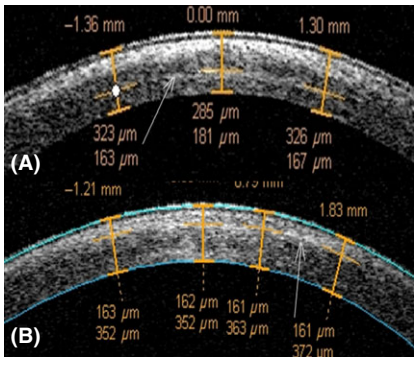


**Fig. 3.** Demarcation line on OCT images 1 month postoperative: (top) after standard corneal crosslinking (CXL) at 285 $\mu$m centrally, (bottom) after ionotophoresis-assisted CXL at 162 $\mu$m centrally.

of keratocyte nuclei in early postoperative period (1–3 months). However, the maximum depth of keratocyte apoptosis was detected at about 240–309 $\mu$m measured from the surface of epithelium in the standard CXL group and at about 150–210 $\mu$m in the transepithelial CXL group. Also in some patients of the transepithelial group, the 'honeycomb' structure was less homogeneous than in standard group. The demarcation line had been identified as a change in tissue reflectivity compared to normal unchanged stroma, caused by stromal lacunar oedema around apoptotic keratocytes (Wollensak & Herbst 2010; Mazzotta et al. 2015). Our quantitative OCT findings regarding the demarcation line corresponded well with our confocal microscopy findings (Table 3, Fig. 3). Repopulation of keratocytes was observed from third month as reoccurrence of cell nuclei and oedema reduction (Fig. 4D). The completion of

**Table 3.** Keratometry and demarcation line depth over time.

| Mean ± SD | | Postoperative | | | | |
|---|---|---|---|---|---|---|
| Variable | Preoperative | 1 Month | 3 Months | 6 Months | 1 Year | 2 Years |
| Standard CXL | | | | | | |
| *K* 1 | 45.94 ± 2.97 | 45.61 ± 2.67 | 45.11 ± 2.73 | 44.98 ± 2.15 | 44.61 ± 2.03* | 44.86 ± 2.24* |
| *K* 2 | 48.83 ± 3.12 | 48.74 ± 2.98 | 47.95 ± 2.98 | 47.68 ± 2.08 | 47.15 ± 2.41* | 46.94 ± 2.62* |
| *Km* | 47.41 ± 3.01 | 47.28 ± 2.96 | 46.82 ± 2.96 | 46.49 ± 2.21 | 45.39 ± 2.34* | 45.46 ± 2.12* |
| Demarcation line depth (μm) | | 287 ± 15/294 ± 22 | 280 ± 12/286 ± 19 | 277 ± 11/283 ± 23 | 264 ± 11/278 ± 21 | – |
| OCT/CM | | 96% of patients | 66% of patients | 48% of patients | 7% of patients | |
| Transepithelial CXL via iontophoresis | | | | | | |
| *K* 1 | 44.89 ± 2.96 | 44.97 ± 1.83 | 44.68 ± 2.34 | 44.51 ± 2.81 | 44.01 ± 2.15* | 44.02 ± 2.68* |
| *K* 2 | 48.28 ± 3.14 | 48.11 ± 1.94 | 48.02 ± 2.68 | 47.94 ± 3.02 | 47.62 ± 2.64* | 47.54 ± 2.93* |
| *Km* | 46.92 ± 3.28 | 46.68 ± 1.97 | 46.18 ± 2.54 | 45.98 ± 2.65 | 45.93 ± 2.46* | 45.95 ± 2.79* |
| Demarcation line depth (μm) | | 169 ± 18/173 ± 24 | – | – | – | – |
| OCT/CM | | 47% of patients | | | | |

D = diopters, μm = micrometers, *K*1 = corneal dioptric power in the flattest meridian for the 3-mm central zone, *K*2 = corneal dioptric power in the steepest meridian for the 3-mm central zone, *Km* = mean corneal power in the 3-mm central zone, OCT = optical coherence tomography, CM = confocal microscopy, CXL = corneal crosslinking.
Values are mean ± SD. *$p < 0.05$ change from preoperatively to 1 year postoperatively (paired Student *t* test).

repopulation was observed after 6–12 months postoperative in both the groups, with slower tendency in the standard group. After standard CXL, scattered microstria were identified within 3–12 months postoperative [Fig. 4D(s)], which had not been observed in the transepithelial group. In the standard group, the haze of anterior stroma was the common finding as hyperdensity of extracellular tissue. In most patients, stromal oedema resolved completely within 6–12 months, however in four patients with developed permanent haze, strong hyperreflective tissue had been found during follow-up period.

No endothelial damage was observed at any time during the follow-up period, and the endothelial cell density remained unchanged in both groups within 2764 ± 91 cells/mm$^2$ and 2756 ± 69 cells/mm$^2$, respectively.

# Discussion

Corneal collagen crosslinking is widely used for halting the progression of corneal ectasias such as keratoconus and pellucid marginal degeneration (Wollensak et al. 2003). It can also be effective in the treatment and prophylaxis of iatrogenic keratoectasia resulting from LASIK (Kohlhaas et al. 2006).

The standard CXL has already proved its efficacy with a 10-year follow-up period (Raiskup et al. 2015). However, the necessity of epithelial removal and the long duration of the procedure (1 hr) prompted several modifications of the standard techniques, including transepithelial CXL via iontophoresis (Bikbova & Bikbov 2014; Mastropasqua et al. 2014; Vinciguerra et al. 2014).

Iontophoresis is known to be effective for transcorneal drug delivery to corneal tissues and aqueous humour (Rootman et al. 1988). The transepithelial approach could be helpful for reducing early postoperative pain, preventing vision impairment and risk for infection (Rama et al. 2011), and significantly shortening the CXL procedure. However, the effectiveness of transepithelial CXL via iontophoresis compared to standard CXL is still debatable.

Our study showed that iontophoresis-assisted transepithelial CXL was effective to stop keratoconus progression

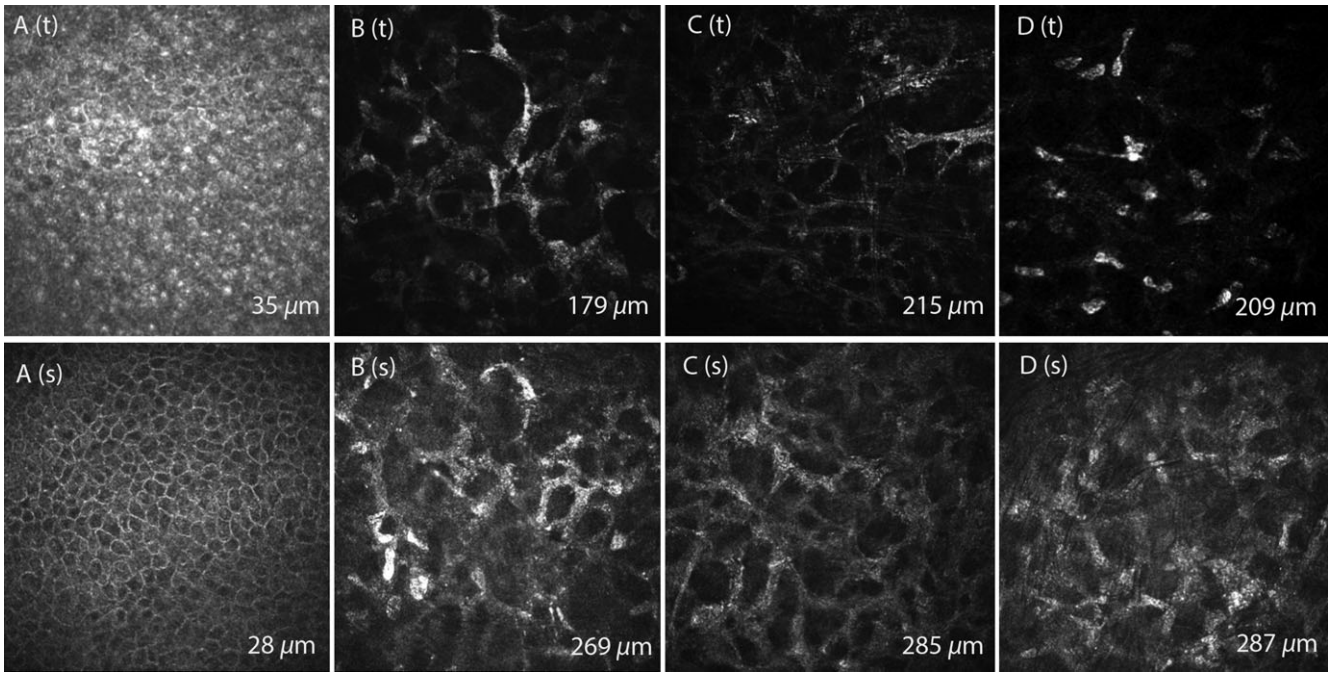


**Fig. 4.** Confocal microscopy images over time; (top) after transepithelial corneal crosslinking (CXL), (bottom) after standard CXL (t) – transepithelial group, (s)-standard group; A – epithelium 1 months after CXL A(t) – hyperreflective cellular appearance, A (s) – normal mosaic epithelial cell structure, B – 1 month after CXL, hyperreflective activated keratocytes with elongated membrane processes are visible, C – 3 months after CXL, stromal oedema with 'trabecular patterned stroma' with decreased number of keratocytes nuclei, D (t) – 6 months after CXL, corneal oedema is reduced, repopulation of keratocytes is detectable. D (s) – 6 months after CXL, microstria reflections and activated keratocytes are visible.

after 2 years, with a statistically significant improvement in the visual and topographic parameters. However with regard to a visible demarcation line, reduced pachymetry, and corneal flattening, our study demonstrated that the demarcation line at 1 month postoperative was more superficial (Seiler & Hafezi 2006; Doors et al. 2009) and pachymetry reduction was less compared to patients with standard CXL treatment. Similar to the findings of Bonnel et al. (2015) and Vinciguerra et al. (2014) a demarcation line was not clearly measurable over time in patients who underwent CXL via iontophoresis, however in their study irradiance of 10 $mW/cm^2$ and only 5 min of iontophoresis were used. Our study showed that a demarcation line was observed in only 45% patients 1 month after transepithelial CXL and was completely absent in all these patients for 3 months post-CXL. Confocal microscopy demonstrated that microstructural corneal changes were more pronounced in the standard CXL group. Similar to confocal microscopy findings of Mazzotta et al. (2015) it was found that after standard CXL, the clear homogenous 'honeycomb' appearance of apoptotic keratocytes and the OCT-based demarcation line were observed within 300 m depth, which is commonly known to be connected with biomechanical improvement and stabilization of the disease. The average keratometry decrease in 2 years in the standard CXL group was more significant (−2.15 D) comparing with the transepithelial group (−0.97 D). These findings conclude that standard CXL is more effective in iontophoresis-assisted transepithelial CXL. The iontophoresis-assisted transepithelial CXL group also had a significant CDVA increase, this difference in the CDVA over time between groups may be explained by the formation of haze in the first group and/or by epithelial remodelling postoperatively (Reinstein et al. 2009; Soeters et al. 2015).

Transepithelial collagen crosslinking by iontophoresis is an effective method for riboflavin impregnation of the corneal stroma, as some experimental studies demonstrated, (Arboleda et al. 2014; Cassagne et al. 2014; Hayes et al. 2015) and was able to stop the progression of keratoconus after 24 months of follow-up. However, the effectiveness of procedure is reduced, probably because of an insufficient supply of riboflavin during UVA irradiation; only charged ions can be moved using iontophoresis, and there is no riboflavin flow after the iontophoresis. Experimental studies demonstrate that riboflavin concentrations in the corneal stroma after 5 min of iontophoresis are only half compared to passive diffusion after epithelial removal: 15 $\mu g/g$ versus 34.1 $\mu g/g$ in a HPLC study performed by Mastropasqua et al. (2014) and 936.2 ± 312.5 ng/ml versus 1708 ± 908.3 ng/m in a HPLC study by Cassagne et al. (2014) . In our study, we used 10 minutes of iontophoresis-assisted delivery of riboflavin, which probably allows to increase riboflavin concentrations in the corneal stroma, as it was demonstrated by Frucht-Pery et al. (2004) for gentamicin, where the drug concentration after corneal iontophoresis delivery increased with a higher current intensity and longer duration of the iontophoresis. Some modifications of iontophoresis riboflavin delivery were described by Hayes et al. (2015), e.g., St Thomas'/Cardiff Iontophoresis protocol A and B, which involves iontophoresis for 5 min (total charge of 300 mC) and left *in situ* for a 20-min soaking time and two 5-min iontophoresis-assisted deliveries of Ricrolin$^+$ with a 15-min soaking time in between respectively, but those methods are also time consuming and do not

solve the question of further riboflavin flow during UV exposure. However, further studies should be performed for evaluating effectiveness of modified protocols. Some studies have reported endothelial damage when thin corneas without sufficient riboflavin were irradiated with UVA (Spörl et al. 2007; Kymionis et al. 2012). However, we did not observe any endothelial damage in the transepithelial CXL group, suggesting adequate UV absorption. Intact epitheliums soaked with riboflavin may also be itself a barrier to UVA irradiation, limiting the depth of keratocyte apoptosis and corneal collagen crosslinks formation (Caporossi et al. 2013). Nevertheless, even with a smaller effect, stabilization of the disease was achieved for 24 months, thus this procedure may be recommended preferably for use with thin corneas, in pain-intolerant patients, and in older patients with slowly progressing keratoconus. However, standard epi-off CXL remains the gold standard in treatment of progressive keratoconus.

A longer follow-up period is needed to assess the long-term results. Additional investigation is recommended for determining ways to supply riboflavin to the corneal stroma during UVA irradiation.

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