Comparison of two annular photorefractive intrastromal cross-linking protocols in high oxygen for low-grade myopia through 24-month follow-up

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ABSTRACT.

Purpose: **To compare two annular epithelium-on (epi-on) high oxygen photorefractive intrastromal cross-linking (PiXL) illuminations protocols for treatment of low-grade myopia.**

Methods: **In this randomized, single-masked, intra-individually comparative study, healthy individuals with bilateral low-grade myopia (manifest refractive spherical equivalent (MRSE) −0.75 diopters (D) to −2.50 D) were treated with high oxygen epi-on PiXL. One eye was randomized to receive pulsed accelerated 365-nm ultraviolet-A illumination in a central annular zone of 4.0 mm (1 second on, 1 second off; 30 mW/cm^2), and the fellow eye in a 3.5 mm annular zone (0.5 second on, 1 second off; 45 mW/cm^2). Uncorrected distance visual acuity (UDVA), MRSE, low-contrast visual acuity (LCVA), best spectacle corrected visual acuity (BSCVA), endothelial cell count (ECC) and Scheimpflug light scattering depths were assessed through 24-month follow-up.**

Results: **Twenty-seven participants (54 eyes) were included. The 3.5 mm protocol rendered less subjective ocular discomfort posttreatment and a larger improvement than the 4.0 mm protocol in UDVA: −0.52 (−0.72, −0.32) logMAR (medians and interquartile ranges, IQR) and −0.38 (−0.50, −0.22), p = 0.003 and MRSE: +1.25 D (0.75, 1.50) and +1.0 (0.75, 1.0), p = 0.037. The transient reduction in LCVA was larger with the 3.5 mm protocol (p < 0.01). No adverse events, and no reductions in ECC or BSCVA were noted.**

Conclusion: **Epi-on PiXL in high oxygen reduces myopia in healthy eyes. A larger reduction of myopia and less early posttreatment subjective ocular discomfort can be seen with a smaller treatment zone, but likely at the expense of a transient decrease in low-contrast visual acuity.**

Key words: myopia – corneal collagen cross-linking – refractive cross-linking – annular PiXL

Avedro, Inc., Waltham, Massachusetts, USA, provided the cross-linking device and the riboflavin for use in this study. The authors have no other financial interest in any procedure or equipment mentioned.

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Introduction

Photorefractive intrastromal cross-linking (PiXL) has been introduced as a modification of the initial cross-linking (CXL) protocols designed for keratoconus and related ectatic corneal disorders (Wollensak et al., 2003a, 2003b; Roberts & Dupps 2014; Yildirim et al. 2014). In PiXL, customized energy patterns are used to remodel and locally flatten the corneal curvature, which has been shown to render favourable refractive and visual outcomes in keratoconus (Nordstrom et al. 2017; Kamiya et al. 2020; Mazzotta et al. 2020). Apart from keratoconus treatment, flattening of the corneal curvature is also of value in refractive correction of myopia. By customizing the illumination protocol to only include a treatment zone of the central 4–6 mm, the resulting flattening effect has been shown to reduce myopia (Kanellopoulos 2014), also in healthy eyes (Elling et al. 2017, 2018; Lim et al. 2017; El Hout et al. 2019; Fredriksson et al. 2019; Näslund et al. 2020; Sachdev et al. 2020). However, the treatment effect of PiXL is limited to a refractive change of about one dioptre (D) in manifest refractive spherical equivalent (MRSE). Therefore, PiXL has mainly been explored as an alternative to refractive surgery in eyes with low-grade myopia.

In contrast to the intrastromal weakening that could be seen after excimer laser ablation in refractive surgery, CXL actually strengthens the corneal stroma (Wollensak et al., 2003a, 2003b). More specifically, the cross-linking

effect of CXL stabilizes the corneal stroma using ultraviolet-A (UV-A) light and the photosensitizer riboflavin to create cross-linked bonds within the corneal tissue through two different biomechanical reactions. The type 1 reaction is favoured in a low oxygen environment, forming free radicals through hydrogen or electron transfer to induce stromal cross-linking. However, if available, oxygen from the ambient air reacts with the excited riboflavin molecule to form reactive oxygen species, rendering photo-oxidation of stromal proteins and collagen cross-linking bonds in a type 2 reaction (Kamaev et al. 2012).

Cross-linking (CXL) without epithelial debridement (epi-on) has been suggested as a way to possibly facilitate the healing process and lower the risks for some complications and shorten the ocular discomfort associated with epi-off cross-linking (Ng, Ren et al. 2021). However, the treatment effect in such epi-on cross-linking has been shown to be restricted due to limited stromal oxygen supply and possibly also because of less penetration of riboflavin through the epithelium (Richoz et al. 2013; Fredriksson et al. 2019; Rubinfeld et al. 2019; Hill et al. 2020). To avoid hampering of the treatment effect, chemical enhancers facilitating epithelium penetration has been developed to increase the intrastromal concentration of riboflavin in transepithelial CXL. Furthermore, supplemental oxygen during treatment has been introduced to maintain a hyperoxic environment in the corneal stroma, with a resulting significant enhancement of the treatment effect of epi-on PiXL in myopia (El Hout et al. 2019; Fredriksson et al. 2019; Näslund et al. 2020; Sachdev et al. 2020).

From previous studies, it appears that early postoperative central haze can affect visual quality after CXL (Beckman Rehnman et al. 2014; Nordstrom et al. 2017). Previous PiXL protocols have included a central homogenous treatment zone, which in turn cause haze in the visual axis. Notably, the CXL effect has been shown to extend beyond the treatment area (Webb et al. 2019), which opens the possibility to treat myopia leaving a small central area untreated to diminish the central haze, while still getting enough cross-linking of the central cornea (Näslund et al. 2020).

Thus, in a previous study, we compared a 4.0 mm homogenous illumination protocol to a 4.0 mm annular protocol for the treatment of myopia, demonstrating similar visual improvement and refractive correction 24 months posttreatment (Näslund et al. 2020). Additionally, our findings showed less early discomfort with the annular protocol, which is in line with another previous study where a smaller treatment zone meant less postoperative pain and light sensitivity (Fredriksson et al. 2019). With these previous findings in mind, we wanted to evaluate the results of an even smaller treatment zone. Thus, the aim of this study was to compare the treatment effects of two epi-on PiXL protocols in high oxygen: a 4.0 mm and a 3.5 mm annular protocol, through 24-month follow-up.

Methods

Study design and participants

This prospective, randomized, single-masked intra-individually comparing study, performed at the Department of Clinical Sciences/Ophthalmology, Umeå University, Umeå, Sweden (NCT03987880; http://ClinicalTrials.gov) was a further development with a methodological resemblance to a previous study for myopia treatment (Näslund et al. 2020). Participants were included between January 22 and April 5 2018. Written informed consent was obtained prior to inclusion according to the tenets of Declaration of Helsinki, and approval was obtained from the Regional Ethics Committee in Umeå.

Men and women aged 18–35 years with a stable (defined as a maximum change of 0.50 D in 2 years) bilateral myopia of −0.75 D to −2.50 D measured as MRSE and a refractive astigmatism of ≤0.75 D were included. In addition, the central corneal thickness (CCT) had to exceed 440 µm and the best spectacle corrected visual acuity (BSCVA) ≥0.0 logarithm of minimum angle of resolution (logMAR) with only spherical refraction. Participants with a past or present disease, allergy, surgery or medication with ocular effects that could affect the outcomes of the treatment were not included.

Pre- and posttreatment assessments

The following parameters were assessed at baseline and at 1, 3, 6, 12 and 24 months follow-up: uncorrected and best corrected visual acuity (UDVA and BSCVA) assessed with the ETDRS-fast protocol (Camparini et al. 2001), MRSE, low-contrast visual acuity (LCVA) at 10% and 2.5% contrast in logMAR with the Sloan letter logarithmic translucent contrast chart, autorefraction (Oculus Park 1, Oculus, Inc., Lynnwood, WA, USA), endothelial cell count (ECC) assessed by specular microscopy (Topcon, Inc., Livermore, CA, USA), keratometry readings assessed with a rotating Scheimpflug camera (Oculus Pentacam HR®, Oculus, Inc, Lynnwood, WA, USA), ocular higher order aberrations measured with iTrace (Tracey Technologies, Inc., Houston, Texas, USA), intraocular pressure (IOP) measurement with Goldmann applanation tonometry and slit lamp examination.

From the Pentacam HR® measurements, the occurrence and depth of the posttreatment stromal light scattering were assessed from the 'Corneal Optical Densitometry' function as a modification of previously published techniques (Beckman Rehnman 2011; Thorsrud et al. 2017; Näslund et al. 2020); the cornea is scanned manually from the surface and back, frame by frame, until no sign of increased corneal light scattering is seen. The depth below which no visible light scattering was seen was defined as the light scattering depth. Three consecutive measurements were performed and the mean depth was calculated. The mean value of the depth assessments by two independent observers was used for the analysis. From iTrace measurements, the total higher order and spherical aberrations (total HOA and SA, respectively) and pupil diameters were extracted.

To assess the subjective experience at each follow-up visit, all participants answered the questions: 'If there is a difference in visual acuity between the eyes, which eye has *the better* visual acuity?', and 'If you experience any ocular discomfort, which eye has *the least* ocular discomfort?' Throughout the study any adverse events were registered and a loss of more than two lines in BSCVA was defined as a deterioration.

PiXL treatment

Tetracaine 1.0% drops were applied three times. After positioning a lid speculum, an oxygen applicator mask was applied to create an oxygenated environment over the ocular surface. The mask has a central opening, allowing for application of riboflavin and UV-treatment. The corneal epithelium was not removed, but was lightly brushed with a riboflavin-soaked cellulose sponge. Transepithelial riboflavin (0.25% riboflavin with addition of EDTA, benzalkonium chloride and hydroxylpropyl methylcellulose, ParaCel Part 1, Avedro, Inc.) was added at 90 seconds interval for 4 min and was then continued with Riboflavin (0.22%, ParaCel Part 2, Avedro, Inc.) at the same rate for additional 6 min. Humidified 100% oxygen with a flow rate of 2.5 L/min was delivered to the application mask 2 min prior to UV illumination and was continued throughout the treatment. An oxygen concentration of at least 95% over the corneal surface was confirmed using a Model 901 oxygen meter device (Quantek Instruments, Grafton, MA) before and directly after UV-treatment. Prior to illumination, superfluous riboflavin was rinsed from the corneal surface with 0.9% saline. A pupil-centred zone of the cornea was illuminated with either a 4.0 mm annular zone with a central 2.0 mm sparing, or a 3.5 mm annular zone with a central 1.5 mm sparing, for 16:40 min with 365-nm pulsed UV light (1 second on, 1 second off at 30 mW/cm^2 and 0.5 second on 1 second off at 45 mW/cm^2, respectively) (Avedro Mosaic system, Avedro, Inc., Waltham, Massachusetts, USA) at a total fluence of 15 J/cm^2 (Fig. 1). The Mosaic device contains a system for setting the energy distribution, which together with a pupil tracker ascertains that the specified energy is delivered in the specified area. During illumination, the corneal surface was protected from dehydration by the humidified oxygen flow and if the cornea became too dry for the eye tracker to detect the pupil border, a drop or two of 0.9% saline were given. One or two drops of tetracaine were added during treatment if the participant reported pain or discomfort. Treatments were performed bilaterally: one eye was randomized to the 4.0 mm annular protocol and the fellow eye to the 3.5 mm annular protocol with one single randomization block. Which eye received which treatment was randomized and masked to the participants. Posttreatment, ofloxacin drops were administered three times daily for three days and lubricating eye drops *ad libitum*. No bandage contact lens or anti-inflammatory eye drops were used.

Statistical analysis

A power analysis based on the UDVA and MRSE in healthy subjects with low-grade myopia showed that a group size of 21 + 21 eyes at pairwise comparison would be able to detect a difference of 0.24 logMAR in UDVA and 0.50 D in MRSE, with 90% certainty and alpha set at 0.05, which is considered as clinically relevant differences in treatment effect.

The normal distribution was assessed using the Shapiro-Wilk test and visual inspection of distribution histograms. Since most of the variables were not normally distributed, a Wilcoxon signed-rank test for dependent variables was used for all between-group comparisons. A Friedman test for dependent variables was used for within-group comparisons to assess the change from baseline through 24 months posttreatment and the stability of the treatment effect from 1 month to 3, 6, 12 and 24 months posttreatment. *Post hoc* analysis with Wilcoxon signed-rank test was performed. A Bonferroni correction for multiple test comparison was then applied, resulting in a significance level set at $p < 0.01$ for all variables using within-group comparisons. Fisher's exact test was used for investigating the association between reported best subjective visual acuity and least subjective postoperative ocular discomfort at each follow-up.

In all variables, except light scattering depths, subjective best visual acuity and least ocular discomfort, multiple imputation was used to handle missing data and the results from the analyses of imputed data were presented consistently in the figure and tables in the article. Observed data was summarized in Table S1–S3. For significance testing after multiple imputation, the median of the p-values from the five imputed data sets were used (Eekhout et al. 2017). IBM SPSS statistics v. 25 (Armonk, New York, USA) was used for data analysis. For the multiple imputation, the default setting was used. A p-value of <0.05 was considered statistically significant. Continuous data were expressed as medians and interquartile ranges (IQR) unless stated otherwise.

Results

Patient characteristics

Twenty-seven healthy myopic individuals (17 females) with a mean age of 28 years ± 4 (SD) (range 19–35 years) were treated bilaterally (54 eyes). The pretreatment UDVA was 0.46 (0.32, 0.66) logMAR and MRSE −1.25 (−2.00, −1.00) D for the 3.5 mm protocol and 0.46 (0.34, 0.60) logMAR and −1.25 (−2.00, −1.00) D for the 4.0 mm protocol. At 1 month one patient was lost to follow-up; at 3 months one patient missed the return visit; at 12 months one patient missed the return visit and 3 participants were lost to follow-up; at 24 months 7 participants missed their return visit. Hence, complete data were retrieved for 14 participants, and 13% of all values were missing.

Visual acuity

Table 1 and Figure 2 summarize the UDVA outcomes. A significant improvement in UDVA was seen at 1 month, with a larger improvement for the 3.5 mm protocol compared to the 4.0 mm protocol, −0.52 (−0.72, −0.32) and −0.38 (−0.50, −0.22) logMAR, respectively ($p = 0.003$). The treatment effect remained stable over time in both groups. Both treatments showed an initial reduction at 1 month for LCVA at 10% (Table 1), with a larger impairment with the 3.5 mm protocol; +0.16 (0.08, 0.30) compared with +0.12 (0.02, 0.18) logMAR with the 4.0 mm treatment ($p = 0.001$). For LCVA at 2.5%, there were reductions with the 3.5 mm protocol up to 12 months posttreatment (+0.42 (0.16, 0.89) logMAR at 1-month posttreatment), compared with the 4.0 mm protocol where reductions were seen 1 month (+0.12 (−0.02, 0.50) logMAR, although not statistically significant), and at 3 months posttreatment (+0.14 (0.04, 0.34) logMAR). However, both 10% and 2.5% LCVA reverted and no statistically significant reductions were

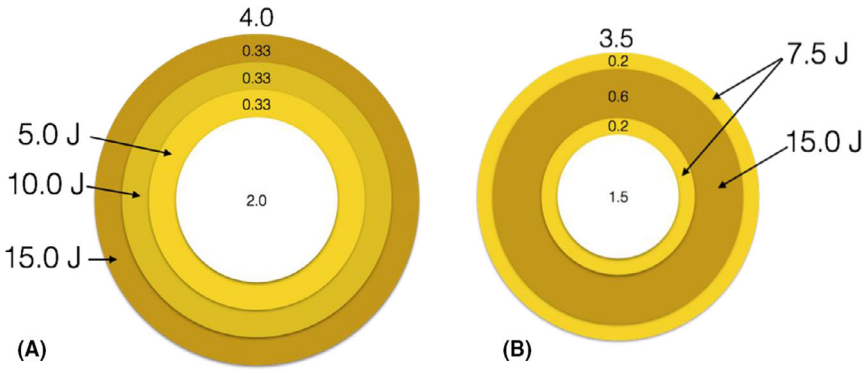

Fig. 1. (A) 4.0 mm pupil-centred annular zone with a 2.0 mm central sparing. Energy distribution (from outer ring): 15.0 J; 10.0 J; 5.0 J. Total illumination area 9.4 mm^2; total energy 1.0 J. (B) 3.5 mm pupil-centred annular zone with a 1.5 mm central sparing. Energy distribution (from outer ring): 7.5 J; 15.0 J; 7.5 J. Total illumination area 7.9 mm^2; total energy 0.9 J.

seen at 24 months compared with baseline in either protocol. The results of the observed data were similar (Table S1).

Manifest refractive spherical equivalent (MRSE)

Table 1 and Figure 2 summarize the MRSE outcomes. After a month, there was a larger reduction in myopia for the 3.5 mm treatment compared with the 4.0 mm treatment, with a median change of +1.25 D (0.75, 1.50) and +1.0 D (0.75, 1.0), respectively (p = 0.037). The MRSE improvement was stable through 24 months for both groups. The 3.5 mm group reached a median emmetropia at every time-point post-treatment, contrary to the 4.0 mm protocol where a median rest myopia of −0.50 D to −0.25 was seen at most posttreatment visits. The results of the observed data were similar in these respects (Table S1).

Other outcomes

Table 2 shows the light scattering depths in absolute values and as a percentage of CCT. The light scattering depths for the 3.5 mm protocol exceeded those of the 4.0 mm protocol at 3, 6 and 12 months.

The assessment of ocular higher order aberrations including total HOA and SA over time, summarized in Table 3, showed that total HOA remained unaltered for both protocols. A positive SA was induced at 1 month for the 3.5 mm protocol, +0.09 (0.02, 0.14) μm, but not at any other time-point. For the 4.0 mm treatment, there was an increase at 1 month compared with baseline, + 0.04 (0.01, 0.16) μm, although not statistically significant. The observed data was similar, except an increase in SA for both protocols at 1 month (Table S2). Keratometry measurements detected no consistent difference with either protocol post-treatment and the IOP was unaltered for both treatments throughout the follow-up (data not shown).

Safety data (BSCVA, ECC, adverse events) and subjective vision and ocular discomfort

Table 4 summarizes the safety outcomes (BSCVA, ECC) for both treatments up to 24 months posttreatment. There was no change in BSCVA or ECC for either group over time and none of the participants had lost two or more lines of BSCVA at 24 months, which was similar in the observed data (Table S3). No adverse event occurred for any patient.

Figure 3 displays the registered eye with best subjective vision and least ocular discomfort at every follow-up visit. After 6 months, the 3.5 mm treatment was perceived as giving a better subjective vision than the 4.0 mm treatment. No difference was reported at any other time-point. The 3.5 mm treatment gave less subjective ocular discomfort at 1 day. At visits after 1 week, no patient reported any ocular discomfort, except one patient reporting ocular discomfort with the 3.5 mm at 3 months and one patient reporting ocular discomfort with the 4.0 mm protocol at 12 months post-treatment.

Discussion

PiXL is a refractive procedure that reshapes the corneal stroma without the need for epithelium debridement or stromal removal. This study employing epi-on PiXL in high oxygen compared the treatment effects, safety outcomes and subjective ocular discomfort of two different treatment zone designs, sizes and light pulse intervals with a follow-up of 24 months.

In this study, the 3.5 mm protocol superseded that of the 4.0 mm protocol, with slightly better visual and refractive outcomes through 24-month follow-up and less early ocular discomfort. The treatment effect for the 3.5 mm protocol resulted in emmetropia more frequently than the 4.0 mm protocol, as shown in Figure 2. This may be explained by the energy distribution in the illumination protocol, where the 3.5 mm protocol had 15 J/cm^2 more centrally located, with more central cross-linking and a potentially better treatment effect. On the other hand, the 3.5 mm protocol in this study also seems slightly better than a 4.0 mm protocol using 15 J/cm^2 in a homogenous treatment zone (Näslund et al. 2020). It may also be that the 2 mm untreated central zone in the 4 mm protocol is a bit too large, and that a 1.5 mm zone is better from the treatment effect perspective. Previous research has suggested that the cross-linking effect increases with a pulsed illumination (Mazzotta et al. 2014). Thus, another explanation may be that the longer pulse intervals relative to the UV-illumination time in the 3.5 mm protocol augments the intrastromal

Table 1. Visual and refractive outcomes

		Baseline	1 month	p-value*	3 months	p-value*	6 months	p-value*	12 months	p-value*	24 months	p-value*
UDVA (logMAR)	3.5-mm	0.46 (0.32, 0.66)	−0.06 (−0.10, 0.04)	<0.001	−0.08 (−0.14, 0.02)	n.s.	−0.04 (−0.10, 0.06)	n.s.	−0.04 (−0.12, 0.14)	n.s.	−0.02 (−0.10, 0.22)	n.s.
	4.0-mm	0.46 (0.34, 0.60)	0.08 (−0.04, 0.24)	<0.001	0.14 (−0.10, 0.27)	n.s.	0.06 (−0.10, 0.22)	n.s.	0.02 (−0.08, 0.26)	n.s.	0.04 (−0.06, 0.56)	n.s.
	p-value†	n.s.	<0.01		<0.01		<0.01		n.s.		n.s.	
MRSE (D)	3.5 mm	−1.25 (−2.00, −1.00)	0.00 (−0.50, 0.00)	<0.001	0.00 (−0.25, 0.00)	n.s.	0.00 (−0.25, 0.00)	n.s.	0.00 (−0.50, 0.00)	n.s.	0.00 (−0.72, 0.00)	n.s.
	4.0 mm	−1.25 (−2.00, −1.00)	−0.50 (−0.75, 0.00)	<0.001	−0.25 (−0.50, 0.00)	n.s.	0.00 (−0.75, 0.00)	n.s.	−0.25 (−1.00, 0.00)	n.s.	−0.50 (−1.42, 0.00)	n.s.
	p-value†		<0.05		<0.05		<0.05		<0.05		<0.05	
LCVA 10% (logMAR)	3.5-mm	0.10 (0.10, 0.22)	0.34 (0.20, 0.40)	<0.001	0.24 (0.20, 0.34)	<0.01	0.24 (0.20, 0.30)	<0.01	0.22 (0.14, 0.32)	n.s.	0.24 (0.14, 0.35)	n.s.
	4.0-mm	0.12 (0.10, 0.22)	0.26 (0.20, 0.32)	<0.01	0.24 (0.12, 0.30)	n.s.	0.14 (0.12, 0.20)	n.s.	0.20 (0.10, 0.26)	n.s.	0.24 (0.12, 0.38)	n.s.
	p-value†	n.s.	<0.01		n.s.		<0.001		<0.01		n.s.	
LCVA 2.5% (logMAR)	3.5-mm	0.50 (0.42, 0.60)	0.94 (0.66, 1.49)	<0.001	0.80 (0.64, 1.00)	<0.01	0.68 (0.52, 0.88)	<0.01	0.68 (0.56, 0.88)	<0.01	0.64 (0.50, 0.88)	n.s.
	4.0-mm	0.50 (0.40, 0.70)	0.72 (0.54, 0.88)	n.s.	0.72 (0.56, 0.80)	<0.01	0.56 (0.44, 0.74)	n.s.	0.60 (0.48, 0.70)	n.s.	0.60 (0.46, 0.92)	n.s.
	p-value†	n.s.	<0.001		n.s		<0.01		<0.01		n.s.	

D = Diopters, LCVA 10%, 2.5% = Low contrast visual acuity at 10% and 2.5% contrast, logMAR = Logarithm of minimum angel of resolution, MRSE = Manifest refractive spherical equivalent, n.s. = not statistically significant, UDVA = Uncorrected distance visual acuity.
* Within-group comparisons between 1 month and each other time point.
† Between-group comparisons at each time point.
‡ Within-group comparisons between baseline and each other time point.

oxygen uptake and thereby the cross-linking effect.

This study suggests a stable treatment effect with both protocols, although it should be taken into account that axial myopia has a tendency to increase slightly over time. The findings are in line with our previous study on PiXL (Näslund et al. 2020). To date, a number of studies have confirmed the refractive treatment effect for PiXL in low-grade myopia (Lim et al. 2017; Elling et al. 2018; El Hout et al. 2019; Fredriksson et al. 2019; Näslund et al. 2020; Sachdev et al. 2020). In 2018, Elling et al. presented similar results as in our present study with epi-off PiXL in low-grade myopia. Recently, Sachdev et al. showed that epi-on PiXL with supplemental oxygen using a 4 mm central treatment zone achieved a mean reduction in myopia of +1.27 D 3 months posttreatment (Sachdev et al. 2020), a result similar to that of the 3.5 mm protocol in our present study.

An initial reduction in LCVA was seen with both protocols, and for the 3.5 mm treatment it was linked to an increase in SA. There was an increase in SA also with the 4.0 mm protocol at 1 month compared with baseline, albeit not statistically significant. The smaller central sparing in the 3.5 mm protocol, resulting in posttreatment haze closer to the optical centre, may have contributed to the transient deterioration in LCVA. Still, a more plausible explanation for the deterioration in LCVA is that it owes to a combination of an initial increase in posttreatment central haze and SA, the latter

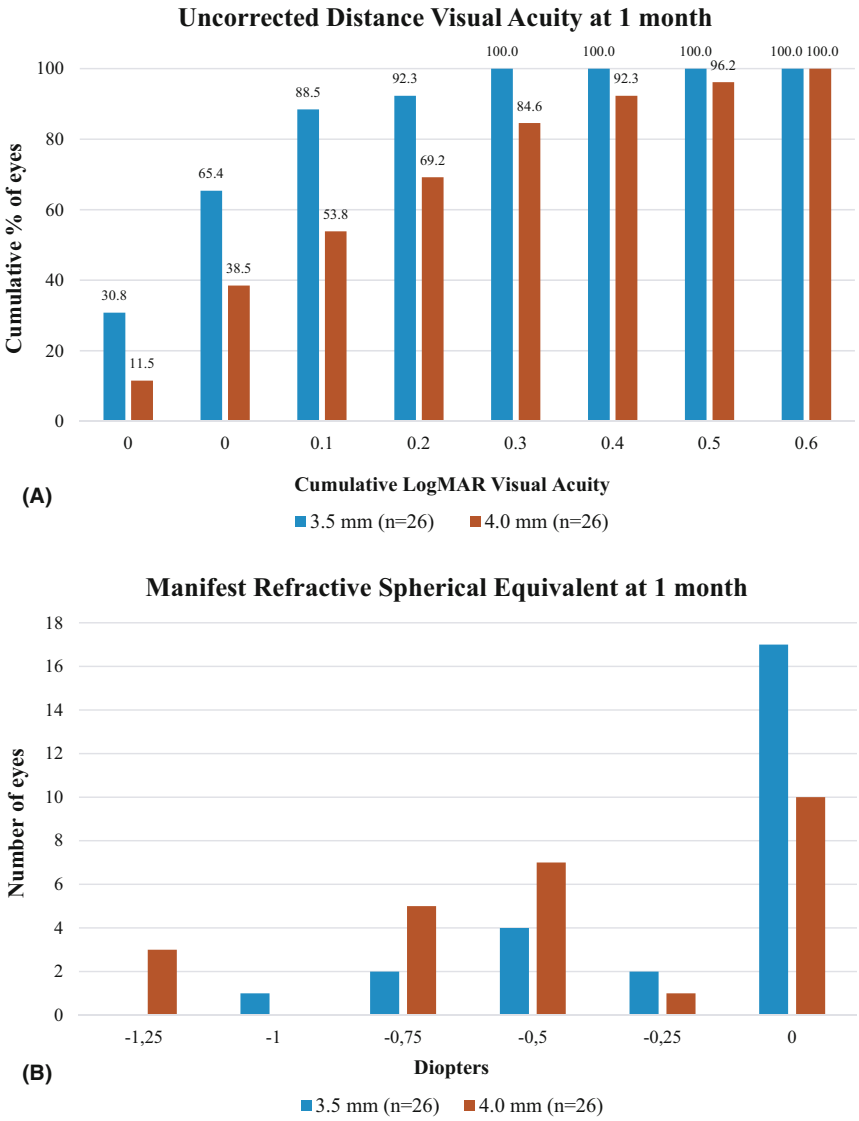

Fig. 2. Between-group comparisons of uncorrected distance visual acuity (A) and manifest refractive spherical equivalent (B) at 1 month. LogMAR = Logarithm of minimum angel of resolution.

Table 2. Light scattering depths

		1 month	3 months	6 months	12 months	24 months
	n =	26	24	25	18	16
3.5 mm	0, N/A, Max	1, 2, 1	2, 1, 0	1, 1, 0	5, 6, 0	9, 1, 0
	Depth (µm)	322	313	272	310	160
		(266, 430)	(262, 434)	(242, 398)	(237, 381)	(81, 217)
	% of CCT	60	56	49	55	30
		(51, 82)	(51, 81)	(46, 75)	(45, 76)	(14, 41)
4.0 mm	0, N/A, Max	2, 2, 0	5, 0, 0	5, 1, 0	8, 8, 0	9, 2, 0
	Depth (µm)	281	253	224	218	94
		(252, 384)	(228, 364)	(199, 332)	(188, 318)	(43, 179)
	% of CCT	51	48	39	39	16
		(46, 71)	(42, 67)	(37, 60)	(36, 59)	(8, 33)
	p-value*	n.s.	<0.01	<0.001	<0.01	n.s.
	p-value†	n.s.	<0.01	<0.001	<0.01	n.s.

% of CCT = Depth as percentage of central corneal thickness, 0, N/A, Max = Number of eyes with no detectable light scattering (0), immeasurable line (N/A), light scattering measured through cornea (Max), Depth = Light scattering depth in absolute values, n.s. = not statistically significant.
* Between-group comparisons in depths.
† Between-group comparisons in % of CCT.

Table 3. Higher order aberrations

		Baseline	1 month		3 months		6 months		12 months		24 months	
			-	p-value*		p-value*		p-value*		p-value*		p-value*
Total HOA (μm)	3.5 mm	0.23 (0.14, 0.30)	0.27 (0.22, 0.31)	n.s.	0.25 (0.21, 0.31)	n.s.	0.21 (0.17, 0.27)	n.s.	0.23 (0.18, 0.31)	n.s.	0.23 (0.16, 0.33)	n.s.
	4.0 mm	0.21 (0.17, 0.32)	0.28 (0.21, 0.35)	n.s.	0.26 (0.19, 0.28)	n.s.	0.25 (0.17, 0.31)	n.s.	0.20 (0.15, 0.29)	n.s.	0.18 (0.12, 0.22)	n.s.
	p-value†	n.s.	n.s.		n.s.		<0.05		n.s.		<0.05	
SA (μm)	3.5 mm	−0.01 (−0.04, 0.12)	0.10 (0.07, 0.17)	<0.01	0.10 (−0.01, 0.14)	n.s.	0.06 (0.03, 0.13)	n.s.	0.09 (0.05, 0.13)	n.s.	0.06 (0.01, 0.11)	n.s.
	4.0 mm	0.02 (−0.03, 0.20)	0.14 (0.04, 0.22)	n.s.	0.07 (0.04, 0.16)	n.s.	0.10 (0.04, 0.21)	n.s.	0.09 (0.05, 0.16)	n.s.	0.04 (0.02, 0.13)	n.s.
	p-value†	n.s.	n.s.		n.s.		<0.05		n.s.		n.s.	
Pupil diameter (mm)	3.5 mm	5.33 (4.68, 6.69)	5.70 (4.40, 6.42)	n.s.	5.29 (4.55, 6.17)	n.s.	5.52 (4.32, 5.90)	n.s.	5.11 (4.17, 6.02)	n.s.	5.37 (3.97, 6.02)	n.s.
	4.0 mm	5.65 (4.99, 6.57)	5.77 (4.84, 6.40)	n.s.	5.48 (4.36, 6.20)	n.s.	5.51 (4.50, 6.22)	n.s.	5.24 (4.39, 6.24)	<0.01	4.91 (3.73, 6.55)	n.s.
	p-value†	n.s.	n.s.		n.s.		n.s.		n.s.		n.s.	

Total HOA = total higher order aberrations, SA = spherical aberrations, n.s. = not statistically significant.
* Within-group comparisons between baseline and each other time point.
† Between-group comparisons at each time point.

Table 4. Safety outcomes

		Baseline	1 month		3 months		6 months		12 months		24 months	
			-	p-value*		p-value*		p-value*		p-value*		p-value*
ECC (cells/mm^2)	3.5 mm	3056 (2872, 3326)	3078 (2816, 3178)	n.s.	2981 (2815, 3220)	n.s.	3062 (2912, 3198)	n.s.	3107 (2857, 3367)	n.s.	2934 (2644, 3163)	n.s.
	4.0 mm	3146 (0.17, 0.32)	3092 (0.21, 0.35)	n.s.	3069 (0.19, 0.28)	n.s.	3101 (0.17, 0.31)	n.s.	3089 (0.15, 0.29)	n.s.	2940 (0.12, 0.22)	n.s.
	p-value†	n.s.	n.s.		n.s.		n.s.		n.s.		n.s.	
BSCVA (logMAR)	3.5 mm	−0.08 (−0.10, 0.00)	−0.06 (−0.10, −0.02)	n.s.	-0.08 (−0.12, −0.03)	n.s.	−0.06 (−0.10, 0.00)	n.s.	−0.08 (−0.12, 0.04)	n.s.	−0.06 (−0.11, −0.01)	n.s.
	4.0 mm	−0.06 (−0.10, 0.00)	−0.04 (−0.08, 0.00)	n.s.	−0.06 (−0.12, 0.00)	n.s.	−0.06 (−0.12, −0.02)	n.s.	−0.06 (−0.10, −0.04)	n.s.	−0.06 (−0.09, −0.02)	n.s.
	p-value†	n.s.	<0.05		n.s.		n.s.		n.s.		n.s.	

BSCVA = Best spectacle corrected visual acuity, ECC = Endothelial cell count, logMAR = Logarithm of minimum angel of resolution, n.s. = not statistically significant.
* Within-group comparisons between baseline and each other time point.
† Between-group comparisons at each time point.

potentially being more pronounced with a smaller treatment protocol, analogous to the decreased contrast sensitivity and increase in SA seen after some corneal laser refractive surgery protocols (Fan-Paul et al. 2002; Özülken & Kaderli 2020). Notably, however, the deterioration in LCVA diminished over time, and at

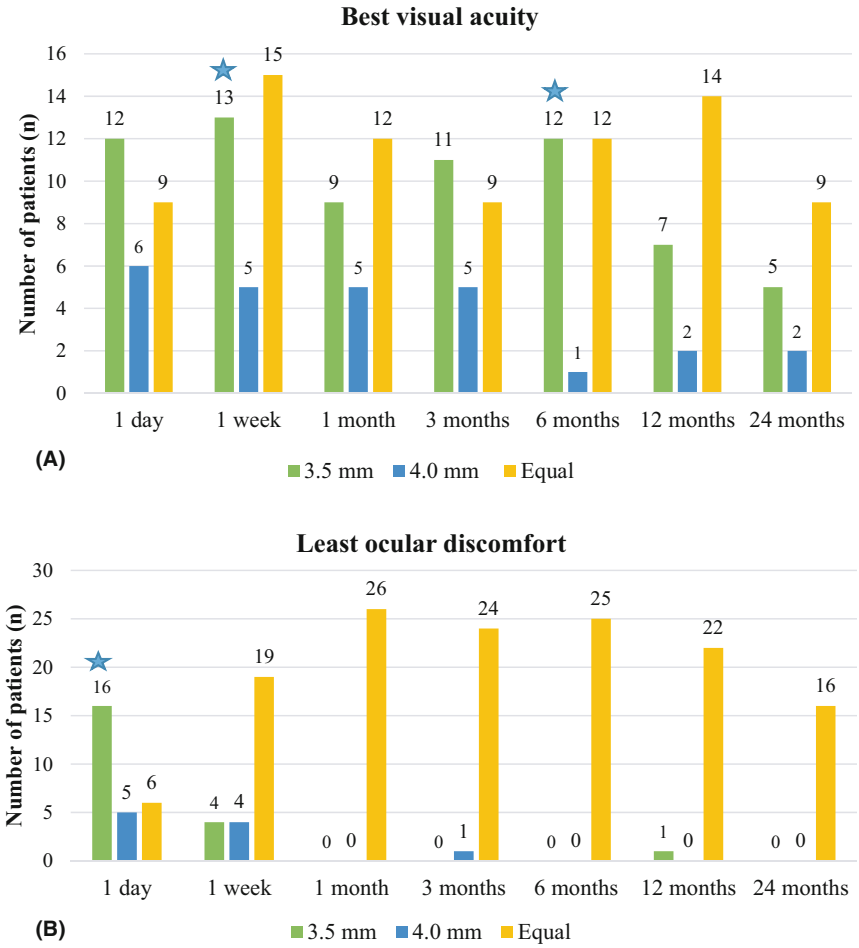

Fig. 3. Subjectively assessed best visual acuity (A) and subjectively assessed least ocular discomfort (B) through 24 months posttreatment. Number of patients (*n*) that preferred the homogenous protocol, annular protocol or deemed the eyes equal. Blue asterisk denotes $p < 0.05$ for between-group comparisons at the time-point in question.

24 months the LCVA:s were not statistically significant different from baseline values. Likely, this is explained by a combination of corneal remodelling and reduced posttreatment haze and possibly other factors such as neural adaptation.

Light scattering was assessed manually from the Pentacam HR® Scheimpflug photos using the 'Corneal Optical Densitometry' display in Pentacam HR® in an attempt to assess and compare the treatment effects, in accordance with the demarcation line assessments used in other studies to evaluate the treatment effect in CXL (Emanuele et al. 2016). The light scattering depths were deeper with the 3.5 mm protocol at 3 to 12 months which correlates to the better treatment effect, although a deeper depth also can be explained by higher illumination closer to the corneal centre due to the corneal curvature (Koller et al. 2013; Rehnman et al. 2015), in accordance with the results in our previous study, where deeper light scattering in a 4.0 mm homogenous protocol did not correspond to larger treatment effect (Näslund et al. 2020). The light scattering depth was deeper for the 4.0 mm protocol in the previous study compared with this study, which reasonably can be explained by a variable individual reaction to the cross-linking and also likely supports that the Scheimpflug-based method we used may not be so accurate and reproducible in assessing the treatment effects. Possibly, AS-OCT or confocal microscopy may give more reliable results (Thorsrud et al. 2017). In our present study, however, each subject was treated with both protocols, which likely reduces the individual variations, and we still feel that our light scattering data may be of some use for comparing our two treatment protocols within this study. In addition, no flattening effect in keratometry readings using Pentacam HR® was detected with the two present protocols, which is in alignment with the results from our previous study using a 4.0 mm annular protocol. This discrepancy lends further support to that these findings are limitations in the keratometry algorithm of the Pentacam HR®, since the myopia is obviously reduced despite unaltered keratometry values. It could be that the formation of central haze, in the border of the illumination zones, influences the ability of the Pentacam HR® to detect a posttreatment flattening of the corneal curvature (Shetty et al. 2017). Although, other explanations may exist due to the disagreement between unchanged keratometry values at later visits and the decline of posttreatment haze. Unfortunately, the same findings were seen with the unaltered Pentacam HR® values and obvious change in myopia in this study, and

as we did not have access to an AS-OCT, the Pentacam HR® data was therefore chosen not to be included. Further studies are needed to evaluate the possible limitations of Pentacam HR® in measuring the treatment effects after corneal cross-linking.

No adverse events, and no reductions in ECC or BSCVA were noted, which confirm previous findings that PiXL at 15 J/cm^2 is a safe refractive treatment for low-grade myopia (Elling et al. 2017, 2018; Lim et al. 2017; Nordstrom et al. 2017; El Hout et al. 2019; Fredriksson et al. 2019; Näslund et al. 2020; Sachdev et al. 2020).

A limitation of the study was the large lost-to follow up. Unfortunately, the outbreak of the Covid-19 pandemic, which interfered with the last visit in our study, may have contributed to this. Hence, the multiple imputation model was chosen to handle missing data. Another limitation is that we did not have an AS-OCT to assess the treatment effect in both protocols.

In conclusion, PiXL appears to be an efficient treatment to improve uncorrected vision and reduce low-grade myopia in healthy eyes without any adverse events. An augmented treatment effect and a favourable reduction in posttreatment discomfort may be achieved with a smaller treatment zone, but likely at the expense of a transient decrease in low contrast visual acuity. Future studies with larger cohorts are needed to optimize the treatment parameters for PiXL as a refractive treatment.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Visual and refractive outcomes.
Table S2 Higher order aberrations.
Table S3 Safety outcomes.