



# Comparison of the effects of femtosecond laser energy on corneal endothelium at two different dissection levels in femtosecond laser-assisted deep anterior lamellar keratoplasty for keratoconus

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## Abstract

**Purpose** The objective of this study is to compare the endothelial safety of femtosecond laser (FSL) energy at two different dissection depths in FSL-assisted deep anterior lamellar keratoplasty (FSDALK) for keratoconus.

**Methods** This prospective double-armed interventional study included 25 eyes from 21 patients with advanced keratoconus who underwent FSDALK (big bubble technique) at a trephination depth of 110  $\mu\text{m}$  in group I (11 eyes) and 80  $\mu\text{m}$  in group II (14 eyes)—all of which were anterior to the Descemet's membrane (DM). Visual acuity measurement, anterior and posterior segment examination, corneal tomography, and specular microscopy were performed preoperatively and at 3, 6, and 12 months, postoperatively. Endothelial cell density, coefficient of variation, percentage of cell hexagonality, and pachymetry were used to evaluate endothelial safety.

**Results** No statistically significant difference in any of the endothelial safety parameters was found between the two groups. The mean 12-month postoperative endothelial cell loss rate was 17.46% and 12.91% in group I and II, respectively ( $P = 0.345$ ). Most of the endothelial cell loss occurred during the

first 3 months after surgery. Group II showed statistically greater improvement in the mean keratometry values at all follow-up visits.

**Conclusion** The endothelial safety profiles of lamellar FSL cuts at 110  $\mu\text{m}$  and 80  $\mu\text{m}$  anterior to the DM are comparable. Cuts as deep as 80  $\mu\text{m}$  anterior to the DM can be safely applied without causing significant injury to the endothelium. Further studies are needed to compare the endothelial safety profiles of different FSL platforms available in the market. *Trial registration* PACTR201901615323963. Registered 24 November 2018—retrospectively registered.

**Keywords** Keratoconus · Deep anterior lamellar keratoplasty · Femtosecond laser · Corneal endothelium · Specular microscopy

## Introduction

Keratoconus is a non-inflammatory progressive ectatic corneal disorder, characterized by thinning and conical protrusion of the corneal stroma, which results in the development of progressive myopia and irregular astigmatism [1]. The annual incidence of keratoconus in the general population is approximately 1 per 2000, and the prevalence is estimated to be 138 per 100,000 [2, 3]. Despite comparable visual outcomes, deep anterior lamellar keratoplasty (DALK) has surpassed penetrating keratoplasty (PK) in the management of

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advanced cases of keratoconus for many reasons [4, 5]. It is a closed-globe surgery that preserves the patient's healthy endothelium with a lower cell loss rate compared to that of a graft preserved in culture medium. Furthermore, it abolishes the risk of endothelial rejection and induces less ocular hypertension due to a shorter course of topical steroids [6]. Moreover, DALK offers better structural integrity in the setting of potential blunt trauma when compared to PK [7]. Besides, the median predicted graft survival period is 49.0 years in DALK versus 17.3 years in PK [8].

The main disadvantage associated with DALK is the difficulty of the surgical procedure and the steep learning curve. The technique is more challenging due to the need to remove the recipient's corneal stroma down to the Descemet's membrane (DM). This is essential to obtain a smoother interface, which is critical to optimize visual outcomes [6].

Anwar and Teichmann described the big bubble technique for DM baring in DALK. This technique made DM exposure easier and less time-consuming than the manual layer-by-layer dissection technique [9]. Dua et al. classified the types of big bubbles that can be achieved in DALK. Type 1 bubble separates the pre-Descemet (Dua's) layer from the deep stroma. It is a central, well-circumscribed bubble, with a diameter of 8–9 mm, and surrounded by white edges. Type 2 bubble separates the DM from the posterior surface of the Dua's layer. It is a larger, thin-walled bubble, with clear edges, that spreads from the periphery to the center. Moreover, both types of big bubbles may coexist resulting in a mixed bubble [10, 11].

The advent of femtosecond laser (FSL) technology has added precision and reproducibility to corneal surgery and keratoplasty. Predefined corneal incisions with customized graft edges for both donor and recipient corneas can be created by FSL-assisted trephination [12]. The advantages of such interfaces include an improved donor-recipient alignment, a potential reduction in the number of sutures, a reduction in induced astigmatism, and a faster post-operative recovery [7].

FSL-assisted DALK (FSDALK) has permitted the standardization of the procedure by increasing its safety and reproducibility [7]. The precise depth of the laser-assisted side cuts may increase the success rate of DM baring using the big bubble technique. It allows accurate identification of tissue depth and insertion of the air needle by following the plane between the

lamellar and posterior laser side cuts. Injection of air at this precisely predefined pre-Descemet plane facilitates the big bubble formation. Moreover, FSDALK abolishes the risk of inadvertent perforation of the DM during trephination [13, 14]. However, the rough interfaces of the deep lamellar FSL incisions and the potential notorious effect to endothelial cells if the cuts are too close to DM remain a major concern. Thus, stromal-DM separation with air or other modalities may still be a necessary part of FSDALK surgery to optimize visual outcomes and preserve the host endothelium [15, 16].

To the best of our knowledge, this is the first prospective study to compare the effects of FSL on the corneal endothelium at two different dissection depths in FSDALK for keratoconus.

## Materials and methods

This is a prospective, double-armed, interventional clinical study that was conducted at Ain Shams University Hospital, Cairo, Egypt, from May 2018 to December 2019. The recruited cohort included eyes with grade III or IV keratoconus according to the Amsler–Krumeich classification [17]. The patients' age group was 18–40 years. This study adhered to the tenets of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (Approval No. MD133/2018). Additionally, an informed consent form was signed by all participants after they received a detailed explanation of the procedures.

The exclusion criteria included eyes with associated ocular diseases, such as Fuch's endothelial dystrophy, glaucoma, uveitis, or retinitis pigmentosa. Patients with a history of ocular surgery or trauma were also excluded. Moreover, eyes with apical corneal scarring or very steep cones that precluded the preoperative specular microscopy were discarded.

Preoperatively, uncorrected distance visual acuity (UDVA) and corrected distance visual acuity (CDVA) were measured using a Snellen chart and then converted to a logarithm of minimum angle of resolution (logMAR) notation for statistical analysis. All eyes underwent a detailed slit-lamp examination, funduscopy, corneal tomography using Sirius (Costo-ruzione Strumenti Oftalmici, Florence, Italy) and specular microscopy using non-contact autofocus

SP-1P (Topcon corp., Tokyo, Japan). Three corneal endothelial photographs were taken from each eye, and the average endothelial cell density (ECD), coefficient of variation in cell size (CV), and percentage of hexagonal endothelial cells (%Hex) were reported.

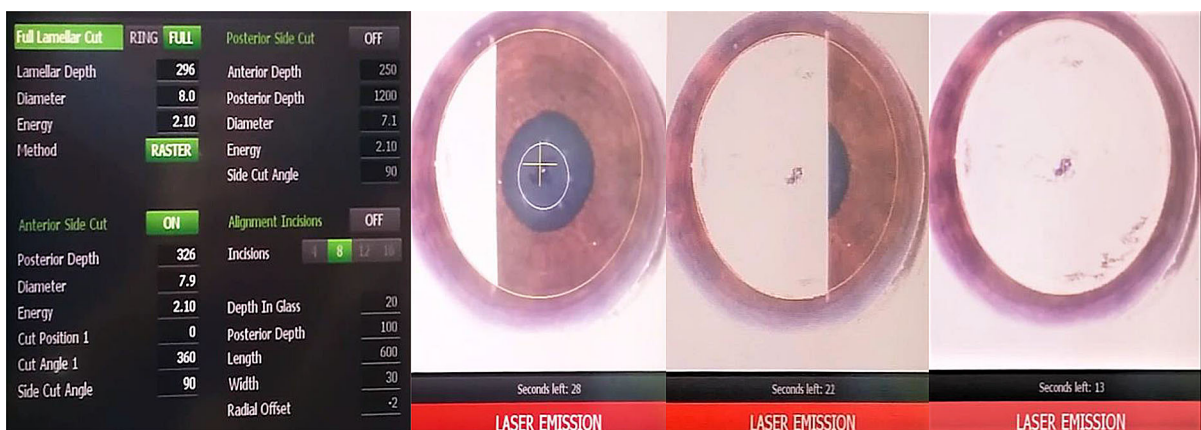
### Surgical technique

All procedures were performed by a single experienced surgeon (S.A.H). The surgical technique was performed in two steps. The first step was carried out in the laser room, where the 150 kHz IntraLase iFS (Johnson & Johnson Inc., Santa Ana, CA, USA) was used to cut the recipient cornea (Fig. 1). Under topical anesthesia (benoxinate 0.4% eye drops administered three times, one drop every 5 min), the recipient eye was stabilized with a disposable suction cone positioned at the limbus. After the docking, FSL was used to create a lamellar cut in the cornea, and according to its depth, the participants were divided into two groups. Random allocation of the eyes into the two groups was done using a coin toss (heads—group I, tails—group II). In group I, the FSL cut was created at a depth of 110  $\mu\text{m}$  anterior to the DM at the thinnest location; in group II, the depth was 80  $\mu\text{m}$  anterior to the DM at the thinnest location. The thinnest locations were determined in the two groups according to the pachymetric maps of the preoperative corneal tomography study. Anterior side cuts were created in both groups at a diameter of 7.9 mm and a cut angle of 90°. The FSL energy used was set to 2.10  $\mu\text{J}$  in all cases.

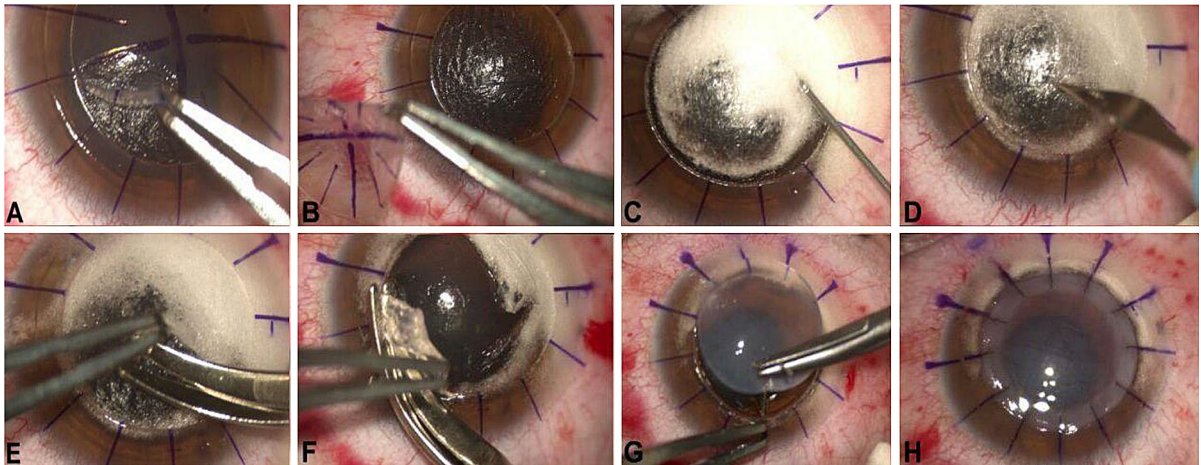
In the second step, the patients were transferred to the surgery room, and the remaining surgery was performed under retrobulbar block anesthesia using 3–5 ml of a 1:1 mixture of lidocaine 1% and bupivacaine 0.5% with light sedation. The patients were then draped in the usual manner. A screw speculum was used to open the lids, and 5% betadine was instilled in the conjunctival sac for 3 min and then thoroughly washed by a balanced salt solution. The previously dissected stromal cap was removed, and an anterior chamber (AC) paracentesis was made. A 27-gauge bent needle attached to a 5-mL syringe filled with air was inserted bevel-down into the deep stroma to create a big bubble. A spatula was used to decrease the intraocular pressure (IOP) through the paracentesis. The roof of the big bubble was slashed using a 15-degree disposable knife, and the cut was extended in four directions using blunt corneal scissors. Then, the stromal quadrants were excised to expose the DM.

The donor tissue was soaked in trypan blue 0.06% stain for 3 min before peeling off the DM; it was then punched using an 8 mm (0.1 mm oversize) donor non-vacuum trephine (Tecfen Medical, Santa Barbara, CA, USA). The donor lamella was then placed over the host recipient bed and sutured using 12 interrupted 10/0 nylon sutures (Fig. 2).

Postoperatively, the patients received topical moxifloxacin 0.5% eyedrops 3 times daily for 10 days and prednisolone acetate 1% eyedrops 6 times daily for a week, which were gradually tapered over a year. A follow-up was performed on postoperative days 1, 7, 14, and 28. It was then performed on a monthly basis for 6 months and then every 3 months for a year.



**Fig. 1** FSL-assisted recipient cornea trephination



**Fig. 2** **a** and **b** Peeling of the stromal cap. **c** Creation of the big bubble. **d** Slashing the roof of the big bubble. **e** Extending the cut using blunt corneal scissors. **f** Excision of the stromal quadrants.

**g** Placement of the first cardinal suture. **h** Securing the donor tissue by 12 interrupted 10/0 nylon sutures

The laser settings and any intraoperative complications were recorded. Postoperative evaluation, including visual acuity, corneal tomography, and specular microscopy, were reported at 3, 6, and 12 months, postoperatively. Corneal tomography was used to assess pachymetry, graft-host alignment, and to evaluate for selective suture removal. Total suture removal was performed between 12 and 15 months, postoperatively. Specular microscopy was used to assess the host corneal endothelium as described in the preoperative phase.

#### Statistical analysis

The Kolmogorov–Smirnov test was used to assess the normality of data. Parametric continuous variables were expressed as mean and standard deviation (SD). Independent samples Student's *t* test was used to compare the quantitative variables of the two study groups, and paired samples Student's *t* test was used to compare variables within the same study group. Qualitative variables were described in the form of numbers and percentages. Chi-square test was used to compare the qualitative data. Nonparametric quantitative data were expressed as median and interquartile range (IQR) with a 95% confidence interval for the mean. A comparison between the variables of dependent samples was performed using Wilcoxon test, and comparisons between variables of two independent samples were performed using the Mann–Whitney

test. Spearman's correlation coefficient was used to assess the correlation between different variables. The level of significance was set at a *P*-value  $\leq 0.05$ . Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS Statistics for Windows, version 23.0. Armonk, NY: IBM Corp.)

#### Results

Twenty-five eyes of twenty-one patients with advanced keratoconus underwent FSDALK using the big bubble technique. FSL was used to create lamellar cuts aiming for a residual stromal thickness of 110  $\mu\text{m}$  in group I ( $n = 11$ ) and 80  $\mu\text{m}$  in group II ( $n = 14$ ), all of which were anterior to DM at the thinnest location. The mean patient age was  $26.27 \pm 8.71$  years in group I and  $22.43 \pm 4.60$  years in group II; 60% of them were female. All preoperative parameters assessed were comparable in the two groups (Table 1). The FSL energy was set to 2.10  $\mu\text{J}$  in both groups, and the mean FSL time was  $29.27 \pm 0.79$  and  $28.93 \pm 1.14$  s in groups I and II, respectively ( $P = 0.852$ ). Type 1 bubble was achieved in 81.8% and 85.7% of the eyes in group I and II, respectively ( $P = 0.792$ ). DM baring was performed in the remaining eyes—either by creating a type 2 bubble or by manual dissection.

**Table 1** Comparison of the preoperative data in the two groups

Preoperative parameter	Group I <i>n</i> = 11	Group II <i>n</i> = 14	<i>P</i> -value
Age (years)	26.27 ± 8.71	22.43 ± 4.60	0.168
MRSE (diopters)	- 12.86 ± 7.39	- 10.22 ± 4.26	0.607
UDVA (logMAR)	1.1 ± 1.1	1.1 ± 1.4	0.304
CDVA (logMAR)	0.8 ± 0.96	0.77 ± 1.1	0.285
K1 (diopters)	54.57 ± 5.26	57.89 ± 9.41	0.306
K2 (diopters)	61.71 ± 7.14	63.54 ± 10.21	0.619
K mean (diopters)	57.91 ± 5.94	60.52 ± 9.58	0.438
K max (diopters)	73.57 ± 10.37	72.80 ± 11.61	0.864
Topographic astigmatism (diopters)	7.15 ± 3.10	5.65 ± 3.48	0.275
Pachymetry at apex (μm)	405.64 ± 37.79	384.79 ± 52.37	0.278
Thinnest location (μm)	368.73 ± 43.42	339.07 ± 52.56	0.145
ECD (cells/mm <sup>2</sup> )	3310.82 ± 411.00	3326.86 ± 239.86	0.904
CV	39.73 ± 3.64	39.07 ± 3.15	0.634
% Hex	40.55 ± 5.59	42.21 ± 4.92	0.436

All values are given in mean ± standard deviation  
*MRSE* Manifest refractive spherical equivalent, *UDVA* Uncorrected distance visual acuity, *CDVA* Corrected distance visual acuity, *ECD* Endothelial cell density, *CV* Coefficient of variation, *%Hex* Percentage of hexagonality of endothelial cells

### Functional outcome

CDVA improved to  $0.2 \pm 0.7$  logMAR and  $0.21 \pm 0.7$  logMAR at 12 months postoperatively ( $P = 0.698$ ) in groups I and II, respectively. The mean topographic astigmatism was  $3.79 \pm 2.01$  diopters (D) and  $4.16 \pm 2.27$  D at 12 months postoperatively in groups I and II, respectively. The mean keratometric power improved from  $57.91 \pm 5.94$  D and  $60.52 \pm 9.58$  D, preoperatively, to  $44.82 \pm 1.47$  D and  $43.13 \pm 1.30$  D at 12 months, postoperatively ( $P = 0.006$ ) in groups I and II, respectively. Group II showed a greater statistically significant improvement in the mean keratometric power at all follow-up visits. Table 2 summarizes the progression of the CDVA and

the mean keratometric power, preoperatively, and at 3, 6, and 12 months postoperatively, in the two groups.

Figure 3 shows the anterior sagittal maps and Scheimpflug images of a case, preoperatively, and at 3, 6, and 12 months postoperatively—all of which demonstrate a marked improvement in mean keratometric power and topographic astigmatism.

### Endothelial safety

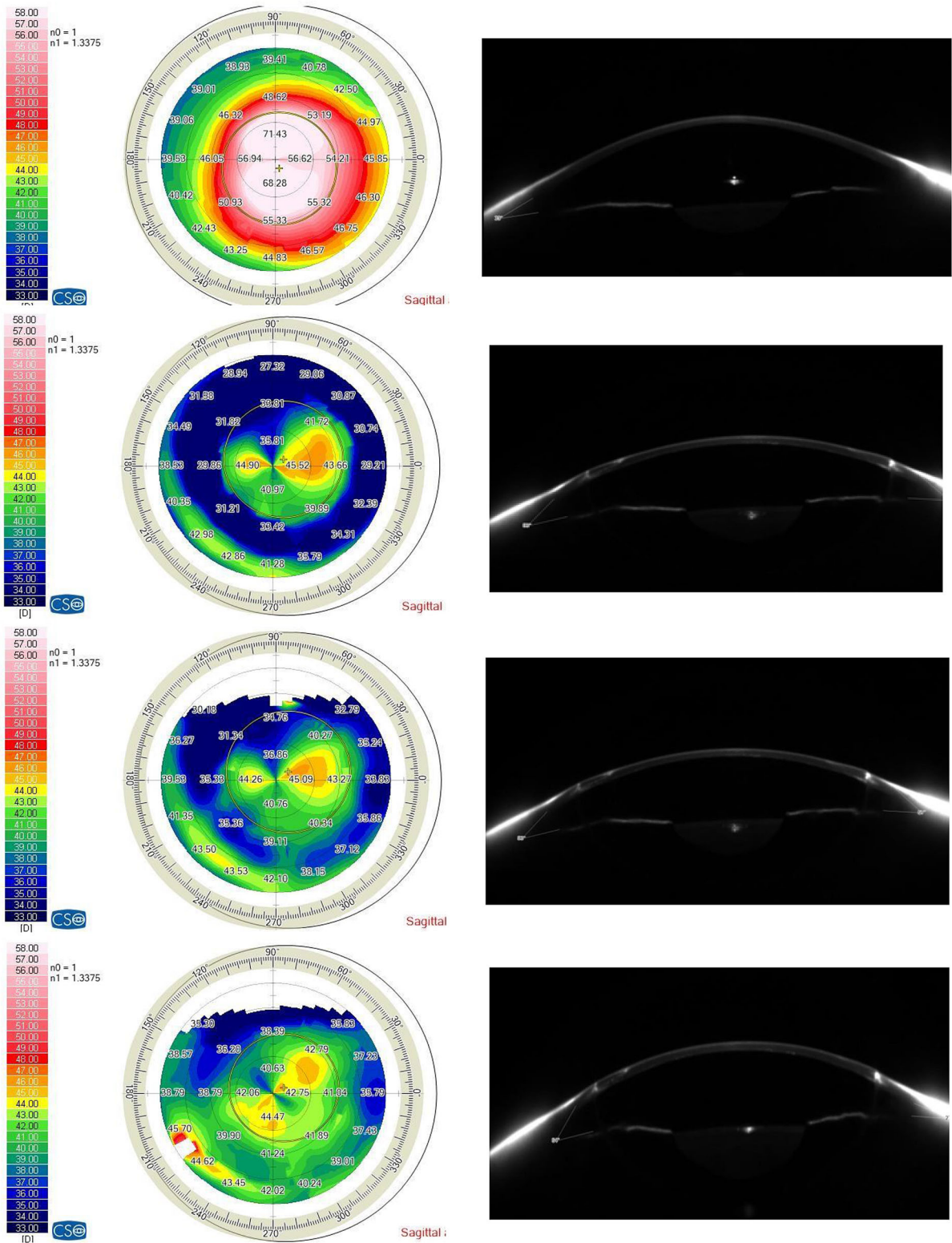
The 12-month postoperative ECD loss rate was 17.46% and 12.91% in groups I and II, respectively ( $P = 0.345$ ). Most of the endothelial cell loss occurred during the first 3 months after surgery in both groups. Moreover, there were no statistically significant differences in the mean ECD, CV, or %Hex

**Table 2** Progression of the functional outcome

	CDVA (logMAR)			<i>K</i> mean (D)		
	Group I	Group II	<i>P</i> -value	Group I	Group II	<i>P</i> -value
Preoperative	0.8 ± 0.96	0.77 ± 1.1	0.285	57.91 ± 5.94	60.52 ± 9.58	0.438
M3	0.55 ± 0.85	0.55 ± 0.89	0.911	44.26 ± 2.19	42.49 ± 2.03	0.048
M6	0.38 ± 0.68	0.41 ± 0.8	0.522	44.46 ± 2.27	42.80 ± 1.51	0.039
M12	0.2 ± 0.7	0.21 ± 0.7	0.698	44.82 ± 1.47	43.13 ± 1.30	0.006

All values are given in mean ± standard deviation

*CDVA* corrected distance visual acuity, *logMAR* logarithm of the minimum angle of resolution, *K mean* mean keratometric power, *D* diopter, *M3* 3rd postoperative month, *M6* 6th postoperative month, *M12* 12th postoperative month



**Fig. 3** Anterior sagittal maps and Scheimpflug images of a case, preoperatively, and at 3, 6, and 12 months postoperatively

progression between the two groups during the follow-up period. The mean central corneal thickness (CCT) was  $541.82 \pm 29.58$  and  $538.00 \pm 26.70$   $\mu\text{m}$  at 12 months postoperatively ( $P = 0.738$ ) in groups I and II, respectively. All grafts remained well-compensated, and pachymetric measurements were stable in both groups throughout the follow-up period. Table 3 summarizes the progression of the endothelial safety parameters in the two groups at 3, 6, and 12 months, postoperatively.

#### Adverse effects

Two patients (8%) experienced DM microperforations during surgery; one occurred in each group, but they did not convert to PK. Five patients (20%) had a double AC during their first postoperative visit; they were all successfully managed by AC rebubbling on the same day. One patient had an acute graft melting 1 month, postoperatively, and was excluded from the study and statistical analysis. Seven patients (28%) developed steroid-induced elevation in the IOP—three in group I and four in group II; they were managed medically with anti-glaucoma eyedrops. Eight patients (32%) were found to have single or multiple loose sutures during the postoperative follow-up period, which required removal using the slit-lamp.

#### Discussion

DALK has become the ‘gold standard’ for the management of advanced cases of keratoconus. It achieves comparable visual outcomes to PK but with a superior safety profile [18]. We found a statistically and clinically significant improvement in the CDVA, mean keratometry values and topographic astigmatism in both groups at all follow-up visits. There was no statistically significant difference in the visual or refractive outcomes between the two groups. Group II showed statistically, but not clinically, greater improvement in the mean keratometric powers at all postoperative follow-up visits. We believe that this difference is largely a suture-related phenomenon.

Successful exposure of DM is essential to optimize visual outcomes in DALK [19]. The use of FSL in DALK added the advantage of precision and reproducibility to the procedure. We noticed a high probability (96%) of achieving a successful big bubble

in our study. This may be attributed to the precise depth of the FSL cuts that guide air injection deep into the posterior stroma. Farid and Steinert [13] and Price et al. [20] reported similar results. They described a FSDALK technique using a zigzag wound configuration and a residual stromal thickness of  $70$   $\mu\text{m}$ . DM baring was achieved using the big bubble technique. Both studies suggested that the precise depth of the laser-assisted posterior side cuts might increase the chances of successful DM baring using the big bubble technique. However, no assessment of postoperative endothelial cell loss was performed in either study. We also noticed a slightly higher rate of successful type I bubble in group II of our study (85.7%). However, the difference between the two groups was not statistically significant ( $P = 0.792$ ).

Buzzonetti et al. [14] described the ‘intra-bubble’ technique where the 60 kHz IntraLase FSL was used to create an additional channel  $50$   $\mu\text{m}$  anterior to DM through which the air is injected deep into the posterior stroma to facilitate big bubble formation. However, no postoperative endothelial assessment was performed in their study to ascertain the safety of such deep FSL cuts.

Kimakura et al. [21] conducted an experimental study on rabbit corneas where they used FSL to create deep cuts leaving behind  $70$ ,  $100$ , or  $150$   $\mu\text{m}$  of residual stroma. In vivo confocal microscopy revealed that endothelial lesions were more frequently observed when the thickness of the residual stroma was less than  $70$   $\mu\text{m}$ . Therefore, this study sets up a limit to the depth of FSL cuts that can be safely applied without injuring the corneal endothelium. However, comparing the effects of FSL energy on the corneal endothelium at different dissection depths in human eyes remains an under studied topic.

We constructed our study to compare the effects of FSL cuts applied at a depth of  $110$   $\mu\text{m}$  (Group I) and  $80$   $\mu\text{m}$  (Group II) anterior to the DM in eyes with keratoconus. The mean postoperative endothelial loss rate was 15.2% after 1 year of surgery. There was neither a statistically nor a clinically significant difference between the loss rates in our two study groups at all follow-up visits. Bleriot et al. [7] reported similar results with an ECD loss rate of 14.7% in their big bubble FSDALK group using LDV Z6 (Ziemer Ophthalmic Systems, Port, Switzerland) with a residual stromal thickness ranging from  $90$  to  $120$   $\mu\text{m}$ .

**Table 3** Progression of endothelial safety parameters

	ECD (cells/mm <sup>2</sup> )			ECD loss rate (%)			CV		
	I	II	P	I	II	P	I	II	P
Preoperative	3310.82 ± 411.00	3326.86 ± 239.86	0.904	–	–	–	39.73 ± 3.64	39.07 ± 3.15	0.634
M3	2957.36 ± 590.09	3027.07 ± 311.82	0.707	10.7 ± 13.64	9.07 ± 5.32	0.679	41.09 ± 3.70	38.86 ± 2.11	0.069
M6	2838.36 ± 591.97	2950.07 ± 294.53	0.543	14.13 ± 15.17	11.34 ± 5.50	0.529	40.73 ± 4.45	38.71 ± 2.27	0.155
M12	2730.09 ± 613.74	2897.71 ± 287.88	0.374	17.46 ± 16.63	12.91 ± 5.46	0.345	40.55 ± 4.18	39.00 ± 1.92	0.230
	%Hex								
				CCT (µm)					
	I	II	P	I	II	P	I	II	P
Preoperative	40.55 ± 5.59	42.21 ± 4.92	0.436	405.64 ± 37.79	384.79 ± 52.37	0.278	384.79 ± 52.37	384.79 ± 52.37	0.278
M3	37.64 ± 5.48	40.79 ± 2.42	0.066	545.64 ± 37.81	539.14 ± 30.24	0.637	539.14 ± 30.24	539.14 ± 30.24	0.637
M6	38.64 ± 5.03	41.29 ± 2.02	0.084	538.18 ± 31.63	538.07 ± 27.02	0.993	538.07 ± 27.02	538.07 ± 27.02	0.993
M12	38.73 ± 4.92	41.00 ± 1.66	0.118	541.82 ± 29.58	538.00 ± 26.70	0.738	541.82 ± 29.58	538.00 ± 26.70	0.738

All values are given in mean ± standard deviation

ECD endothelial cell density, CV coefficient of variation, %Hex percentage of hexagonal endothelial cells, CCT central corneal thickness, M3 3rd postoperative month, M6 6th postoperative month, M12 12th postoperative month

Our study showed higher endothelial loss rates than those reported by Lu et al. [22] (6.4%) and Chen et al. [19] (9.1%). This may be partially attributed to the difference in the FSL platforms used. We used the low frequency/high energy pulse (1000nJ) iFS IntraLase (Johnson & Johnson Inc., Santa Ana, CA, USA), whereas they used the high frequency/low energy pulse (170nJ) VisuMax (Carl Zeiss Meditec AG, Jena, Germany) [12]. Higher energy FSL platforms may induce more endothelial cell damage—especially with the deep cuts applied in FSDALK. However, Bleriot et al. [7] reported a mean endothelial loss rate comparable to that of our study despite using a low energy FSL platform, LDV Z6 (< 50 nJ). More studies are needed to compare the effects of different FSL platforms on the postoperative endothelial cell loss.

We reported a rate of 8% for intraoperative microperforations without conversion to PK. Shehadeh-Mashor et al. [23] reported a similar rate of 8.3%, whereas Chen et al. [19] reported a rate of 12%. A higher rate was reported by Fung et al. [24] where 3 out of 9 cases (33%) had microperforations with two conversions to PK. It is possible that a surgeons' learning curve and experience level are major factors in the occurrence of intraoperative microperforations. We noticed that the two cases with intraoperative microperforations had significantly higher endothelial loss, especially when the perforation was central. Kubaloglu et al. [25] reported similar findings in their study.

We noticed that some cases showed postoperative improvement in the endothelial cell pleomorphism and polymegathism indices. There was a slight improvement in the CV and %Hex in these cases. We believe that the improved corneal back-surface curvature and biomechanics after DALK may result in an apparent improvement in endothelial cell shape and symmetry. Salouti et al. [26] reported that ECD may show a slight increase in some cases after an uncomplicated DALK. Linear regression showed a weak but significant correlation between this increase and the change in keratometry, postoperatively. They attribute this apparent increase in ECD to crowding of endothelial cells in the smaller posterior graft surface. This was supported by the fact that these cases showed a decrease in the mean cell area. Nevertheless, very steep cones that preclude preoperative specular microscopy may be the reason why this paradox occurred in only a few cases. Moreover, postoperative

suture-induced corneal astigmatism may affect corneal endothelial image quality and lead to increased cellular pleomorphism and polymegathism.

Given the advantages of FSL in DALK, the cost-effectiveness of FSDALK remains a major concern. In our study, we proposed a novel technique where the donor tissue was prepared manually using a regular non-vacuum trephine, and only the recipient cornea was prepared using laser-assisted trephination. This is achieved by creating the FSL anterior side cuts in the recipient cornea at an angle of 90°. We oversized the donor graft by 0.1 mm by creating the recipient FSL side cuts at a diameter of 7.9 mm and using an 8-mm punch trephine for the donor tissue. Scheimpflug imaging showed good graft-host alignment in all cases. By saving the cost of single-use artificial anterior chambers and disposable FSL-machine cones, this hybrid technique renders FSDALK a more cost-effective procedure without losing the advantage of the precise depth of FSL cuts.

#### Study limitations

The study sample is small and the follow-up period is relatively short. Inherent errors in the automated specular microscope may affect the results. The postoperatively altered corneal curvature and biomechanics may confound the outcomes. Some endothelial cell polymegathism indices were not reported such as the mean cell area and its standard deviation. Various forms of surgical trauma that may affect the endothelial outcome, such as the number of air injection attempts during the big bubble creation, were not reported.

#### Conclusion

Our study reveals that deep FSL-assisted trephinations may increase the chance of successful big bubble formation and facilitate DM baring in DALK surgery for keratoconus. The endothelial safety profiles of lamellar FSL cuts at 110  $\mu\text{m}$  and 80  $\mu\text{m}$  anterior to the DM are comparable. Cuts as deep as 80  $\mu\text{m}$  anterior to the DM can be safely applied without causing significant injury to the endothelium. Further studies are needed to compare the safety profiles of different FSL platforms available in the market.

**Authors' contribution** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mustafa Saber, Ashraf Soliman and Walid El-Zawahry. The first draft of the manuscript was written by Mustafa Saber, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Data availability and material** All data and material are available if required.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This study adhered to the tenets of the Declaration of Helsinki (1964) and was approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (Approval No. MD133/2018).

**Informed consent** Informed consent was obtained from all individual participants included in this study after they received a detailed explanation of the procedures involved.

**Consent to publish** Participants provided informed consent for publication of the images in Figs. 1, 2, and 3. All participants have consented to the submission of the study to the journal.

**Code availability** Available if required "Sirius (Costruzione Strumenti Oftalmici, Florence, Italy)"

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