

Clinical Outcomes Of Descemet Membrane Endothelial Keratoplasty Using The Bonfadini-Todd Injector For Graft Insertion

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Division of Cornea, Cataract and External Diseases, Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA **Purpose:** To evaluate the clinical outcomes of using an Alcon intraocular lens (IOL) B cartridge for graft insertion during Descemet membrane endothelial keratoplasty (DMEK). **Patients and methods:** We retrospectively reviewed medical charts of patients who underwent DMEK using the Bonfadini-Todd injector, composed of an Alcon IOL B cartridge connected to plastic tubing and a syringe, for graft insertion between May 2016 and August 2018. Patient demographics, diagnoses, donor information, visual acuity, intraocular pressure (IOP), graft position and attachment status, pachymetry, and postoperative complications were recorded.

Results: Twenty-four eyes of 23 patients with an average age of 72.8 ± 10.0 years (range, 48-87 years) were included. Mean follow-up duration was 13.3 ± 6.6 months (range, 3-26 months). Twenty-one (87.5%) patients had a primary diagnosis of Fuchs endothelial dystrophy, two (8.3%) patients had bullous keratopathy and one (4.2%) had Peter's anomaly. Sixteen (66.7%) cases combined phacoemulsification and IOL implantation. Best-corrected visual acuity improved from a median of 0.398 logMAR preoperatively to 0.097 logMAR (P <0.001) at last follow-up examination, and central corneal thickness decreased from a median of 651 μ m to 533.5 μ m (P <0.001). Nine of 24 patients (37.5%) required re-bubbling due to partial graft detachment with a mean time of 12.1 \pm 9.2 days (range, 5–35 days). One patient (4.2%) developed graft failure after re-bubbling and underwent Descemet stripping endothelial keratoplasty.

Conclusion: The Alcon IOL B cartridge for DMEK graft insertion is safe and simple. **Keywords:** Descemet membrane endothelial keratoplasty, corneal transplantation, DMEK injector

Introduction

Endothelial keratoplasty has become the leading surgical procedure for treating corneal endothelial disorders such as Fuchs' endothelial dystrophy (FED) and bullous keratopathy (BK) as it has introduced selective tissue replacement.^{1,2} While both Descemet stripping endothelial keratoplasty (DSEK) and Descemet membrane endothelial keratoplasty (DMEK) are commonly utilized,^{3,4} DMEK selectively allows for an exchange of dysfunctional Descemet membrane (DM) and endothelium with donor corneal tissue. Compared with DSEK, DMEK results in faster visual recovery, greater visual acuity, and lower graft rejection rate.^{5–7}

Non-FDA approved DMEK injectors have been used by ophthalmic surgeons in the safe and controlled delivery of the DMEK scroll into the anterior chamber (AC). An

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ideal DMEK injector is a closed system that maintains AC volume, consists of a material safe for the endothelium, and allows easy loading with minimal handling of the graft. Modified intraocular lens (IOL) injection cartridges such as the Viscoject IOL injector (Bausch & Lomb, Aliso Viejo, CA, USA), the AMO Emaerald One Series IOL cartridge (Abbott Laboratories Inc., Abbott Park, IL, USA), and Alcon IOL B cartridge (Alcon®, Forth Worth, TX, USA) and glass injectors such as the Geuder glass tube (Geuder® AG, Heidelberg, Germany) and Jones glass tube (Gunther Weiss Scientific Glassblowing Co., Inc., Portland, OR, USA) are closed-system injectors that allow for the slow, controlled delivery of the donor tissue.⁸⁻¹⁴ The Bonfadini-Todd injector, which includes an Alcon IOL B cartridge, has an advantage of being easily assembled with affordable, disposable parts that are easily transported and resistant to breaking.⁹ Injection of the donor tissue in a scroll or tri-folded configuration are two standardized techniques for the safe delivery of the DMEK graft using an Alcon IOL B cartridge. 15,16 Clinical outcomes of scroll-based DMEK using the Alcon IOL B injection cartridge are unknown. In this study, we present clinical outcomes of a series of DMEK surgeries in which the grafts are delivered into the eye with an Alcon IOL B cartridge.

Materials And Methods

Patients

This study was approved by the Institutional Review Board (IRB) at the Johns Hopkins University School of Medicine and conducted in accordance with the tenets of the Declaration of Helsinki. The IRB at the Johns Hopkins University School of Medicine waived the necessity for acquiring patient consent to review medical records retrospectively as the investigators guaranteed and assured the confidentiality of the collected data. Medical charts of patients who underwent a DMEK or combined DMEK, phacoemulsification, and IOL implantation (triple-DMEK) using the Alcon IOL B cartridge for graft insertion performed by a single ophthalmic surgeon (A.O.E) at the Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore, Maryland, USA between May 2016 and August 2018 were reviewed retrospectively. Patients, over the age of 18 years, with ≥3 months of follow-up were included. Collected data included patient demographic information, history of ocular pathology, corneal donor characteristics, pre-operative and post-operative data such as best-corrected visual acuity

(BCVA), manifest refraction, intraocular pressure (IOP), graft position and attachment status, and corneal thickness. Post-operative complications such as detachment, rejection, or failure of the graft, corneal edema, and IOP elevation, as well as the need of additional procedures such as re-bubbling or repeat keratoplasty, were recorded. Eyes in which a primary graft failure occurred during the follow-up period were included until an additional procedure was performed. Donor endothelial characteristics were supplied by the eye banks.

Surgical Technique

All pre-cut donor tissue was provided by Keralink International Eye Bank, Baltimore, USA (n=23) and SightLife Eye Bank, Seattle, WA, USA (n=1). Procedures were performed under topical anesthesia and intravenous (IV) sedation (n=22) and sub-Tenon's block with a local anesthetic mixture of 2% lidocaine and 0.75% bupivacaine (n=2) by a single ophthalmic surgeon (A.O.E). In cases with combined phacoemulsification, a paracentesis was made at 3:00 and 12:00 o'clock and intracameral lidocaine and epinephrine were injected into the AC with satisfactory dilation of the pupil. Healon® ophthalmic viscoelastic device containing 10 mg/mL of sodium hyaluronate dissolved in sodium chloride phosphate buffer (Abbott Medical Optics, Inc., Santa Ana, CA, USA) was then placed into the AC and the 2.75 mm steel keratome (Alcon®, Fort Worth, TX, USA) was used to create a triplanar corneal wound. The cystotome was used to incise the anterior capsule and a continuous circular capsulorhexis was completed using the Utrata forceps without difficulty. Hydrodissection was performed. The lens nucleus was removed using phacoemulsification and remaining cortical material was removed using irrigation and aspiration. Viscoelastic was placed in the capsular remnant and AC and the three-piece IOL was selected from preoperative calculations, placed in the B cartridge injector system, and inserted into the capsular remnant through a 3.0 mm clear corneal incision.

An 8.0 mm John DXEK/DSAEK single-ended marker (ASICO® LLC., Westmont, IL, USA) was used to create a centered mark on the epithelial surface of the cornea. Through one paracentesis, viscoelastic was placed in the AC. Through the two paracentesis incisions 90° to either side of the main wound, the inverted Sinskey hook (D.O. R.C. International, Zuidland, The Netherlands) was used to score DM for 360° along the mark previously placed on the epithelial surface. The central edge of DM was then Dovepress lghani et al

reflected and pulled into the center. Using the Sheets forceps through the main wound, an 8.0 mm diameter disc DM and pathologic endothelium were stripped and removed. Descemet membrane was scored and stripped, but the stromal bed was not scraped and an inferior peripheral iridotomy was produced using a 25-gauge needle in all cases. The scraper was then inserted through the main wound and used to roughen the peripheral 2 mm of the defect in DM. Irrigation and aspiration were used to completely remove remaining viscoelastic and the AC was reformed with balanced salt solution (BSS) PLUS® (Alcon®, Forth Worth, TX, USA).

The Bonfadini-Todd injector was assembled using an Alcon IOL B cartridge (Alcon[®], Forth Worth, TX, USA), standard IV tubing (part number MX451FL; Smiths Medical, Inc., Dublin, OH, USA), and cut with a bevel using drape scissors approximately 1.91 cm from the Luer-lock. The Luer-lock end of the cut IV tubing was attached to a BSS PLUS® filled 3-5 cm³ syringe. The cut end of the IV tubing was wedged into the loading end of the Alcon IOL B cartridge. Later, the donor DM was grasped using fine tying forceps. The donor DM-roll was stained with a 0.06% trypan blue solution (VisionBlueTM, D.O.R.C. International, Zuidland, The Netherlands) for 20 seconds and then placed back into a reservoir of BSS PLUS[®]. Fluid aspiration was used to aspirate the donor scroll into the injector cartridge. The injector cartridge was then inserted into the AC through the clear corneal incision and the donor tissue was slowly injected, after which incisions were sutured. The donor tissue was unrolled using a combination of fluid injection through the paracentesis incisions and tapping and sweeping on the epithelial surface. Once the graft was fully unfolded, the AC was filled with 20% sulfur hexafluoride (SF6) gas (n=23) or air (n=1) placed posterior to the graft. At this point, a total of 10 mins was counted, during which time the graft tissue was allowed to affix. At the end of 10 mins, the paracentesis incisions were sealed with stromal hydration. BSS PLUS® was injected through a paracentesis to reduce the 20% SF6 gas or air bubble to approximately 90% of the vertical corneal diameter. Graft loading and insertion are apparent in Video 1.

Post-Operative Management

After being transferred to the recovery room, patients remained supine for two hours and were kept upright for 10 mins, after one hour. All patients received standard post-operative topical corticosteroids and antibiotics, and were asked to spend 24 hrs supine, with breaks allowed.

Postoperatively, patients used moxifloxacin ophthalmic solution 0.5% (VIGAMOX®; Alcon®, Fort Worth, TX, USA) four times a day for one week and tapered prednisolone acetate 1.0% (Pred Forte®; Allergan, Inc., Irvine, CA, USA) over the course of one year, starting with eight times a day for the first two weeks, then four times a day up to one month postoperatively, three times a day between one and three months, twice a day between three and six months, and daily after six months.

Re-bubbling was conducted for detachment of any size or location in the graft seen postoperatively at one day, one week, two weeks, and one month. A 1 cm³ syringe attached to a 30-gauge needle was used to inject 0.1 cm³ of air superotemporally at the slit-lamp microscope, with a corresponding amount of aqueous released inferotemporally afterwards. If detachment was inferior, requiring a larger bubble, this process was repeated.

Outcomes

BCVA, manifest refraction, IOP, and central corneal thickness (CCT) were collected pre-operatively as well as at one week (range, 5-14 days), one month (range, 2 weeks - 2 months), three months (range, 2-4 months), and last exam (range, 3-26 months) after surgery. The Last Observation Carried Forward (LOCF) method was used for imputing any missing data. Follow-up appointments incorporated BCVA testing using Snellen charts, slit-lamp and funduscopic examinations, IOP measurement using slit-lamp-mounted Haag-Streit Goldmann applanation tonometer (Model AT 900 C/M; Haag-Streit, Bern, Switzerland), corneal thickness measurement using ultrasound pachymetry, and corneal topography using Scheimpflug imaging (Oculus[®], Inc., Arlington, WA, USA). Snellen BCVAs were converted to logarithm of the minimum angle of resolution (logMAR) units and graft detachment was defined as partial or full lack of adherence of the DMEK graft from the recipient's stroma bed.

Statistical Analysis

Collected data were recorded in an electronic, encrypted database generated using Microsoft Excel® 2016 (Microsoft®, Inc., Redmond, WA, USA). Demographic and clinical characteristics were analyzed for normality by Shapiro-Wilk testing, which illustrated that most variables were of non-normal distribution. Additionally, prospective dependent-independent variable relationships were analyzed for linearity and equal variance by visual inspection of data plots, which demonstrated that most relationships were not clearly linear and with equal

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variance. Therefore, non-parametric testing was performed. Changes between pre-operative and post-operative outcomes and differences in the outcomes of subgroups (FED vs BK, FED vs Peter's anomaly, BK vs Peter's anomaly, DMEK vs triple-DMEK, re-bubbling procedure vs no re-bubbling procedure) were analyzed using the Wilcoxon rank-sum test. Statistical analyses were carried out using IBM[®] SPSS[®] Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA) with *P* values <0.05 considered significant.

Results

Demographics

Twenty-four eyes of 23 patients with an average age of 72.8 ± 10.0 years (range, 48-87 years) of which 18 (75%) were females underwent DMEK surgery between May 2016 and August 2018. In 16 (66.7%) cases, combined phacoemulsification with IOL implantation was performed before the DMEK surgery. Mean incision to case finish time was 83.7 ± 40.5 mins with a range of 38 to 222 mins. FED was the predominant pre-operative indication for DMEK in 21 (87.5%) eyes, of the remaining three patients, two (8.3%) had BK and one (4.2%) had Peter's anomaly. Patient demographic and clinical characteristics as well as donor characteristics are summarized in Table 1. All surgeries were uneventful.

Clinical Outcomes

Mean follow-up duration was 13.3 ± 6.6 months (range, 3–26 months). At last examination, six (25%) patients had a follow-up duration of 3–6 months, 12 (50%) patients had a follow-up duration of 12–17 months, and six (25%) patients had a follow-up duration of 19–26 months. The median BCVA increased from 0.398 logMAR (Snellen equivalent \sim 20/50) pre-operatively to 0.748 logMAR (Snellen equivalent \sim 20/100) at one week (P = 0.02) and improved to 0.176 logMAR (Snellen equivalent \sim 20/30) at one month (P <0.001) post-operatively. Snellen BCVA improved to \geq 20/25 in 2 (8.3%) cases at one week, 9 (37.5%) cases by one month, 9 (37.5%) cases by three months, and 13 (54.2%) cases at last examination (Table 2).

There was an insignificant difference between the baseline (median, 14; range, 6–18 mmHg) and the last follow-up examination (median, 13.5; range, 9–22 mmHg) IOP (P = 0.77).

Prior to DMEK surgery, the median spherical equivalent (SE) was -1.25 diopters (D; range, -9.5-1.875 D). At last follow-up examination, a median shift of -0.438 D (range, -4-2 D) was illustrated which was statistically insignificant (P = 0.07).

Table I Demographic And Clinical Characteristics Of The Full Cohort

	All DMEK (n = 24)
Donor characteristics	-
Donor age, mean ± SD [years] Sex	64.4 ± 5.6
Female, No. (%) Male, No. (%)	10 (41.7) 14 (58.3)
Race	
Caucasian, No. (%)	20 (83.3)
Asian, No. (%)	I (4.2)
Hispanic, No. (%)	2 (8.3)
Unknown, No. (%)	I (4.2)
Graft diameter, mean ± SD [mm]	8.0 ± 0
Death to preservation time, mean ± SD [hours]	11:12 ± 3:45
Preservation to surgery time, mean ± SD [days]	4.1 ± 1.1
Pre-processing ECC, mean ± SD [cells/mm ²]	2880.1 ± 166.7
Post-processing ECC, mean ± SD [cells/mm ²]	2808.0 ± 158.5
Demographic/clinical characteristics	
Age, mean ± SD [years]	72.8 ± 10.0
Sex	
Female, No. (%)	18 (75)
Male, No. (%)	6 (25)
Race	
Caucasian, No. (%)	24 (100)
Operated eye	
OD, No. (%)	10 (41.7)
OS, No. (%)	14 (58.3)
Lens status	
Phakic, No. (%)	16 (66.7)
Pseudophakic, No. (%)	8 (33.3)
Indication for surgery	
Fuchs' endothelial dystrophy, No. (%)	21 (87.5)
Bullous keratopathy, No. (%)	2 (8.3)
Peter's anomaly, No. (%)	I (4.2)
Surgical procedure	
DMEK, No. (%)	8 (33.3)
Triple-DMEK, No. (%)	16 (66.7)

Abbreviations: DMEK, Descemet membrane endothelial keratoplasty; SD, standard deviation; OD, oculus dexter; OS, oculus sinister; ECC, endothelial cell count.

CCT increased from a median of 651 μ m (range, 523–834 μ m) pre-operatively to 665 μ m (range, 523–884 μ m) at one week (P = 0.74), and improved to 610 μ m (range, 467–884 μ m) at one month (P = 0.22), 558 μ m (range, 419–878 μ m) at three months (P = 0.04), and 533.5 μ m (range, 390–688 μ m) at last examination (P = 0.19).

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 Fable 2
 Clinical Outcomes Of DMEK Patients At All Time Intervals

Variable	Pre-Operative	I-Week Post-Operative	I-Month Post-Operative	3-Months Post-Operative	Last Examination
BCVA, median (range) [logMAR]	0.398 (0.097–1.398)	0.748 (0–2.3)	0.176 (0–0.875)	0.176 (0–1)	0.097 (0-0.796)
Spherical equivalent, median (range) [Diopters]	-1.25 (-9.5-1.875)	-1 (-9.5–1.875)	-0.875 (-3.125-1.625)	-0.188 (-3 - 2)	-0.438 (-4 - 2)
IOP, median (range) [mmHg]	14 (6–18)	14.5 (7–26)	12.5 (7–29)	13 (7–34)	13.5 (9–22)
Pachymetry, median (range) [μm]	651 (523–834)	665 (523–884)	610 (467–884)	558 (419–878)	533.5 (390–688)

Abbreviations: BCVA, best-corrected visual acuity; DMEK, Descemet membrane endothelial keratoplasty; IOP, intraocular pressure; logMAR, logarithm of the minimum angle of resolution Note: The mean follow-up duration was 13.3 ± 6.6 months for all patients and the range of last follow-up examination was 3–26 months.

Post-Operative Complications

Post-operative complications consisted of graft failure, graft rejection, corneal edema, and corticosteroid-induced IOP elevation. Nine (37.5%) of patients underwent a rebubbling procedure within a mean time of 12.1 ± 9.2 days (range, 5-35 days) after DMEK surgery due to partial peripheral graft detachment. In two (8.3%) eyes, we repeated the re-bubbling procedure twice. No graft dislocation was observed during follow-up. In one patient (4.2%), a repeat endothelial keratoplasty was necessary due to graft failure. This patient had undergone a re-bubbling procedure at eight days post-operatively, but received a DSEK surgery 73 days later due to persistent corneal edema.

A subgroup analysis of cases undergoing DMEK (n=8) vs triple-DMEK (n=16) revealed a significantly higher IOP at three months (P = 0.03) in patients who received cataract surgery (Table 3). A suspected open angle glaucoma patient (4.2%) who underwent triple-DMEK experienced an IOP elevation of 34 mmHg at three months postoperatively secondary to corticosteroid response. The patient was advised to reduce prednisolone use to once daily and prescribed dorzolamide hydrochloride 2.0%-timolol maleate 0.5% ophthalmic solution (Cosopt®; Merck & Co., Inc., Whitehouse Station, NJ, USA) to be utilized twice daily. No significant IOP differences were observed at other time intervals.

Discussion

In this study, the largest series of DMEK using the Alcon B cartridge as an injector, we demonstrate significant improvement in visual acuity and restoration of corneal thickness in 24 eyes of 23 patients. There is a demand for affordable DMEK injectors that can be utilized in a variety of limited-resource settings. The Alcon IOL B cartridge injector is a single-use affordable technique to load and introduce the DMEK graft into the AC. At last follow-up examination, the median Snellen BCVA was approximately 20/25 which is comparable with the visual acuity results typically reported for DMEK. 17-22

Re-bubbling rates vary by surgeon and are related to preference regarding how often to re-bubble. 23-26 Our approach considers re-bubbling any partial detachment as it is felt that air also helps contribute to more rapid visual recovery, although a low threshold results in a higher rebubbling rate.

Pre-loaded DMEK has increased in frequency as it provides less intraoperative donor tissue manipulation

Table 3 Subgroup Analyses Of All DMEK Patients

	DMEK alone (N=8)	Triple-DMEK (N=16)	P-Value	Re-bubbling (N=9)	No Re-bubbling (N=15)	P-Value
BCVA, median (range) [logMAR]						
Pre-operative	0.544 (0.176–1.398)	0.398 (0.097–0.875)	60:0	0.301 (0.097–0.699)	0.477 (0.176–1.398)	0.01*
Post-operative, I week	0.651 (0.398–1.477)	0.796 (0–2.3)	0.56	1.301 (0–2.3)	0.699 (0–1.477)	0.27
Post-operative, I month	0.176 (0-0.875)	0.176 (0-0.875)	86:0	0.301 (0-0.875)	0.176 (0-0.875)	0.45
Post-operative, 3 months	0.301 (0.097–1)	0.137 (0-0.796)	90.0	0.176 (0-0.796)	0.176 (0–1)	0.38
Post-operative, last examination	0.176 (0–0.796)	0.097 (0–0.544)	0.08	0.097 (0-0.544)	0.097 (0–0.796)	0.85
Spherical equivalent, median (range) [Diopters]	iopters]					
Pre-operative	-I (-I.375–I.375)	-I.563 (-9.5-I.875)	0.07	-1 (-2.5-1.5)	-I.375 (-9.5-I.875)	1.00
Post-operative, I week	-I (-I.375–I.375)	-1.313 (-9.5-1.875)	0.23	-0.875 (-2.375-1.5)	-1.125 (-9.5-1.875)	0.63
Post-operative, I month	-0.688 (-2.25-0.625)	-0.938 (-3.125-1.625)	0.62	-1 (-3.125-0.125)	-0.75 (-2.25-1.625)	0.30
Post-operative, 3 months	-0.688 (-3-1.125)	0 (-2.125-2)	0.37	0 (-1.625-0.75)	-0.375 (-3-2)	89.0
Post-operative, last examination	-0.438 (-1.375-1.625)	-0.25 (-4-2)	0.78	-0.5 (-1.625-0.75)	-0.375 (-4-2)	1.00
IOP, median (range) [mmHg]						
Pre-operative	14 (6–16)	14.5 (11–18)	91.0	15 (12–18)	14 (6–18)	0.21
Post-operative, I week	14 (7–21)	15 (7–26)	0.56	13 (7–18)	16 (7–26)	0.44
Post-operative, I month	12 (7–14)	13 (10–29)	0.22	12 (10–29)	13 (7–22)	0.93
Post-operative, 3 months	12 (7–20)	14.5 (10–34)	0.03*	13 (12–17)	13 (7–34)	0.81
Post-operative, last examination	12.5 (10–21)	14 (9–22)	0.83	13 (10–17)	14 (9–22)	0.70
Pachymetry, median (range) [μm]						
Pre-operative	674 (534–834)	636.5 (523–749)	0.48	649 (523–749)	653 (534–834)	0.55
Post-operative, I week	718 (525–884)	620 (523–749)	0.13	649 (523–749)	681 (525–834)	0.84
Post-operative. I month	711 (467–884)	604.5 (479–878)	0.22	616 (523–884)	608 (467–834)	0.53
Post-operative, 3 months	569 (462–834)	557 (419–878)	0.90	560 (419–878)	557 (462–834)	0.79
Post-operative, last examination	512.5 (390–673)	533.5 (464–688)	0.43	538 (464–688)	494 (390–673)	0.27

Notes: Median (range) values shown. Wilcoxon rank-sum testing for comparisons between groups. *, P < 0.05. The mean follow-up duration was 13.3 ± 6.6 months for all patients and the range of last follow-up examination was 3–26 months.

Abbreviations: BCVA, best-corrected visual acuity; DMEK, Descemet membrane endothelial keratoplasty; IOP, intraocular pressure; logMAR, logarithm of the minimum angle of resolution.

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and donor endothelial cell loss.^{27–31} It is unclear if this cartridge may be used for pre-loaded DMEK. However, we have begun loading the grafts at the beginning of cases to prepare for use later in the case and the grafts have remained stable throughout.

Limitations to the study include that it took place in a single center and procedures were performed by a single surgeon; a larger cohort treated by various surgeons at multiple centers is necessary to further assess outcomes. Second, as a retrospective study, it is dependent on the availability and accuracy of the medical records.

In summary, we present the clinical outcomes of the largest series of DMEK using an Alcon B cartridge to date. Larger and longer-term data on endothelial loss and refinements in technique are needed. These findings offer an affordable, disposable injector similar in use to the Jones tube, but for a fraction of the cost. This approach is a simple and inexpensive method for the treatment of corneal endothelial dysfunction.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

Dr Eghrari is supported by the Prevention of Blindness Sybil B and Harrington Special Scholar Award and has ownership interest in Treyetech and LuckyVision, LLC. Dr Eghrari reports personal fees from Keralink, outside the submitted work. In addition, Dr Eghrari has a patent DMEK insertion device pending. The authors report no other conflicts of interest in this work.

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