Three-Year Outcomes of Tri-Folded Endothelium-In Descemet Membrane Endothelial Keratoplasty With Pull-Through Technique

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• PURPOSE: To assess the 3-year outcomes of tri-folded endothelium-in Descemet membrane endothelial keratoplasty (DMEK) using bimanual pull-through delivery technique.

• DESIGN: Interventional case series.

• METHODS: In this single-center study, we included 153 consecutive eyes that underwent DMEK for various indications (Fuchs endothelial corneal dystrophy [FECD]: n = 111; bullous keratopathy [BK]: n = 24; and failed graft: n = 18). DMEK grafts were loaded into a disposable cartridge in a tri-folded, endothelium-in configuration and delivered using bimanual pull-through technique. Main outcome measures were graft preparation and unfolding times, best spectacle-corrected visual acuity (BSCVA), endothelial cell density (ECD), and graft survival.

• RESULTS: Mean graft preparation time was 5.9 ± 1.1 minutes; and mean graft unfolding time was 2.9 ± 0.9 minutes. Excluding eyes with comorbidities, logarithm of minimum angle of resolution BSCVA improved significantly from baseline preoperative values of 0.92 ± 0.58 to 0.02 ± 0.07 at 1 year ($P < .001$) and remained stable up to 3 years. Mean postoperative ECD decreased significantly ($P < .001$) from eye bank values to 1,818 ± 362, 1,675 ± 372, and 1,580 ± 423 cells/mm^2 at 1, 2, and 3 years, respectively. No significant differences in ECD were observed between eyes with FECD and BK, but ECD was significantly lower in eyes with previous failed graft ($P < .05$). Three-year cumulative graft survival rate was significantly ($P < .001$) lower for eyes with previous failed graft (71%) than for FECD (97%) and BK (92%).

• CONCLUSIONS: Tri-folded endothelium-in DMEK requires minimal time for graft unfolding, which is the surgical step considered most challenging by corneal surgeons. Visual outcomes and complication rates are not adversely affected by the modification of the surgical technique. (Am J Ophthalmol 2020;219:121–131. © 2020 Elsevier Inc. All rights reserved.)

ENDOTHELIAL KERATOPLASTY (EK) CURRENTLY REPRESENTS the gold standard treatment for corneal endothelial failure.[1] Because of its advantages over penetrating keratoplasty (PK), several EK methods have been developed and can broadly be divided into Descemet stripping automated endothelial keratoplasty (DSAEK) and Descemet membrane endothelial keratoplasty (DMEK).

Based on the 2019 statistical report from the Eye Bank Association of America, DSAEK currently remains the most popular EK technique.[2] Although DMEK is associated with faster visual rehabilitation and significantly lower rates of immunologic rejection,[3] its adoption rate by cornea surgeons has been relatively slow,[2] mainly because of the challenges in tissue preparation and subsequent graft unfolding. Both surgical techniques have been identified as significant hurdles for broad acceptance among novice DMEK surgeons.[4] Moreover, in eyes with complex anterior segment anatomy (e.g., abnormalities of the iris-lens diaphragm or in eyes with previous glaucoma surgery or pars plana vitrectomy), poor control of the DMEK graft within the anterior chamber during unfolding and centration increases the technical complexity of the procedure and often results in excess graft manipulation.[5]

In an attempt to overcome these issues, methods involving the preparation of tri-folded, endothelium-in donor tissue were proposed.[5,6] *Ex vivo* and early clinical outcomes of endothelium-in methods were comparable to those of endothelium-out DMEK,[7-9] but no longer term data are currently available. Thus, we present the 3-year

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outcomes of tri-folded endothelium-in DMEK using a contact lens–assisted bimanual pull-through delivery technique in eyes with different surgical indications.

METHODS

THIS SINGLE-CENTER INTERVENTIONAL CASE SERIES OF DMEK surgeries included eyes with corneal endothelial decompensation secondary to Fuchs endothelial corneal dystrophy (FECD), bullous keratopathy (BK), and previous failed graft. Tri-folded, endothelium-in DMEK was performed by a single surgeon (M.B.) at a single tertiary level center (Ospedali Privati Forlì, Forlì, Italy) between January 2015 and December 2016. No outcomes of any case included in this series were reported previously. The study adhered to the tenets of the 2013 Declaration of Helsinki and was prospectively approved by the local institutional review board and/or ethics committee, Comitato Etico Ospedali Privati Forlì (Forlì, Italy). Detailed informed consent for surgery and research was obtained from all participants.

Preoperatively, all patients underwent complete ophthalmologic examination including slit-lamp examination, best spectacle-corrected visual acuity (BSCVA), manifest refraction, applanation tonometry, and funduscopy. In addition, optical biometry (Lenstar LS900; Haag-Streit, Bern, Switzerland) was performed for intraocular lens (IOL) power calculation of cases that required combined cataract surgery. Follow-up visits were scheduled at least once every year for 3 years after DMEK. All patients had the potential for 3-year follow-up.

Main outcome measures were graft preparation and graft unfolding times, BSCVA, endothelial cell density (ECD), graft survival, and complication rates, expressed as mean ± SD or percentage. Graft preparation and graft unfolding times were evaluated using video recordings of all surgeries. Graft preparation time was defined as the time from the beginning of donor tissue preparation to loading onto the cartridge, whereas graft unfolding time was considered as the time between graft insertion and full intracameral air injection.[7] BSCVA was assessed using the Snellen visual acuity chart and converted to logarithm of the minimum angle of resolution (logMAR) units. Baseline donor ECD was measured through light microscopy after vital staining with trypan blue by the provider eye bank (Veneto Eye Bank Foundation, Venice, Italy). The postoperative ECD was evaluated via noncontact specular microscopy (EM-3000, Tomey Gmbh, Erlangen, Germany) using automatic focusing and digital capture of 15 images of the central cornea.[10]

• **SURGICAL TECHNIQUE:** The previously described procedure for performing tri-folded, endothelium-in DMEK with pull-through technique was slightly modified with regard to type of access for graft delivery (Supplemental Video 1).[5] Instead of a clear corneal incision, a scleral tunnel was prepared and extended into clear cornea; then a 9-mm descemetorhexis was performed under air. Pre-marked, pre-stripped donor tissue was stained with trypan blue (Vision blue, D.O.R.C., Zuidland, The Netherlands) and punched to 8.25 mm (Barron corneal donor punch, Katena Products, Inc., Denville, New Jersey). All donor grafts were tri-folded, endothelium-in, and transferred via a sterile therapeutic soft contact lens (Sooft, Montegiorgio, Italy) into an IOL cartridge (MDJ Company, La-Monniere-le-montel, France) intraoperatively. After performing an inferior peripheral iridotomy, the corneal end of the scleral tunnel was opened using a 2.75-mm keratome, and the DMEK graft was delivered bimanually under continuous, low-flow irrigation from a dedicated anterior chamber maintainer (Moria SA, Antony, France) usually placed at the 12 o'clock position. Air was injected to tamponade the graft against the recipient cornea, and the side entries were sealed airtight by stromal hydration or a single 10-0 nylon suture, if necessary. Additional procedures such as cataract surgery, pupilloplasty, phakic IOL explantation, and secondary scleral-fixated IOL insertion were performed, as indicated, immediately before the DMEK procedure.

• **POSTOPERATIVE MANAGEMENT:** Triamcinolone acetonide and gentamicin sulfate 0.3% were injected subconjunctivally at the end of the procedure. A fixed combination of dexamethasone phosphate 0.1% and netilmicin sulfate 0.3% (Netildex, SIFI, Catania, Italy) ophthalmic solution was started every 2 hours daily and tapered off to 4 times daily over the first postoperative month. Subsequently, antibiotic treatment was discontinued while dexamethasone was changed to fluorometholone and slowly tapered to once daily indefinitely. Steroid-induced ocular hypertension was treated with intraocular pressure–lowering agents, beginning with dorzolamide and timolol ophthalmic solution, then subsequent addition of brimonidine and/or prostaglandin inhibitors, as required.

• **DATA ANALYSIS:** All data collected in the study were entered into an electronic database via Microsoft Excel 2013 (Microsoft Corp., Redmond, Washington) and analyzed with IBM SPSS (version 26.0; IBM, Armonk, New York). Eyes with pre-existing ocular comorbidities or poor visual potential, including medical retinal disease (n = 7; 4.6%), post-pars plana vitrectomy (n = 2, 1.3%), advanced medical glaucoma (n = 1; 0.7%), surgical glaucoma (n = 7; 4.6%), and amblyopia (n = 3; 2.0%) were excluded from the BSCVA analysis. In addition, all eyes that underwent repeat keratoplasty (repeat DMEK n = 4; secondary DSAEK n = 3) after the DMEK procedure evaluated in this series (7 of 10 total cases of graft failure, 2 in the first year, 3 in the second year, and 2 in the third year) were also excluded. Patients who remained phakic after

DMEK (n = 2; 1.3%) were included in the analysis of visual outcomes.

Endothelial cell loss was calculated by subtracting postoperative ECD from baseline donor ECD, dividing by baseline donor ECD and multiplying by 100. Analysis of repeated measures using linear mixed models was used to assess the changes in BSCVA and ECD over the 3-year follow-up. Analysis of variance was performed to determine if there were significant differences in the mean BSCVA and ECD of surgical indications. Adjustment with Bonferroni's correction was applied to multiple pairwise comparisons. The significance threshold was set at 5%. Cumulative probability curves of graft rejection and survival were generated by Kaplan-Meier analysis with log-rank test. Sensitivity analysis was also performed to evaluate the influence of the inclusion of the second operated eye of some patients (n = 8; 5%) on the results.[11]

As applied by Price et al.[12], we used the criteria from the Cornea Preservation Time Study,[13] which defines graft failure as all and any graft that required repeat transplantation, regardless of tissue attachment status. According to these criteria, "early failure refers to a graft with cloudy or equivocal recipient stroma on the first postoperative day, that does not clear or requires a regraft within 8 weeks and is associated with intraoperative and/or perioperative complications, while nonrejection refers to a graft that on the first postoperative visit had a clear central recipient stroma and becomes cloudy because of causes other than an immune event (eg, surface failure, infection, glaucoma/hypotony, endothelial decompensation, interface irregularity/opacity, stromal scarring, blunt or penetrating trauma, or other causes)."[13]

RESULTS

THIS STUDY INCLUDED 153 EYES OF 145 PATIENTS WITH corneal endothelial decompensation who underwent DMEK. Average follow-up was 33 ± 7 months. Patient demographics and indications for surgery are summarized in Table 1. Follow-up data was obtained for 153 (100%), 149 (97%), and 141 (92%) eyes at 1, 2, and 3 years, respectively. Mean graft preparation time was 5.9±1.1 minutes, and mean graft unfolding time was 2.9±0.9 minutes.

• **VISUAL OUTCOMES:** There was a significant improvement in BSCVA at 1 year ($P < .001$) compared with baseline preoperative values (Figure 1). No further significant changes in BSCVA were observed at all subsequent time points (year 1 vs year 2; $P = 1.00$; year 1 vs year 3; $P = .21$). Table 2 summarizes the Snellen BSCVA distribution (BSCVA ≥ 20/20, ≥ 20/25, and ≥20/40) over the 3-year follow-up after DMEK (Figure 2). Mean 3-year logMAR BSCVA was 0.01 ± 0.06, 0.03 ± 0.06, 0.12 ± 0.10 for cases with FECD (n = 89) and BK (n = 15), and previous failed graft (n = 10) as indications, respectively (Table 2).

TABLE 1. Baseline Characteristics

No. of eyes	153
No. of patients	145
Recipient age, y mean ± SD (range)	68 ± 11 (31-90)
Recipient sex, male, n (%)	68 (47%)
Indication for DMEK	
FECD	111 (73%)
BK (pseudophakic, aphakic, phakic IOL)	24 (16%)
Failed previous graft	18 (12%)
Failed DSAEK	12
Failed PK	6
Combined procedures	
Cataract surgery, IOL implantation, n (%)	91 (59%)
Phakic IOL explantation, cataract surgery, IOL implantation, n (%)	2 (1%)
Secondary scleral-fixated IOL implantation, n (%)	1 (0.7%)
Pupilloplasty, n (%)	1 (0.7%)

BK = bullous keratopathy; DMEK = Descemet membrane endothelial keratoplasty; DSAEK = Descemet stripping automated endothelial keratoplasty; FECD = Fuchs endothelial corneal dystrophy; IOL = intraocular lens; PK = penetrating keratoplasty

Comparing surgical indications, statistically significant differences in mean BSCVA between FECD and BK eyes were observed in the first year after DMEK ($P < .05$), but no significant differences were found when comparing 1-year BSCVA with subsequent time points for these 2 indications. At all examination times, eyes with FECD and BK achieved higher mean BSCVA than eyes with previous failed grafts ($P < .001$ and $P < .05$, respectively). Exclusion of the second operated eye (n = 8) in the statistical analyses did not appreciably change the observed stabilization of BSCVA after the first year (year 1 vs year 2; $P = 1.00$; year 1 vs year 3; $P = .29$) nor the results of the pairwise comparisons.

• **ECD:** Mean preoperative ECD was 2,580 ± 103 cells/mm^2 (range: 2,300 to 2,900 cells/mm^2), which decreased to 1,818 ± 362 cells/mm^2 at 1 year, 1,675 ± 372 cells/mm^2 at 2 years and 1580 ± 423 cells/mm^2 at 3 years (Figure 3). There was a significant decrease in postoperative ECD every year ($P < .001$). Mean endothelial cell loss (ECL) rate was 29.6 ± 14.3%, 34.6 ± 13.8%, and 38.6 ± 16.8% at 1, 2, and 3 years, respectively. After the first year, average annual ECL was 4.5%. Mean 3-year ECD was 1,657 ± 378, 1,557 ± 405, 906 ± 223 cells/mm^2 for cases with FECD (n = 103), BK (n = 18), and previous failed grafts (n = 13) as indications, respectively (Table 3). Annual mean ECD was significantly lower in eyes with previous failed grafts compared with FECD and BK ($P < .001$), whereas outcomes did not significantly

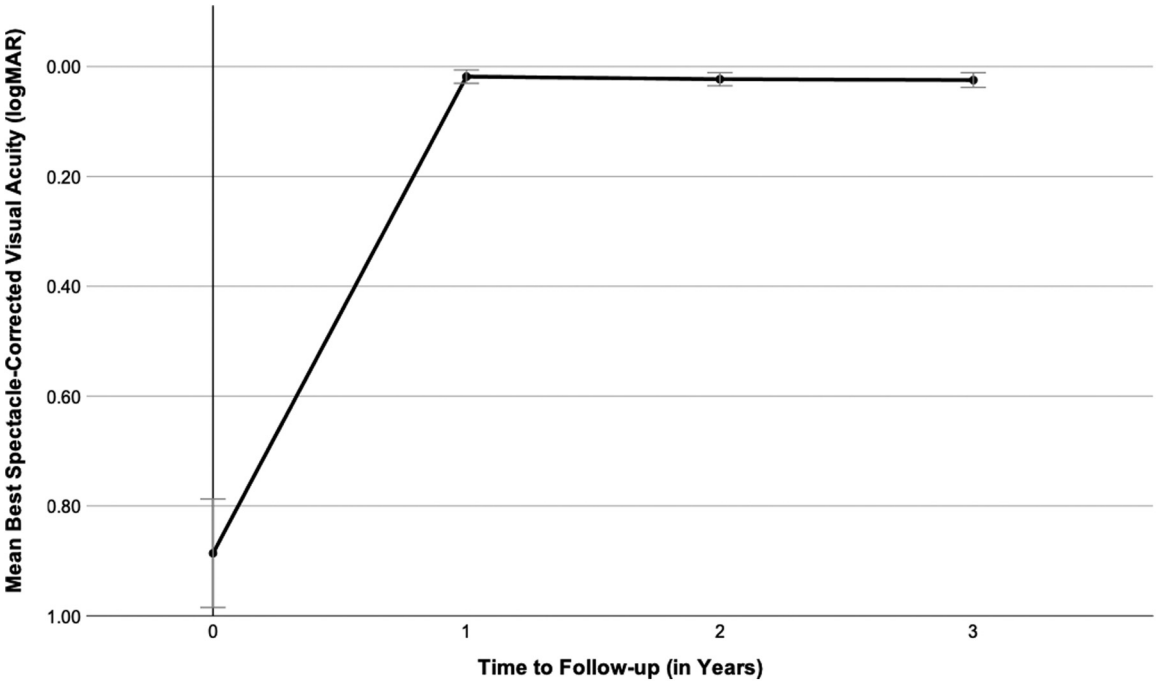

FIGURE 1. Mean best spectacle-corrected visual acuity over 3 years after Descemet membrane endothelial keratoplasty with 95% confidence interval. logMAR = logarithm of the minimum angle of resolution.

differ between FECD and BK. Sensitivity analysis with the exclusion of the second operated eye (n = 8) demonstrated no changes in the results of the ECD analysis ($P < .001$ every year and eyes with previous failed grafts vs FECD or BK).

• **POSTOPERATIVE COMPLICATIONS:** Table 3 summarizes the postoperative adverse events. The most common postoperative complication was graft detachment, which was observed in 42 (27.4%) cases that all subsequently underwent re-bubbling, once in 38 cases and twice in 4 cases. Re-bubbling was not associated with ECD decrease ($P = .29$) nor graft failure ($P = .36$). Two cases (1.3%) of persistent graft detachment after 2 re-bubblings required repeat surgery (DSAEK n = 1; DMEK n = 1). During the first 6 postoperative months, cystoid macular edema (CME) occurred in 3 cases (2.0%) that underwent combined DMEK and cataract surgery,. All these eyes were successfully treated with topical nonsteroidal anti-inflammatory drugs, topical corticosteroid, and oral acetazolamide. A persistent epithelial defect occurred in 1 case (0.7%) within the 2 weeks from surgery and resolved with application of bandage contact lens and topical medication.

• **GRAFT REJECTION AND SURVIVAL:** The Kaplan-Meier cumulative graft rejection rate was 0.7%, 1.3%, and 2.8% at 1, 2, and 3 years after DMEK, respectively (Figure 4A). Overall, 4 eyes experienced an episode of immunologic rejection, only 1 (0.7%) of which required repeat grafting.

Using the definitions in the Cornea Preservation Time Study,[11] graft failure, which includes eyes that require a re-graft for all and any reason, occurred in 10 eyes (6.5%). Of these, 7 (4.6%) grafts showed progressive ECL without signs of immune rejection, 2 (1.3%) failed after repeated re-bubbling for recurrent graft detachment, and 1 (0.7%) had evidence of immune rejection in the form of endothelial precipitates. No primary donor failures were observed. Repeat EK (DMEK n = 4; DSAEK n = 3) was performed in 7 of these 10 eyes. The 3 remaining patients were offered repeat keratoplasty but have not undergone surgery at our institution.

Kaplan-Meier cumulative graft survival rate was 99% at 1 year, 97% at 2 years, and 93% at 3 years (Figure 4B). When graft detachment was excluded as cause of graft failure, the cumulative graft survival rate was 100%, 98%, and 94% at 1, 2, and 3 years, respectively.

The 3-year cumulative graft survival rate was 97%, 92%, and 71% after DMEK surgery for FECD, BK, and failed previous grafts, respectively. Mean survival time was greatest among FECD cases (35.5 ± 3.7 months) and significantly higher than among eyes with previous failed grafts ($P < .001$), but comparable with BK eyes ($P = .16$) (Figure 4C).

Excluding the second eye of 8 patients with FECD who underwent bilateral DMEK, Kaplan-Meier estimates for immune rejection were 0.7%, 1.4%, and 3.0% at 1, 2, and 3 years, respectively, whereas the annual graft survival probabilities over 3 years of the entire cohort and FECD eyes alone, as well as the results of log-rank analysis were unchanged.

TABLE 2. Three-Year Clinical Outcomes Following Descemet Membrane Endothelial Keratoplasty

	Baseline	Year 1	Year 2	Year 3
BSCVA				
No. of eyes analyzed	131/153 (86%)	131/153 (86%)	124/153 (81%)	114/153 (75%)
No. of eyes excluded		22/153 (14%)	29/153 (19%)	39/153 (25%)
Lost to follow-up		0	4	12
Re-graft		2	5	7
Low visual potential due to ocular comorbidity		20	20	20
Mean ± SD (logMAR)	0.916 ± 0.582	0.018 ± 0.069	0.021 ± 0.066	0.022 ± 0.072
Fuchs endothelial corneal dystrophy	n = 96, 0.72 ± 0.42	n = 96, 0.01 ± 0.06	n = 92, 0.01 ± 0.06	n = 89, 0.01 ± 0.06
Bullous keratopathy	n = 21, 1.28 ± 0.61	n = 21, 0.05 ± 0.05	n = 19, 0.03 ± 0.07	n = 15, 0.03 ± 0.06
Failed previous graft	n = 14, 1.66 ± 0.10	n = 14, 0.08 ± 0.10	n = 13, 0.07 ± 0.10	n = 10, 0.12 ± 0.10
No. of eyes ≥ 20/40	39 (25%)	131 (100%)	124 (100%)	114 (100%)
No. of eyes ≥ 20/25	0 (0%)	121 (92%)	113 (92%)	102 (89%)
No. of eyes ≥ 20/20	0 (0%)	87 (66%)	81 (65%)	69 (61%)
No. of eyes ≥ 20/17	0 (0%)	15 (11%)	13 (10%)	12 (11%)
Endothelial cell density				
No. of eyes analyzed	151/153 (99%)	151/153 (99%)	144/153 (94%)	134/153 (88%)
No. of eyes excluded		2/153 (1%)	9/153 (6%)	19/153 (12%)
Lost to follow-up		0	4	12
Re-graft		2	5	7
Mean ± SD (cells/mm^2)	2,580 ± 103	1,818 ± 362	1,675 ± 372	1,580 ± 423
Fuchs endothelial corneal dystrophy	n = 110, 2,577 ± 103	n = 110, 1,893 ± 320	n = 106, 1,750 ± 319	n = 103, 1,657 ± 378
Bullous keratopathy	n = 24, 2,588 ± 103	n = 24, 1,774 ± 289	n = 22, 1,640 ± 352	n = 18, 1,557 ± 405
Failed previous graft	n = 17 2,606 ± 111	n = 17, 1,398 ± 430	n = 16, 1,186 ± 393	n = 13,906 ± 223

BSCVA = best spectacle–corrected visual acuity; logMAR, logarithm of the minimum angle of resolution

• **OUTCOMES IN COMPLICATED EYES:** Complicated cases included post-glaucoma surgery (trabeculectomy: n = 4 [2.6%], glaucoma drainage device: n = 3 [2.0%]) and post-pars plana vitrectomy (n = 1; 0.7%) eyes, as well as combined procedures (combined phakic IOL explantation and cataract surgery: n = 2 [1.3%]; combined secondary scleral fixated IOL insertion for aphakia (n = 1; 0.7%); and combined pupilloplasty (n = 1; 0.7%]. Re-bubbling was required in 4 of the 12 cases (33%). Graft failure occurred in 2/12 cases (17%), 1 due to persistent major graft detachment and another due to graft rejection; both required repeat keratoplasty.

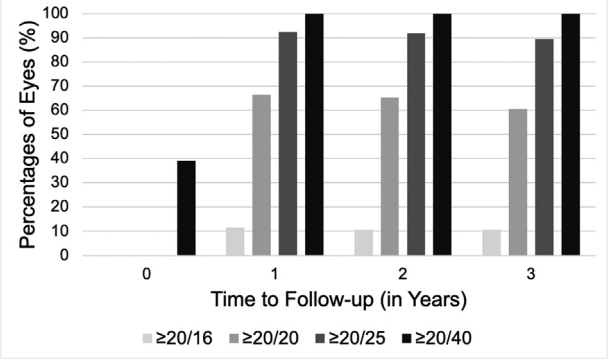

FIGURE 2. Distribution of Snellen best spectacle-corrected visual acuity up to 3 years following Descemet membrane endothelial keratoplasty.

DISCUSSION

DESPITE THE SUCCESS OF DMEK IN TERMS OF RAPID SPEED OF visual rehabilitation and low immunologic rejection rates, the initial high incidence of postoperative complications has prompted many surgeons to refine the surgical technique.[5,6,14,15]

In our initial report, we have demonstrated that using tri-folded, endothelium-in grafts with bimanual pull-through technique addresses several key problems during loading and delivery of the DMEK graft.[5] Folding the graft endothelium-in allows spontaneous unfolding within the anterior chamber following the tissue's natural tendency to roll endothelium outward. In addition, it prevents

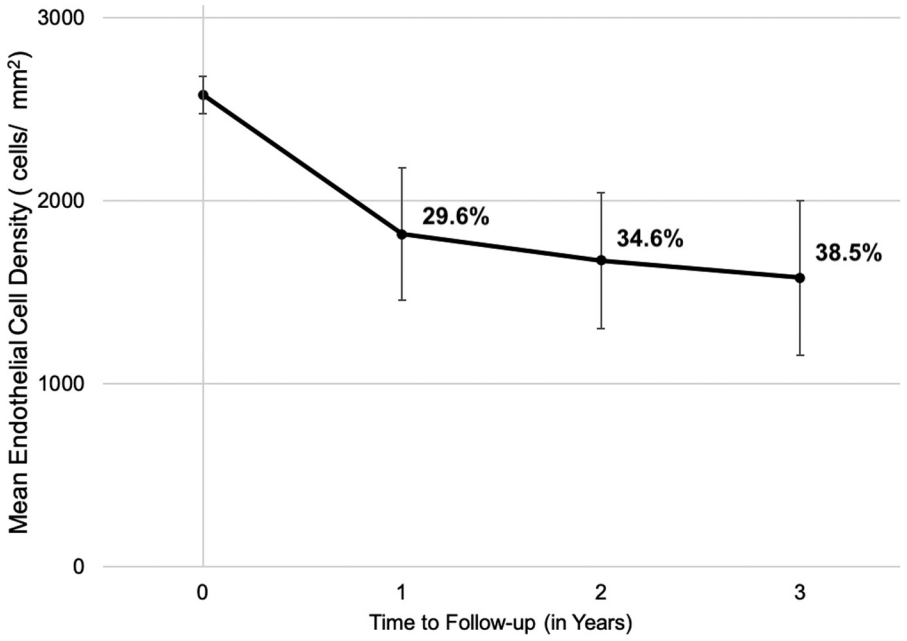

FIGURE 3. Mean endothelial cell density over 3 years following Descemet membrane endothelial keratoplasty. Vertical bars represent SD. Percentage of endothelial cell loss at annual postoperative follow-up is shown in bold.

TABLE 3. Postoperative Complications

Graft detachment	42 (27.4%)
One re-bubbling procedure	38
Two re-bubbling procedures	4
Graft rejection	4 (2.6%)
Graft failure [a]	10 (6.5%)
Early failure [a]	2
Primary donor failure [a]	0
Graft rejection [a]	1
Nonrejection [a]	7
Refractive/visual [a]	0
Repeat graft	7 (4.6%)
Cystoid macular edema	3 (2.0%)
Persistent epithelial defect	1 (0.7%)

[a]Definitions were based on the criteria from the Cornea Preservation Time Study.[11]

possible deleterious contact of the endothelial cells with any device used for graft delivery.

The scleral tunnel incision allows the cartridge to protrude less into the anterior chamber during graft delivery, which results in more space for the forceps to complete the pull-through maneuver (Supplemental Video 1). Thanks to the self-sealing surgical access, simple removal of the cartridge while holding the graft with the forceps results in spontaneous graft unfolding under a closed system condition. The incision does not require suturing in most cases (129/153 cases in this series [84%]).

The current results of DMEK using this technique confirm the previous observation of excellent visual results and demonstrates that these outcomes are maintained for at least 3 years postoperatively. In contrast to published reports that demonstrated better BSCVA outcomes in FECD eyes than in BK eyes for as long as 7 years postoperatively,[10,16,17] no significant differences were observed as early as 1 year after DMEK in this series. This is possibly related to differences in baseline severity of corneal edema and associated stromal changes, which can cause variations in the time required to achieve corneal complete clearance. The suboptimal visual performance among eyes with previous failed grafts could be explained by the presence of larger amounts of higher-order aberrations after keratoplasty and concomitant extensive subepithelial fibrosis.

Techniques that use endothelium-in grafts can optimize graft unfolding,[5,6,14,15] which is perceived as one of the most significant challenges among novice DMEK surgeons.[4] Delivering the graft bimanually provides total control throughout the procedure and minimizes any prolonged unnecessary manipulation.[5] As demonstrated in this series, the ease of graft unfolding can compensate for longer graft preparation time, thereby maintaining an average total surgical time of <20 minutes in most cases. Our mean graft unfolding time (2.9 ± 0.9 minutes) compares favorably with those previously reported for both endothelium-out (6.0 ± 3.5 minutes) and endothelium-in insertion methods (6.0 ± 3.5 minutes).[7] This may be due to slight differences in our technique that influences the predictability of graft unfolding. In particular, it is crucial that the unfolded part of the Descemetic surface of the graft is positioned on the

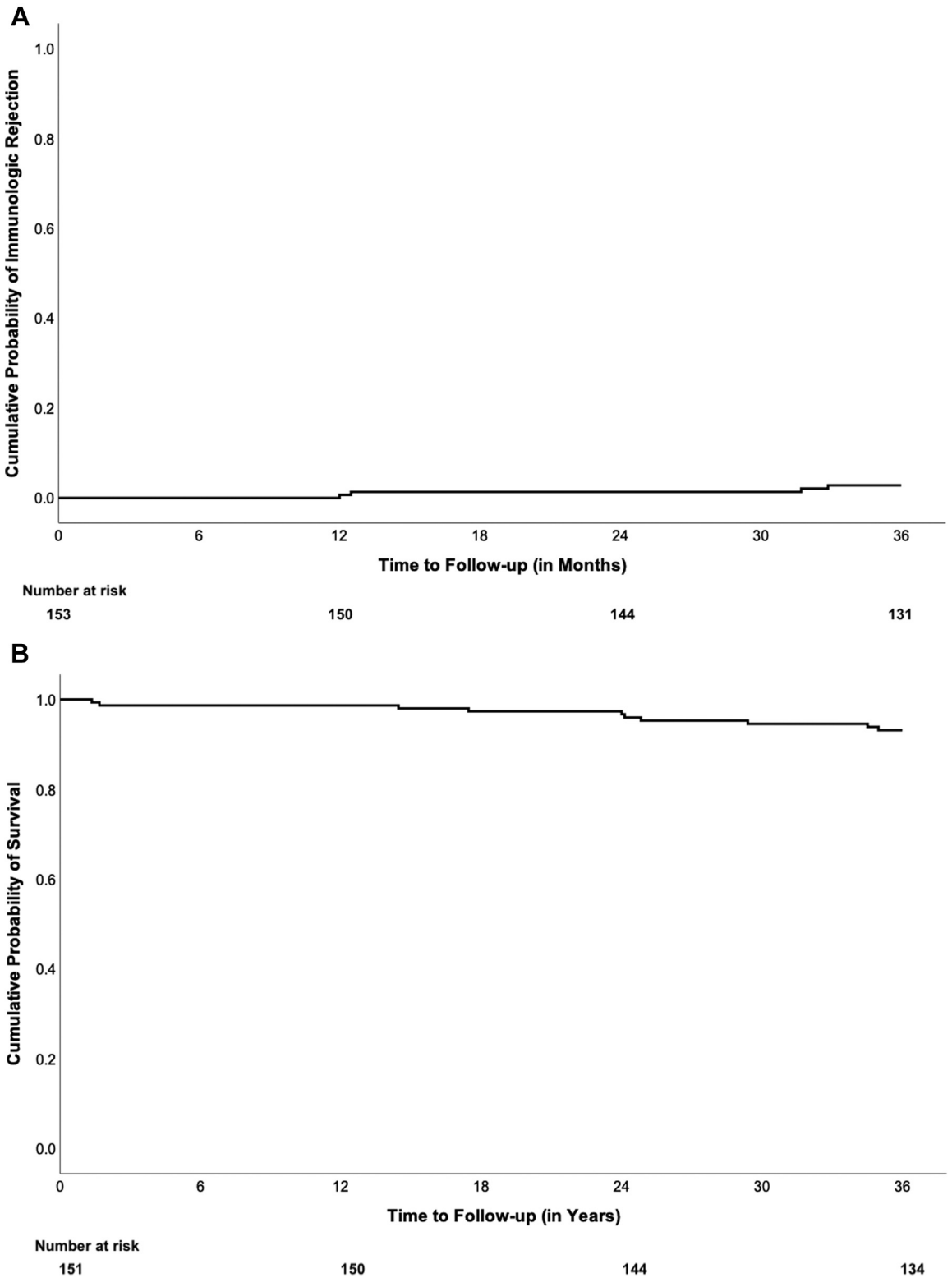

FIGURE 4. Kaplan-Meier curves up to 3 years following Descemet membrane endothelial keratoplasty. (A) Cumulative probability of graft rejection; (B) graft survival of the entire cohort; and (C) graft survival according to surgical indication.

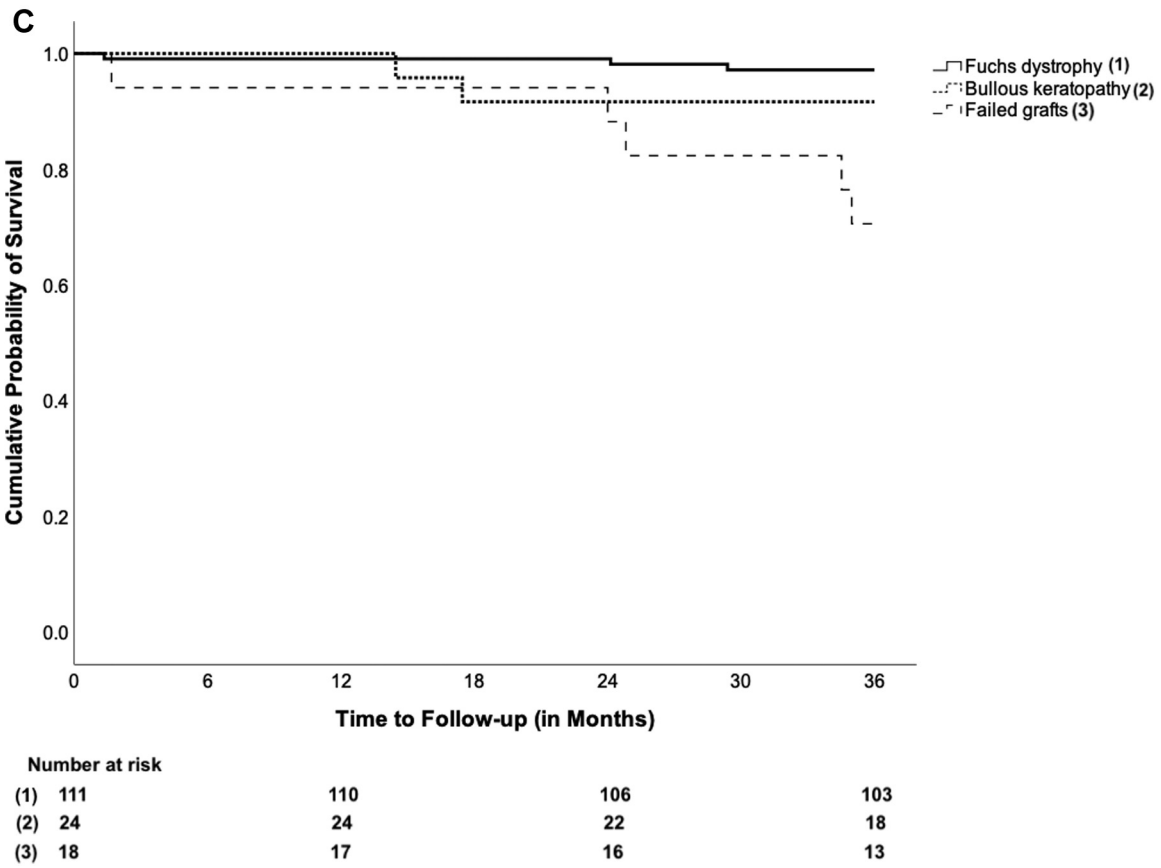

FIGURE 4. (*Continued*).

floor of the cartridge and that an endothelium-in scroll is formed during advancement of the graft into the cartridge funnel with balanced salt solution. It is equally important to rotate the cartridge by 180°, such that the floor becomes the ceiling of the cartridge funnel upon entry into the anterior chamber. This allows spontaneous unfolding with proper graft orientation during the pull-through maneuver. Attention to these details minimizes graft unfolding time, a factor reported to influence surgeon preference toward the trifolded endothelium-in method.[7]

Direct control of the graft also prevents undesired scrolling or graft inversion during the procedure. Although it may be argued that grasping the DMEK graft with forceps may result in greater ECL, we previously showed that as many as 18 forceps bites are necessary to destroy approximately 1% of the total amount of endothelial cells. Consequently, with the peripheral crown of the graft containing endothelial cells invariably damaged during punching, the effect on ECL of the 3 to 4 forceps bites commonly required during DMEK is essentially negligible.[5]

Evaluating the ECD trend, our present data demonstrate the gradual decline of ECD after endothelium-in DMEK and compare favorably to previous published models for PK.[18,19] This suggests the possibility of longer graft survival than the 3-year period considered in the series. In comparison with values recorded after DMEK using the endothelium-out technique, the 3-year ECL (39%) was similar to that reported by Price et al. (40%)[12] and lower than those published by the Melles group (Ham et al.[10]: 48.1%; Birbal et al.[20]: 56.6%).

Although ECL at 1 year was <15% in 25 of 131 FECD eyes (23%) from this series, the mean cell loss of the entire cohort was greater possibly due to inclusion of a greater number of eyes with more advanced endothelial dysfunction. Multinomial regression analysis of various surgical parameters has demonstrated that the severity of disease is associated with greater endothelial cell loss and may also account for the variability observed in this series.[21] In our practice, when DMEK was increasingly performed in cases that were previously indicated for DSAEK, higher ECL was observed. This might also have been affected by the modifications in the DMEK technique using the scleral tunnel incision. In addition, after an interventional series in partnership with our provider eye bank, we reverted to using non-preloaded grafts as per our standard technique due to less intense trypan blue staining in preloaded grafts and a greater ECL observed in the early postoperative period.[22]

As previously reported after DSAEK,[23] no differences in ECD outcomes in eyes with FECD and BK as indications were observed. This was in contrast to data published after

endothelium-out DMEK using the no touch technique.[10,16,17,20] The discrepancy might be explained by differences in the preoperative condition of the host cornea, wherein a varying percentage of DMEK was performed in FECD eyes without significant corneal edema.

Even with de-epithelialization of the recipient cornea and staining of the DMEK tissue, corneal haze from long-standing BK impedes visualization of the graft. When using the endothelium-out technique in these eyes, achieving proper graft orientation and centration may often require prolonged graft manipulation within the anterior chamber, which can result in increased endothelial cell damage. In contrast, tri-folding the graft endothelium-in uses the natural tendency of the tissue to spontaneous unfold with the correct orientation upon graft insertion. The comparable ECD outcomes for both indications support our claim that controlled surgical manipulation using our technique not only facilitates DMEK but also makes it equally feasible in eyes with poorer anterior chamber visibility.

With regard to the higher ECL after DMEK in previous failed grafts over other indications, the same predisposing factors that led to accelerated cell loss during the previous keratoplasty probably contributed to greater ECL after DMEK, as was also observed in cases of repeat PK[24] or DSAEK after PK.[25]

Graft detachment was the most common major complication that occurred in the early postoperative period, but its incidence was within the wide range found in previous literature (0.2%-76%).[3] Moreover, we routinely performed re-bubbling for any case of graft detachment and did not wait for spontaneous clearance because a significant number of our patients resided in remote areas and even in foreign countries, which made a long perioperative period of observation often prohibitive. In our clinical practice, we routinely use air tamponade for all types of lamellar surgery due to concerns of potential endothelial toxicity of sulfur hexafluoride and conflicting evidence of the latter's efficacy over 100% air fill.[26-29]

Immunologic rejection was a rare occurrence within 3 years from DMEK (<3%), which confirm the outcomes of other series.[10,16,17,20] Our rejection rate was lower than those reported after standard DSAEK (0%-45%)[3] or ultrathin DSAEK (5%).[30] The rate of CME within 1 year after DMEK (1.9%) was lower than rates reported after DSAEK (11%-13%)[31-33] and within the range reported after DMEK (0.7%-13%).[34] Despite iatrogenic iris trauma from routine peripheral iridectomy, the incidence of CME remained low, possibly due to our postoperative subconjunctival and topical steroid regimen.

The 3-year cumulative survival rate in our series included eyes with diagnoses other than FECD, which was 2 to 3 times higher in number than previously published studies (28% vs 9%-11%).[10,16,20] This could explain why our 3-year graft survival rate (93%) was slightly lower than those published for endothelium-out DMEK (94% to 96%).[10,16]

Conversely, when considering only FECD cases in our series, the 3-year cumulative graft survival rate (97%) was slightly higher than that reported by Price et al. (94%)[12] and Birbal et al. (94%).[20] Moreover, this was consistent with previous observations that FECD eyes tended to have better graft survival probabilities after DMEK than eyes with other indications.[10,12,16,20]

Although majority of the graft failures in this series were observed in eyes with failed previous grafts, the 3-year graft survival (71%) after DMEK was comparable to survival estimates reported for DSAEK (74%)[25] and within the wide range reported for PK.[25,35] The inherent risk for subsequent failure in eyes with previous failed grafts might account for the significantly increased risk of failure observed.[25]

As with any longitudinal study, the limitations of the study were an increasing number of patients lost to follow-up and the noncomparative study design. However, our dropout rates at all follow-up examination times compared favorably with those of other DMEK studies.[10,16,17] As a tertiary center, because most of our patients were referred to us for surgery, the main reason for loss of compliance to scheduled examinations was the difficulty, both in terms of cost and logistics, for older adult patients to return for routine postoperative visits. Nevertheless, the use of statistical methods to account for the loss to follow-up support the validity of the findings in this study. Notably, all patients who were not included in the present analysis but contacted telephonically reported satisfaction with their visual outcomes.

Finally, unlike the published clinical trials with more stringent inclusion criteria,[36,37] our study analyzed a wide range of cases including advanced endothelial decompensation and complex clinical situations that represent the breadth of indications encountered in routine clinical practice. Although there is an apparent advantage of DMEK for uncomplicated FECD, there are limited data on the outcomes of grafts in a heterogenous population. Tri-folded endothelium-in DMEK is a valuable tool in the cornea surgeon's armamentarium, not only for those planning to transition to DMEK, but also for more advanced ones that seek to broaden the surgical indications, including challenging cases with poor anterior chamber visualization.

CONCLUSION

IN CONCLUSION, TRI-FOLDED, ENDOTHELIUM-IN DMEK MINIMIZES the time required for graft delivery, which is the surgical step that is considered the most challenging by corneal surgeons. Visual outcomes and complication rates are not adversely affected by the modification of surgical technique.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

ANGELI CHRISTY YU: CONCEPTUALIZATION, METHODOLOGY, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **James Myerscough:** Investigation, Formal analysis, Data curation, Writing - review & editing. **Rossella Spena:** Investigation, Writing - original draft. **Fiorella Fusco:** Investigation, Writing - original draft. **Sergiu Socea:** Investigation, Writing - original draft. **Luca Furiosi:** Investigation, Writing - original draft. **Luigi De Rosa:** Investigation, Writing - original draft. **Cristina Bovone:** Investigation, Writing - original draft. **Massimo Busin:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - review & editing.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST.

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