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

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ABSTRACT:

Purpose: To report 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, 153 consecutive eyes undergoing DMEK for various indications (Fuchs endothelial corneal dystrophy (FECD) n=111; bullous keratopathy (BK) n=24; failed grafts n=18) were included. DMEK grafts were loaded into a disposable cartridge in 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, logMAR BSCVA improved significantly from baseline preoperative values of 0.92±0.58 to 0.02±0.07at 1 year ($p<0.001$) and remained stable up to 3 years. Mean postoperative ECD decreased significantly ($p<0.001$) from eye bank values to 1818±362 cells/mm^2, 1675±372 cells/mm^2 and 1580±423 cells/mm^2 at 1, 2 and 3 years respectively. No significant differences in ECD was observed between eyes with FECD and BK, but ECD was significantly lower in eyes with failed grafts ($p<0.05$). Three-year cumulative graft survival rate was significantly ($p<0.001$) lower for eyes with failed grafts (71%) than for FECD (97%) and BK (92%).

Conclusions: Tri-folded, endothelium-in DMEK requires minimal time for graft unfolding, the surgical step considered most challenging by majority of surgeons. Visual outcomes and complication rates are not adversely affected by the modification of surgical technique.

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

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Short title: Pull-Through Technique for Tri-folded DMEK: 3-Year Outcomes

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Abbreviations and Acronyms
BK = bullous keratoplasty; BSCVA = Best spectacle-corrected visual acuity; CME = cystoid macular edema; DMEK = Descemet Membrane Endothelial Keratoplasty; DSAEK = Descemet Stripping Automated Endothelial Keratoplasty; ECD = endothelial cell density; ECL = endothelial cell loss; EK = endothelial keratoplasty; FECD = Fuchs endothelial corneal dystrophy; logMAR = logarithm of minimum angle of resolution; PK = penetrating keratoplasty.
This manuscript contains 1 video as an additional online-only material. (Supplemental Material available at AJO.com)

Endothelial keratoplasty (EK) currently represents the gold standard for the treatment of corneal endothelial failure.[1] Owing to 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 remains currently the most popular EK technique.[2] Although DMEK is associated with faster visual rehabilitation and significantly lower rates of immunologic rejection,[3] its adoption by cornea surgeons has been relatively slow,[2] mainly because of challenges in tissue preparation and subsequent graft unfolding. In fact, both surgical steps have been identified as significant hurdles for broad acceptance among novice DMEK surgeons.[4] Moreover, in eyes with complex anterior segment anatomy such as 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 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 for endothelium-in methods were comparable to those for endothelium-out DMEK,[7-9] but no longer-term data is currently available. Thus, we present herein the 3-year outcomes of tri-folded, endothelium-in DMEK using 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 graft failure. 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 have been reported previously. The study adhered to the tenets of the 2013 Declaration of Helsinki and was prospectively approved by the local Institutional Review Board/ ethics committee, Comitato Etico Ospedali Privati Forlì in Forlì, Italy. Detailed informed consent for the 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. Additionally, optical biometry (Lenstar LS900; Haag-Streit, Bern, Switzerland) was performed for intraocular lens power measurement for cases requiring combined cataract surgery. Follow-up visits were scheduled at least once every year for up to 3 years after DMEK. All patients had the potential for 3-year follow-up.

Main outcome measures included graft preparation and graft unfolding times, BSCVA, endothelial cell density (ECD), graft survival and complication rates, expressed as mean ± standard deviation or proportion. All surgical procedures were video-recorded for evaluation of both graft preparation and graft unfolding times. Graft preparation time was defined as the time from the beginning of donor tissue preparation to loading onto the cartridge while 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 non-contact 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 tri-folded endothelium-in DMEK with pull-through technique described previously was slightly modified with regards 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 9mm 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, NJ). All donor grafts were tri-folded, endothelium-in; and transferred via a sterile therapeutic soft contact lens (Sooft, Montegiorgio, Italy) into an intraocular lens 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 2.75mm keratome and the DMEK graft was delivered bimanually under continuous, low-flow irrigation through 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 means of stromal hydration or 10-0 nylon single stitches, if necessary. Additional procedures including cataract extraction via bimanual phacoemulsification and posterior chamber intraocular lens implantation, pupilloplasty, phakic IOL explantation, secondary scleral fixated IOL insertion were performed as indicated immediately prior to DMEK.

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 by means of intraocular pressure-lowering agents, beginning with dorzolamide and timolol ophthalmic solution with subsequent addition of brimonidine and/or prostaglandin inhibitors as required.

Data Analysis

All data collected in the study was entered into an electronic database via Microsoft Excel 2013 (Microsoft Corp., Redmond, WA) and analyzed with IBM SPSS (version 26.0; SPSS Inc, Chicago, Illinois, PA). 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%), amblyopia (n=3, 2.0%) were excluded from the analysis of BSCVA. 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. Phakic patients (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 and then dividing by baseline donor ECD and multiplying by 100. Analysis of repeated measures using linear mixed models was used to assess changes in BSCVA and ECD over 3-year follow-up. Analysis of variance was performed to determine significant differences in mean BSCVA and ECD among surgical indications. Adjustment with Bonferroni correction was applied to multiple pairwise comparisons. Significance threshold was set at 5%. Cumulative probability curves for graft rejection and survival was generated by Kaplan-Meier analysis with log-rank test. Sensitivity analysis was also performed, in order 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] for the definition of graft failure, 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 could be 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 of BSCVA at 1 year ($p<0.001$) compared to 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 $p=0.21$). 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), BK (n=15) and failed grafts (n=10) as indication, respectively (Table 2).

Comparing surgical indications, statistically significant difference in mean BSCVA between FECD and BK eyes was observed in the first year after DMEK ($P<0.05$), 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 failed grafts ($p<0.001$, $p<0.05$ 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 $p=0.29$) nor the results of the pairwise comparisons.

Endothelial Cell Density

Mean preoperative ECD was 2580±103 cells/mm^2 (range: 2300 to 2900) which decreased to 1818±362 cells/mm^2 at 1 year, 1675±372 cells/mm^2 at 2 years and 1580±423 cells/mm^2 at 3 years after surgery (Figure 3). There was a significant decrease in postoperative ECD every year ($p<0.001$). Mean 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 1657±378 cells/mm^2, 1557±405 cells/mm^2, 906±223 cells/mm^2 for cases with FECD (n=103), BK (n=18) and failed grafts (n=13) as indication, respectively (Table 3). Annual mean ECD was significantly lower for failed grafts compared with both FECD and BK ($p<0.001$), while outcomes did not significantly 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 ($p<0.001$ every year and failed grafts versus 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, all of which underwent re-bubbling, once in 38 cases and twice in 4 cases. Re-bubbling was not associated with ECD decrease ($p=0.29$) nor graft failure ($p=0.36$). Two cases (1.3%) of persistent graft detachment after 2 rebubblings required repeat surgery (DSAEK n= 1; DMEK n = 1). Cystoid macular edema (CME) occurred in 3 cases (2.0%), all after DMEK combined with cataract surgery and during the first 6 postoperative months. All these eyes were successfully treated with topical NSAID, 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 a bandage contact lens and topical medication.

Graft Rejection and Survival

The Kaplan-Meier cumulative graft rejection rate was 0.7%, 1.3%, 2.8% at 1, 2 and 3 years after DMEK (Figure 4, part A). 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 included 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 repeat air injections to treat recurrent graft detachment and 1 (0.7%) had endothelial precipitates as evidence of immune rejection. No primary donor failures were observed. Repeat EK (DMEK n=4; DSAEK n=3) was performed in 7 of these 10 eyes. The remaining 3 patients have been offered repeat keratoplasty but have not undergone the surgery at our institution.

Kaplan-Meier cumulative graft survival rate 99% at 1 year, 97% at 2 years and 93% at 3 years (Figure 4, part B). 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 that of eyes with failed grafts ($p<0.001$), but comparable to that of BK eyes ($p=0.16$), as illustrated in Figure 4, part C.

Excluding the second eye of 8 FECD patients 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, while 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.

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%]). Rebubbling was required in 4 of the total 12 cases (33%). Two of 12 cases (17%) developed graft failure, one due to persistent major graft detachment and another due to graft rejection, both requiring repeat 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 tri-folded endothelium-in grafts with bimanual pull-through technique addresses several key problems during DMEK graft loading and delivery.[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 possible deleterious contact of the endothelial cells with any device utilized for graft delivery.

The scleral tunnel incision allows the cartridge to protrude less into the anterior chamber during graft delivery, resulting in more space for the forceps to complete the pull-through maneuver (Supplemental Video 1). In addition, 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 closed system condition. The incision does not require suturing in the majority of cases (129 of 153 cases in this series [84%]).

The results of DMEK using this technique confirm the previous observation of excellent visual results and currently demonstrate that these outcomes are maintained up to at least 3 years postoperatively. In contrast to published reports demonstrating 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 may be possibly related to differences in baseline severity of corneal edema and associated stromal changes causing variations in the time required to achieve corneal complete clearance. The suboptimal visual performance among eyes with previous failed grafts as surgical indication can be explained by the presence of larger amounts of higher-order aberrations after keratoplasty and concomitant extensive subepithelial fibrosis.

Techniques utilizing delivery of endothelium-in grafts optimize graft unfolding,[5,6,14,15] which is perceived as one of most significant challenges among beginning 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 compensates for the longer graft preparation time, thereby maintaining the average total surgical time below 20 minutes in the

majority of cases. Our mean graft unfolding time (2.9±0.9 minutes) compared 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 the technique that we believe improves predictability of graft unfolding. In particular, it is crucial that the unfolded part of the Descemetic surface of the graft is positioned on the floor of the cartridge and that an endothelium-in scroll is formed during advancement of the graft into the cartridge funnel containing 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, thereby allowing 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 tri-folded endothelium-in method.[7]

Direct control of the graft prevents undesired scrolling or graft inversion during the procedure. Though it may be argued that grasping the DMEK graft with forceps results in greater ECL, we have previously shown that as many as 18 forceps bites are necessary to destroy around 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 current data demonstrates gradual decline of ECD after endothelium-in DMEK and compares 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%)[20] and lower than those published by the Melles group (Ham et al = 48.1%[10]; Birbal et al = 56.6%[21]).

Although ECL at 1 year was less than 15% in 25 of the 131 FECD eyes (23%) from this series, the mean cell loss of the entire cohort was greater possibly due to inclusion of greater number of eyes with more advanced endothelial dysfunction. Since the 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.[22] In our practice, as DMEK was increasingly performed in cases which were previously indicated for DSAEK, higher ECL was observed. This may also have been affected by the modifications in DMEK technique using the scleral tunnel incision. Additionally, after an interventional series in partnership with our provider eye bank, we have reverted to using non-preloaded grafts as per our standard technique due to a greater ECL observed in the early postoperative period and mainly because of the less intense trypan blue staining in preloaded grafts.[23]

As previously reported after DSAEK,[24] no differences in ECD outcomes in eyes with FECD and BK as indications were observed. This is contrast to data published after endothelium-out DMEK using the ‘no touch’ technique.[10,16,17,21] The discrepancy may be explained by differences in the preoperative condition of the host cornea, wherein a greater percentage of DMEK is 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 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 utilizes 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 used in our technique not only facilitates DMEK but also makes it equally feasible in eyes with poorer anterior chamber visibility.

With regards to the higher ECL after DMEK performed in failed grafts over other indications, the same predisposing factors that led to accelerated cell loss during the prior keratoplasty probably contribute to greater ECL after DMEK, as has also been observed in cases of repeat PK,[25] or DSAEK after PK.[26]

Graft detachment was the most common major complication occurring in the early post-operative period, but its incidence was within the wide range found in previous literature (0.2-76%).[3] Moreover, we routinely perform rebubbling for any case of graft detachment and do not wait for spontaneous clearance because a significant number of our patients reside in remote areas and even in foreign countries, thus making 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 with conflicting evidence on its superior efficacy compared to 100% air fill.[27-30]

Confirming the outcomes of other series,[10,16,17,21] immunologic rejection was a rare occurrence within 3 years from DMEK (<3%), with rates lower than those reported after standard DSAEK (0-45%)[3] or ultrathin DSAEK (5%).[31] The rate of CME within 1 year after DMEK (1.9%) was similarly lower than rates reported after DSAEK (11-13%)[32-34] and within the range reported after DMEK (0.7%-13%).[35] Despite iatrogenic iris trauma from routine peripheral iridectomy, the incidence of CME remained low possibly due to our postoperative subconjunctival and topical steroid therapy.

The 3-year cumulative survival rate in our series included patients with diagnoses other than FECD, 2 to 3 times higher in number than previously published studies (28% versus 9-11%).[10,16,21] This could explain why our 3-year graft survival rate (93%) was slightly lower than that published for endothelium-out DMEK in these series (94% to 96%).[10,16]

Conversely, when considering only FECD cases also in our series, the 3-year cumulative graft survival rate (97%) was slightly higher than that reported by Price et al. (94%)[20] and Birbal et al. (94%).[21] Moreover, this is consistent with previous observations that FECD eyes tend to have better graft survival probabilities after DMEK than eyes with other indications.[10,16,20,21]

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 is comparable to survival estimates reported for DSAEK (74%)[26] and within the wide range reported for PK.[26,36] The inherent risk for subsequent failure in eyes with failed grafts may account for the significantly increased risk of failure observed.[26]

As with any longitudinal study, limitations of the study are an increasing number of patients lost to follow-up and the noncomparative study design. However, our drop-out rates at all follow-up examination times compare favorably with those of other DMEK studies.[10,16,17] As a tertiary center, since most of our patients are referred to us for

surgery, the main reason for loss of compliance to scheduled examinations is the difficulty both in terms of cost and logistics for elderly patients to return for routine postoperative visits. Nevertheless, the use of statistical methods to take 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 current analysis but contacted telephonically reported satisfaction with their visual outcomes.

Finally, unlike the published clinical trials with more stringent inclusion criteria,[37,38] our study sample has 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. Despite the apparently clear advantage of DMEK for FECD, there is 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 seeking to widen the surgical indications including challenging cases with poor anterior chamber visualization.

In conclusion, tri-folded, endothelium-in DMEK minimizes the time required for graft delivery, the surgical step considered most challenging by majority of surgeons. Visual outcomes and complication rates are not adversely affected by the modification of surgical technique.

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LEGENDS

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

Figure 2. Distribution of Snellen best spectacle-corrected visual acuity over 3 years following Descemet membrane endothelial keratoplasty

Figure 3. Mean endothelial cell density over 3 years following Descemet membrane endothelial keratoplasty. Vertical bars represent standard deviation. Percentage of endothelial cell loss at annual post-operative follow-up is shown in bold.

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

Table 1. Baseline characteristics

Number of eyes	153
Number of patients	145
Recipient age, years, 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, 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 membrane endothelial keratoplasty FECD, Fuchs endothelial corneal dystrophy; IOL, Intraocular lens; PK, Penetrating keratoplasty; SD, standard deviation

Table 2. Three-year clinical outcomes following Descemet membrane endothelial keratoplasty

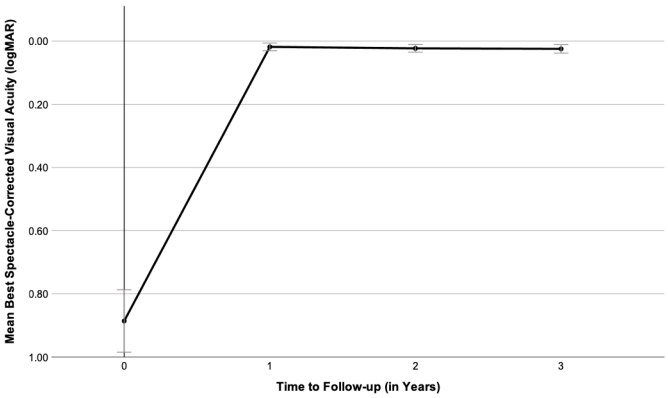
	Baseline	Year 1	Year 2	Year 3
BSCVA				
Number of eyes analyzed (%)	131 of 153 (86%)	131 of 153 (86%)	124 of 153 (81%)	114 of 153 (75%)
Number of eyes excluded (%)		22 of 153 (14%)	29 of 153 (19%)	39 of 153 (25%)
Lost to follow-up		0	4	12
Re-graft		2	5	7
Low visual potential due to ocular comorbidity		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
number of eyes ≥ 20/40 (%)	39 (25%)	131 (100%)	124 (100%)	114 (100%)
number of eyes ≥ 20/25 (%)	0 (0%)	121 (92%)	113 (92%)	102 (89%)
number of eyes ≥ 20/20 (%)	0 (0%)	87(66%)	81(65%)	69(61%)
number of eyes ≥ 20/17 (%)	0 (0%)	15 (11%)	13 (10%)	12(11%)
Endothelial cell density				
Number of eyes analyzed (%)	151 of 153 (99%)	151 of 153 (99%)	144 of 153 (94%)	134 of 153 (88%)
Number of eyes excluded (%)		2 of 153 (1%)	9 of 153 (6%)	19 of 153 (12%)
Lost to follow-up		0	4	12
Re-graft		2	5	7
mean ± SD (cells/mm^2)	2580±103	1818±362	1675±372	1580±423
Fuchs endothelial corneal dystrophy	n=110, 2577±103	n=110, 1893±320	n=106, 1750±319	n=103, 1657±378
Bullous keratopathy	n=24, 2588±103	n=24, 1774±289	n=22, 1640±352	n=18, 1557±405
Failed previous graft	n=17 2606±111	n=17, 1398±430	n=16, 1186±393	n=13, 906±223

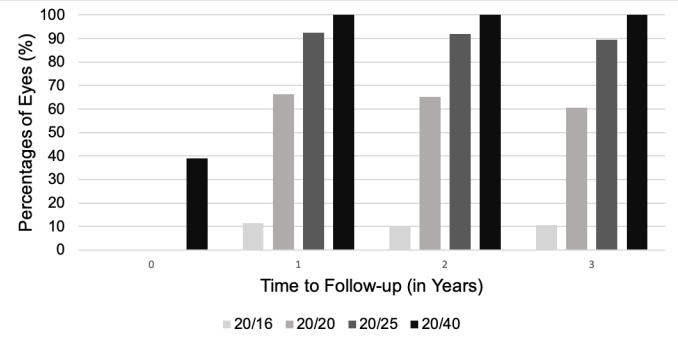
BSCVA, Best spectacle corrected visual acuity; logMAR, logarithm of the minimum angle of resolution; SD, standard deviation

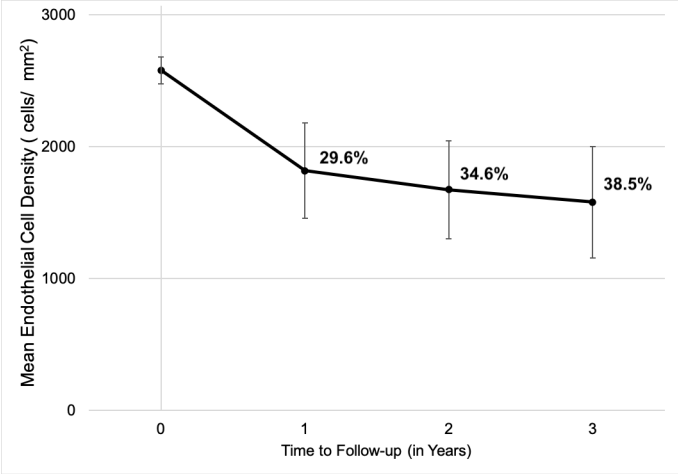
Table 3. Postoperative Complications

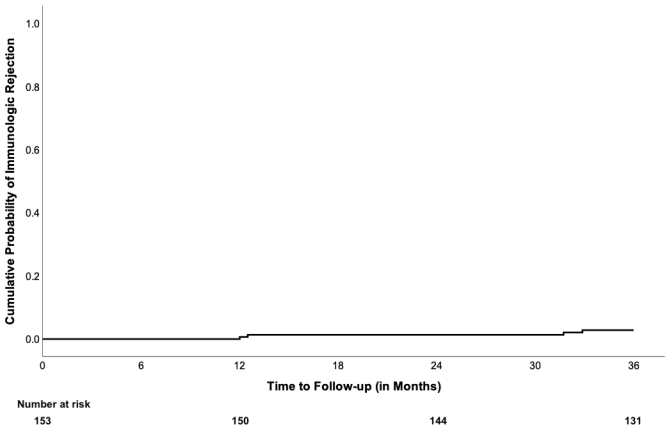
Graft Detachment	42 (27.4%)
One Rebubbling Procedure	38
Two Rebubbling Procedures	4
Graft Rejection	4 (2.6%)
Graft Failure*	10 (6.5%)
Early Failure*	2
Primary Donor Failure*	0
Graft Rejection*	1
Nonrejection*	7
Refractive/Visual*	0
Repeat Graft	7 (4.6%)
Cystoid Macular Edema	3 (2.0%)
Persistent Epithelial Defect	1 (0.7%)

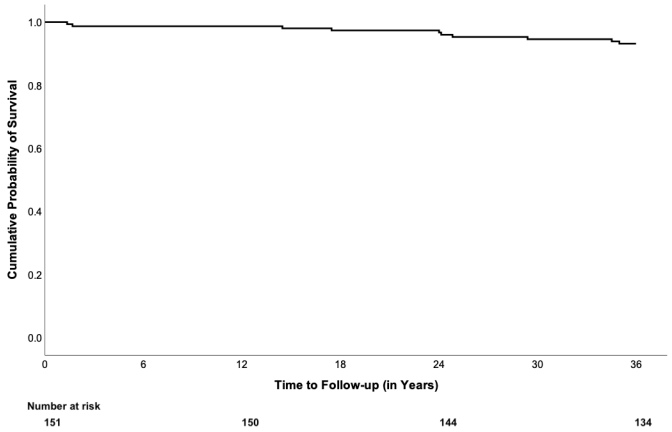
*Definitions were based on the criteria from the Cornea Preservation Time Study[11]

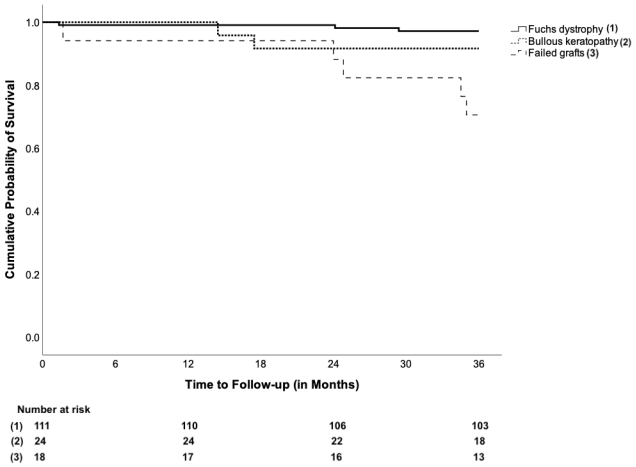












Tri-folded endothelium-in Descemet membrane endothelium keratoplasty with bimanual pull-through delivery technique facilitates graft unfolding with excellent 3-year outcomes regardless of preoperative eye status.