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Contact Lens-Assisted Pull-Through Technique for Delivery of Tri-Folded (Endothelium in) DMEK Grafts Minimizes Surgical Time and Cell Loss

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Purpose: To evaluate the feasibility and outcomes of contact lens-assisted bimanual pull-through delivery of Descemet membrane endothelial keratoplasty (DMEK) tissue trifolded with the endothelium inward.

Design: Prospective, noncomparative, interventional case series.

Participants: Forty-two consecutive eyes of 42 patients with Fuchs endothelial dystrophy with or without cataract.

Intervention: Standardized DMEK was performed as a single procedure (n = 9) or in combination with phacoemulsification and implantation of a posterior chamber intraocular lens (n = 33) using prestripped donor tissue punched to a diameter of 8.25 mm and then trifolded with the endothelium in. Using a sterile soft contact lens as scaffold, the tissue was loaded in this configuration into a disposable cartridge and delivered into the anterior chamber under continuous irrigation using a bimanual pull-through technique to facilitate spontaneous proper unfolding.

Main Outcome Measures: Surgical time, intraoperative and postoperative complications, visual acuity 3 and 6 months after surgery, and endothelial cell loss 6 months after surgery.

Results: Surgery was uneventful in all cases and the time required for the DMEK procedure (from Descemet scoring until final air filling) never exceeded 20 minutes (average, 17.1±1.6 minutes). The only complication observed after surgery was graft detachment (10 of 42 eyes [23.8%]), successfully managed in all cases by single rebubbling within 6 days from surgery. In all eyes with a minimum postoperative follow-up of 6 months (n = 20), best spectacle-corrected visual acuity was 20/25 or better and the average endothelial cell density (±standard deviation) was 2363.8±82.7 cells/mm^2 (range, 2258−2490 cells/mm^2). The cell loss calculated as a percentage of the preoperative value determined at the eye bank (range, 2500−2700 cells/mm^2) was 9.9±2.1% (range, 4.1%−11.9%).

Conclusions: Delivering DMEK tissue, trifolded with the endothelium inward, reduces surgical trauma to donor cells and facilitates spontaneous unfolding, thus minimizing surgical time. *Ophthalmology 2016;123:476-483 © 2016 by the American Academy of Ophthalmology.*

Supplemental video available at www.aaojournal.org.

During the last decade, endothelial keratoplasty has become the gold standard for the treatment of endothelial decompensation. The annual report of the Eye Bank Association of America showed that in only 2 years, between 2005 and 2007, the number of Descemet stripping endothelial keratoplasty procedures performed in the United States increased by 10 fold and that this number has been constantly higher than 20 000 since 2011. Instead, Descemet membrane endothelial keratoplasty (DMEK) has gained popularity much more slowly, and in 2014, fewer than 3000 surgeries were counted. Despite the appeal of DMEK in terms of its minimally invasive nature, the fast recovery of optimal vision,[1–3] and the extremely low incidence of postoperative immunologic rejection,[4] the technique still offers major challenges, mainly related to delivery, unfolding, and positioning of the graft.[5,6] In addition, with current techniques, donor tissue is rolled with the endothelium outward, thus exposing it to friction against the device walls during both loading and delivery.[1]

To minimize endothelial damage, Muraine et al[7] modified the DMEK technique by trifolding the stripped donor tissue with the endothelium inward, which then was injected into the anterior chamber in a manner similar to that used with conventional tissue rolls (endothelium outward). However, transferring the tissue roll from the

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donor cornea onto the cartridge in its modified configuration is difficult, and unfolding, as well as proper positioning, were not standardized. In an attempt at overcoming the limitations of the technique reported by Muraine et al, we used a sterile soft contact lens as scaffold to load the graft in its trifolded configuration into a cartridge for delivery by means of a bimanual pull-through technique. We present herein the outcomes of the first 42 consecutive eyes operated on with this technique.

Methods

We reviewed the charts of all patients with decompensated endothelium who underwent surgery according to the technique described in detail below and were included in a prospective clinical study undertaken at our institution in June 2014 and still in progress. The study followed the tenets of the 1964 Declaration of Helsinki and was approved by the local ethics committee; detailed informed consent was provided to all patients undergoing surgery. Best spectacle-corrected visual acuity better than 20/30 and peripheral endothelial cell density (ECD) higher than 2000 cells/mm^2 in the absence of central corneal edema were the only exclusion criteria.

Before surgery, demographic data were recorded and every patient underwent a complete ophthalmologic evaluation including slit-lamp examination, best spectacle-corrected visual acuity, refraction, tonometry, funduscopy, as well as central (when possible) and peripheral endothelial microscopy (EM-3000; Tomey, Erlangen, Germany). In addition, the power of the intraocular lens to be implanted was determined by means of optical biometry (Lenstar LS900; Haag-Streit, Bern, Switzerland).

All surgical procedures were video-recorded and the time elapsing between the beginning of descemetorhexis and the final air filling was noted (Video 1, available at www.aaojournal.org). Patients were scheduled for assessment of best spectacle-corrected visual acuity 3 and 6 months after DMEK and assessment of ECD 6 months after DMEK. Postoperative ECD was compared with that measured before surgery by the eye bank for the donor corneas using light microscopy after vital staining with trypan blue, and cell loss was determined as a percentage of the preoperative in vitro value. Intraoperative and postoperative complications also were recorded.

Surgical Procedure

In all patients, anesthesia and akinesia were obtained by means of peribulbar injection of 10 ml of a 0.75% ropivacaine solution. Epithelial edema affecting visualization of the intraocular structures was managed by removal of the epithelium from the central area approximately 8 mm in diameter. Then, when necessary (n = 33 eyes), bimanual phacoemulsification was performed using a 0.5-mm long and 2.75-mm wide clear-cornea tunnel, located inferotemporally in all right eyes and supranasally in all left eyes. In all cases, a hydrophobic intraocular lens (iSert 250; Hoya, Tokyo, Japan) was implanted into the capsular bag expanded by the injection of viscoelastic substance (IAL-F; Fidia Farmaceutici, Abano Terme, Italy), which then was removed carefully from the anterior chamber by prolonged irrigation and aspiration. The endothelium–Descemet complex was scored with a Price hook (Moria SA, Antony, France) and removed under air from the central 9 mm of the recipient cornea, possibly in a single piece. An inferior peripheral iridotomy was performed using vitreoretinal guillotine scissors under continuous irrigation from a specially designed anterior chamber maintainer (ACM; Moria SA) inserted at the 12-o'clock position.

According to the technique described by Terry et al,[8] each donor cornea (donor age range, 55–64 years) was prestripped at the eye bank over a 9.5-mm central area, with the exception of the peripheral edge for approximately 1 clock hour, which was marked on the scleral rim using gentian violet. The stripped endothelium was repositioned onto the stromal bed, and liquid was removed from the peripheral endothelial surface to facilitate adherence, before gently reimmersing the tissue into the storage medium. During surgery, the cornea was laid onto the trephination block of an 8.25-mm Barron punch (Katena Products, Inc., Denville, NJ) stained with trypan blue (VisionBlue, D.O.R.C., The Netherlands) to outline better the edge of the stripped area and punched partial thickness. The crown of detached endothelium outside of the punched area included the hinge of incomplete stripping and was removed. The tips of a dedicated anatomic forceps (Moria SA) were used to lift the edge of the DMEK graft, which then was trifolded with the endothelium inward (Fig 1A, B) and stained again with trypan blue. A sterile therapeutic soft contact lens commercially available (Sooft, Montegiorgio, Italy) was laid next to the trifolded graft, which was grasped at its very edge of the unfolded part with the same forceps and dragged onto the contact lens in its trifolded architecture (Fig 1C). As shown in Fig 1D, the contact lens was moved onto the back entrance of the funnel of a commercially available intraocular lens cartridge (MDJ Company, La-Monniere-le-montel, France), which was filled with balanced salt solution (BSS) from its distal part. A dedicated anatomic microincision forceps (Moria SA) was inserted into the distal entrance of the cartridge to reach the contact lens surface and grasp the edge of the DMEK graft at the center of its unfolded part (Fig 2A). The graft then was pulled into the funnel, taking care to make the unfolded part slide onto the floor of the funnel (Fig 2C). As shown schematically in Figure 2B, D, coming in contact with the BSS solution, the DMEK roll opened up partly, thus adhering to the funnel wall but maintaining the endothelium in its facing inward configuration, and therefore preventing possible damage resulting from the contact with the plastic. The back entrance of the cartridge funnel was sealed with a silicone plug mounted on the prototype of a dedicated handle to avoid reflux of liquid and graft loss during delivery.

Figure 3 shows intraoperative pictures (Fig 3A, C) and drawings (Fig 3B, D) illustrating that the cartridge then was turned by 180°, thus making the floor become the ceiling of the funnel, and was inserted into the main wound. An additional side entry was created supranasally in all right eyes and inferotemporally in all left eyes for insertion of the microincision forceps. Then, similar to the Descemet stripping endothelial keratoplasty technique, the DMEK graft was delivered bimanually through the clear-cornea tunnel under low-flow continuous irrigation from a dedicated ACM with a lateral 0.5-mm port, which, unlike conventional ACMs, would prevent creation of a jet fluid stream directed against the DMEK graft, and therefore would eliminate possible interference with tissue unfolding. After delivery into the anterior chamber, the descemetic surface of the unfolded part of the DMEK graft, initially in contact with the cartridge ceiling, was now facing the internal surface of the recipient cornea, as required for proper attachment (Fig 4A). Gentle tapping onto the cornea surface was used to facilitate unfolding of the lateral folds (Fig 4B, C), which invariably occurred because of the natural tendency of the tissue to roll with the endothelium outward from its initial inward position. In some cases, also twisting the forceps or gently moving the graft inside the anterior chamber was used to unfold it. As soon as

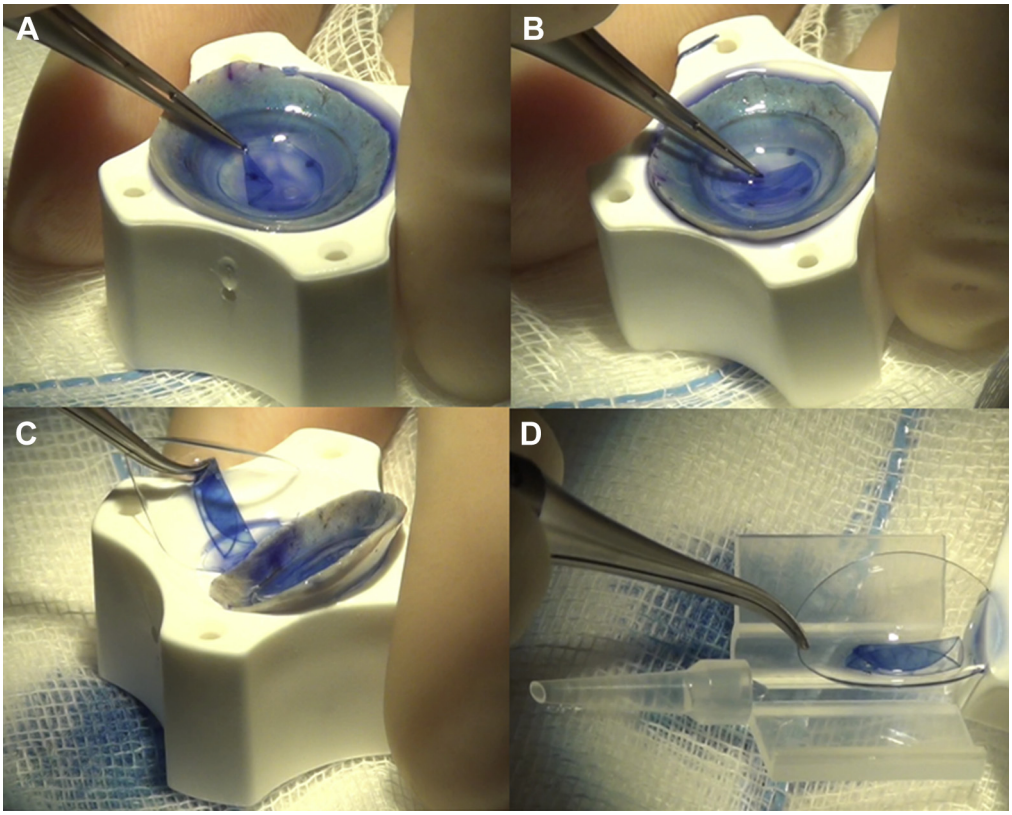

Figure 1. Photographs showing (**A**, **B**) trifolding of the Descemet membrane endothelial keratoplasty graft with dedicated toothless fine forceps, which (**C**) are also used to drag the tissue onto the soft contact lens. **D**, The contact lens carrying the donor tissue is brought onto the cartridge groove.

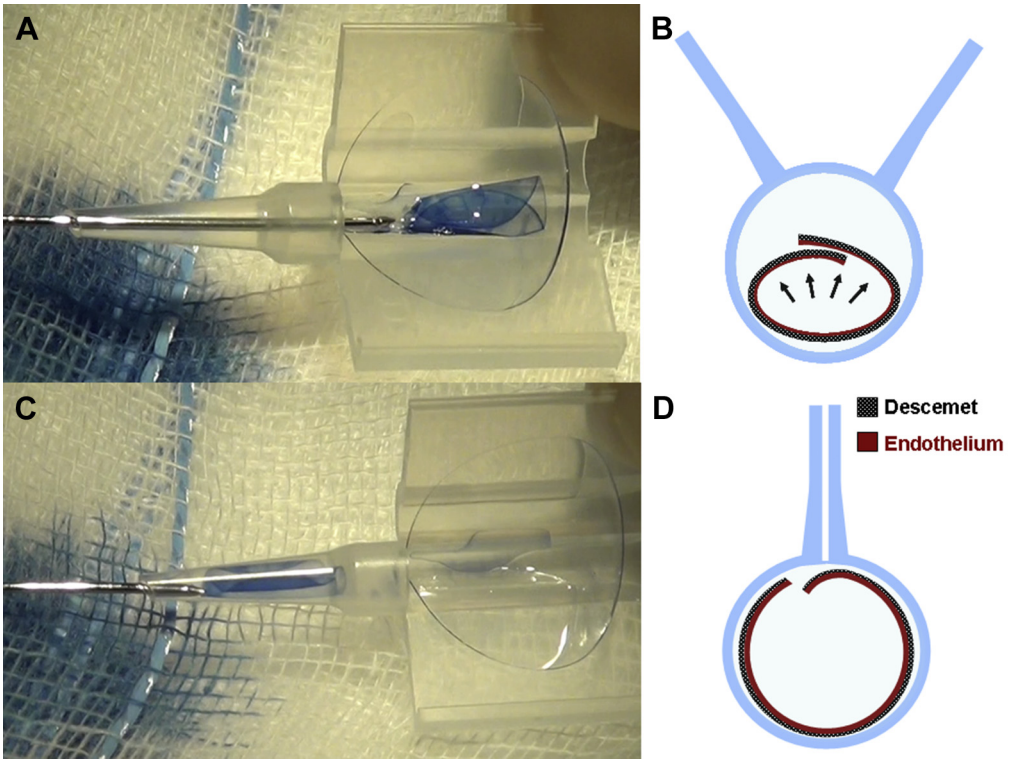

Figure 2. **A**, **C**, Intraoperative photographs and (**B**, **D**) schematic representations illustrating the loading maneuver using dedicated microincision forceps. **A**, **B**, The tissue roll, coming in contact with the liquid inside the funnel, opens up, (**C**, **D**) making the descemetic side adhere to the plastic wall.

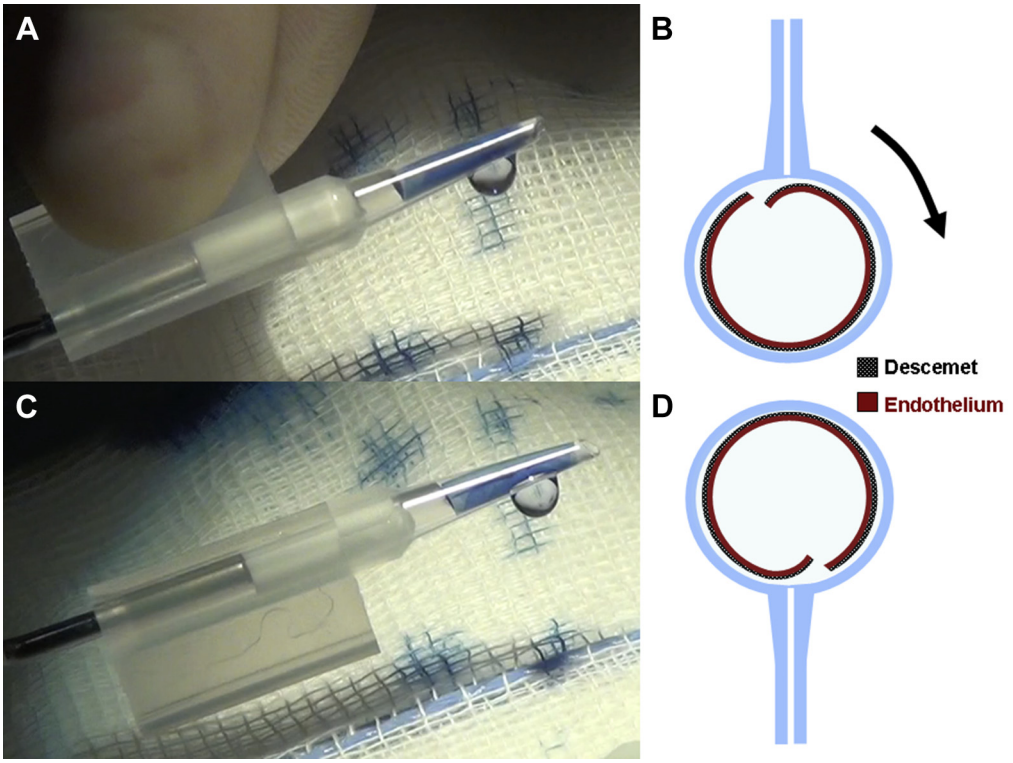

Figure 3. **A**, **C**, Intraoperative photographs and (**B**, **D**) schematic representations illustrating proper positioning of the graft in preparation for delivery. **A**, **B**, Rotating the cartridge by 180° makes (**C**, **D**) the descemetic side of the unfolded part of the graft face upward; that is, after delivery, it faces the internal surface of the recipient cornea.

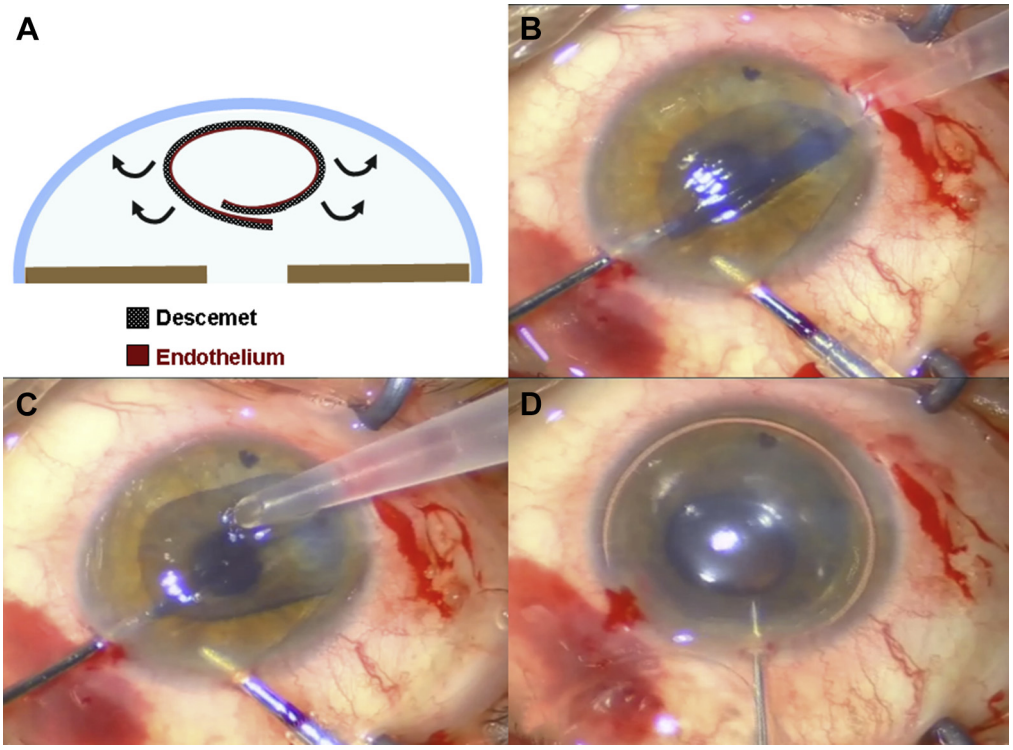

Figure 4. **A**, Schematic representation and (**B**, **C**, **D**) intraoperative photographs illustrating graft delivery and attachment. The natural tendency of the Descemet membrane endothelial keratoplasty (DMEK) graft to roll with the endothelium outward (**B**, **C**) makes it unfold spontaneously under minimal irrigation from the anterior chamber maintainer. **D**, After unfolding is completed, air is injected under the DMEK graft until the anterior chamber is completely full.

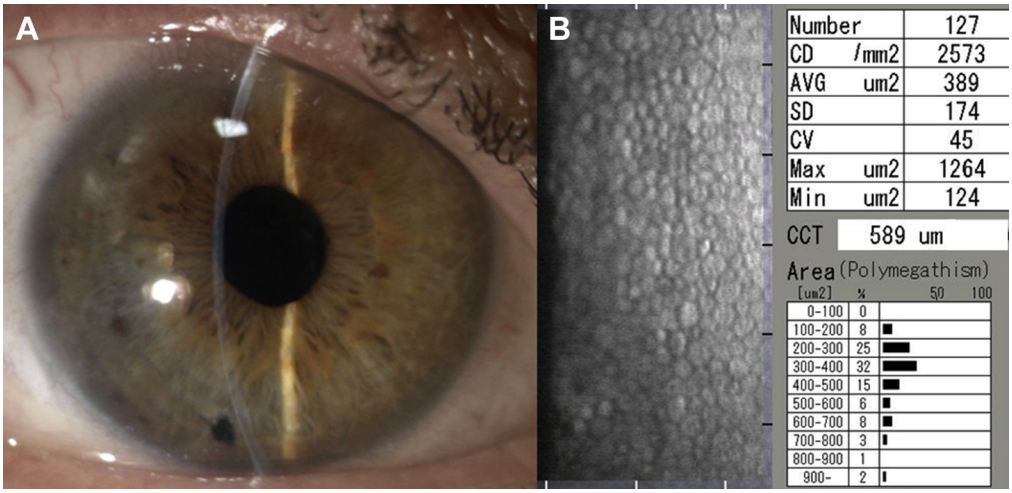

Figure 5. **A**, Clinical photograph and (**B**) endothelial specular microscopy results of the right eye of a 59-year-old patient obtained 6 months after combined Descemet membrane endothelial keratoplasty and phacoemulsification with implantation of a posterior chamber intraocular lens. Uncorrected visual acuity was 20/20 and endothelial cell loss was 4.7% of the 2700 cells/mm^2 counted before surgery. AVG = average; CCT = central corneal thickness; CD = cell density; CV = cell volume; Max = maximum; Min = minimum; SD = standard deviation.

unfolding was achieved, the BSS flow in the ACM was stopped and the forceps were removed. In only 2 of 20 cases, additional maneuvers, also described by other authors,[2,9] were necessary to unfold the donor tissue completely. A 27-gauge cannula then was inserted through the 12-o'clock side entry and advanced under the donor tissue up to approximately the center of the pupil before filling the anterior chamber with air and obtaining this way proper attachment of the donor tissue onto the posterior surface of the recipient cornea (Fig 4D). Surgery was completed by air-tight suturing of the main wound as well as the side entries with 10-0 nylon. Finally, air was injected under pressure into the anterior chamber by means of a 32-gauge needle inserted obliquely through the limbus and rapidly retracted to prevent reflux.

Triamcinolone acetonide and gentamicin sulfate 0.3% were injected subconjunctivally at the end of the procedure. After surgery, a pressure patch was applied and patients were instructed to lie on their backs for 2 hours before being checked at the slit lamp. If a pupillary block was present, air was released from the main wound. Beginning the next morning, dexamethasone phosphate 0.1% and tobramycin sulfate 0.3%, both antibiotic eye drops, were administered every 2 hours, then tapered over 3 to 4 months to a single daily steroidal administration, which then was discontinued only in steroid responders. In every patient, all sutures were removed 4 to 6 weeks after DMEK.

Results

At the time of this review, 42 eyes of 42 patients had been operated on with the technique described above, but only 20 eyes had reached 6 months of follow-up. There were 24 women and 18 men with an age range of 47 to 86 years (average ± standard deviation, 69.1±10.6 years). Surgery was uneventful in all cases. The time required to perform DMEK, from scoring of Descemet membrane to final air filling, never exceeded 20 minutes (average ± standard deviation, 17.1±1.6 minutes). After surgery, no primary failures were observed, but partial detachment was seen in 10 of 42 eyes (DMEK alone, n = 2; DMEK and phacoemulsification, n = 8). All of the eyes were managed successfully by single rebubbling within 6 days from surgery. Best-spectacle-corrected visual acuity was 20/25 or better in 34 of 35 eyes with a minimum follow-up of 3 months and in 20 of 20 eyes with a minimum follow-up of 6 months.

Six months after DMEK, the average ECD ± standard deviation was 2363.8±82.7 cells/mm^2 (range, 2258−2490 cells/mm^2). The cell loss calculated as a percentage of the preoperative value determined at the eye bank (range, 2500−2700 cells/mm^2) was 9.9±2.1% (range, 4.1%−11.9%). Figure 5 illustrates the outcome of combined DMEK and phacoemulsification in the right eye of a 59-year old woman with Fuchs' dystrophy; 6 months after surgery, uncorrected vision was 20/20 and endothelial cell loss was 4.7% of the 2700 cells/mm^2 counted before surgery.

Discussion

Within 10 years from its introduction in 2004,[10] Descemet stripping endothelial keratoplasty rapidly became the keratoplasty procedure performed most frequently in the United States and other countries. The main reasons for this include the ease of preparation of donor tissue in the eye bank or during surgery, standardization of the surgical technique, and outcomes far superior to those of conventional penetrating keratoplasty. However, although the initial report of successful DMEK dates back to 2006,[1] the 2014 annual report of the Eye Bank Association of America showed that in the United States, its use is still limited to only approximately 10% of eyes with endothelial decompensation, notwithstanding the strong appeal of the procedure in terms of visual outcomes and speed of visual rehabilitation. The need for high surgical skills and lack of standardization of DMEK, as well as the high rate of intraoperative and postoperative complications, all have contributed to slowing down the spread of this technique among corneal surgeons.

However in recent years, substantial progress has been made in the technique of preparing DMEK grafts, and prestripped tissue is available today from many eye banks. The use of prestripped tissue eliminates the intraoperative

waste during graft preparation, reduces considerably the time required for the procedure, and yields results comparable with those obtained with donor tissue stripped during surgery.[8,11,12]

Instead, graft delivery and positioning remain critical steps that are dealt with by different surgeons in very different ways, including various types of direct and indirect manipulations as well as the use of intracameral air.[2,9,13] More recently, in an attempt at simplifying and standardizing the DMEK technique, Muraine et al[7] advocated the preparation and injection of grafts folded with the endothelium facing inward. We approached the procedure from a different perspective based on the fact that DMEK tissue has a natural tendency to roll onto itself with the endothelium facing outward. We thought that if we could deliver the DMEK tissue and hold it in the anterior chamber with the endothelium facing inward under minimal irrigation, it would unroll spontaneously, following its natural tendency. In addition, avoiding direct contact between donor endothelium and the walls of the cartridge could reduce the cell loss during both loading and delivery of the graft.

Trifolding the prestripped tissue with the endothelium inward was relatively easy while the detached graft was still lying on the donor cornea. However, maintaining the trifolded architecture during transportation into the cartridge initially was a major challenge, because lifting the tissue with forceps made it lose its configuration. Therefore, we resorted to using a soft contact lens for this purpose. The hydrophilic nature of the lens allowed us to drag the graft in its trifolded shape smoothly onto its surface. The soft contact lens was a flexible support that adapted perfectly to both the hollow of the donor cornea and the groove of the cartridge while maintaining the donor tissue adherent in its trifolded architecture. This maneuver succeeded uneventfully in all 42 consecutive patients in this series. Also, controlled loading of the DMEK graft into the cartridge funnel by pulling it with toothless dedicated forceps was achieved successfully in all cases. As a result, proper adaptation of the tissue with its Descemetic surface against the funnel wall was obtained while the endothelium was still facing inward, thus eliminating a potential source for endothelial damage.

The bimanual pull-through technique that we developed in the past for delivery of DSAEK grafts[14] also was used here with minor changes. The main change was the use of a dedicated ACM with a closed end and a laterally located port, which prevented BSS from flowing against the donor tissue and affecting its unfolding. Holding the graft until unfolding was completed was instrumental for the success of the maneuver, which sometimes also required gentle tapping on the cornea or twisting of the forceps. In only 2 of 42 cases (4.8%), inadvertent loss of the grip required completion of unfolding by means of other maneuvers, as commonly used by other authors.[2,9,13,15] Unlike in DSAEK, trifolding DMEK grafts were found to cause minimal and scattered endothelial damage, without correlation with the location of the folds. In particular, after trifolding (endothelium-in) 18 DMEK grafts, 0.63% of cells showed positive staining results for trypan blue and 0.26% of uncovered areas (no cell zone) were seen (unpublished data, Fondazione Banca degli Occhi del Veneto, Venice, Italy, 2015). Also, possible concerns about damage caused by direct grasping of the tissue were ruled out by considering that the tip platform of our forceps has a triangular area of contact of approximately 0.03 mm^2 (base, 200 µm; height, 300 µm). Each forceps bite therefore could damage only approximately 75 endothelial cells of a graft with a preoperative density of 2500 cells/mm^2. In addition, as shown in Figure 6, punching the tissue to the required diameter results in a peripheral crown of dead endothelial cells extending for approximately 50 µm in width at the very edge of the graft. These cells have to be deducted from the 75 cells crashed by the forceps tips, thus reducing the number of cells damaged by each bite to approximately 50 (area of contact between metal platform and endothelium minus the area damaged by punching, 0.02 mm^2), an absolutely negligible amount even if multiple bites are required for the procedure. Finally, most of the time, the tissue is grasped using only the distal part of the platform, thus further reducing the possible endothelial damage.

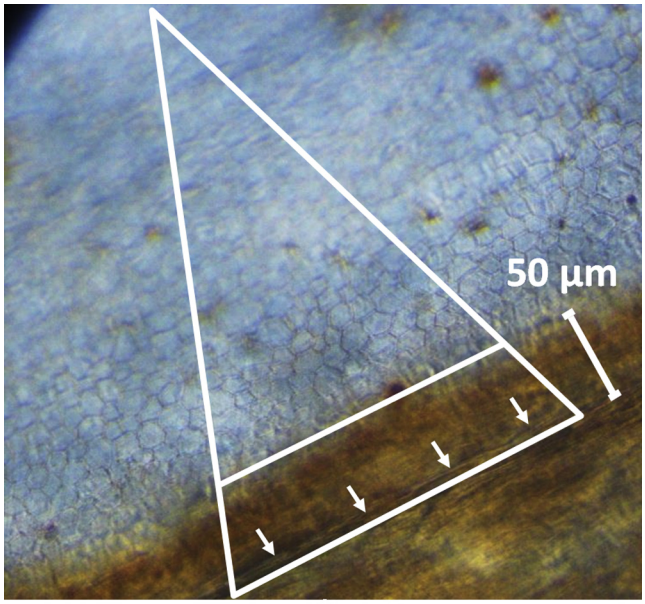

Figure 6. Photograph showing eye bank prestripped tissue after being punched. At the very edge of the tissue (arrows), a crown of damaged endothelial cells approximately 50 µm wide is stained by alizarine red. The triangular grasping platform of the dedicated forceps is represented here in white. The maximum area of possible damage caused by tissue grasping amounts to 0.03 mm^2, that is, approximately 75 cells for a donor tissue with a density of 2500 endothelial cells/mm^2.

The approach that we have developed offers several advantages over those used to date. Most importantly, each surgical step of the procedure is under complete control by the surgeon. The Descemetic and endothelial sides of the graft therefore remain identified throughout the procedure with no need for marking. Primary failure resulting from attachment of the graft upside down, which remains the main cause for primary failure,[8] did not occur in any of the

eyes operated on with our technique. In addition, our initial assumption on which the technique was based revealed that counter-folding the donor tissue against its natural tendency to roll with the endothelium outward resulted in spontaneous proper unfolding during delivery, requiring minimal additional manipulation and shortening the procedure considerably. The relatively old age of the donors in our series was a choice of the eye bank to facilitate Descemet stripping, but also may have avoided a too-pronounced counter-folding at the time of surgery, thus optimizing our procedure. Minimizing surgical manipulation and eliminating friction against the funnel wall while both loading and delivering the DMEK tissue trifolded with the endothelium inward proved instrumental in limiting the endothelial cell loss recorded 6 months after surgery to values less than those reported after both DSAEK and DMEK.[7,14,16,17]

Visual outcomes in the eyes of our series were similar to those published by other authors independent of the surgical technique used, an outcome to be expected after successful DMEK surgery.[7,11,16,18] Postoperative graft detachment occurred in a percentage of eyes similar to that of previous reports,[7,8,15,18] confirming that this complication also is not strictly related to the surgical technique used, but rather may depend on other as-yet unidentified factors.

Although the number of eyes included in our series is too small to allow proper comparison, similar to the results of Chaurasia et al,[19] we did not see any substantial difference between the outcomes of DMEK and those of DMEK in association with cataract surgery. Therefore, a combined procedure remains our procedure of choice whenever indicated.

In conclusion, our initial results indicate that the contact lens-assisted pull-through technique for delivery of DMEK grafts achieves the major goal of allowing complete control of all surgical steps of the procedure. As a consequence, intraoperative complications such as inadvertent inversion of the graft are eliminated, surgical time is minimized, and endothelial cell loss is reduced to less than the values reported with other techniques. We believe that this technique could be instrumental in making DMEK affordable to the average corneal surgeon, thus quickly spreading the use of this procedure.

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Footnotes and Financial Disclosures

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Analysis and interpretation: Leon, Ponzin

Data collection: Leon, Ponzin

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Overall responsibility: Busin, Scorcia

Abbreviations and Acronyms:
ACM = anterior chamber maintainer; **BSS** = balanced salt solution; **DMEK** = Descemet membrane endothelial keratoplasty; **ECD** = endothelial cell density.

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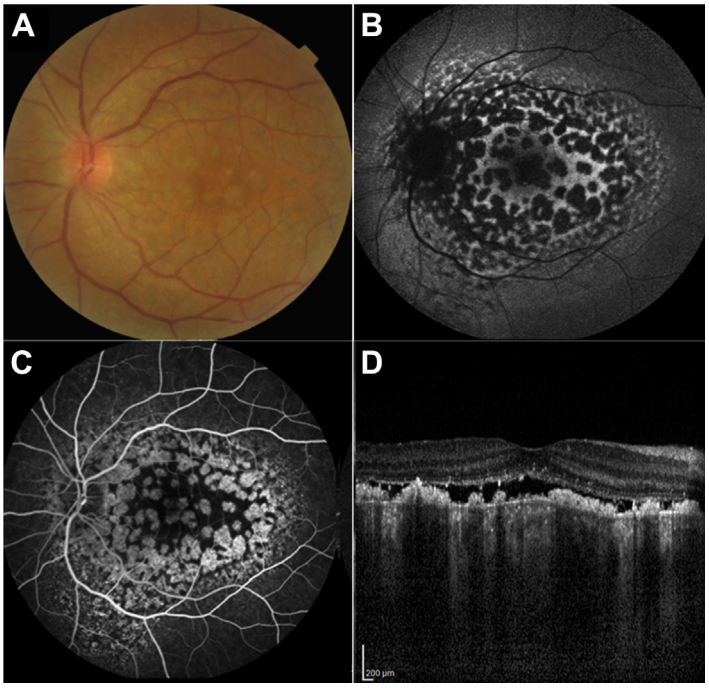
Pictures & Perspectives



Giraffe Pattern of Bilateral Diffuse Uveal Melanocytic Proliferation

A 42-year-old woman with history of ovarian cancer after recent induction chemotherapy was referred for bilateral progressive vision loss of 4 months duration. On presentation, her visual acuity was 20/40 in both eyes. Funduscopic examination (Fig 1**A**) revealed bilateral nummular retinal pigment epithelial (RPE) clumping that with autofluorescence imaging (Fig 1**B**) appeared as islands of decreased autofluorescence surrounded by zones of increased autofluorescence signal in a giraffe-like pattern. The inverse was observed with fluorescein angiography (Fig 1**C**). Optical coherence tomography (Fig 1**D**) revealed irregularity of the RPE layer with associated subretinal fluid, confirming a diagnosis of bilateral diffuse melanocytic proflieration (BDUMP).

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