Two-year clinical outcome after Descemet membrane endothelial keratoplasty using a standardized protocol

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Purpose: The purpose of this study is to evaluate 2-year clinical outcome after Descemet membrane endothelial keratoplasty (DMEK) in a variety of endothelial dysfunctions using a standardized protocol. Methods: From a group of 230 eyes which underwent DMEK for Fuchs’ endothelial corneal dystrophy (FECD), aphakic and pseudophakic bullous keratopathy, failed full thickness corneal transplants, ICE syndrome, failed DSEK, and TASS the clinical outcomes [best spectacle-corrected visual acuity (BSCVA), central endothelial cell density (ECD)] were evaluated before, and at 6, 12, and 24 months and the success rate, failure rate and postoperative complications were also analyzed. Results: Out of 230 eyes, 144 eyes (70%) had BSCVA 6/9 or better 2 years postoperatively. Mean donor ECD was 2692.23 (range, 2300–3436) cells/mm² preoperatively, which was reduced to 1433.64 (range, 619.0–2272.0) cells/mm² 2 years after DMEK surgery, indicating a mean reduction of 1258 cells/mm² (46%) in ECD. Conclusion: DMEK is a highly successful surgical procedure when following a standard protocol for treating diseases of the corneal endothelium providing a near perfect anatomic restoration and a high degree of visual rehabilitation.

Key words: Corneal endothelium, Descemet membrane, Descemet membrane endothelial keratoplasty, Standardized technique

The final iteration of endothelial keratoplasty came with pure anatomic replacement: Descemet membrane (DM) was removed from the recipient and replaced with only donor DM and no stromal carrier tissue compared to its predecessor DSEK/DSAEK (Descemet stripping (automated) endothelial keratoplasty). The first case of this was performed by Melles et al. in 2006 and he named it Descemet membrane endothelial keratoplasty (DMEK).[1-4]

Even after a decade, still DMEK has not yet gained widespread acceptance in India and the number of corneal surgeons performing DMEK remains just a handful because of its steep learning curve encompassing donor preparation, insertion, and positioning inside the eye. Also, there are no long-term established DMEK studies published in Indian literature. Several variations of the surgical technique of DMEK and different DMEK graft injectors have evolved over the years but yet no standardized protocol has been adopted worldwide for DMEK surgery due to its technical difficulty itself, economic practicalities, and increased postoperative complications compared with those of DSEK or DSAEK.[6] Unlike western world, patients in India also present at a later stage during which stromal scarring starts due to chronic corneal decompensation[8] resulting in poor visibility for unfolding the DM scroll in the anterior chamber. Another key issue for surgeons is that in DMEK, a total dislocation of tissue will require replacement of tissue, whereas DSEK requires only rebubbling.[9-11] Since there is a dearth of corneal tissues in India, surgeons are worried about the tissue loss and resort to the safer and standard DSEK procedure.

Even though we started DMEK as early in 2013, we started performing DMEK regularly only after 2015 after refining each and every step involved in the procedure as emphasized by Terry.[12] The current study will evaluate the clinical results of DMEK using our standardized protocol in a wide variety of endothelial diseases.

Methods

Results of the 230 consecutive cases of DMEK performed have been reported. All patients read and signed an informed consent document. The tenets of Declaration of Helsinki were followed. Patients with any type of endothelial dysfunction which included phakic/pseudophakic/aphakic bullous keratopathies, endothelial dystrophies, and toxic endothelial damage were taken for the study. A full history and physical examination were conducted in all patients, and best spectacle corrected visual acuity (BSCVA), slit lamp examination results, specular microscopy of the other eye, and IOP measurements were recorded. Fundus examination or ultrasonography was performed to assess the posterior segment.
Surgical Procedure

Donor preparation

Initial scoring

The donor tissue is placed in the well of the cutting block and a 10-mm trephine is used to gently mark the DM (use very minimal pressure, so that only the DM and a thin layer of stroma are cut) with the endothelial side facing up [Fig. 1a]. The marked DM is scored with a Sinskey hook 360° gently without creating any tags or irregularities at the edge of the DM [Fig. 1b].

The scoring is performed while the donor tissue and the subsequent graft remain submerged in balanced salt solution (BSS). The tissue is scored 0.5–1.0 mm central to the limbus, as more peripherally, the stroma and DM are adherent and an edge cannot be created.

Staining

Trypan blue is used to stain the graft only after the initial trephination. First, the storage media in which the graft has been submerged is largely removed with a Weck-cel ophthalmic sponge so that it will not dilute or prevent adequate staining. After staining with the dye for approximately 2 min, excess dye is decanted and several drops of the media are again used to fill the donor rim. With this process, scored tissue edges are highlighted as dark blue.

Lifting up the edge and DM stripping

The periphery of the scored endothelial edge is elevated with a blunt Sinskey hook to separate the endothelium-Descemet membrane 360° circumferentially. Once the peripheral tissue is separated, an edge is grasped with smooth curved forceps and additional endothelial-Descemet membrane is stripped under BSS using “single-pull technique” [Fig. 1c].

Marking the tissue

After stripping half of the DM from the donor and creating 3 mm punch in the stroma [Fig. 1d], “L”-shaped mark is made through the stromal window on the DM using a stained “L” stamp with the horizontal arm being shorter and vertical arm being longer [Fig. 1e] so the shorter horizontal arm always comes to the right side of the longer vertical arm when oriented endothelial side down. After this, the DM is replaced back on the stroma.

Trephination

The size of trephine used is determined by the host’s underlying corneal pathology and also the horizontal diameter of the cornea. Baron’s vacuum punch was used to cut the tissue.

Storage

After trephination to the desired graft size and complete separation of the DMEK tissue, the tissue scrolls with the endothelium facing outward [Fig. 2a]. The DMEK tissue is then placed in BSS and stained again with trypan blue to allow visualization after insertion.

Recipient preparation

Removal of epithelium

Epithelium was removed with a blunt spatula prior to the intraocular procedure for better visualization in all the cases.

Wound construction

Paracentesis is made almost parallel to the iris plane in the superior and inferior clear corneal limbus. The main incision is made temporally at 3 o’clock in a uniplanar fashion with a 3-mm keratome at the limbus.

Descemet’s membrane stripping

A desired size trephine is gently pressed on the epithelial side to help in the sizing of the descemetorhexis. The edges of the trephine mark are highlighted with a mark in a dot fashion. Using a reverse Sinskey hook the recipient DM is scored and gently removed from the stromal bed avoiding any trauma to the overlying stroma. Peripheral iridectomy is done at 6 o’clock position as peripherally as possible to prevent pupillary block.

Loading

Prototype injector: A regular intraocular lens C-cartridge, IV tubing, and 1-cm³ syringe is assembled [Fig. 2b]. The DM scroll, which was previously scored in the storage medium, is then sucked into the DMEK injector using “no touch technique” [Fig. 2c].

Injection into the anterior chamber

Using our prototype injector, the DM scroll is injected into the anterior chamber anterior chamber at one go [Fig. 3a]. Once the tissue is injected into the anterior chamber, unscrolling or injecting saline into the anterior chamber should be done only after the application of a single 10-0 nylon on the main tunnel incision to prevent the escape of the scroll from the anterior chamber [Fig. 3b].

Unscrolling the tissue

Saline is injected into the anterior chamber to position the DM scroll. Once the DM scroll is positioned to the desired place and the L mark is seen upright confirming the correct orientation of the graft, we use the “hold and release technique” [Fig. 3c] in which the far tip of the scroll is held with a hydro canula and the anterior chamber is shallowed from the main tunnel anterior chamber.

| Table 1. Demographics of DMEK eyes, surgical procedures and donor details |
| --- |
| Number of patients | 230 |
| Mean age±standard deviation (range), years | 62.27±12.14 (23-86) |
| Gender (female: male) | 104:126 |
| Indications for DMEK (no. of eyes) |  |
| Pseudophakic bullous keratopathy | 134 |
| Fuchs’ endothelial dystrophy | 46 |
| Graft failure | 22 |
| Aphakic bullous keratopathy | 10 |
| Phakic bullous keratopathy | 6 |
| Iridocorneal endothelial syndrome | 6 |
| Failed DSEK | 4 |
| TASS | 2 |
| Surgical procedures |  |
| DMEK | 180 |
| DMEK + IOL | 50 |
| Donor age (male: female) | 58.68±8.90 (28-79) |
| Donor sex (male: female) | 158:72 |

DMEK=Descemet membrane endothelial keratoplasty, DSEK=Descemet stripping endothelial keratoplasty, TASS=Toxic anterior segment syndrome, IOL=Intraocular lens
another hydro cannula. This shallowing is key as the tissue unfolds gently without any tapping or violent maneuvers.

**Bubbling**

Once unfolded over the iris, the DMEK graft can be easily lifted onto the recipient posterior stroma by positioning an air bubble underneath the graft. A full-chamber air bubble is injected into the anterior chamber which supports the adherence of the graft to the host stroma. No burping of the air was done postoperatively [Fig. 3d].

**Postoperative medications**

Patients received topical moxifloxacin 0.5% (Vigamox; Allergan) drop every 6 h for 30 days and topical prednisolone 1.0% (Predforte; Allergan) every 4-h tapered over 1 year and was replaced with topical loteprednol 0.5% (L-Pred: Alcon Laboratories) after 1 year. Topical lubricants and homatropine 2.0% eye drop (Homide; Warren) were used for 15 days in the immediate postoperative period. Patients were advised lifelong follow-up at regular intervals.

**Postoperative evaluations**

BSCVA was measured using the standard Snellen chart and was converted to logarithm of the minimum angle of resolution (logMAR) for statistical analysis. Keratometric readings and refraction were measured using an auto-kerato-refractometer (Topcon KR800, Japan). Anterior-segment OCT (Carl Zeiss Meditec, Dublin, California, USA) was performed if subclinical DM detachment was suspected. Specular microscopy (NidekCEM-530, Japan) to evaluate endothelial cell density (ECD) was performed 6 months, 1 year, and 2 years, postoperatively.

**Results**

**Demographics**

Patient demographics, surgical procedures and donor details are summarized in Table 1. Clinical results of a group of 230 consecutive DMEK (male—104, female—126) eyes up to 24 months postoperatively were evaluated using our standardized protocol. The mean patient age was 62.27 ± 12.14 years (range, 23–86 years). FBK was the commonest indication for DMEK in our series. Complicated cases like scarred cornea [Fig. 4a-d] and
TASS [Fig. 5a-d] were also included in this study. Combined surgeries included phacoemulsification with foldable IOLs in Fuchs', phakic BK, and regraft cases and secondary IOL procedure with retro-iris fixated IOL in ABK. The mean donor age was 58.68 ± 8.90.

Visual acuity outcomes
Patients with coexisting noncorneal ocular pathology, such as advanced glaucoma or macular degeneration, were included in this study but were excluded from visual acuity analysis. Two hundred and four of the 230 patients were included in the visual acuity analysis. Twenty-six cases were excluded because of preexisting ocular comorbidities like macular edema and scar, epiretinal membrane, high myopia, amblyopia, and pale disc, which could affect the visual outcome of the study.

Mean BSCVA improved from 1.217 ± 0.53 logMAR preoperatively to 0.373 ± 0.28 logMAR at 6 months, 0.261 ± 0.25 logMAR at 12 months and 0.198 ± 0.23 logMAR at 24 months [Table 2] follow-up. Mean BSCVA across all indications was <0.5 logMAR at 24 months [Table 2]. Postoperatively at 24 months, 41% of the 204 eyes recovered 6/6 or better vision, 70% had 6/9 or better BSCVA, and 97% could be corrected to 6/24 or better vision [Fig. 6a].

Endothelial cell density
The mean donor ECD was 2692.26 ± 242.0 cells/cm². Postoperatively the mean ECD was 1829.67 ± 389.32 cells/mm², 1620.98 ± 406.07 cells/mm², and 1427.08 ± 418.37 cells/mm² at 6, 12, and 24 months [Fig. 6b]. The average endothelial cell loss (ECL) was about 32 ± 12%, 40 ± 13%, and 47 ± 14% at the 6, 12, and 24 months, respectively. The average ECL indication wise ranged between 40% and 60% at 24 months [Table 3].

Complications and reinterventions
During donor DM preparation, 2 grafts were damaged. They were replaced by standby tissue. Graft detachment was the main complication with 10 eyes showing partial detachments at the 2nd and 3rd day postoperatively (4.3%). These detachments settled with rebubbling. Graft failure was seen in 10 eyes (4.3%). They were primary failures due to surgical trauma. Four patients underwent repeat DMEK and six patients had penetrating keratoplasty.

Discussion
In 2006, Melles et al. published the first case report of a patient who achieved 20/20 vision within 1 week after Descemet’s DMEK.[1] They subsequently reported results of the first 50 DMEK cases for treatment of Fuchs’ dystrophy.[4] The visual...
outcomes were impressive, with 75% of the eyes achieving 20/25 vision or better at 6 months, but the regraft rate was high (20%), and donor tissue loss, while stripping DM was an additional concern. Since then various advancements in DMEK techniques have significantly reduced the ECL, regraft rate, and tissue loss encouraging surgeons worldwide to perform this technique as the preferred choice of endothelial keratoplasty.

**Visual outcome**

Mean BSCVA improved from 1.217 ± 0.53 logMAR preoperatively to 0.198 ± 0.23 logMAR at 24 months. The improvement in mean logMAR value at each follow-up, from baseline, was statistically significant (P < 0.001). Van Dijk et al. reported a mean BSCVA improvement from 0.46 (±0.27) logMAR preoperatively to 0.12 (±0.14) logMAR at 3 months and 0.07 (±0.11) logMAR at 12 months postoperatively. After 1 year, the mean BSCVA remained stable up to 2-year follow-up. In our study, we could reach a BSCVA of 0.261 ± 0.25 logMAR at 12 months which improved to 0.198 ± 0.23 logMAR only at the end of 24-month follow-up. Forty-four percent of patients had vision >0.5 at 1 month which increased to 77% at the end of 6 months as compared to 77% of patients with vision of >0.5 at 1 month and 95% at the end of 6 months as seen in Dapena et al. This can be attributed to DMEK being done in the early stages of endothelial dysfunction in western countries, whereas in India, the patients are operated at later stages when there is severe corneal edema possibly causing visually relevant subepithelial fibrosis and the transdifferentiation of keratocytes into fibroblasts or myofibroblasts resulting in disorganization of the stromal collagen structure. Earlier intervention for these eyes may be suggested and patients should be counseled appropriately for a faster visual recovery. Similar to earlier studies, our study also showed clinical outcomes of DMEK may vary according to surgical indications and eyes with FECD (Fig. 7a-c) had achieved better visual outcomes (91.7% achieved 6/9 or better) than other endothelial disorders post-DMEK.

**Endothelial cell density**

The mean donor ECD was 2692.26 ± 242.0 cells/cm² and 1427.08 ± 418.37 cells/mm² postoperatively at 24 months. The mean difference of ECD was 863, 1072, and 1275 cells/mm² at 6 months, 1 year, and 2 years postoperative, respectively. This finding was similar to Peraza-Nieves et al. who reported a donor ECD of 2530 (±210) and 1600 (±490), 1530 (±488) and 1400 (±491) at 6, 12, and 24 months postoperatively. However in Ham et al. and Dapena et al. study, donor ECD was averaged 2700 (±260) cells/mm² and 2614 (±186) cells/mm² before, and 1780 (±390) cells/mm² and 1730 (±400) cells/mm² at 24 months. The average ECD loss was about 32 ± 12%, 40 ± 13%, and 47 ± 14% in our study which was similar in Peraza-Nieves et al. study at the 6, 12, and 24 months, respectively. Ham et al. reported a mean ECL of 19%, 24%, and 34% at 6, 12, and 24 months. In all DMEK studies, FECD was the main indication and our FECD endothelial counts were also better with a mean ECL of 30%, 37%, and 44% at 6, 12, and 24 months compared to other indications.

The major factor determining cell loss in DMEK is surgical trauma and it is considered inevitable and is attributed to surgically induced endothelial damage during DM stripping from the donor or during the process of injection and unfolding in the recipient eye. For novice surgeons cell loss at 6 months was 46% or 47%. In contrast, careful preselection of patients, advanced technique, and surgeon experience can limit ECL to 19%.

Our mean ECL rates are comparable to most studies, in spite of including most complicated endothelial diseases like ICE [Fig. 8a-c], postglaucoma shunt with corneal

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**Table 2: Indication wise-mean pre and postoperative visual acuity at 6, 12, and 24 months**

| Indication | n=204 (%) | Mean BSCVA preop | Mean BSCVA 6 months | Mean BSCVA 12 months | Mean BSCVA 24 months |
|------------|-----------|------------------|---------------------|---------------------|---------------------|
| PBK        | 114 (55.9)| 1.43±0.51        | 0.39±0.27           | 0.28±0.27           | 0.21±0.26           |
| Fuchs’     | 44 (21.6) | 0.85±0.49        | 0.17±0.16           | 0.10±0.13           | 0.06±0.10           |
| Graft failure | 22 (10.8)| 1.36±0.39        | 0.60±0.33           | 0.48±0.22           | 0.41±0.17           |
| ABK        | 10 (4.9)  | 1.44±0.33        | 0.65±0.12           | 0.47±0.10           | 0.32±0.14           |
| ICE        | 4 (2)     | 0.80±0.23        | 0.15±0.17           | 0.09±0.10           | 0.0                 |
| Phakic BK  | 6 (2.9)   | 0.81±0.31        | 0.36±0.35           | 0.12±0.09           | 0.12±0.09           |
| Failed DSEK| 2 (1)     | 1.0±0.0          | 0.18±0.0            | 0.0                 | 0.0                 |
| TASS       | 2 (1)     | 1.0±0.0          | 0.48±0.0            | 0.18±0.0            | 0.18±0.0            |

**Table 3: Indication wise-postoperative endothelial cell loss at 6, 12, and 24 months**

| Indication | n=230 (%) | Mean ECL 6 months (%) | Mean ECL 12 months (%) | Mean ECL 24 months (%) |
|------------|-----------|-----------------------|------------------------|------------------------|
| PBK        | 134 (58.3)| 31.33                 | 38.48                  | 45.95                  |
| Fuchs’     | 46 (20)   | 29.82                 | 37.34                  | 43.98                  |
| Graft failure | 22 (9.6)| 36.42                 | 44.83                  | 50.69                  |
| ABK        | 10 (4.3)  | 37.65                 | 47.52                  | 53.32                  |
| ICE        | 6 (2.6)   | 36.83                 | 43.44                  | 51.75                  |
| Phakic BK  | 6 (2.6)   | 33.57                 | 49.73                  | 52.75                  |
| Failed DSEK| 4 (1.7)   | 28.07                 | 34.48                  | 39.95                  |
| TASS       | 2 (0.9)   | 39.47                 | 54.45                  | 59.63                  |
Figure 7: Fuchs’ corneal endothelial dystrophy (FECD). (a) Preoperative. (b) Postoperative period—2 years. (c) Specular count—2 years

Figure 8: Irido—Corneal endothelial syndrome (ICE). (a) Preoperative. (b) Postoperative period—2 years. (c) Specular count—2 years

Figure 9: Post-glaucoma shunt with aphakia and corneal decompensation. (a) Preoperative. (b) Postoperative period DMEK + posterior iris claw lens—2 years. (c) Specular count—2 years

Figure 10: Failed scarred graft. (a) Preoperative—on table after removing the thickened epithelium. (b) Postoperative day 2. (c) Postoperative period—2 years. (d) Specular count—2 years

decompensation [Fig. 9a-c], and scarred failed grafts [Fig. 10a-d], because we selected older age (>50 years) donors and strictly adhered to our donor peeling methods which resulted in shortened donor DM peel time (3–5 min)\(^{21}\) and immediate utilization of the DM after stripping\(^{22}\) and our simple injector methodology\(^{23}\) and ingenious “L”-marking system\(^{24}\) helped
to ensure a smooth transfer and correct attachment of DM even in hazy corneas.

**Complications**

In our study a total of 10 patients had graft detachment which constitute about 4.3% which is less in comparison with 19 (21%) cases out of 87 patients in Droutsas et al. study.[25] Graft failure is a significant complication after DMEK which almost always requires regrafting. Graft failure was noted in 10 patients (4.3%) in our study. The persisting edema was attributed to surgically induced ECL.

Price et al. reported a primary graft failure rate of 8% in an initial series of 60 eyes[15] and Dapena et al. reported 11 primary failures (9.2%) in their consecutive series of 120 eyes[26] after DMEK. The cause for graft failure in our study was primarily failure and may be attributed to primary donor endothelial dysfunction from suboptimal quality or surgical trauma to the donor endothelium at the time of transplantation. Different devices have been used for measuring ECD in these studies, resulting in technology-based variations in the measured values.

**Conclusion**

In conclusion, to the best of our knowledge our study reports the maximum variety of endothelial disorders ever published in literature and the data shows that the clinical outcome in this varied group remained excellent within the study period, and the rate of retransplantation and major postoperative complications were very low compared to most peer-reviewed journals which have Fuchs’ endothelial dystrophy (which has the best prognosis) as the major indication.[15] The key to this success could be attributed to the strict adherence of a standardized protocol, which reduced the donor DM peeling time, eased the delivery of the DM scroll through a simple in house injector, and the surgical technique which included the “L”-marking over the DM and the “hold and release technique” minimized the trauma to the endothelial cells during the procedure. Our results should motivate all corneal surgeons in India to accept DMEK as a feasible procedure in future for all endothelial disorders.

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**Conflicts of interest**

There are no conflicts of interest.

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