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A simple surgical technique for splitting a single donor cornea for performing deep anterior lamellar keratoplasty and Descemet membrane endothelial keratoplasty without using a microkeratome

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Purpose: To describe a simple manual surgical technique for splitting a single-donor eye for performing both deep anterior lamellar keratoplasty (DALK) and Descemet membrane endothelial keratoplasty (DMEK) without using a microkeratome. **Methods:** Twenty-three eyes with anterior stromal pathology and 23 eyes with irreversible endothelial dysfunction were evaluated for keratoplasty at a tertiary eye care referral center. Twenty-three healthy donor corneas were split into two parts. The Descemet's membrane was stripped and used for DMEK. The stripped stroma was used for DALK. Best-corrected visual acuity (BCVA) of both DALK and DMEK, endothelial cell density, and endothelial cell loss in DMEK were noted at 1-year follow-up, along with any intraoperative or postoperative complications and failures. **Results:** In the DALK group, mean BCVA improved from 1.264 ± 0.25 log Mar preoperatively to 0.355 ± 0.27 log Mar at 12 months follow-up. There were no complications and failures. In the DMEK group, mean BCVA improved from 1.537 ± 0.61 log Mar preoperatively to 0.592 ± 0.67 log Mar and the mean donor ECD was 3071.66 (range, 2783–3487) cells/mm^2 preoperatively, which was reduced to 1989.33 (range, 1546–2543) cells/mm^2 at 12 months follow-up indicating a mean endothelial cell loss of 35%. The failure rate was 21.7%. **Conclusion:** This study demonstrates that with a single donor corneal tissue, both DALK and DMEK can be performed successfully without any complications. Our technique will help corneal surgeons in all developing countries to cost effectively perform more lamellar surgeries and help in reducing the magnitude of corneal blindness without the need for expensive microkeratomes.

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Component surgery of the cornea allows a disease-specific corneal layer replacement with several advantages including decreased risk of graft rejection and avoidance of complications associated with open-sky techniques. Shimmura[1] had conceptualized the idea of this technique in 2004 as they entail the utilization of one donor cornea for multiple recipients. Depending on the depth of the corneal pathology, various surgical treatment options may be undertaken such as the automated lamellar therapeutic keratoplasty (ALTK),[2,3] deep anterior lamellar keratoplasty,[4,5] and Descemet stripping automated endothelial keratoplasty (DSAEK)[6] In all the earlier studies, microkeratomes and more recently femtosecond laser have been used to separate the layers, and no study had mentioned manual techniques to separate the corneal layers for anterior and posterior lamellar keratoplasties. In developing countries where there is a dearth of donor tissues and where patients cannot afford expensive treatment with sophisticated instrumentation, we suggest a modification of manual technique for using a single donor cornea for two recipients.

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Methods

This is a prospective nonrandomized interventional study that used one donor cornea for two patients with anterior stromal pathology and irreversible endothelial dysfunction. After getting approval from the Ethical Committee, the study was conducted between May 2018 and June 2019 at a tertiary eye care referral center. Twenty-three eyes with anterior stromal pathology and 23 eyes with irreversible endothelial dysfunction were included in the study. Anterior stromal pathologies included keratoconus and macular corneal dystrophies (MCDs) and endothelial dysfunctions included pseudophakic bullous keratopathy (PBK), Fuch's endothelial dystrophy, failed grafts, and toxic anterior segment syndrome. Twenty-three good quality donor corneas were retrieved from healthy donors. The age of the donor corneas ranged between 40 and 55 years. We particularly chose this age because this group was ideal for both DALK and DMEK patients, since

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tissues older than this cannot be used for DALK in young keratoconus patients and also separating the DM from younger tissues is difficult. The donor blood samples tested negative for human immunodeficiency virus, Hepatitis B surface antigen, and syphilis antigen. A corneoscleral rim with 4 mm of sclera and an intact epithelium was dissected from the donor eyeball in a lamellar flow hood, under sterile conditions, and was stored in Cornisol medium (Aurolab, India) at 4 degree temperature. These corneas were split into two parts manually without using any automated microkeratomes in the operating room, prior to the surgery. The Descemet's membrane was stripped and used for DMEK. The stripped stroma was used for DALK. Best-corrected visual acuity (BCVA) of both DALK and DMEK, endothelial cell density (ECD) and endothelial cell loss in DMEK were noted at 1 year follow-up, along with any intraoperative or postoperative complications and failures.

In India, where there is a dearth of corneal tissues, the patients who require corneal transplantation are evaluated in the outpatient department and are registered for keratoplasty. When a suitable donor eye is received, they are intimated immediately over the phone to get admitted the next day. For this study, we segregated consecutive patients with anterior stromal pathology and with irreversible endothelial dysfunction into two groups, and both the patients were called simultaneously for surgery. All patients read and signed an informed consent document. The tenets of the Declaration of Helsinki were followed. The split tissues were used for 23 patients with anterior stromal pathology and 23 patients with irreversible endothelial dysfunction. Both surgeries were performed by a single surgeon on the same day.

Preoperative evaluation

A detailed history and physical examination were conducted in all patients preoperatively. The best spectacle corrected visual acuity (BSCVA), slit lamp examination and intraocular pressure(IOP) measurements were recorded. Fundus examination or ultrasonography was performed to assess the posterior segment. In keratoconus patients, preoperative topography was done with Pentacam HR (high resolution) tomographer (Oculus, Wetzler, Germany). Specular microscopy (Tomey, Phoenix, AZ, USA) was done postoperatively for DMEK cases.

Surgical procedure

(See Video.1 which explains the entire procedure of manual dissection in detail).

DMEK

Donor preparation

The donor tissue (A- grade) is placed on a Teflon 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 is cut) with the endothelial side facing up. The marked DM is scored with a sinskey hook 360° gently without creating any tags or irregularities at the edge of the DM. 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. 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 minutes, 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. The periphery of the scored endothelial edge is elevated with a blunt Sinskey hook to separate the Endothelium-Descemet Membrane 360 degrees circumferentially. Once the peripheral tissue is separated, the edge is grasped with a smooth curved forceps and the endothelial-descemet membrane is stripped under BSS using a single pull technique. After stripping half of the DM from the donor a "L" shaped mark is made on the stromal side on the Descemet membrane using a stained "L" stamp with the horizontal arm being shorter and vertical arm being longer .(Fig.1a) Parallelly , a B-grade tissue which is usually discarded is stripped of its DM. [Fig.1b] The half stripped marked DM from the A grade tissue is removed from the rest of the stroma. Once the DM is detached 360 degrees , it is seen to scroll on itself with the endothelium facing outward. (Fig.2) The B-grade tissue in which the DM was previously stripped is used as a support for punching the A-grade DM to the desired size. The stamped DM(Fig.3a) is now transferred into this tissue. (Fig.3b) and is unfolded with saline with the endothelial side facing up.(Fig 3c) Baron's Vacuum punch is used to cut the tissue and its size is determined by the host's underlying corneal pathology and also the horizontal diameter of the cornea. After trephination, the DM is separated completely from the stroma and is placed in an IOL tub filled with BSS and stained again with trypan blue to allow visualization after insertion into the recipient's anterior chamber.

Recipient preparation

Under peribulbar anaesthesia, epithelium is removed with a blunt spatula prior to the intraocular procedure for better visualization in all the cases. 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. 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.

Prototype injector- (See Video 2. which demonstrates the making of a simple cost effective injector).

A regular intraocular lens C-cartridge, IV tubing and 1cc syringe is assembled. The DM scroll which was previously scored in the storage medium is then sucked into the DMEK injector using no touch technique. Using our prototype injector, the DM scroll is injected into the anterior chamber at one go. 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. 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" in which the far tip of the scroll is held with a hydro canula and the anterior chamber is shallowed from the main tunnel with another hydro cannula. This shallowing is

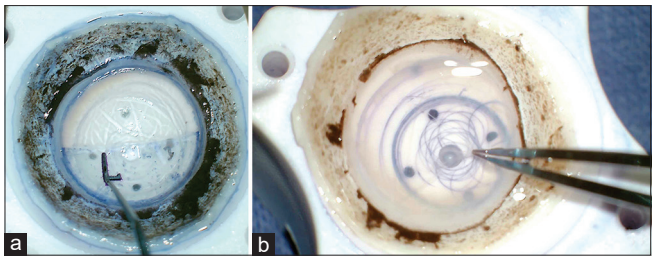

Figure 1: (a) Donor DM marking in A-grade donor tissue (b) DM stripping from B-grade donor tissue

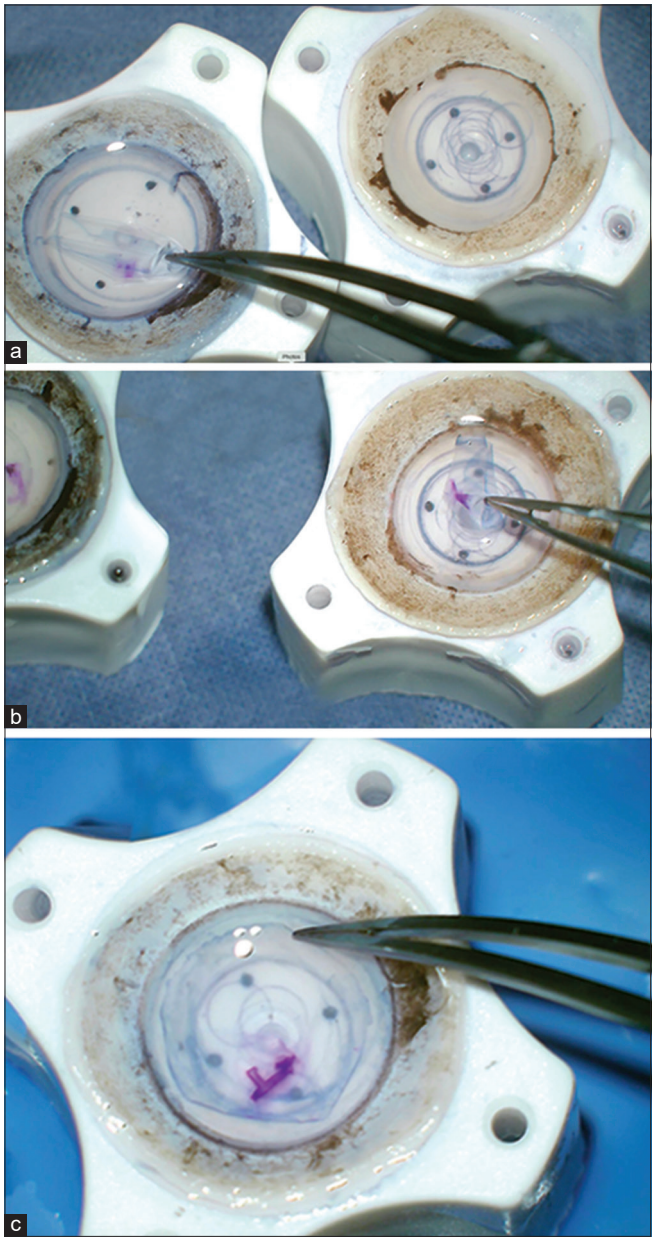

Figure 3: (a) Transfer of DM scroll from A-grade tissue (b) DM scroll placed over B-grade tissue (c) DM scroll unfolded over the B-grade tissue

key as the tissue unfolds gently without any tapping or violent manuovers.

Figure 2: DM scroll in A-grade tissue and stroma barring the DM in B-grade tissue

Once unfolded over the iris, the DMEK graft can be easily lifted onto the recipient's 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 post-operatively.

Postoperative medications

Patients received topical Moxifloxacin 0.5% (Vigamox ; Allergan) drops every 6 hourly for 30 days and topical Prednisolone 1.0% (Predforte; Allergan) every 4 hourly 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.

DALK

Donor preparation

The remaining A grade donor cornea on the Teflon block, now, devoid of the DM was trephined with a baron's vacuum punch to a final size 0.25 mm larger than the recipient's button. After punching, the graft was held at the posterior edge and trimmed in a fashion such that the posterior edge is thin and approximates to the thin host cornea. This provides a better approximation and adherence at the graft host junction.

Recipient's preparation

All surgeries were done under general anesthesia. After marking the cornea with a trephine of desired size, partial trephination with a 350 micron guarded knife was performed. A 27 gauge DALK cannula was used to create a type 1 big bubble to separate the stroma from the DM as described by Anwar and Teichmann.[7] Air is gently injected into the deep stroma until a round, well-demarcated big bubble is formed extending to the borders of trephination. After big-bubble formation, debulking of the anterior two-thirds of the corneal stroma is performed with a crescent blade. This is followed by a peripheral paracentesis to soften the eye. Using a sharp-side port knife, the big bubble is released with a single slash. The separated stroma is dissected using blunt scissors. After complete removal of the recipient's stroma, the pre-DM stripped donor button is punched to desired size and fixed using 16, 10-0 interrupted nylon sutures. The knots of all sutures were tightened so as to provide little flattening of the graft in the immediate postoperative period.

Postsurgical care

Postoperative medications consisted of topical Chloramphenicol – Dexamethasone 0.10% (Dexoren -S :Warren Laboratories) every 6 hourly tapered over 3 months and was replaced with topical Loteprednol 0.5% (L-Pred: Alcon Laboratories). 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

BCVA was measured using the standard Snellen chart and was converted to logarithm of the minimum angle of resolution (log MAR) for statistical analysis. Keratometric readings and refraction were measured using an auto-kerato-refractometer (Topcon KR800, Japan). Specular microscopy (Tomey, Phoenix, AZ, USA) to evaluate ECD was performed at 1 year postoperatively.

Results

All the donor cornea tissues could be successfully split into two tissues. One tissue comprises all the corneal layer except the DM and the other comprises the DM alone with the endothelial cells. No tissue was wasted.

DALK group

The mean age of the patients who underwent DALK was 20 ± 6.186. Mean BCVA improved from 1.264 ± 0.25 log Mar preoperatively to 0.355 ± 0.27 log Mar at 12 months follow-up. The mean pre- and postoperative difference in BCVA was 0.909 ± 0.34. There was a significant improvement in vision postoperatively ($P < 0.001$) at 12 months follow-up. There were no intraoperative complications and postoperative failures, and the grafts remained healthy at the last follow-up.

DMEK group

In the DMEK group, the mean age of the patients was 61 ± 8.683. Mean BCVA improved from 1.537 ± 0.61 log Mar preoperatively to 0.592 ± 0.67 log Mar at 12 months follow-up. The mean donor ECD was 3071.66 (range, 2783–3487) cells/mm^2 preoperatively, which was reduced to 1989.33 (range, 1546–2543) cells/mm^2 at 12 months follow-up, indicating a mean endothelial cell loss (ECL) of 35%. The failure rate was 21.7%.

Discussion

The burden of corneal disease in India is reflected by the fact that 90% of the global cases of ocular trauma and corneal ulceration leading to corneal blindness occur in developing countries.[8] Every year even though the average death is about 10 million, the conversion to actual eye donation stands at 49,000 per year.[9] Among these, only 40% are utilized due to quality issues. The low collection rates are multifactorial and are due to unawareness, illiteracy, orthodox ideas, poor eye banking facilities, and voluntary eye donation are uncommon.[10,11] This wide disparity in demand and supply of donor human eyes in India has created is a huge waiting list of patients requiring donor's eye. In this scenario, component corneal surgery is the only solution to reduce the backlog and that was why we decided to routinely perform these surgeries.

Optimal visual results were obtained in our study with no cases of infections related to surgery in both groups of patients. In the DALK group, 78.2% of patients achieved a BCVA of 20/60 or better which were comparable to published reports on DALK. A BCVA of >20/40 has been reported by Feizi[12] *et al.* in 78% of cases, Kubaloglu[13] *et al.* in 80% of cases, Sarnicola[14] *et al.* in 83% of cases, Coster[15] *et al.* in 61% of cases, Romano[16] *et al.* in 89% of cases, and by Gadhvi[17] *et al.* in 77% of cases.

In the DMEK group, 52.2% of the patients achieved a BCVA of 20/30 and 69.6% achieved 20/40 or better by 6 months. This was comparable to Peraza-Nieves[18] *et al.* who all reported >20/20 in 41% of cases and >20/25 in 75% of cases at 6 months. Guerra *et al.*[19] reported 20/30 in 72% of the cases at 6 months. Deng[20] *et al.* reported 12 studies that achieved 20/20 or better in 17–67% patients at 6 months. Our endothelial loss at 1 year was 35% and was similar to published reports. Ham[21]*et al.* reported an ECL of 36%, Gorovoy[22] *et al.* 19%, Peraza-Nieves[18] *et al.* 40%, and Guerra[19] *et al.* 36% at 1 year. However, we had 21% graft failure, which was significantly higher compared to the average rate of primary DMEK graft failure of 1.9% (range, 0–12.5%). We retrospectively analyzed the five primary graft failure cases and found that these were within our first 10 cases when we were in the process of standardizing the technique. This handling during the learning period resulted in a huge loss of endothelial damage resulting in primary graft failure in these initial cases. As we refined and standardized the technique, the results were comparable to regular DMEK cases . A repeat DMEK surgery was performed in all these primary graft failure cases.

Vajpayee[23] *et al.* in 2007 introduced a new concept of using one donor cornea for three recipients. He used a microkeratome to split the cornea for ALTK in a patient with MCD and for DSAEK in a patient with PBK. The cadaveric limbal stem cells from the corneoscleral rim were transplanted in a patient with limbal stem deficiency postalkali burns. The study suggested that in developing countries more corneal surgeons should convert to techniques of customized component corneal transplantation so that it becomes a standard surgical practice to use a single donor cornea in more than one patient. Sharma[8] *et al.* in 2010 reported the use of a single donor corneal tissue for two recipients. They split 12 healthy donor corneas into an anterior lamellar button (350 micron-thick) and posterior lamellar button (150 micron-thick) using a microkeratome. They successfully performed ALTK surgeries for superficial anterior stromal scarring cases and DSAEK in corneal decompensation cases. In both the studies, automated microkeratomes were used to separate the tissue. In our study, we did not use any expensive machines and further simplified the method by our manual dissection. The only issue was punching the DM of the desired size as the DALK and DMEK recipient diameter may vary from case to case. Since 50% of the tissues are discarded due to quality issues, we utilized those tissues as a support for punching the DM, thus sparing the good quality tissue for DALK. This manual technique allows a perfect anatomical replacement of recipient tissue both in DALK and DMEK.

Limitations of this study include a steep learning curve as the modification can lead to significant endothelial loss and graft failure in DMEK, need for a B-grade tissue for punching the DM, no comparable group without modification, and a relatively small sample size. However, this study emphasizes the need to adapt to techniques that will help in optimal use of donor tissue for multiple recipients, particularly post COVID-19.

Conclusion

In conclusion, our study demonstrates that with a single donor corneal tissue, both DALK and DMEK can be performed successfully without any complications. Our technique will help corneal surgeons in all developing countries to cost effectively perform more lamellar surgeries and help in reducing the magnitude of corneal blindness without the need for expensive microkeratomes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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