# Resource maximization during COVID-19 crunch – A novel graft marking technique to use one cornea for two recipients for either Descemet membrane endothelial keratoplasty or deep anterior lamellar keratoplasty

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During pandemic, eye banks worldwide faced drastic reduction of corneal retrieval. Since precut tissues are uncommon in India, the onus is on corneal surgeons to maximize the donor corneal usage. The stumbling block making the stromal part unusable in the graft preparation of the Descemet membrane endothelial Keratoplasty is the stromal window creation to place the orientation mark on the stromal side of the folded graft. To overcome this, we simply marked directly on the folded Descemet graft, after partly stripping it from the donor cornea. Then, we unfolded, punched, and created two grafts after completely stripping it, retaining the Descemet part for the endothelial Keratoplasty and the stromal part for lamellar Keratoplasty. Initially, we used a special F marker, and later we marked an L using just a spatula. This simple technique needing no extra donor tissue or instrumentation maximizes the tissue utility with proper patient selection.

Key words: DALK, DMEK, donor preparation, F mark, L mark



In India, the pandemic further increased the existing corneal tissue shortage. The concept of precut tissue is not yet popular in India, so mostly corneal surgeons prepare their grafts. Sharing donor corneal layers between recipients became possible after the introduction of lamellar corneal surgery,<sup>[1]</sup> and this has already been published for Descemet stripping automated endothelial keratoplasty (DSAEK) with deep anterior lamellar keratoplasty (DALK)<sup>[2]</sup> and DSAEK with anterior lamellar keratoplasty (ALTK).<sup>[3]</sup> Unlike DSAEK, the DMEK graft scrolls immediately after stripping and an orientation mark placed on its stromal side aides in proper unfolding. A 3 mm circular window is created in the stromal part of the donor through which the orientation mark on the graft is placed and this makes the stromal portion unusable. Recently, Siddharthan et al.<sup>[4]</sup> published on direct marking on the folded DM of an A grade tissue, followed by unfolding it over a B grade tissue to punch, so that the A grade stroma can be preserved for DALK. We report a similar marking of the folded DM, but directly unfolding and punching it followed by complete stripping to produce two grafts of same size. We have no conflict of interest.

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# **Surgical Technique**

A total of eight patients needing either DMEK or DALK underwent surgery and all were operated using only four donor corneas by sharing one donor between two cases, thus forming four pairs. The patients needing DMEK were all male with irreversible endothelial decompensation, one was a 56 year old with iridocorneal endothelial syndrome (ICE) syndrome, two with pseudophakic bullous keratopathy (PBK) were 53 and 52 years old, and one was a 68 year old with primary angle closure glaucoma and a dense cataract. They were all operated using donor cornea from donors of age 26, 21, 30, and 47 years, respectively.

The stromal part of the respective donor cornea was utilized for three DALK procedures, one in a 21-year-old male with post traumatic central cornea scar, a 14-year-old female with advanced keratoconus, a 36-year-old male with progressive post lasik ectasia operated elsewhere, and the fourth donor was used as a tectonic LK for Descemetocele in a 63-year-old female with rheumatoid arthritis [Table 1].

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Donor age (years)	26	21	30	47
Cause of death	Road traffic accident	Head injury	Suicide by paraquat poisoning	Acute pulmonary edema
Specular count (cells/mm <sup>2</sup> )	3049	3077	2890	3597
DALK patient age (years)	21	63	36	14
Reason for DALK	Traumatic scar	Rheumatoid corneal melt	Post lasik ectasia	Advanced keratoconus
Preop BCVA (LogMAR)	20/1200	20/60	20/60	20/1200
Final BCVA (LogMAR)	20/1200	20/60	20/60	20/1200
DALK complications	Spontaneous reattachment	None	None	None
DMEK patient age (years)	56	53	52	47
Reason for DMEK	ICE syndrome	РВК	PBK	PACG
Preop BCVA (LogMAR)	20/1200	20/1200	20/60	20/80
Final BCVA (LogMAR)	20/125	20/25	20/60	20/20
DMEK complications	Rebubbling once	Spontaneous attachment	None	None

#### Table 1: Donor corneal tissue details

All the patients submitted informed consent and underwent preoperative comprehensive eye exam including best-corrected visual acuity (BCVA), IOP, by GAT, slit-lamp bio microscopy, dilated indirect ophthalmoscopy, and posterior segment ultrasound B scan when needed and corneal tomography using pentacam HR for the ectasia. They also had slit-lamp photo and ASOCT (Visante, Carl Zeiss, Germany) for documentation. The study was approved by the institutional review board and followed the principles of Declaration of Helsinki. The donor corneas were from CU Shah Eye bank and were preserved in CORNISOL (Aurolab) [Table 1]. All the grafts were prepared by the operating surgeon (Dr NVN) in the OR. The preselected patients needing same size of the grafts got admitted once the grafts were ready.

The initial DMEK graft preparation was as usual for all the cases, which briefly include epithelial side marking of the center on the donor cornea, placing its epithelial side down on the Janach vacuum donor punch, vacuum activation for steady graft holding, 10 mm initial delimiting trephining with 100 µm guarded Janach punch blade followed by staining with 0.06% trypan blue for 1 min to highlight the punched groove, trypan blue wash and replacing with adequate BSS, undermining the punched groove to sever the residual peripheral attachments with a Fogla cleavage hook, removing the peripheral frill of adherent DM with Epsilon curved titanium tying forceps, circumferential peripheral edge elevation of up to 1 mm of the DM graft using the cleavage hook, substituting BSS with 0.06% trypan blue for 1 min to highlight the released edge, and again replacing trypan blue with BSS followed by slow and steady striping of the DM up to a three-fourths forming a fold using tying forceps.

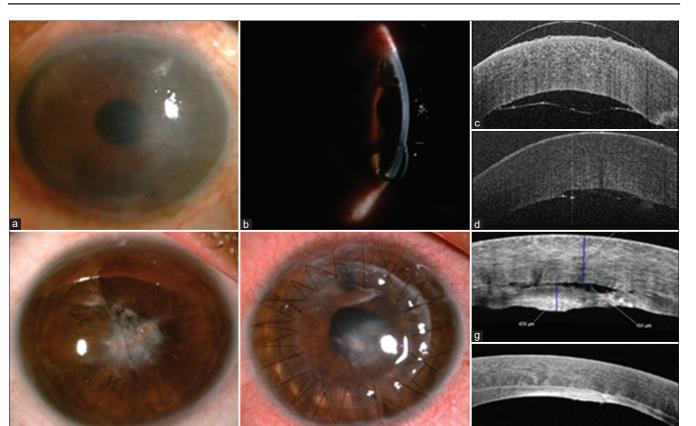
At this stage, the folded DM of the donor cornea had its stromal side exposed, which was gently dried at one spot and marked with an F-shaped stamp in three cases (video supplement 1), and in one case, we used a simple spatula to form an L-shaped mark (video supplement 2) by precoating all markers with a marker pen. Once marked, the grafts were unfolded with a jet of saline, gently dried, and finally punched to 8 mm in three cases and 7.5 mm in one case using Katena corneal trephine. After releasing the residual attachments, the DM scroll is placed on a separate block filled with 0.06% trypan blue for 2 min for staining.

Meanwhile, the stroma was separated without staining for the DALK procedure and placed into a sterile container with CORNISOL. Now, two grafts are ready for use each of equal size, DM for DMEK and the stroma for DALK. The technique of the rest of the surgeries in both DMEK and DALK in detail is beyond the scope of this article. Briefly for the DMEK cases, a Janach glass injector for insertion through a 2.8 mm clear corneal incision, tapping technique to unfold, and large air injection to hold the unfolded graft in position were used. In the DALK cases, a direct manual stromal dissection technique for the center traumatic scar and Descemetocele cases, big bubble technique for the post lasik ectasia, and a failed big bubble converted to manual dissection for the keratoconus were done. For the one DMEK case that needed phacoemulsification, a direct chop technique through a 2.2 mm clear corneal incision was done which was extended to 2.8 mm for DMEK graft insertion like the other cases.

Postoperatively, for the DMEK patients, one tablet of acetazolamide 250 mg was given once at night on the day of surgery. All patients received 1 week of prophylactic antibiotic eye drop 6 times per day and lubricating eye drops 6 times per day for 2 months. Topical prednisolone acetate 1% (Pred Forte; Allergan, Inc., Irvine, California, USA) was started initially at 10 times per day dosage and then with weekly tapering it will be gradually reduced to once a day, which will be continued indefinitely for DMEK and up to complete suture removal at 1 year for DALK cases like the standard regimen followed by the surgeon for her cases. The patient with PACG continued medical management with topical timolol maleate 0.5% eye drops 2 times per day.

#### Results

All the patients were kept under close follow-up of once in 2 days till stability was observed, which is mostly within 10 days, after that, they were on a monthly follow-up and the last review was up to 3 months postsurgery. Slit-lamp biomicroscopy, anterior segment photo, and diagnostic ASOCT were routinely performed [Figs. 1-3]. The post DMEK graft in ICE syndrome showed mild separation initially that widened later but was successfully reattached with a single injection of 10% perfluoropropane [Fig. 1a and b]. The procedure was performed under intraoperative monitoring using hand held



**Figure 1:** (a) Pre-DMEK slit-lamp photo of ICE syndrome. (b) Post-DMEK on day 4, slit view of small air bubble in AC and epithelial bulla. (c) Bioptigen intraop showing detached DMEK graft. (d) Bioptigen intraop of attached graft postperfluoropropane injection. (e) Pre-DALK slit-lamp photo of traumatic corneal scar. (f) Post-DALK on day 6, slit-lamp photo with mild graft edema. (g) ASOCT post-DALK on day 2, showing central limited graft separation. (h) ASOCT post-DALK on day 9, showing spontaneous graft opposition

Bioptigen, (Inc., Research Triangle Park, NC) [Fig. 1c and d]. This patient at 3 months post op had a clear cornea with BCVA of 20/125 improved from the pre op of 20/1200.

The initial DMEK graft undulations of the second pair PBK patient were noted in ASOCT, which settled spontaneously [Fig. 2a-c]. At 3 months, the patient maintained a clear cornea and his BCVA was 20/25 improved from the pre op of 20/1200. The third pair PBK patient post DMEK had an uneventful postoperative period, and at last, the follow-up of 3 months postsurgery maintained the preop BCVA of 20/60 [Fig. 3a and b]. The fourth pair of the patient with PACG who underwent combined DMEK with phaco had an uncomplicated surgery with an uneventful postoperative period and was last seen at 2 months post op with a BCVA of 20/20 improved from the preop BCVA of 20/80 [Fig. 3e and f].

In the post DALK patient of the first pair, a spontaneous resolution of an initial graft separation was noticed [Fig. 1g and h]. Though this patient was happy with the cosmetic outcome, his BCVA remained only at 20/1200 at 3 months due to fixed retinal folds diagnosed by indirect ophthalmoscopy once the cornea cleared post op. The second pair post DALK for keratoconus recovered well [Fig. 2d-f] and maintained the preop BCVA of 20/60 up to 3 months follow-up.

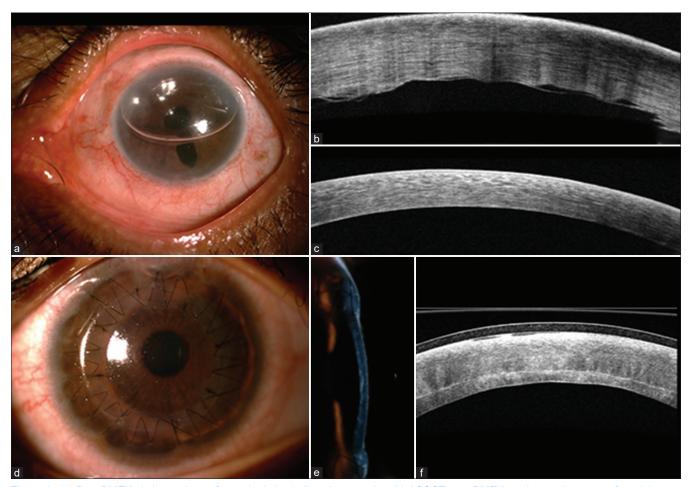
The third pair post DALK for progressive post lasik ectasia had uneventful post op maintaining his BCVA of 20/60, but his refraction dropped from  $-20.00-6.00 \times 50$  preop

to Plano -9.00 × 150 at 3 months postsurgery [Fig. 3c and d]. The final patient who had LK for tectonic purpose recovered well in the initial 10 days with good epithelialization, graft clearing, well-formed anterior chamber, and normal IOP [Fig. 3g and h] and maintained the preop BCVA of 20/1200 up to a 2 months follow-up now. The donor corneal rim bacterial cultures, done as a part of our routine hospital practice, were all negative.

#### Discussion

Though the idea of using one donor cornea for more than one patient became possible due to the introduction of lamellar endothelial Keratoplasty in 1999 by Melles *et al.*<sup>[1]</sup>, the benefits of replacing only the diseased layer of the cornea and producing a smooth surface by blunt separation of the corneal lamellae were understood from the concept of component surgery of the cornea.<sup>[5]</sup>

In Ljubis's<sup>[2]</sup> case report on one donor cornea for DSEK After manual DSEK, the DALK was performed for herpetic stromal melt and DSEK for PBK and both showed good visual outcome. The post DALK pachy was 430  $\mu$ m, which may not render adequate biomechanical support in advanced keratoconus for which DALK is often indicated. Also in keratoconus, in order to reduce the corneal curvature post DALK, graft equal or under sizing is practiced,<sup>[6]</sup> which can further stretch and lower the pachy. In the publication of DSAEK with ALTK combination by Namrata *et al.*,<sup>[3]</sup> initially both the grafts were 10 mm in



**Figure 2:** (a) Post-DMEK slit lamp photo of pseudophakic bullous keratopathy. (b) ASOCT post-DMEK on day 2, showing graft undulations. (c) ASOCT post-DMEK on day 7, showing spontaneous graft opposition. (d) Post-DALK slit-lamp photo of the keratoconus patient. (e) Slit-view post-DALK showing good graft opposition. (f)ASOCT post-DALK showing well-opposed graft

size, the ALTK graft with 350  $\mu$ m thickness was later punched to desired size between 8.5 and 9.5 mm, and the DSAEK graft with 150 pachy was punched between 8.0 and 8.5 size based on necessity. In their report, they also suggested that DALK grafts can easily be stored in glycerol for future usage as cellularity lost during storage in glycerol does not affect the graft quality for DALK. The use of one donor for three patients was published by Vajpayee *et al.*,<sup>[7]</sup> when they additionally used the donor corneal limbal stem cells (LSCs) for a third patient with LSC deficiency.

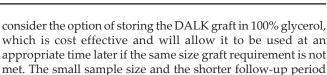
Normally, in both DSAEK and ALTK, the donor cornea is held on a pressurized artificial chamber while the stroma is cut using a blade of preset depth run by a motorized system (ALTK; Moria SA). While the depth of cut here is inversely proportional to the speed of motorized cut, it is directly proportional to the chosen blade depth, the artificial chamber pressure, and the number of cuts done. None of the above-said parameters are precisely predictable, and this leaves the varying thickness of remaining stromal part to be used for anterior lamellar procedures.

On the contrary, DMEK is reproducible. A fellow eye study comparing DSAEK with DMEK showed the benefits of exact anatomical replacement in DMEK-like lesser hyperopic shift, smaller incision needed for graft insertion, lesser induced astigmatism, and lower aberrations.<sup>[8]</sup> As the donor DM stripping is an essential part of DALK graft preparation, once the DMEK graft is prepared, the full thickness donor stroma becomes optimum for DALK if left unaltered. However, the major problem in DMEK graft preparation is the free graft scrolling which makes it necessary to mark the stromal side through a 3 mm stromal window creation, making the stromal part of the donor unusable.

A recent publication from K,S. Sidddharthan *et al.*<sup>[4]</sup> mentioned the direct marking on the folded but partly stripped DM of an A grade donor and then after fully stripping it unfolding the graft onto a separate B grade donor for punching, thus making the A grade corneal stroma suitable for DALK. This method allows us to prepare two grafts from a single A grade cornea. Our method is similar to this in the first part of marking the folded and partly stripped DM. We then unfolded the DM and punched to the desired size producing two grafts of the same size after final stripping of the residual attachments. Instead of using the special alphabet marker, in the last case, we used a spatula to successfully mark an L shape, which was visible even on the day one postop [Fig. 3b].

For the final DMEK graft staining in our study, the released scroll was transferred to a separate Teflon block filled with trypan blue. This avoids undesired stromal staining which we faced in С

TACAM 1 Large Color Map



### Conclusion

This simple marking technique in DMEK graft preparation will enable us to use one cornea for two patients optimizing the available resources. The L mark using just a spatula eliminates the need to buy additional specific instruments for marking. Successful graft preparation from younger donor age of up to 21 years widens the range of donor age suitable for DMEK graft preparation. More patients receiving this technique and longer duration of follow-up will let us understand this possibility better.

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are our also study inadequacies.

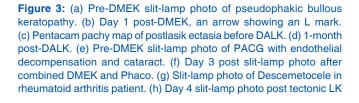
Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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our first case, but it spontaneously cleared on the first postop day. In short, our technique is simple, faster with minimal graft manipulations, and does not require a special marker or an extra B grade donor cornea. This technique was possible even with younger age donor corneas of up to 21 years of age.

The fact that our technique needed careful patient selection requiring the same size of the grafts is a limitation. We should