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Review article

DMEK surgical training: An instructional guide on various wet-lab methods

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ABSTRACT

Descemet membrane endothelial keratoplasty (DMEK) is a partial-thickness corneal transplantation procedure that involves selective transplantation of the Descemet membrane and endothelium. DMEK offers significant advantages over other keratoplasty techniques, such as faster visual rehabilitation, better final visual acuity due to minimal optical interface effects, lower risk of allograft rejection, and less long-term dependence on topical steroids. Despite all its advantages, DMEK has been found to be more challenging than other corneal transplantation techniques, and its steep learning curve appears to be an obstacle to its widespread use and adoption by corneal surgeons worldwide. DMEK surgical training laboratories (wet labs) provide a window of opportunity for surgeons to learn, prepare, manipulate, and deliver these grafts in a risk-free environment. Wet labs are a significant learning tool, especially for those institutions that have limited tissue availability in their local centers. We provide a step-by-step guide for preparing DMEK grafts using different techniques on human and nonhuman models with instructional videos. This article should eventually help the trainees and the educators understand the requirements for performing DMEK and conducting a DMEK wet lab and develop their skills and interests from a wide variety of available techniques.
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1. Introduction

In the past two decades the development of posterior lamellar keratoplasty techniques, especially Descemet membrane endothelial keratoplasty (DMEK), has revolutionized corneal transplantation. DMEK outclassed penetrating keratoplasty and Descemet stripping automated endothelial keratoplasty (DSAEK) as gold standard treatment for endothelial failure, thanks to a more rapid visual recovery, lower rejection rate, and superior refractive outcomes.[7,26,28,41,72] Notwithstanding all its advantages, the DMEK learning curve slows down its adoption among ophthalmic surgeons.[77] Controlling an extremely thin graft during preparation, loading and delivery remain as challenges. Intraoperative complications and endothelial cell loss (ECL) have been found to be directly related to the surgeon's experience and may lead to primary or early graft failure[69] and restrict long-term survival. Such factors lead to an increasing need of creating DMEK surgical training laboratories (dry and wet labs).[3,36,66,86] Wet labs have shown to be a successful method for developing surgical skills, where surgeons are introduced to the technical aspects of surgery step-by-step in a risk-free setting. This hands-on experience is necessary for developing proficiency and confidence to improve surgical performance. Both human and nonhuman models are available for learning DMEK. Each model is characterized by its own unique aspect, which makes it more suitable for use in specific situations.

Ex vivo human models involve the use of donor corneoscleral rims that are not suitable for transplantation. It is more expensive than any other available model but allows the practice of complete DMEK procedure, from graft preparation to delivery, in a context as close to reality as possible. To achieve this, a human donor cornea is usually mounted on the artificial anterior chamber (AAC)[78] to create an artificial environment that is similar to the actual surgical setup. Handling human tissues with the same instruments that are used in the surgery provides a real-time graft handling experience. In addition, tissues from donors > 60 years, which tend to be less stiff, are used to reduce early learning hurdles. Nonhuman models, such as animal tissues (pig eyes),[21] vegetable matter (onion model),[46] or synthetic material [artificial eye (AE)], have also been used for surgical training, as the former two are less expensive and have better availability than the human corneas; however, they have different consistency and size, and they may not be able to simulate all DMEK surgical steps but serve as a good model for experiencing surgical maneuvers. AEs for surgical training are also available, some of them being very specific for DMEK surgical training. AE presents a synthetic cornea made of soft plastic with its posterior lining comprising of a thin opaque gel layer that simulates the endothelium.

This review, therefore, aims to provide guidance and instructional materials, including videos, for enhanced DMEK learning, using human and nonhuman models.

2. Human model

2.1. *Graft harvesting/preparation*

Several surgical techniques have been described for harvesting a DMEK tissue. The trend is toward techniques that allow dissection with minimal manipulation during tissue harvesting to minimize graft damage.[10] The tissue wastage following DMEK graft preparation ranges between 2% and 20%, and it is directly correlated to surgeons' or more recently

to the eye bank technicians' experience.[37,52,70,74,80,85] Debellemanière and coworkers[18] reported that the number of procedures required for an experienced ophthalmic corneal surgeon to reach 90% of the learning curve plateau was 68 cases for graft preparation. They also noted that increased surgical experience led to shorter times in harvesting the graft with less ECL. Interestingly, neither ECL nor the learning curve affected the patients' best-corrected visual acuity gain at 1 week and 6 months. Venice eye bank[55] audited the performance of standardized graft preparation techniques of their technicians. Four technicians prepared 645 DMEK grafts using the double-trephine technique between 2014 and 2017, and all showed a decrease in tissue wastage/failed graft preparation from 2% to 0%, 8% to 6%, 6% to 2.4%, and 8% to 2.5% respectively.[51] Recently, Din and coworkers confirmed a learning curve involved in graft preparation but highlighted that ECL and tissue wastage could be reduced with practice and a standardized DMEK peeling technique.[19] Thus, using a standardized technique following a learning curve could reduce graft wastage and make DMEK more accessible.

2.2. *Parameters to check before graft preparation*

Carefully inspect the cornea for various defects, such as foreign bodies, scar tissue, old surgical scars, and residual iris tissue attached to the trabecular meshwork (TM). If fluffy iris tissue is localized on the TM, initiate by removing it with the hockey knife or forceps to have a clean preparation field. In pseudophakic corneas, scars on the DM caused by the paracenteses and main incisions may already be visible at this point. During the learning curve, avoid pseudophakic or diabetic corneas, as these can be more challenging. After removing the cornea from the storage medium, rinse it in a balanced salt solution (BSS) to remove any media remnants for effective staining with trypan blue during the procedure. Proceed further as follows.

2.3. *Method 1: No-touch technique (stripping from the TM)*

1. Place the cornea, endothelial side up, on a supportive holder. Suction is not necessary. Before starting and during the preparation, add a few drops of BSS/ preferably storage media on the endothelium to keep it moist throughout the procedure.
2. Inspect the anatomy of the cornea, focusing on the pigmented band of the TM, and the Schwalbe line. The Schwalbe line is an important landmark that appears just inside the nonpigmented band of the TM (Fig. 1).
3. Gently push the TM toward the center of the cornea using the hockey knife. When Schwalbe's line is passed, it can be usually seen as an elevated fold in the DM, which reassures that the DM is intact and detached. After the first clock hour is detached, stripping becomes easier as the loose end of the TM can be used for further extension of the detachment (Fig. 2A).
4. Repeated trypan blue staining can be performed during the process to allow better visibility, particularly during the learning curve. After staining, the detached DM margins become visible, as the color is effective only on the stromal side of the DM (Fig. 2B).
5. Note: This step is very important. Make a second 360° inspection of the detached margin to check for any residual peripheral adhesion. The adhesion can be released using a hockey knife or by gentle pulling with the forceps. These can vary from very light to dense adhesions and are usually present only around the TM. Peeling without releasing the adhesion can lead to peripheral tears.
6. Before initiating the peel, inspect for peripheral tears.

a. Small tears can be ignored as they are typically far away from the trephination zone. The diameter inside the TM is around 11–12 mm, while the trephination varies from 7.5 to 9.0 mm. If preferred, small tears can also be secured by pulling on one side and creating a chip in the margin, which prevents the extension of the tear.
b. larger radial tears should not be manipulated. If the peeling is otherwise successful, the trephination can be completed in a safe way, possibly a bit decentered, by avoiding or incorporating the radial tear on the graft, provided that the part is not too large. In our experience, small peripheral radial tears in the final DMEK roll do not create any impediment during surgery.

7. Peeling:

a. Start from a region away from the tear.
b. Grasp both TM and the peripheral DM with the forceps; otherwise, the TM will be peeled away, without the DM. Detaching the TM attached to the DM must be avoided; otherwise, the tissue will curl spontaneously, and it will be complicated to trephine it. If TM is detached, switch to "stripping by scoring the peripheral endothelium" technique.
c. Use "sweeping" movements by applying gentle pressure (Fig. 2C). Depending on the ease of peeling, this can be done in one step, or it can be stopped in the middle and continued from another site. The outer 1/3rd of the DM has strong adhesions with the stroma; thus, it is better to achieve complete detachment in this area prior to stripping the rest of the tissue.
d. In cases with multiple peripheral tears, the forceps can be used to grasp the DM tissue more centrally than the tear, and in such a case, it is safer to create a chip out of the tear. Avoid attempting to peel by grasping one margin of the tear, as this will make the defect larger.

8. The peeled DM is then allowed to float in BSS to create space for the contact lens (CL). Sliding the CL under the graft can be tricky during the learning curve. The CL must be moist, and a two-forceps approach, one for holding the lens and the other for tilting the tissue at an angle for sliding, is ideal. The entire margin of the floating DM must be on the CL, which can be easily achieved by adding BSS drops and/or by moving the floating graft with the forceps using the TM margin.
9. When the graft is well-centered on the CL, use vitreous sponges to absorb the fluid between the CL and the stroma, as well as between the stroma and the cornea holder. Less fluid at this step significantly increases

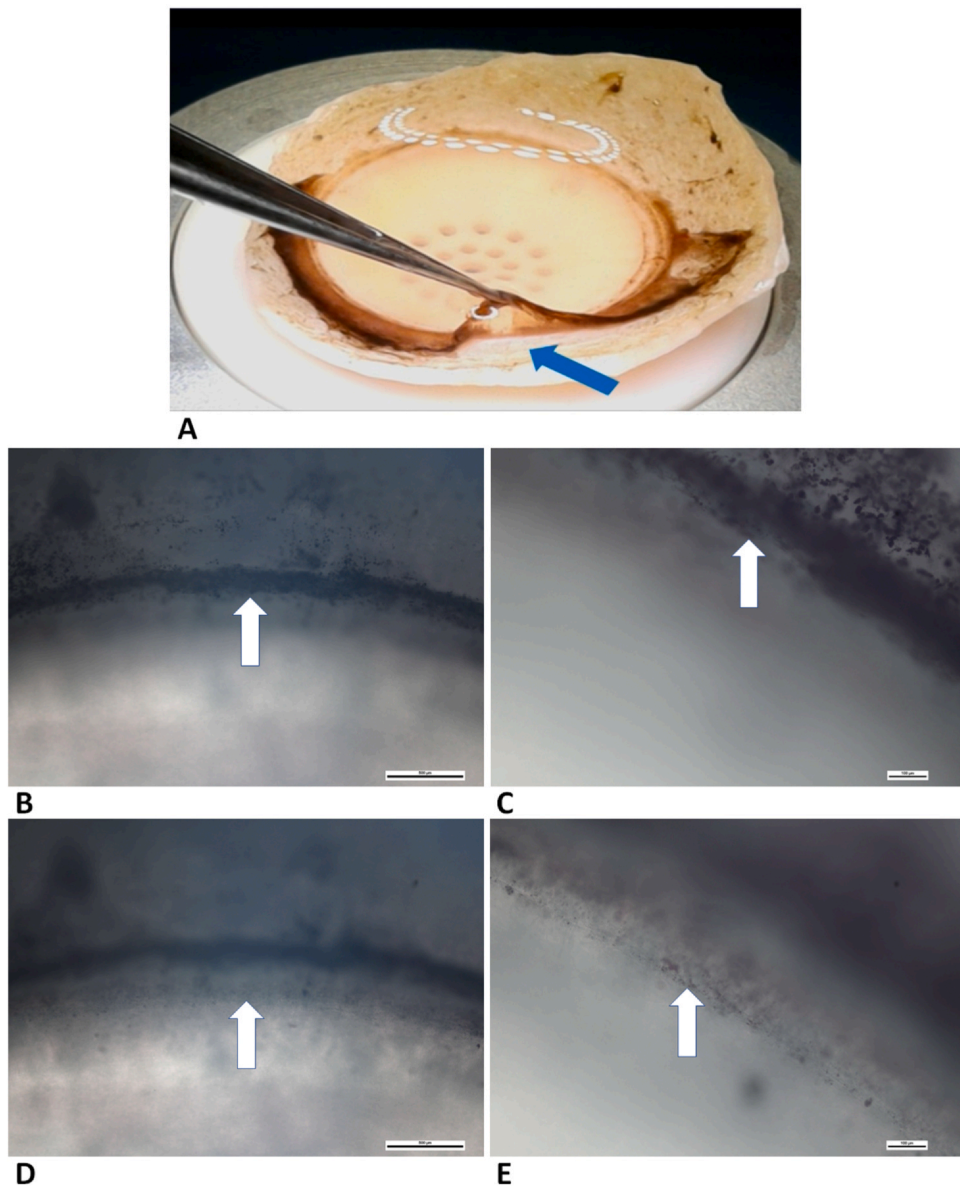

Fig. 1 – Anatomy of the cornea showing (*A*) trabecular meshwork and Schwalbe's line indicated by the blue arrow on the corneoscleral rim. *B*: low magnification and *C*: high magnification of trabecular meshwork. *D*: low magnification and *E*: high magnification of Schwalbe's line. Both trabecular meshwork and Schwalbe's line indicate a key landmark for DMEK preparation.

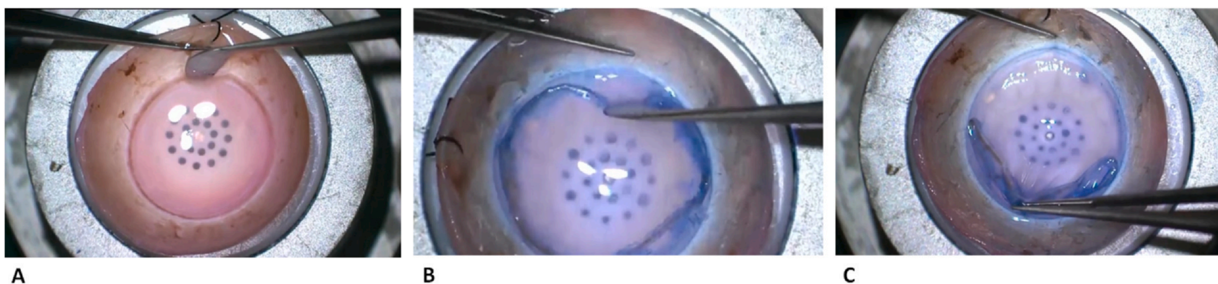

Fig. 2 – No-touch technique (stripping from the trabecular meshwork). *A*: stripping from the TM using the hockey knife. *B*: staining with trypan blue makes detached DM margins more visible. *C*: peeling of both TM and DM together.

control of the movements. While holding the CL in place, the stroma can be pulled with the second forceps.

10. Removing excess fluid is not easy during the learning curve, as the sponge might stick to the graft. Use vitreous sponges on the margin of the CL, which uses surface tension to remove the fluid while avoiding direct contact with the graft.
11. Trephination is usually straight forward as the tissue and underlying CL are very thin. While holding the trephine in place with one hand, move the peripheral rim of the cut CL to ensure that all margins are free. When removing the trephine, excised CL rim can be removed with the TM on top. This will provide a clean preparation field.
12. After adding drops of BSS, the flat graft will tend to make a roll.

a. Use the smooth-ended forceps to gently grasp the margin of the graft, and place it in the culture medium.
b. The graft can also be transferred to the Petri dish on the CL, avoiding any touch at all. The Petri dish with BSS can be closed and used for further tissue quality evaluation. If the graft is used directly for surgery, the Petri dish should be replaced by a glass container for further staining and aspiration in the injector.

2.4. Outcomes

The technique was described by Groeneveld et al.[27] It can allow obtaining large grafts (up to 12 mm), leaving the corneal stroma intact to perform a deep anterior lamellar keratoplasty, if needed. The authors also noticed better tissue handling during the stripping phase and moving it on the CL. Punching the DMEK donor on a soft CL results in fewer areas of bare Descemet membrane (DM) at the margin of trephination compared to direct punching onto the donor stroma.[1,23] It may be due to the compression that occurs when the trephine is pressed into the stromal bed (when punching the DMEK graft on the stroma). Lyon eye bank described the learning curve of their technicians using the no-touch technique.[42] An ECL of 3.3% was noted in the first 19 donor corneas. Similar results were obtained by Droutsas and coworkers[20] where the authors reported that a standardized "no-touch" technique could successfully be implemented in a clinical setting without an in-house eye bank facility.

2.5. *Method 2: stripping by scoring the peripheral endothelium*

1. Place the donor cornea with the endothelial side up on the corneal punch block. Suction is not mandatory for this technique.
2. Use a Sinskey hook to create a partial break on the peripheral corneal endothelium, about 1 mm from the TM.
3. After a quick wash in BSS, trypan blue dye is applied for 30 s to stain the cut edge, and then, the cornea is rinsed again in BSS to remove the excess trypan blue.
4. Use a DM cleavage hook to separate the peripheral cut edge of DM from the underlying stroma throughout the 360° circumference (Fig. 3A).
5. A few drops of BSS are applied to the endothelium to avoid drying.
6. Using the tying forceps, the free edge of the DM is gently grasped, and the peeling is performed toward the opposite end from the point of initiation. The peeling is performed in quadrants, and the DM peel is interrupted leaving a central hinge (Fig. 3B).
7. At this point, the graft is restored to its original position using BSS, and a vacuum punch trephination is performed (Fig. 3C).
8. Graft marking is performed (detailed later), if necessary.
9. The graft is punched with a diameter, which can be flexible between 7.5 and 9.5 mm.
10. The free edge of the donor graft is grasped, and the peeling is completed to obtain a free-floating graft. Trypan blue dye (0.06%) is then applied for 3 min to stain the donor tissue for visualization during graft delivery.

The details can be found in supplementary video 1.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

2.6. Outcomes

In 2018, Parekh and coworkers,[52] comparing 5 DMEK harvesting techniques, recommended the stripping by scoring the peripheral endothelium technique as the best option for surgeons who prepare their own graft in the theater, considering all the parameters analyzed, such as cell death, ECL, the time required to prepare the graft, and the associated costs. The authors reported

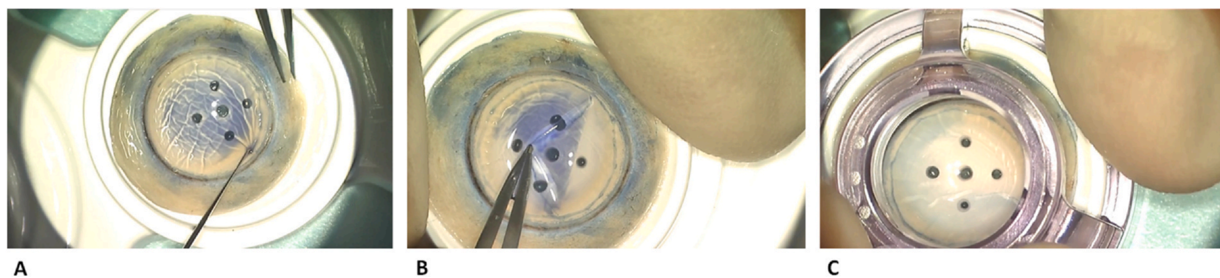

Fig. 3 – Stripping by scoring the peripheral endothelium. *A*: Descemet membrane cleavage hook is used to separate the peripheral cut edge of DM from the underlying stroma all around 360°. *B*: tying forceps are used to grasp the free edge of the DM to perform the DM peeling toward the opposite side from the start. C: the vacuum punch trephination is performed to obtain the final graft.

a mean DMEK preparation time of 7.5 min with an ECL of 2.7%. However, it must be noted that the tissues were prepared by expert technicians in this study.

Observation and evaluation of the graft are important parameters for successful DMEK procedures. Fogla and coworkers[23] and Parekh and coworkers[57] suggested using a device with the capacity to retroilluminate/transilluminate the tissue, which makes it easier to identify the peripheral edges of the DM during the peeling process, and preventing peeling at the abnormal areas of adherence. Fogla and coworkers[23] described a faster process of separating the peripheral edge of DM from stroma circumferentially using retroillumination both for experienced and a trainee surgeon. In addition, a shorter duration of DM peeling to achieve a free-floating DM graft using retroillumination has also been reported. DM tears were described only in the grafts obtained without retroillumination: peripheral radial tears occurred in 20% of corneas for the experienced surgeon, whereas, for the trainee surgeon, 40% developed DM tears with 30% peripheral radial tears and 10% central DM tears.[23] In addition to these benefits of retroillumination, transillumination has also been described as a useful tool to visualize donor corneal endothelial cells.[57] Transillumination using a stereomicroscope helps reduce corneal drying time during graft preparation, allows evaluation of the global health of the corneal endothelium after each manipulation step accurately, and provides better visibility of the tissue with or without staining, thus guiding the surgeon to avoid the area with scars, tight adherences, or tears.

2.7. *Method 3: double-trephination technique*

1. Place the cornea onto the trephine block, and center it using a 8 mm reference ring and limbus. Once centered, apply vacuum to the cornea using the suction syringe. Note that, for the trephination technique, the cornea must be centered and fixed on the block. If the tissue moves, it may result in a decentralized cut.
2. Place 9.5 mm guarded trephine onto the block, and press/tap gently to perform a partial superficial trephination. Strong tapping or full-thickness punches can result in endothelium margins incarcerated in the corneal stroma increasing the overall preparation time (Fig. 4A).
3. Carefully remove the trephine and tilt the cornea to remove storage media with a swab spear.
4. Stain the tissue with trypan blue and remove the stain after 30–60 s using a swab spear. Rinse the stain with BSS.
5. Using tying forceps, remove the peripheral endothelium, leaving only the central endothelium intact. (Fig. 4B). This is to ensure that there is no hindrance during graft peeling.
6. Insert the tying forceps or a cleavage hook between the DM and stroma, and slide the forceps along the edge. Allow the tip of the forceps to act as a wedge between the layers. Perform the peripheral DM separation along the entire edge (Fig. 4C).
7. Cover the endothelial side with BSS, and grasp the dissected edge of the DM. Peel the membrane using a single- or four-quadrant method from superior to inferior position. The entire process may take a few to several minutes depending on the adherence of DM to the underlying stroma. Be careful to release the membrane 2 mm before complete detachment.
8. The peripheral hinge protects the tissue from free-floating or forming a roll in the media. It is also helpful to allow marking of the DMEK tissue on the DM side to avoid the tissue being transplanted with the endothelium side

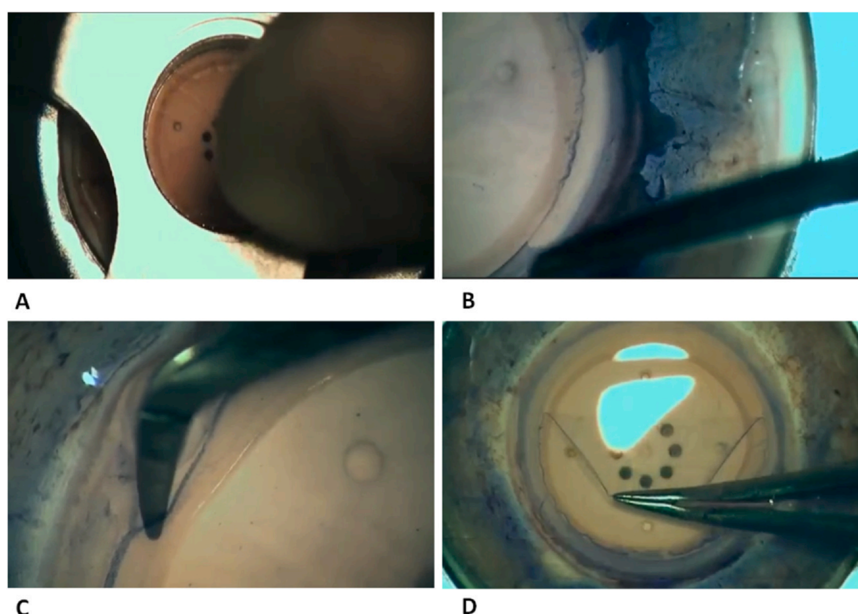

Fig. 4 – Double-trephination technique. *A*: a 9.5 mm guarded trephine is used to perform a partial trephination. *B*: tying forceps are used to remove the endothelium peripheral to the trephine score, leaving only the central endothelium. *C*: tying forceps are inserted between the DM and stroma, and slided along the edge, allowing the dissection along the entire edge. *D*: the dissected edge is grasped, and the membrane is peeled straight back.

up. Marking the tissue is not mandatory, but it will ease the unfolding of the DMEK (Fig. 4D).

9. Proceed to the final graft punch only if the tissue is not intended for “prestripped” graft: place back the corneal tissue onto the trephine base, and center the cornea and apply suction. Place the second trephine (8.0–8.5 mm depending on the requirement) onto the cornea, and press harder just enough to create a superficial cut.
10. Remove the trephine and stain the endothelium (stain for 20–30 s) followed by a washing step. As the hinge will be outside the trephined zone, the tissue when placed in BSS releases as a roll.

The details can be found in supplementary video 2.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

2.8. *Outcomes*

In a comparative study, Parekh and coworkers[52] showed that the double-trephine technique resulted in lesser cell death evaluated as trypan blue positive cells (1.2%) versus 2.9% in stripping from the TM, 0.21% in stripping by scoring the peripheral endothelium, 8.71% in submerged hydroseparation, and 11.0% in pneumatic dissection method.[52] Less endothelial cell damage in terms of uncovered areas was observed with the double-trephine technique (1.18%) versus 2.71% in stripping from the TM, 2.96% in stripping by scoring the peripheral endothelium, 3.34% in submerged hydroseparation, and 4.06% in pneumatic dissection method. A lesser probability of peripheral tears compared to other manual stripping techniques was shown[52]; however, the technique is time consuming and, as it utilizes two trephine blades, it is relatively expensive, but it is possible to obtain DMEK grafts from diabetic donors as this technique allows decentralized peeling. Borroni and coworkers[11] found a correlation between the speed of stripping, scroll width, and ECL. It was reported that slow-peeled DMEK grafts result in a wider scroll width but a greater ECL, which is contradictory to our general observation.

2.9. *Method 4: double-trephination technique–Muraine technique*

1. Place the cornea with the endothelium upward on the concave surface of a disposable AAC or another suitable concave holder (Fig. 5A).
2. A trephination blade with a diameter of 8–8.5 mm should be broken to produce a 3–4 mm-long fragment.
3. The blade is then held in the Halstead forceps and pressed against the corneal endothelium to dissect the DM circumferentially, producing a 330° cut on DM instead of a 360° cut. It is important to curve the blade slightly outward to avoid DM tearing at the transition between the trephined section and the peripheral cornea (30°) (Fig. 5B).
4. The circular trephination blade should be held upright, and the graft should be well-centered and flattened. No rotational force should be applied to avoid tearing the

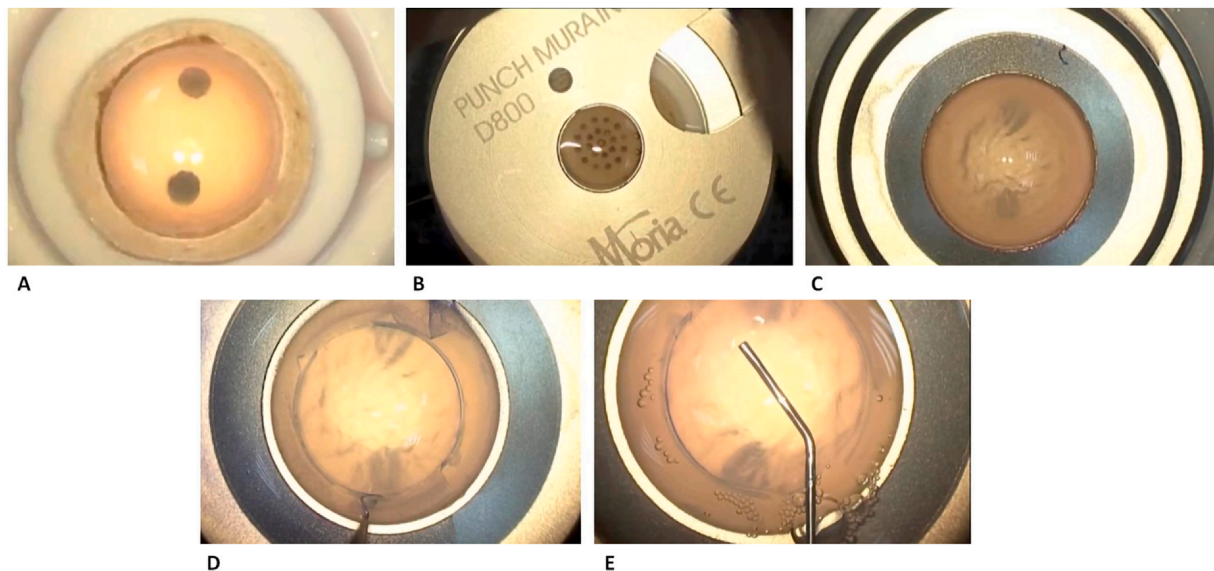

Fig. 5 – Muraine technique. *A*: the cornea is placed with the endothelium upward on the concave surface of a disposable artificial anterior chamber or another suitable concave holder. *B*: the blade is pressed on the corneal endothelium to dissect the DM circumferentially, producing a 330° cut on the DM. *C*: the artificial anterior chamber is then closed with the endothelium still on the top, and the air is insufflated into the anterior chamber, resulting in an inversion of the cornea, with the endothelium well stretched upward. *D*: the peripheral endothelium can be detached very easily, in a single movement, with forceps. The peripheral DM should be grasped at the precise point of trephination to avoid contact with the central endothelium. *E*: the 27-gauge cannula mounted on a 2.5 mL syringe filled with storage medium or BSS is threaded under the endothelium, toward the center of the cornea. Injecting liquid at this point easily detaches the endothelium by hydrodissection in front of the cannula.

membrane. The depth of trephination is not important, but a full-thickness cut should be avoided.

5. The AAC is then closed. With the endothelium still facing the top, the air is insufflated into the anterior chamber resulting in an inversion of the cornea, with the endothelium well stretched upward (Fig. 5C).
6. The excess storage medium is then removed from the side with a swab spear, and the endothelium is stained with trypan blue to ensure perfect visualization of the DM trephination zone. To remove excess trypan blue, the graft can be rinsed with BSS.
7. A cohesive viscoelastic drop is placed on the endothelium to prevent it from drying out, particularly at the apex.
8. At the level of the 30° uncut area, there is a continuity region between the central endothelium and peripheral endothelium: on either side of this zone of continuity, the peripheral endothelium can be detached very easily, in a single movement, with forceps. The peripheral DM should be grasped at the precise point of trephination to avoid contact with the central endothelium. Providing sufficient pressure in the anterior chamber allows the DM to be easily detached (Fig. 5D).
9. The peripheral endothelium is torn in the continuity area in such a way as to create a small flap that is easily lifted.
10. Thus, by inserting a small spatula or the jaws of a pair of Troutman forceps into the opening, proper detachment of the DM over a length of 2–3 mm can be ensured.
11. The remainder of the dissection is then performed with a 27-gauge cannula mounted on a 2.5 mL syringe filled with culture medium or BSS. The 27-gauge cannula is threaded under the endothelium, toward the center of the cornea. Injection of the culture medium or BSS at this point easily detaches the endothelium by hydrodissection in front of the cannula (Fig. 5E).
12. Once the center of the endothelium is reached, it is easy to detach the DM on both sides, from right to left, to the trephination zone. The hydrodissection is directly extended to the opposite zone of DM trephination followed by detaching the endothelium on both sides to the periphery.
13. At the end of the dissection, the graft remains in contact with the underlying stroma because it is simply resting on top, not actually submerged. Nevertheless, it is important to make sure that it does not slide too far to the side and, if necessary, recenter it.

The video can be accessed at https://www.youtube.com/watch?v=avAWGamavms.

2.10. Outcomes

Muraine and coworkers[47] showed that this technique is simple and can be performed even by an inexperienced surgeon because it avoids trephination over 360°, which makes it very difficult to differentiate the DM plane from the general trephination zone. The authors claim that it is easier to detach the DM at the edge of the trephination zone. The suggestion, therefore, was to perform a trephination only over 330°, leaving a zone of continuity between the periphery and the center. Another advantage reported for this technique is that it allows obtaining a graft rolled up with the endothelium on the inside of the roll (endo-in conformation) rather than on the outside, which provides better protection of the endothelium during graft manipulation.

Brissette and coworkers[12] compared this technique with the standard submerged cornea using backgrounds away (SCUBA) peeling technique. In a wet-lab setting, 20 donor corneas were prepared for DMEK using the former and 20 donor corneas using the latter technique. In each of the technique groups, 10 corneas were prepared by a corneal surgeon, and 10 were prepared by a corneal fellow. Time to prepare donor grafts was similar between the 2 techniques for both the corneal surgeon (301 ± 85 s for SCUBA versus 359 ± 83 s for Muraine) and the corneal fellow (523 ± 58 s for SCUBA versus 543 ± 44 s for Muraine). Also, the Muraine technique showed central staining in 2 grafts, whereas the SCUBA technique showed peripheral positive trypan blue staining in 2 grafts. This pattern of staining may represent the area of the donor tissue that is most manipulated during the tissue preparation. Five grafts (2 made by a surgeon and 3 by a fellow) showed tears following the Muraine technique, while no graft tears from the SCUBA technique were observed, which was statistically significant. The authors assumed that this could be attributed to the learning curve of adopting a new graft harvesting technique for both the corneal surgeon and the corneal fellow.[47]

2.11. Method 5: DM separation by injection

a. ***Pneumodissection***[15]

1. After washing with BSS, the corneal tissue is placed on a sterile wire gauze with the endothelium facing upward.
2. A 10 cc syringe is connected to a 30-gauge needle prefilled with air.
3. The needle is inserted into the peripheral cornea with the bevel up and advanced tangentially from approximately 1 mm beneath the limbus up to approximately 2 mm inside the stroma-DM interface (Fig. 6A).
4. Air is injected subsequently to achieve detachment of DM.
5. The bubble is enlarged as far as possible up to the corneal periphery.
   Note: stromal edema can occur if the air leaks from the peripheral limbus or the insertion site is completely in the stroma. A different site can be chosen to complete the separation (Fig. 6B).
6. Depending on the requirement, the insertion site can be selected to obtain a type I or type II bubble.

The details can be found in supplementary video 3.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

b. ***Submerged hydroseparation***[56]

1. Submerge the corneoscleral rim in a 90 mm Petri dish half-filled with the storage media to keep the endothelium moist during the entire procedure.

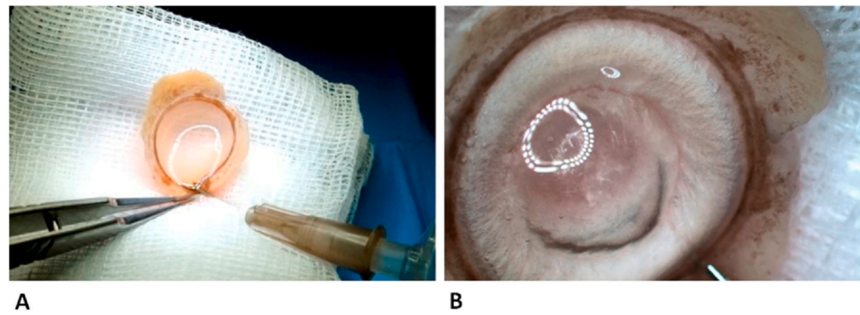

Fig. 6 – Descemet membrane separation by injection. *A*: pneumatic dissection technique—a pair of forceps are used to hold the tissue at the sclera, and the needle is inserted beneath the trabecular meshwork and moved beneath the endothelium in the posterior stroma or in the stroma-DM interface. *B*: inject air in order to obtain a central Type 1 big bubble that is formed in the center of the cornea with a diameter of around 8 mm.

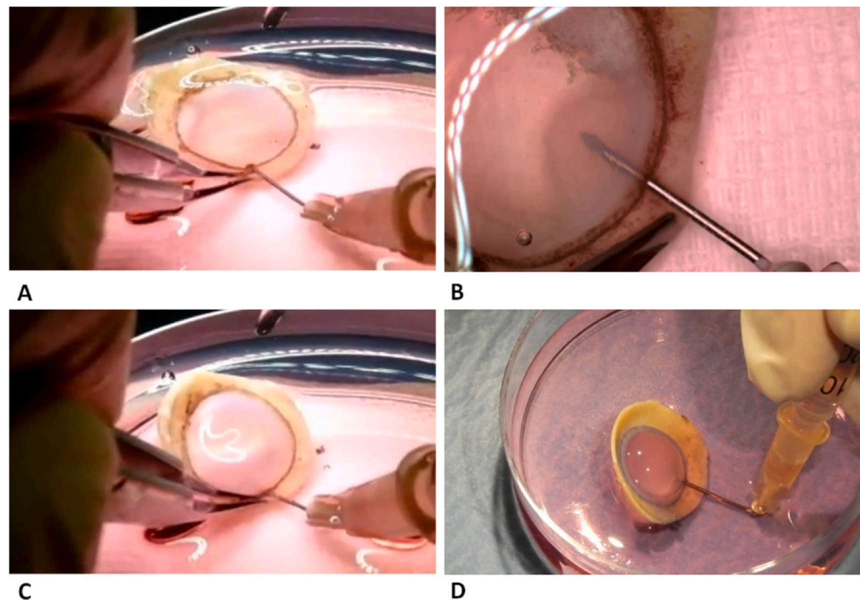

Fig. 7 – Submerged hydroseparation technique. *A*: a pair of forceps are used to hold the tissue at the sclera, and the bent needle is inserted beneath the trabecular meshwork and moved radially beneath the endothelium in the posterior stroma or the stroma-DM interface. *B*: the needle is inserted into the peripheral cornea approximately 1 mm from the limbus beneath the endothelium up to approximately 2 mm. *C*: storage media is injected into the tissue with increased pressure in order to achieve a full 10 mm-diameter bubble. *D*: a complete 10 mm liquid bubble is, thus, achieved.

2. A 30-gauge needle is bent by 90° with its bevel upward and connected to a 1 cc syringe, which is prefilled with the storage media.
3. Using a pair of toothed forceps, the tissue is held tightly at the sclera and pushed on the bottom of the Petri plate to maintain the grip.
4. The free hand is used to insert the bent needle beneath the TM (Fig. 7A) and advanced beneath the endothelium in the posterior stroma or in the stroma-DM interface until the bevel of the needle is visible inside the tissue. This can be considered as an insertion threshold, and it is recommended not to advance further (Fig. 7B).
5. The storage media is then injected in the DM-stroma interphase with pressure (moderate) enough to separate the layers. A small, clear, visible bubble will be produced, thus ensuring that the procedure is correct (Fig. 7C).
6. If the liquid bubble unknowingly initiates in the stroma, then more liquid must be injected (a greater force may be required) to fill the stroma and puncture the posterior stroma leading the liquid to enter the stroma-DM interphase (Fig. 7D).
7. Once the bubble is complete, the liquid can be removed using the same syringe. The tissue is punched with the desired diameter trephine and released in BSS for washing and aspirating in the injector.

The details of bubble preparation can be found in supplementary video 4, and preparation of the graft after bubble collapse can be found in supplementary video 5.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

c. ***Pre-Descemet endothelial keratoplasty (PDEK)***[2,48]

1. After placing the tissue on a block, inject air to obtain a central dome-shaped Type 1 big bubble that characteristically spreads from the center to the periphery with a diameter of around 8 mm, provided that the bevel is correctly positioned in the cleavage plane (as described in pneumodissection technique). If the bevel is in the posterior stroma, the air will spread in the posterior stroma primarily followed by creating a cleavage through the stroma at its weakest point and entering the DM-stroma interphase, which is a similar phenomenon to the submerged hydroseparation method.
2. Once the bubble is achieved, puncture the extreme periphery of the graft with a side-port blade.
3. Inject trypan blue inside the pocket created by the side port to stain the graft.
4. Cut the tissue circumferentially with a Vannas scissor held flat or cut with a desired sized trephine.
5. Once the tissue is separated following the bubble, the tissue is deflated using the same syringe, and the tissue is punched with a desired diameter trephine. The separated DMEK tissue is either peeled off or placed in sterile BSS and shaken gently to remove it from the underlying stroma.

The details can be found in supplementary video 6.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

2.12. Outcomes

Submerged hydroseparation and pneumatic dissection techniques are cheaper as they do not require any special instruments. The aim of these techniques is to separate the DM-endothelium complex from the stroma, injecting either the storage medium or air in the posterior stroma or in the DM-stroma interface to create a bubble.[15,56,64,68] As a large DMEK graft can be obtained by inflating the bubble to its max capacity (10 mm), a desired (large or small) diameter graft can be prepared for transplantation. Submerged hydroseparation is recommended for the tissues that are preserved in organ culture storage media, which have a thickness of over 800 µm at the time of bubble formation. The bubble formation is relatively smoother due to the presence of fluid inside the cornea. Although the bubble can be formed in a thinner graft, it may lead to higher cell mortality (8.71% and 11% of dead cells, respectively, for liquid and air dissection) compared to the conventional stripping technique due to retrograde pressure generated on the DM.[52] Agarwal and coworkers[2] proposed PDEK that refers to the transplantation of pre-Descemet's layer (PDL) or the Dua layer. The DM and the endothelial graft can be obtained with a thickness of approximately 28 ± 5.6 µm. The PDEK graft is prepared by injecting air into a donor corneoscleral rim to get a Type 1 big bubble. This is characterized by a cleavage plane that is formed between the PDL, DM, and endothelium on one side and the stroma on the other side. It differs from a Type 2 big bubble, which is essentially a DMEK graft. A DMEK graft is formed when the injected air cleaves a plane between the DM and endothelium on one side and the DM on the other side. A Type 2 graft starts from the periphery and expands over to the other side, and it has low bursting pressure. A Type 1 graft, on the other hand, starts at the center, expands circumferentially, and has a higher bursting pressure.

Compared with DMEK, PDEK is reported to be easier in lenticular preparation, graft scrolling, and handling with comparable visual recovery time.[32–34,48] Furthermore, PDEK allows the use of donor tissue younger than 40 years of age; however, in the case of DMEK, the edges of the grafts are more difficult to unscroll when grafts are prepared from younger donors. This is possible due to the presence of PDL in the case of the PDEK graft, which has a splinting effect resulting in less curling of the donor tissue.[76]

2.13. Method 6: using asymmetrical trephines

a. ***Yogurt technique (using Tzamalis DMEK punch)***

1. The donor corneoscleral disc is grasped carefully with toothed forceps from the scleral rim, and it is positioned endothelial side up on the cutting block.
2. The donor disc is properly centered on the cutting block ensuring that the limbus is equally distanced from the peripheral markings of the cutting block 360°.
3. Vacuum is applied by means of a spring-loaded syringe attached to the cutting block (applying negative pressure) to secure the position and stabilization of the corneoscleral disc.
4. Trypan blue solution 0.04% is applied on the endothelial side and left for at least 30 s in place to stain the endothelium/DM and facilitate better visualization of the procedure.
5. Trypan blue solution is rinsed off with BSS and cleared using a sponge.
6. A partial-thickness trephination with the DMEK-guarded punch blade is performed avoiding any rotational movements.
7. The above-described DMEK punch has a circular guarded blade missing 1 clock hour, creating an uncut hinge/tab on the donor cornea.
8. The uncut hinge of approximately 40° arc is being identified and brought opposite to the surgeon's field at the 12 clock hours (Fig. 8A).
9. A nonsharp-pointed instrument (e.g., Sinskey hook) or crescent blade is used to identify the end of the DM at the level of Schwalbe's line in the uncut tab area (Fig. 8B).
10. DM with overlying endothelium is peeled off from the underlying corneal stroma using a crescent blade.
11. The DM peeling is performed carefully beyond both angles of the hinge (the two ends of the circular cross-section) taking care to avoid inducing any tears on the graft (Fig. 8C).

Fig. 8 – Yogurt technique (using Tzamalis DMEK punch). *A*: the punch has a circular blade missing 1 clock hour, creating an uncut hinge of approximately 40° brought opposite to the surgeon's field at the 12 clock hours. *B*: Sinskey hook is used to identify the end of Descemet's membrane at the level of Schwalbe's line in the uncut tab area, and then, a crescent blade is used to peel off the DM with overlying endothelium from the underlying corneal stroma. *C*: DM peeling is performed carefully beyond both the angles of the hinge carefully to avoid any tear on the graft.

12. The peeled edge is placed back using BSS, and thereafter, the graft is stained again with trypan blue and rinsed off.
13. The detached hinge is being cut with the crescent blade to leave only an orthogonal triangle part, which will act as a marking when the graft is inserted into the wet-lab eye model allowing identification of correct graft orientation. The hypotenuse of the orthogonal triangle created lies clockwise to the right (90°) angle so that, when inserted in the anterior chamber and unfolded, it appears anticlockwise as the endothelial side should be facing downwards.
14. The DMEK graft is grasped with forceps (tying, jewelers, or other DMEK forceps) from the triangle marking and further stripped in a single-peel technique.

The details can be found in supplementary video 7.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

2.14. *Outcomes*

Proposed by Tzamalis and coworkers, this technique aims to prepare the graft using a specific partial-thickness hinge punch.[76] The novel punch has a circular guarded blade missing 1 clock hour, creating an uncut hinge on the donor cornea. In addition, 2 straight cuts are made by the punch perpendicular to the edge of trephination toward the TM in the hinge area, resembling a "yogurt cup." After the donor corneoscleral rim is positioned with the endothelial side upward, a partial-thickness trephination is performed avoiding any rotational movements. The DM is lifted from Schwalbe's line at the hinge, and the DMEK graft is peeled after the desired marking without further manipulation, as the opening of a "yogurt cup." The advantages of this technique are the relatively less preparation time of the graft (6.1 min on average) and a short learning curve. In addition, no significant differences in the failure rate or tissue loss among study participants (senior surgeon, independent surgeon, and fellow) were reported. Also, the authors described no ECL before and after peeling.[76]

b. *Kite technique*

1. Place the donor tissue on the block in such a manner that the punch would create a pedicle along the long axis of the cornea and would result in the longest possible pedicle graft.
2. Mark the sclera on the endothelial side with a skin-marking pen to orient the long axis.
3. Stain the TM for 30 s with trypan blue (0.06%), and rinse with BSS.
4. Separate the DM-endothelial complex at the TM up to 270° using the Rootman-Goldich DMEK dissector (Fig. 9A).
5. It is easier to start the dissection at the short axis where the adhesions are weaker. Stain the interface of the dissected area in the periphery for 30 s periodically to

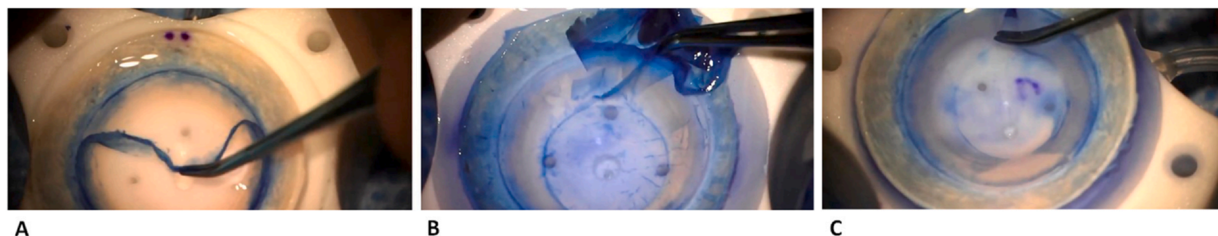

Fig. 9 – Kite technique. *A*: using nontoothed forceps, peel 60%–70% of the DM-endothelial complex. *B*: carefully remove the external part of the DM-endothelial complex without damaging the pedicle. *C*: grasp the graft using nontoothed forceps near the pedicle–body junction, and then, peel completely. Stain with trypan blue 0.06% for 3 min.

visualize any strong fibrous attachments between the DM and the stroma.

6. Detach 75% of the TM and release the DM-endothelial complex 1–2 mm past Schwalbe's line into the central cornea.
7. Apply vacuum to the donor button to hold the stroma in place. Using nontoothed forceps, peel 60%–70% of the DM-endothelial complex (Fig. 9B).
8. Restore the DM-endothelial complex to the original position on the stroma (Fig. 9C). Draw out the fluid from the interface between the peeled DM-endothelial complex and the stroma by tilting the graft (unpeeled side up and peeled side down), and draw any excess fluid using spear sponges near the TM without touching the graft.
9. If there is a wrinkle on the graft, eliminate it by pulling the TM more peripherally along the stroma toward the edge of the button. Position the donor button in such a way that the tip of the punch pedicle just overlaps with the TM on the long axis of the cornea (endothelium facing up).
10. Apply pressure on the punch along the rim in either clockwise or anticlockwise direction in a rocking motion to cut the graft and remove any trapped interface fluid.
11. Grasp the graft using nontoothed forceps near the pedicle–body junction, and then, peel it completely. Stain with trypan blue (0.06%) for 3 min.

The video can be accessed at https://www.youtube.com/watch?v=Dg-zCkJk_Ls.

2.15. Outcomes

In 2020, Bala and coworkers[6] proposed using a graft with a pedicle that allows better control of orientation, centration, and unrolling of the DMEK scroll. The "Bala Asymmetric Corneal Vacuum Punch" allows to obtain a 7.5 mm graft with a pedicle of 3.0 mm length and a 1.2 mm width at the tip. The donor button position must be adjusted before punching so that the TM can be included as part of the pedicle. The authors described a short learning curve where the manipulation time decreased significantly after the fifth case with a significant decrease in ECL. The orientation of the graft can be recognized by 4 different methods: inking the tip of the graft pedicle at an angle using the circumferential scleral fibers on the pedicle (indicating the nonendothelial surface), the orientation notch, and the Veldman Venn technique, which involves observing the movement of the overlapping edges of the graft stained with trypan blue. In addition, instead of injecting the graft into the anterior chamber, the graft is placed using the pedicle, reducing the likelihood of achieving retropupillary placement by avoiding direct contact with the graft. The pedicle also facilitates holding the graft in place during the gas injection process.

2.16. Donor marking techniques

Incorrect anterior-posterior orientation of the graft is one of the reasons for graft detachment and failure in DMEK.[61] Relying only on the natural rolling tendency of the graft in the anterior chamber can be risky, as there is no certainty that the endothelial layer will face outward in all cases; sometimes, it does not roll at all. For this reason, several marking techniques have been developed over the years to identify the correct endothelial side of the graft during a DMEK transplant.

a. ***Shaped marking***

1. Bhogal and coworkers introduced a single triangular mark using a 30° incision knife.[8] The knife is used to excise a small right-angle triangular segment of DM after partial peeling and trephination, from within the trephined graft edge opposite to where the peel was initiated; it is performed in an area where DM is still attached (Fig. 10A).
2. The rest of the graft is peeled under BSS so the last portion to be detached is at the point of the mark. This reduces the risk of initiating a radial tear at the site of the mark (Fig. 10B).

The details can be found in supplementary video 8.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

b. ***Asymmetrical letter marking***

Common marking techniques rely on "S" or "F" letters being stamped with a dermatological biopsy punch through a stromal window created in the donor tissue.[79]

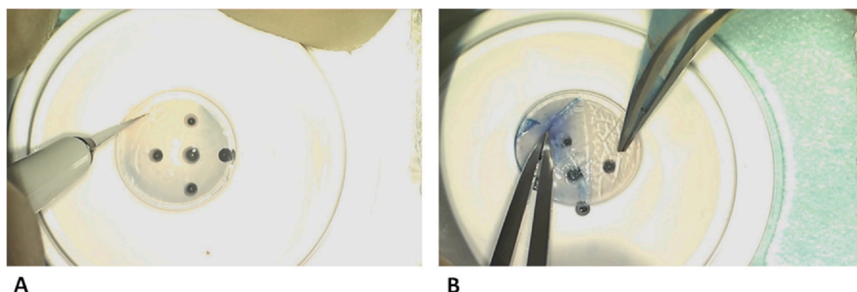

Fig. 10 – Single triangular mark using a 30° incision knife. *A*: a small right-angle triangular segment of DM is excised after partial peeling and trephination. *B*: the second half of the graft is peeled under BSS, and the last portion detached is the marked edge.

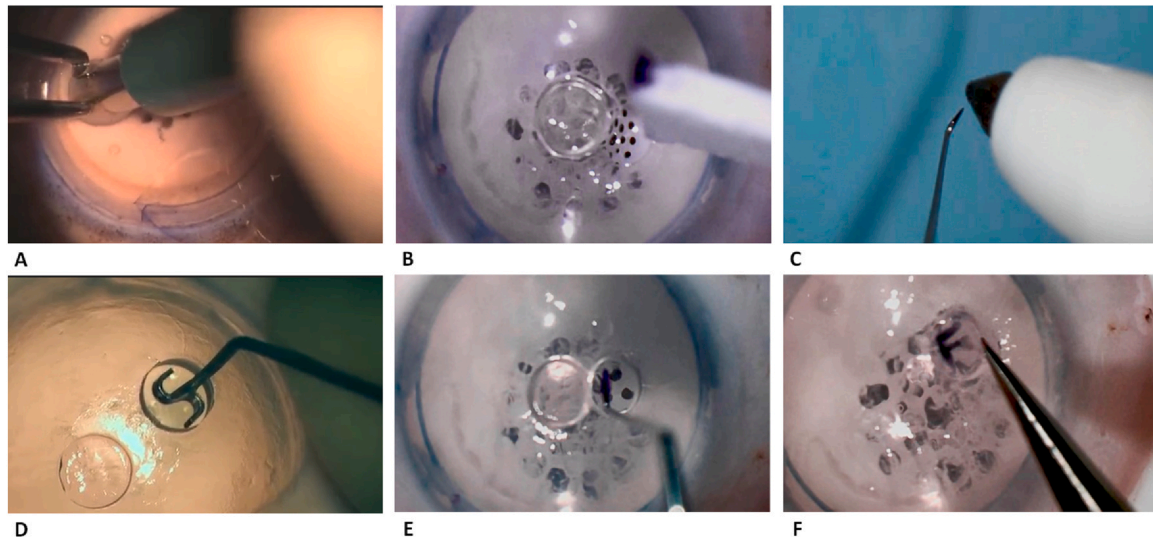

Fig. 11 – The “S” and “F” marking techniques. *A*: perform a 2 mm punch through the stroma. *B*: use BSS to restore the peeled DMEK back on the stroma and invert the corneal tissue. Remove the 2 mm stromal plug and dry Descemet’s layer through the 2 mm hole. *C*: ink the stamp/cleavage hook using a skin marker. *D*: either stamp with letter “S” or *E*: manually mark with letter “F” on the DM. *F*: Allow the ink to dry, and replace the stromal plug.

1. To mark the graft, the peeled tissue must be left peeled with a hinge “on” and should not be restored back on the stroma to expose the stroma.
2. Perform a 2 mm punch through the stroma (Fig. 11A).
3. Use BSS to restore the peeled tissue back on the stroma. Tilt the tissue holder, and use swab spears to remove as much BSS as possible.
4. Dry the scleral rim and mark the hinge. Then, disconnect the suction syringe from the trephine block, if needed, to release any remaining suction.
5. Flip the cornea over to apply either “S” or “F” stamp:

– Remove the 2 mm stromal plug and dry Descemet’s layer through the 2 mm hole (Fig. 11B).
– Ink the stamp/cleavage hook with the skin marker (Fig. 11C).
– Gently mark the DM using the skin marker with a letter S (Fig. 11D) or letter F (Fig. 11E).
– Allow the area to air dry for several seconds, and dry it gently with a swab spear.
– Place a drop of BSS into the hole, replace the 2 mm stromal plug (Fig. 11F), and then dry with a swab spear, securing the plug.

The details can be found in supplementary videos 9 (“S” stamp) & 10 (“F” stamp).

Another method to determine the orientation of the donor graft may be the use of intraoperative ocular coherence tomography.[65,71] However, such devices are expensive and are not readily available in all operating rooms.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

c. *I/II marking*

A relatively new marking technique based on stamping roman numericals “I and II” has been introduced recently (unpublished).

1. After peeling, perform the final graft trephination, and remove the excess peripheral endothelium.
2. Use the tying forceps stained with a dermatological marker to apply 2 adjacent marks on the endothelium and a single mark 2–3 h clockwise directly on the peripheral endothelium (Figs. 12A and 12B).
3. The graft when inserted must be oriented from I to II and not otherwise to ensure the correct orientation of the graft.

2.17. Outcomes

Although the marking is quicker than the other techniques as it avoids additional steps of obtaining a stromal biopsy, inverting the tissue, and stamping, this technique may induce peripheral ECL. Hence, a larger diameter graft may be needed; however, as this technique is still at a very early stage of development, it is true that clinical advantages have not been completely evaluated. As the footprint of the “S” stamp is approximately 45% larger than the “F” stamp, a study by Newman and coworkers[50] showed that the S stamp causes an average ECL of 1.9%, whereas the smaller F stamp caused an average ECL of 1.0%.

In addition, the Moutsouris sign can be useful to identify graft orientation only in the case of grafts with endothelial

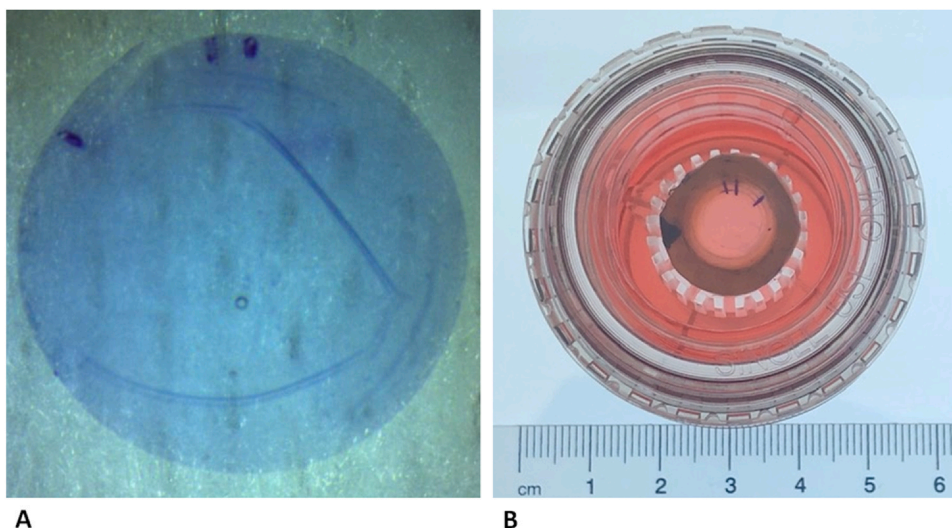

Fig. 12 – Graft marking (I and II) technique. *A*: DMEK graft peeled and marked with roman numbers and placed with endothelial side facing down to mimic the graft orientation after implantation. *B*: Entire corneal tissue with the endothelial side facing up in the viewing chamber.

rolling. When the graft is oriented correctly within the anterior chamber (edges facing upward), the tip of a cannula can be positioned inside a peripheral curl so that the tip appears blue because of the overlying blue donor tissue (Moutsouris sign positive). When the graft is positioned upside down (edges facing downward), the tip of the cannula cannot find the curls, so the tip will not change color (Moutsouris sign negative).[17]

Bachmann and coworkers used three marks of 1.0 mm trephined asymmetrically on the edge of the graft.[5] The marks have no adverse effect on donor attachment or postoperative corneal transparency. A possible drawback of this method is when the graft is dislocated and a part thereof is hidden behind the angle, or when the graft is partially folded at the edge. In these cases, using only three asymmetric marks, graft orientation cannot be determined. Matsuzawa and coworkers proposed two pairs of asymmetrical semicircular marks of 1.0 and 1.5 mm in diameter placed on the edge of the donor graft using dermatological biopsy punches.[43] Both pairs of marks are readily recognizable during surgery, and one pair is always visible when the other is obscured for various reasons. When the surgeon observes them during and after the surgery from the epithelial side, the large and small pair of marks are always observed in the opposite orders that were made during preparation with the endothelial side up. If the graft is attached on the wrong side, the pair of marks can be recognized in the same order as that observed during graft preparation. A new marking technique, based on the punch proposed by Tzamalis and coworkers,[76] provides an asymmetrical mark outside the usual circular tissue. The knife is used to shape the inch of the yogurt technique as a small right-angle triangular segment of DM after a partial peeling and trephination (Fig. 13). The details can be found in supplementary video 11.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

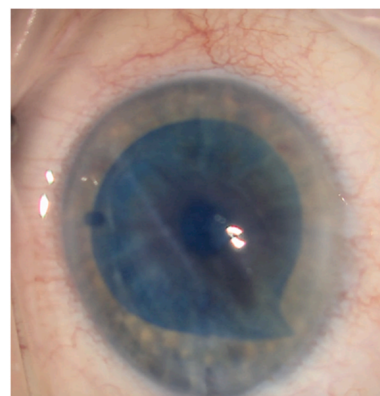

Fig. 13 – Asymmetrical marking technique by Tzamalis et al. An asymmetrical mark outside the usual circular tissue is created using a knife to create a right-angle triangular segment of DM after a partial peeling and trephination.

Unlike methods that rely on gentian violet marking/stamping/inking, the graft orientation can be confirmed at the slit lamp at any time postoperatively using these techniques.

However, the stamping/inking of the DM-endothelial complex has some disadvantages that include increased local ECL (5.8% for asymmetrical semicircular marks and 2.5% for asymmetrical three marks), higher possibility of the lamella to become entangled in intraocular structures at the punched edge, and the difficulty in visualizing the marks when the corneal periphery is opacified.[50,79]

Wasielica-Poslednik and coworkers proposed to apply a braille “R” letter dot by dot onto the stromal surface of the graft by injecting an air bubble into the interface between the

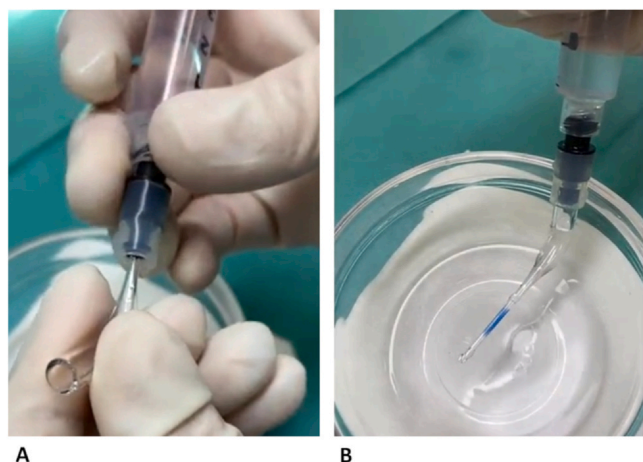

Fig. 14 – Endo-out graft loading. *A*: the connector is attached to a syringe, then the glass injector is secured to the connector. *B*: the wide end of the injector is attached to the connector.

endothelial surface of the partially stripped graft, resulting in a minor ECL (approx. 0.3%).[81] In contrast to other methods determining the donor graft orientation, this cost-saving technique does not require any specific instrument, such as a dermatological biopsy punch or intraoperative ocular coherence tomography.

2.18. *Graft loading*

The peeled DM is likely to roll up into a cylindrical structure with the endothelial side facing outward when in contact with BSS; however, manual folding of the DM-endothelial complex is performed for endothelium facing inward. In general, only these two orientations have been published in the literature.

a. ***Endo-out graft loading***

1. Attach an appropriate connector to a syringe and connect the syringe with a preferred glass injector (Fig. 14A): Straiko Modified Jones Tube (Gunther Weiss Scientific Glass, Portland, OR, USA), DORC injector (DORC, Zuidland, The Netherlands) (Fig. 14), Geuder injector (Geuder AG, Heidelberg, Germany) (Fig. 15), or the Janach injector (eJanach, Como, Italy).
2. Transfer the cornea to the Petri dish filled with the storage media (for preloaded DMEK) and BSS (for immediate transplantation). Allow the peeled graft to float off naturally from the cornea by gentle shaking. The graft will scroll with the endothelium outward when placed in the fluid.
3. Submerge the tip of the injector near the scrolled graft, and aspirate the graft in the injector (Fig. 15A).
4. Either the injector can be inverted and used directly for graft delivery (Fig. 14B), or the device can be capped (Fig. 15B), clipped (Fig. 15C), and shipped as a preloaded graft with endo-out conformation (Fig. 15D).
5. During the transport of preloaded DMEK grafts, one end of the injector must always remain sealed to prevent accidental graft ejection.

The details can be found in supplementary videos 12 & 13.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

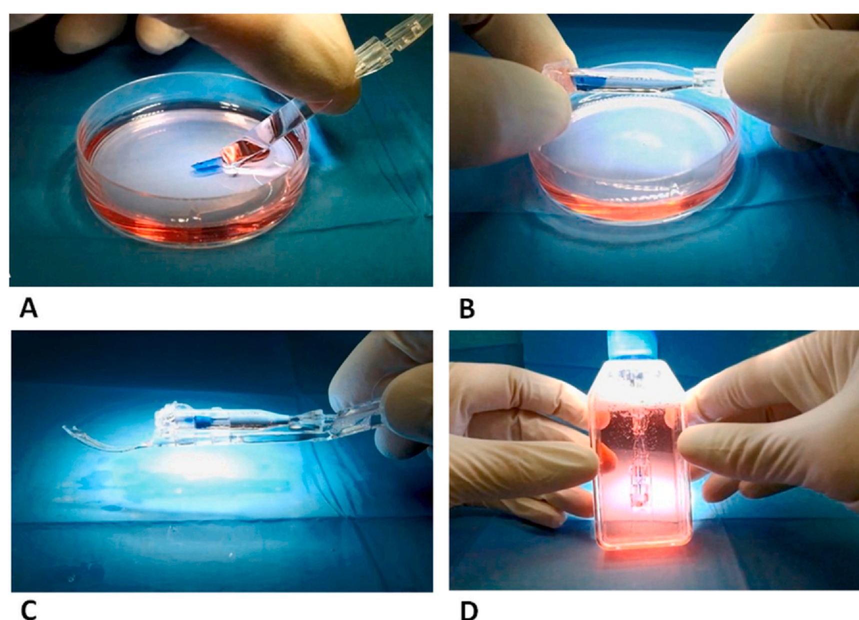

Fig. 15 – Endo-out graft loading in DMEK Rapid device. *A*: using the wider end of the injector, aspirate the tissue inside the cartridge. *B*: remove the syringe and cap the device from its front and rear ends and *C*: clip it on the cartridge holder. *D*: the entire unit is placed in a flask prefilled with the storage media as a preloaded graft with endo-out conformation.

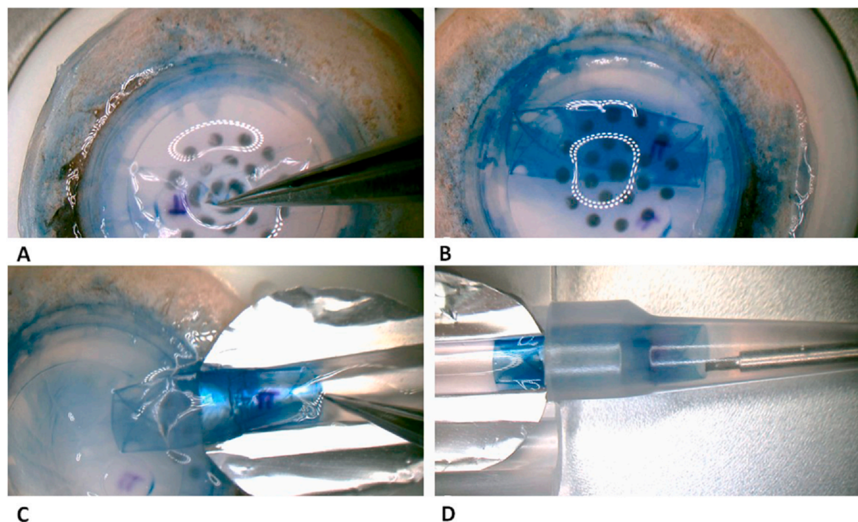

Fig. 16 – Endo-in graft loading. *A*: using anatomic forceps, the membrane is manually trifolded with the endothelium inward and *B*: stained with trypan blue. *C*: the graft is dragged onto a sterile aluminum foil or a therapeutic soft contact lens for transferring the tissue on the cartridge. *D*: dedicated anatomic microincision forceps are inserted into the distal entrance of the cartridge to reach the edge of the DMEK graft and pull it into the funnel. The unit is now ready for shipment with endo-in conformation.

b. ***Endo-in graft loading***

1. Following the peeling and marking of the DMEK graft, use the tips of an anatomic/nontoothed acute forceps to lift the edge of the graft (Fig. 16A).
2. Trifold the DMEK graft with the endothelium inward from the two opposite ends and stain with trypan blue (Fig. 16B).
3. Drag the tissue onto a sterile therapeutic soft CL or aluminum foil (Fig. 16C) for a better grip.
4. Transfer the foil with the graft onto the rear entrance of an intraocular lens cartridge filled with the storage media or BSS from its distal part.
5. Insert a dedicated anatomic microincision forceps into the distal entrance of the cartridge to reach the aluminum foil surface and grasp the edge of the DMEK graft. Pull the graft into the funnel (prefilled with the storage media or BSS) ensuring that the unfolded part slides onto the floor of the funnel (Fig. 16D).
6. The folded tissue opens partly when in contact with the storage media inside the funnel, thus adhering to the funnel wall but maintaining the endothelium in its inward-facing configuration. Endo-in conformation, thus, prevents any possible damage to the endothelial cells resulting from any contact with the cartridge wall.
7. Seal the rear end of the cartridge funnel with a silicone plug to avoid liquid reflux and graft loss during delivery.
8. Turn the cartridge by 180°, thus turning the floor to become the ceiling of the funnel, and insert it into the main wound for graft delivery.

The details can be found in supplementary video 14.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

2.19. *Outcomes*

Regardless of the loading device or technique used, it is crucial for surgeons to be aware of the natural tendency of the DM (endo-out) to adopt this shape and to be able to manoeuver it accordingly in the anterior chamber. It is crucial to engraft the tissue with minimum handling to avoid any possible operative or postoperative complications. The other graft orientation is instead with the endothelium facing inward, the “endo-in” conformation. These two techniques have similar ECL at six months, between 29.5% and 30.9%. The endo-in technique has a lower unfolding time, whereas the endo-out takes less time for graft preparation.[13,49,54,60] Donor graft preparation, insertion, unwinding, identifying the correct orientation, and holding the graft in place using air or gas are all technically challenging steps. If the DM is manipulated extensively during surgery, normally, the loss of endothelial cells will be greater, decreasing the likelihood of long-term graft survival and facilitating primary transplant failure.[29]

2.20. *Graft delivery*

Several techniques have been reported for delivering an endo-out graft, for example, the one described by Fogla and coworkers and the “no-touch.”[25,40] The lack of comparative studies, however, prevents to assess the superiority of one technique over the other.

2.21. *Endo-out conformation*

a. ***Standard technique***[25]

1. In the recipient's eye, a 2.8 mm corneal incision is made, along with 2-hour paracentesis on either side of the main incision.
2. If the chosen wet-lab method allows it, execute Descemetorhexis.
3. A lower peripheral iridectomy is then performed using a vitrector or surgically through a paracentesis. The viscoelastic material is completely flushed from the AC, and a 25-gauge anterior chamber maintainer (ACM) is placed in one of the paracentesis incisions and then turned on, maintaining the height of the bottle at 40 mmHg.
4. At this point, the glass cartridge tip can be introduced into the AC and rotated to bring the donor DM roll to an appropriate configuration, with the open end facing up.
5. The ACM is then turned off, and the intravenous tubing is connected to the ACM. This allows fluid to exit the anterior chamber using the ACM and facilitates injection of the donor DM roll into the AC in a controlled manner without any back pressure of fluid into the AC. The chamber is kept shallow so that the orientation of the donor DM roll can be well maintained.
6. The corneal incision is secured with a 10–0 nylon suture, and the ACM can be removed by paracentesis.
7. Deployment of the DM roll at this point can be achieved by tapping the surface using two 27-gauge cannulas.
8. After complete deployment and central placement of the DM donor, a medium-sized air bubble is placed under the donor graft using a 30-gauge cannula, followed by a complete air filling of the AC.

b. ***DMEK Rapid technique***

1. This device acts as a storage and delivery system mainly for preloaded DMEK tissues. The cartridge with front and rear plugs attached is securely removed from the holder.
2. As the cartridge is made of glass, it is easier to monitor the tissue and check for any deformities. (Note: the "F" stamp can be checked inside the cartridge.)
3. The silicone tube with a syringe containing vision blue is attached to the front plug, and the vision blue dye is slowly pushed though the device, allowing the DM tissue to be stained inside the cartridge.
4. The architecture of the device allows the staining and restaining the tissue to flush out.
5. Using the same technique, the tissue can be washed inside the cartridge with BSS.
6. The syringe along with the silicone tube is removed, and the rear plug is replaced directly with a fresh 5 cc syringe. The tissue is advanced gently to reach the loading edge.
7. The front plug is removed just before inserting the cartridge in the anterior chamber.
8. After performing a Descemetorhexis and using the syringe-generated pressure, the DMEK graft is injected into the anterior chamber and gently manipulated to obtain correct graft conformation.
9. The surface of the eye is gently tapped to ensure graft opening and correct the orientation. Once unscrolled completely, the DMEK graft is attached to the stroma using air tamponade.

c. ***The "no-touch" technique***

1. Using the glass injector, the DMEK roll is inserted into the anterior chamber through the main incision at 12 o'clock position. During insertion, the size of the incision should allow sufficient BSS leakage so that the receiving anterior chamber is not overpressurized.
2. Once the correct orientation of the graft has been verified with the edges facing up, an air bubble is injected between the double rolls. The air bubble will be trapped between the rolls, and manipulating it using a cannula on the outer corneal surface can be used to facilitate graft unrolling ("Dapena manoeuver").[40]
3. When the double roll is almost fully opened, the air bubble can be expanded until the center portion of the DMEK graft flattens over the iris. Throughout the deployment process, direct contact between the graft and the cannula should be carefully avoided.
4. Once the graft is unrolled, additional BSS should be injected into the anterior chamber to counteract the downward force from the air bubble pushing the graft against the iris; alternatively, the air bubble can be reduced.
5. The graft should then be centered within the anterior chamber with gentle tapping of the ocular surface using a cannula. Slight offsets are acceptable; this does not worsen the likelihood of successful transplantation or the final visual outcome. In fact, excessive manipulation to center the graft should be avoided to minimize damage to donor endothelial cells.
6. After centering, the air bubble above the DMEK graft should be enlarged, and after approximately 10 s, it should be aspirated from the anterior chamber, keeping the tip of the cannula in the center of the air bubble. Without exiting the anterior chamber, the cannula should then be slowly moved under the graft, positioned centrally over the pupillary area, avoiding contact with the endothelial cells.
7. A small air bubble should be injected under the DMEK graft to lift the graft upward toward the recipient cornea, slowly widening it and carefully observing the edges of the graft. Not infrequently, peripheral inward folds (i.e., an inward curl with the endothelium facing the recipient stroma) may be present. These folds can be flattened by "bubble-bumping manoeuver,"[17] gently tapping the cannula on the outer corneal surface overlying the fold to create a flow of aqueous solution by which the residual folds resolve.
8. Once the DMEK graft is fully deployed, the anterior chamber should be filled with air for 45–60 min at approximately 20 mmHg. This presses the graft onto the receptive posterior stroma.
9. Next, a partial air-BSS exchange should be performed to leave the eye presaturated with 30%–50% air filling in the anterior chamber. In phakic eyes, the air bubble should be

reduced to 20%–30% at the end of surgery to avoid air-induced displacement of the iris diaphragm after surgery. If air tends to move behind the iris during surgery, remove all air from the anterior chamber at the end of the procedure, as these eyes may be prone to catching the air bubble behind the iris after surgery.

2.22. *Outcomes*

During the insertion process, it may occur that the donor DM roll may follow the fluid thrust due to pressure in the AC and leak through the main incision or side ports, resulting in partial or complete expulsion of the donor graft.[38,75] To reduce the risk, the donor graft can be injected with a short, strong jet of fluid; however, this ploy results in the placement of the donor graft in AC in a poorly controlled manner with possible loss of donor roll orientation. Another option is to insert the graft into the anterior chamber at lower pressure. It must be remembered, however, that insertion of the injector tip into an eye with a lower AC can be challenging, increasing the risk of bleeding caused by iris trauma.

For the conformation of the graft, regardless of being endo-out or endo-in, the aim is to minimize the graft manipulation.[8] To understand how the learning curve affects surgical outcomes, Debellemanière and coworkers[18] evaluated the first 109 DMEK procedures performed by a single surgeon (98 eyes and 84 patients) between March 2012 and November 2014. The authors reported the learning curve of a single surgeon for graft preparation and performance, as well as the impact of experience on visual acuity gain and percentage of ECL. In this study, the number of procedures required for a surgeon to reach 90% of the learning curve plateau was 68 cases for graft preparation and 46 for the unwinding procedure. As would be expected, increased surgical experience has led to shorter times in graft preparation, unrolling, and ECL; however, neither the cell loss nor the learning curve affected the patients' best-corrected visual acuity gain at 1 week and 6 months.

Dapena and coworkers[16] evaluated the DMEK learning curve in a multicentric retrospective study and reported that their postoperative visual outcomes and ECD did not correlate with their learning curve but rather with graft detachment. Pereira and coworkers also did not report a correlation between the learning curve and visual outcome but highlighted the importance of supervision and mentoring for cornea fellows during their initial learning curve.[59]

Wojcik, Parekh and coworkers recorded the surgical time of 6–25 min with no immediate surgical complication. Rebubbling was observed in 7/26 cases with one graft failure within 15 days post-op. The mean CDVA on day 1 was 0.64 logMAR, which was improved to 0.18 logMAR at the last follow-up. ECL was 27%. CCT significantly dropped from 694 to 502 μm by the last follow-up. The authors in this study concluded that the DMEK Rapid device is quick, easy, and efficient for preloading and shipping DMEK grafts internationally in endothelium outward orientation.[82,83]

2.23. *Endo-in conformation*

a. ***Pull-through technique***

1. Execute Descemetorhexis if the chosen wet-lab method allows.
2. Create an additional side entry.
3. Rotate the delivery device by 180° before delivering the graft.
4. Deliver the DMEK graft 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 the creation of a jet fluid stream directed against the DMEK graft and, therefore, would eliminate possible interference with tissue unfolding.
5. After inserting the device into the anterior chamber, the Descemet surface of the unfolded part of the DMEK graft, initially in contact with the cartridge ceiling, would now face the stromal surface of the recipient cornea, as required for proper attachment.
6. Gentle tapping onto the corneal surface facilitates the unfolding of the lateral folds, which invariably occurs because of the natural tendency of the tissue to roll with the endothelium outward from its initial inward position.

2.24. *Outcomes*

For delivering the endo-in graft, a pull-through technique can be used, which is characterized by reproducibility, better control over the graft, a lesser learning curve, and overall lower ECL.[14,54] Pull-through techniques rely on folding the graft into a trifold configuration with the endothelium inward, protecting the endothelial cell layer during loading and delivery.[4,14,62,63,73,84] These techniques also offer advantages including transplants in eyes with an anatomically altered anterior segment, such as aphakic eyes or in which a previous glaucoma drainage device has been implanted or eyes that have undergone vitrectomy in which AC shallowing could be challenging. Pull-through techniques frequently involve a tissue loading protocol involving a CL or an aluminum foil with an intraocular lens cartridge[14,53] or various inserters, such as the EndoGlide[4,73,84] (Coronet). An AC maintainer is required in such cases. Jabbour and coworkers[31] suggested using 2 surgical instruments for easier loading of the trifolded tissues, avoiding the use of ACM. The donor tissue loading is performed in one step on a special loading spoon, which allows the surgeon to directly observe the orientation of the graft. Second, novel microforceps attached to the BSS infusion tube can be used to controllably pull the trifold donor tissue from this loading spoon into the AC while maintaining the endothelium downward throughout the entire pull-in procedure. The infusion cannula connects to the BSS syringe, allowing the surgeon to manually control the BSS infusion, AC depth, and AC jet throughout the procedure avoiding the use of ACM, which can cause unwanted fluid turbulence compared to previously described techniques.

b. ***The "kite" technique***[6]

1. Make four side-port incisions with a 1.2 mm keratome directed posteriorly at the limbus (45°, 135°, 225°, and 315°).
2. Execute the Descemetorhexis.
3. Seize the temporal main incision to fit the Straiko modified Jones tube, and place a 10–0 nylon suture.
4. Make a fifth side-port incision opposite to the main incision at the nasal limbus using the 1.2 mm keratome. Keep this incision as short as possible and enlarge it to a 1.8 mm width.
5. Place an AC maintainer through the side-port incision, closest to the surgeon's dominant hand.
6. The graft pedicle must not be kept at the tip of Straiko modified Jones tube but a few millimeters inside the tube as the introduction of the tube into the AC can result in the pressure differential leading to push (high) the graft into the tube or pull (low) the graft out of the tube in an uncontrolled manner.
7. Introduce the Tan DSAEK 23 G forceps with the dominant hand through the nasal incision, and grasp the pedicle. The introduction of the forceps causes the AC to shallow, which can be compensated by further increasing the AC maintainer flow. The thumb and index fingers control the opening or closing of the forceps, and the ring finger can be used to rest the hand on the nasal bridge.
8. Grasp the pedicle with the forceps in the Straiko modified Jones tube, and drag it across the AC. When the pedicle is about to be extravasated, turn off the AC maintainer. Fluid will escape around the forceps, and the chamber will start to shallow.
9. Extravasate the pedicle through the small incision, and open the forceps immediately on exiting the eye. Be careful to not accidentally pull the graft out of the eye with the forceps through the small nasal incision.
10. Remove the Straiko modified Jones tube and the AC maintainer, and close the main incision with the preplaced 10–0 nylon suture.
11. The graft position can be adjusted by pulling, pushing, or pivoting the pedicle using wet instruments, such as wet forceps or Rycroft cannula.

2.25. *Outcomes*

The kite technique instead requires loading the body of the graft first so that the pedicle could be grasped by the Tan EndoGlide™ Loading Forceps (Coronet) during surgery. Therefore, orientation landmarks should be checked inside the Straiko modified Jones tube.

3. Nonhuman models

3.1. *Method 1: porcine*

Droutsas and coworkers[21] reported an *ex vivo* wet-lab model, using 20 porcine eyes to create pseudografts from the lens capsule and successfully implanted them into intact porcine globes using the standardized "no-touch" technique. This simple model allows the performance of essential surgical steps that are required to confirm the orientation of the endothelium, and unfold and apposition the DMEK graft in a repeatable and reproducible manner.

The components used for this procedure (e.g., porcine globes and disposable instruments) are low-cost and readily available worldwide. In fact, the use of animal-derived corneas makes it possible to create wet labs when human-derived material is in limited supply; however, as performing Descemetorhexis in a porcine globe is not possible because the DM is very sticky and prone to tearing, the authors used the lens capsule, which forms a roll when submerged in liquid, like a human DMEK graft.

Porcine eyes are only an approximate simulation of the size and thickness of tissues compared to the surgeries performed on human eyes. The porcine pseudograft has a significantly larger diameter, and the depth of the anterior chamber during surgeries is more controllable, facilitating the execution of most, if not all the manoeuvers.

3.2. *Method 2: onion*

An interesting and useful tool to improve surgical skills.

1. Begin by simulating the corneoscleral button. First, shape a circular section 18–25 mm wide on a single onion layer (OL) using a trephine, and fix this layer on a stable surface with the concave side facing up.
2. Next, simulate the initial marking of the DM. Make an impression in the center of the OL using an 8 mm trephine, applying minimal pressure so that only the surface layers are cut. Highlight the newly created impression and the transparent membrane by applying trypan blue dye (0.4%).
3. At this point, the membrane should be immersed in the fluid (lactated Ringer's/BSS). A Sinskey hook or curved binder clamp is used to create a protrusion between the inner membrane and the underlying fleshy part all around.
4. Stain the stromal side of the DM to improve visualization. Inject trypan blue dye again between the membrane and the underlying fleshy part.
5. Pull the membrane gently with the curved forceps until two-thirds of the membrane separates from the underlying stroma. The direction of the applied force should be toward the opposite end of the membrane. Pay attention to immediately recognize tears on the edge of the membrane during peeling. If any adhesion is encountered, the force vector should be changed to a circumferential one.
6. Dry the newly separated area with a Q-tip or sponge, and use a 3 mm skin biopsy punch to create a full-thickness cut in the dry OL stroma, away from the edge of the separated membrane.
7. Reposition the membrane using fluid. Once unfolded, adjust the peripheral edges using a surgical sponge. This step is like stripping the DM from the cornea, the only difference being that the OL membrane tends to roll in the opposite direction to its "stroma," whereas the DM rolls toward the corneal stroma. It is challenging to keep the membrane in place (compared to human DM) due to its thickness.

8. Flip the OL with the convex side up to proceed with the marking. The perforated stromal cylinder is removed with toothed forceps.
9. The membrane is exposed from the stromal side and is ready to be marked. Dry the membrane to avoid dye smears and coat the tip of the Sinskey hook with gentian violet. Place the desired mark (P, F, or S) on the membrane.
10. The stromal cylinder is placed back using toothed forceps. No additional pressure should be applied to push it in.
11. The OL is flipped back to its original position. After placing a drop of fluid, the remaining membrane is completely detached.
12. After the membrane has been peeled off completely, it curls onto itself in a double roll. The stromal side of the membrane faces outward (in contrast to human DM where the endothelial side tends to face outward from the roll).
13. Stain the membrane again with trypan blue and transfer it to a Petri dish.
14. Aspire the membrane roll into the DMEK injector. It can be injected into an AE to learn the problems associated with the injection.

3.3. *Outcomes*

Mittal and coworkers[46] proposed to use the inner transparent membrane of the onion (Allium cepa) as a simulation model for human DM. The transparent membrane over the single OL has some similarities compared to human DM: microscopically, it consists of a sheet of large rectangular interlocked epithelial cells similar to the human corneal endothelium, which is made up of a tightly packed sheet of hexagonal cells. This model can be useful for DMEK surgeons to learn the steps of DM donor graft harvesting in a repeatable cost-effective way and especially for those who are limited by the availability of the human corneal tissues.

3.4. *Method 3: goat eye*

Gupta et al. have recently described an ex-vivo nonhuman model (goat eyes) for DMEK training that closely matches the experience of human DMEK surgery. The model requires 2 goat globes: the anterior lens capsule of one goat eye is used to create a pseudograft and then implanted into an intact goat globe. The goat eye model mimics the human eye in its structure, even if a bit larger in size. It is able to simulate all DMEK surgery steps, except the Descemetorhexis. The anterior lens capsule used as a DMEK pseudograft can be stained and fixed creating scrolls that behave similarly to the human DMEK grafts from older donors. In addition, the model is cheap and cost-effective.[30]

3.5. *Method 4: latex glove*

1. 15 mm latex disc is obtained by trephination of a sterile latex glove. Then, the circlet is placed on the top of an AAC to reproduce the human iris diaphragm.
2. The cornea is properly positioned upon the latex and then secured firmly by screwing on the AAC holder.
3. Once the AAC is assembled, the space between the cornea and latex diaphragm must be filled with BSS through a peripheral paracentesis to pressurize the anterior chamber. Any air bubbles trapped in the anterior chamber are removed using a syringe.
4. Subsequently, two more inlets are created in the corneal periphery. The first one is made using the 15° knife, while the second by means of the 2.75 mm slit knife.
5. The smaller inlet is used to adjust the volume of liquid inside the chamber during the following steps, while the larger one will serve as the insertion site for the cartridge containing the preloaded membrane.
6. A helpful practice consists of using a skin marker to lightly stain the instrument blades before cutting. This will make the inlets easily visible and simplify tissue handling during the surgery.[67]

The details on DMEK simulation can be found in supplementary video 15.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

3.6. *Method 5: AE*

1. A paracentesis inlet is created in the AE cornea.
2. The synthetic endothelial membrane is incised starting from its periphery using a hook.
3. For Descemetorhexis, consequently, the artificial membrane is grabbed and gently pulled toward the corneal inlet ensuring no tearing. This step could be extremely useful to novice surgeons since they can understand and train the right movements to perform and apply correct pressure on the ocular surface to extract the endothelium in the best possible way avoiding membrane ruptures.
4. The extraction of the artificial membrane is carried out using the hook or any other tiny instrument, such as the 23-gauge coaxial forceps.

3.7. *Outcomes*

AE represents a good alternative to simulate the first phase of DMEK surgery, but, on the other hand, the grafting simulation cannot be properly reproduced. Indeed, due to the intrinsic plastic properties of the device, the anterior chamber pressure maintenance and the water removal from the inlets can be extremely tricky and difficult to control. Furthermore, the creation and removal of the air bubble for DM grafting are difficult as well. Two different types of iris diaphragm simulations are outlined in the literature for DMEK wet labs with AE[22,39].

The details can be observed in supplementary video 16.

Supplementary material related to this article can be found online at doi:10.1016/j.survophthal.2023.06.008.

3.8. *Outcomes of the nonhuman models used for surgical training*

Famery and coworkers[22] described a model using human corneas mounted on an AAC with a 3-D-printed iris, comparing the performance time and scores between beginners

and experienced anterior segment surgeons, performing 10 successful procedures. The performance score correlated with the surgeon's experience, and except for the Descemetorhexis, all procedure steps of a DMEK surgery were performed with close resemblance to reality. The diameter chosen for the artificial iris was 11 mm, and the radius curvature ranged between 5.78 and 7.30 mm in order to give a vaulting that ranges between 2.5 and 4 mm, obtaining an anterior chamber depth of 3.1 and an anterior chamber width of 11 mm. Otherwise, as reported by Sales,[67] a 15 mm diameter of latex circlet can be placed beneath a cornea mounted in an AAC to mimic the human iris and prevent the graft from falling during the manipulation. This solution will favor the right holding of the air bubble necessary for DM engrafting as well.

To modulate the intrachamber pressure, the posterior segment of the AAC is insufflated with air using a syringe connected to the AAC back. To correctly insert the DM into the AAC and avoid its swift expulsion, some saline solution must be removed from the chamber through the paracentesis (by means of a syringe or by increasing the intrachamber pressure) before the injection of the graft.

Fogla and coworkers[24] recommended the application of anhydrous glycerine (99.5%) on the cornea for 10 min to improve clarity, especially in the case of stromal corneal edema. It has also been proposed to use a light color 17 mm disc obtained by latex glove to simulate the intraoperative behavior of the iris diaphragm during DMEK surgery in the human eye. Being stretchable, it could be moved up and down by injecting or withdrawing methylcellulose (e.g., K-Y jelly) or air through one of the ports of the AAC.[24,67] Although the AE comes with a good size, which simulates a real human eye, as it does not have a pupil or posterior segment, and they are usually expensive, they do not make a convenient model for DMEK teaching. However, as multiple models and systems are available for DMEKs, customizing the technique based on the need would be ideal.

4. Conclusions

In recent years, wet labs have emerged as an extremely useful and important learning tool where surgical training can be achieved with minimal risks. Wet labs are particularly helpful for training in a risk-free environment with no complications or failures. In addition, wet labs help the surgeon to develop microsurgical skills with higher reproducibility while executing critical steps of surgery. It has been assumed that, upon completion, a trainee is confident and competent in performing surgeries; however, only a few centers, which are well-equipped and well-sustained, have the capacity to provide such training, thus leaving only a decent majority of trainees with limited hands-on training. It is also important to understand if a surgeon has developed a skill set for complicated cases. Unfortunately, wet labs can only offer the development of basic skills, as they are not equipped for complicated cases. As wet labs provide a safe and standardized method for training without the risks, the trainees will be better equipped to handle surgeries with confidence further instilling and enhancing psychomotor skills, hand-eye coordination, and ambidexterity, which are important traits in an ophthalmic surgeon.[44]

One of the most difficult parts of setting up a wet lab is the finances associated with it, especially for simulators and surgical machines. Although grants and financial institutes can be involved, it is recommended to share the space and skills with the neighboring institute to avoid a repeatable financial burden. Various models can be used, such as humans, animals, or artificial entities for surgical practices. Virtual simulators may be a viable solution in the future. Prior to COVID-19, approximately 10% of U.S. ophthalmology residents struggled surgically,[9] which is assumed to be much higher in developing countries. Although 80% of trainees reported that COVID-19 had no impact on their surgical training, over 50% have reported increased stress levels.[35,45] Long-term impacts of surgical and psychological changes among the ophthalmology residents are yet to be seen; however, it is predicted that global scenarios be it pandemic or competition will continue to affect the trainees in the future.[58] Hence, a safe and globally accessible method that could not only help a trainee to understand the difference between the techniques but helps identify the best adaptable technique for the trainee would be ideal.

Currently, wet labs in general focus on one technique with specific instruments that may or may not be present in the trainees setting. Once completed, the trainee must recollect the steps and practice on their own. Traveling restrictions and multiple options for different wet labs can also result in decision-making. Hence, to avoid complications and help in decision-making, we gathered most of the techniques for preparing, marking, loading, and delivering DMEK grafts. Although this article is just a guide to provide information on the primary selection of the technique a trainee would like to learn, wet-lab hands-on practice will be needed to master surgical manoeuvers. We have focused on DMEK wet lab in a risk-free, inexpensive, online/offline, travel-free, future pandemic-proof, step-by-step guided instructional program with the intention to help trainees globally to learn and develop their skills from a wide range of available techniques.

Methods of literature search

Literature search was conducted on PUBMED and Google Scholar for the topic "DMEK surgery." The authors analyzed original studies, reviews, and case reports. Keywords used were Descemet membrane endothelial keratoplasty (DMEK), learning curve, dry lab, wet lab, and teaching corneal surgery.

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This study discusses standardizing DMEK technique using specific tools and simple techniques.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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