Donor Descemet Membrane Preparation for Descemet Membrane Endothelial Keratoplasty (DMEK) – Review of current techniques

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Introduction
Descemet membrane endothelium keratoplasty (DMEK) was first reported in 2006 by Melles. Since then, technique of replacing diseased corneal endothelium with Descemet scroll consisting of healthy endothelial cells is constantly evolving. Compared to DSAEK (Descemet stripping automated endothelial keratoplasty), DMEK allows faster visual recovery, and greater percentage of patients achieve 20/25 or better. DMEK also seems to have significantly reduced risk of rejection compared to DSAEK and PK (Penetrating keratoplasty). Based on these advantages, DMEK seems to be the appropriate choice of procedure for endothelial replacement surgery.

Donor preparation
Melles technique
Donor corneoscleral rim is removed from storage solution and mounted endothelial side up on a custom-made fixation device. With a hockey stick, DM is cut just anterior to the trabecular meshwork and scleral spur and the edge of the membrane is gently pushed centrally. Using the same movement, DM is loosened over 180 degrees. With tying forceps holding the outer edge, DM is slowly stripped from the posterior stroma until approximately two thirds of the stroma is denuded. The entire rim is then submerged in a vial with saline to flush DM in its original position. On the rim holder, a superficial trephination is made centrally. With forceps holding the inner edge, DM is completely stripped from the posterior stroma. After detachment, DM rolls up with the endothelium on the outside.

SCUBA technique
The Submerged Cornea Using Backgrouns Away (SCUBA) technique was developed by Art Giebel. In this technique the donor corneoscleral rim is first placed on a sterile flat surface endothelial side facing up. While stabilizing the rim with toothed forceps, the corneal periphery is gently scored just inside the trabecular meshwork using blunt instrument such as nontoothed forceps, or a Y-hook. The scored edge of DM is grasped with nontoothed forceps and slowly stripped away.

Peripheral donor corneo–scleral rim is then stained with trypan blue to highlight the scoring mark. Following this the tissue is placed in a corneal viewing chamber containing corneal storage solution (Optisol; Bausch & Lomb, Rochester, NY). The viewing chamber suspends the cornea below the fluid surface and away from the bottom of the container, significantly improving DM visualization when the microscope is focused on the donor cornea. The scoring mark was examined carefully for any jagged edges and irregular tags are torn off with blunt forceps to help make a smooth perimeter that would resist tearing as the membrane is being stripped.

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from the stroma. DM stripping is performed in a 3-step process: (1) DM is stripped approximately 20% to 40% of the way across and then laid back down on the stroma; (2) the donor cornea is placed on a punch block where the endothelial side was tapped lightly with a trephine (8.0-9.0mm), cutting through DM, but only partially through the stroma; and (3) the donor cornea is placed back in the viewing chamber and DM stripping completed across the trephinated center. When using the 3-step technique, a mark is placed on the peripheral corneal rim to identify the starting point of DM stripping. A partial stripping prior to the central trephine cut, makes it much easier to lift the edge away from an intact stromal bed.

After complete stripping, the donor DM typically curls up into a scroll with the endothelial side outward. It is stained with trypan blue to improve subsequent visualization while inserting and positioning it in the recipient eye.

In both Melles technique and SCUBA technique, modifications in peeling DM have been described. Two forceps assisted peeling, helps distributes pressure forces and reduces incidence of DM tears. Specialized forceps with larger surface area at the tip, allow for a larger grip on DM and thereby help prevent iatrogenic tears during peeling.

Our Modified technique (Fig 1, A-Y) , Fogla

Donor corneoscleral rim is placed on endothelial punch, endothelial side facing upwards. A 9.5 / 10.0mm trephine (Barron Punch, Katena, USA) is taken and the cutting edge marked with gentian violet (A). Partial thickness trephination is performed with light pressure (B). BSS is then placed to cover the endothelial surface. Gentian violet highlights the cut edge (C). Fogla DMEK cleavage hook (Janach J2891E, Italy) is used to identify the inner edge of DM at site of trephination (D), following which inner edge of DM is separated 3600. Fogla DMEK stripping-peeling forceps (Janach J2892E, Italy) are used to grasp one edge of DM (E) and tooth forceps to stabilise the corneo-scleral rim. Using gentle pressure, DM peeling is performed for slightly over 1800 (F). Donor rim is tilted towards the side of peeling and excess fluid is absorbed using merocel sponge (G). 3mm trephine (Aurolab, India) is used to punch the central bare stroma (H,I). BSS is used to move the peeled donor DM back into its original position (J,K,L), following which donor rim is tilted towards the initial site of peeling, and excess fluid once again absorbed (M). Donor corneo-scleral rim is gently flipped with the epithelial side facing upwards. Stromal disc from 3mm trephination is removed and excess fluid absorbed using merocel sponge (N). Donor DM is marked on the stromal side “S” / “L” using marker stained with Gentian violet ink (O). Stromal disc is replaced into position. Donor corneoscleral rim is flipped back into position with endothelial side facing upwards (P). 8mm endothelial punch (Barron Punch, Katena, USA) is used to make a partial central trephination (Q). BSS is again placed on the endothelial surface. Peripheral rim of donor DM between 9.5 & 8mm trephination is removed first (R), followed completion of peeling of central donor DM using fine tying forceps (S,T,U). The donor DM scroll is then stained with trypan blue (V,X). Donor DM scroll is ready for DMEK surgery (Y).

Pneumatic dissection

Donor corneoscleral rim is placed on sterile gauze with the endothelial side facing up. A 27 / 30 G needle connected to a 10-cc, air-filled syringe is inserted bevel up into the peripheral cornea approximately 1 mm from the limbus and advanced in a tangential direction immediately beneath the endothelium for approximately 2 mm. Air is subsequently injected to achieve detachment of Descemet’s membrane (formation of big bubble) and the bubble enlarged as far as possible into the corneal periphery. Calipers used to measure diameter of the bubble. The needle is introduced into the big bubble from the scleral part of the corneoscleral rim to collapse the bubble by aspirating the air. Finally, a Barron punch (7.5 – 8.5 mm in diameter) is used to punch donor DM from endothelial side.

In another study, pneumatic dissection techniques on histological analysis revealed that a thin layer of stroma remained on DM in all the samples. The average central stromal thickness was 12.4 μm (range 6.5 - 20.0μm). Authors concluded that pneumatic dissection prepared donors were structurally more like DSAEK donors than DMEK donor tissue.

Recently Ruzza et al described comparison of air versus fluid injection for separation of DM in eyebank tissues. DM and endothelium could be separated exclusively using air (80%) or liquid bubble (100%). However, liquid bubble seems to have certain advantages over air such as the generation of larger diameter and higher maintenance of endothelial cell density.

Hybrid technique of DMEK donor preparation has also been described. Pneumatic dissection is used to create a big bubble, followed by manual or automated stromal dissection to
allow a residual rim of stroma around the exposed DM. Although this technique makes it easier to handle the donor DM, the extent of endothelial damage is higher compared to other techniques. 15

Muraine Technique

Donor corneoscleral rim is centered on the Muraine Punch block (Moria, Antony, France) with the endothelium facing up. Balanced salt solution (BSS) is placed on the center of the graft. Suction applied and donor cornea is punched using the Muraine punch (Moria, Antony, France). This punch is 8 mm diameter, a preset depth of 200 mm, and 2 opposite 3-mm segments of the circumference that do not cut and leave intact, hinged areas of DM. The donor cornea is removed from the punch, flipped “inside out,” and placed endothelial side up on the artificial anterior chamber. The chamber is gently inflated with an air syringe and the valve closed to stabilize the chamber. A lower pressure is maintained in the artificial anterior chamber than would be used in DSAEK graft preparation to allow for easier dissection of the donor tissue from the stroma. Vision Blue is used to stain the area of the punch while cohesive viscoelastic is applied to the center of the graft for protection. Fine tying forcesps used to peel the peripheral DM outside the punch area. A delamination plane was initiated under the hinged graft area with the use of curved forcesps. DM was hydrodissected with a 27-gauge cannula (FCI, Paris, France) on a 2.5 mL syringe of BSS. Once the apex was reached, hydrodissection was extended to the left and right of the trephination edges to completely detach DM.

A recent study comparing the SCUBA technique and Muraine technique found increased incidence of graft tears with the Muraine technique. 17

Donor characteristics

Donor DM is harvested from corneo-scleral rims in donor storage medium, obtained from eyebanks. Tissue requirements for endothelial cell density are similar compared to DSAEK, except that for DMEK it is preferable to have tissues from donors above the age of 40 years. DM from younger donors are most likely to develop tight spontaneous scrolls and predispose to difficult unfolding and subsequent detachments postoperatively. Its just the reverse with older donors, (>80 years), DM fails to form a spontaneous scroll and it may become difficult to identify the right side unless marking is performed on the stromal side.

Donor DM preparation has a definite learning curve, and donor loss rate reduces significantly with greater experience. In the hands of an experienced surgeon, the donor loss rate may vary from 1-3%. 19,21

An analysis at Price Vision Group recently showed that there is no difference in endothelial cell counts for donors prepared on the same day, 1 day before or 2 days before surgery. 22 Ham et al stored all of their DM scrolls in organ culture solution for 1–2 weeks before insertion with good cell counts. 2 This makes it possible for eyebanks to provide donor corneo-scleral rims wherein the DM has been pre-stripped almost 90% or even provide completely stripped DM scrolls for DMEK surgery.

In conclusion, DMEK donor tissue preparation has evolved since its original description. Current techniques allow for a safe and reliable harvesting of donor DM. Minimal endothelial cell loss and reduced risk of damage during donor preparation play an important role in successful DMEK surgery.

REFERENCES