Video Article

Corneal Donor Tissue Preparation for Descemet's Membrane Endothelial Keratoplasty

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Abstract

Descemet's Membrane Endothelial Keratoplasty (DMEK) is a form of corneal transplantation in which only a single cell layer, the corneal endothelium, along with its basement membrane (Descemet's membrane) is introduced onto the recipient's posterior stroma[3]. Unlike Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK), where additional donor stroma is introduced, no unnatural stroma-to-stroma interface is created. As a result, the natural anatomy of the cornea is preserved as much as possible allowing for improved recovery time and visual acuity[4]. Endothelial Keratoplasty (EK) is the procedure of choice for treatment of endothelial dysfunction. The advantages of EK include rapid recovery of vision, preservation of ocular integrity and minimal refractive change due to use of a small, peripheral incision[1]. DSAEK utilizes donor tissue prepared with partial thickness stroma and endothelium. The rapid success and utilization of this procedure can be attributed to availability of eye-bank prepared precut tissue. The benefits of eye-bank preparation of donor tissue include elimination of need for specialized equipment in the operating room and availability of back up donor tissue in case of tissue perforation during preparation. In addition, high volume preparation of donor tissue by eye-bank technicians may provide improved quality of donor tissue. DSAEK may have limited best corrected visual acuity due to creation of a stromal interface between the donor and recipient cornea. Elimination of this interface with transplantation of only donor Descemet's membrane and endothelium in DMEK may improve visual outcomes and reduce complications after EK[5]. Similar to DSAEK, long term success and acceptance of DMEK is dependent on ease of availability of precut, eye-bank prepared donor tissue. Here we present a stepwise approach to donor tissue preparation which may reduce some barriers eye-banks face in providing DMEK grafts.

Video Link

The video component of this article can be found at https://www.jove.com/video/51919/

Introduction

Endothelial Keratoplasty (EK) has rapidly replaced penetrating keratoplasty (PKP) as the procedure of choice for treatment of endothelial diseases accounting for over 50% of all transplants performed in the United States in 2012[6]. PKP is limited by prolonged visual rehabilitation with high astigmatism and refractive instability, loss of globe integrity and suture-related complications. Early generation EK procedures, including Deep Lamellar Endothelial Keratoplasty (DLEK) involved manual dissection of corneal stroma with transplantation of partial thickness donor tissue. These procedures were successful in transplanting donor endothelial tissue while maintaining an intact corneal surface but were technically challenging and resulted in limited best corrected visual acuity due to an irregular corneal lamellar interface[1]. The current EK procedure of choice is Descemet's Stripping Endothelial Keratoplasty (DSEK) which offers corneal clarity with faster recovery of vision, minimizes postoperative refractive error and astigmatism, and decreases risk of traumatic wound dehiscence while maintaining the corneal surface[7]. This procedure involves removal of the diseased Descemet's membrane and endothelium and replacement with partial thickness donor corneal tissue comprising a new endothelial cell layer. The donor cornea is dissected with a microkeratome to prepare the posterior tissue being transplanted[8]. Preparation of the donor tissue by the eye-banks has been instrumental in the rapid growth and, arguably, the success of this procedure. The near 16-fold increase in DSEK procedures in the United States between 2005 and 2012 corresponds with almost half of the tissue currently being prepared by the eye-banks[6].

Although DSEK can improve visual acuity to 20/40 or better, most patients do not achieve 20/20 vision due to stromal interface haze and increased light scatter from the addition of donor stromal tissue[7]. The EK surgical technique has progressed to Descemet's Membrane Endothelial Keratoplasty (DMEK) which involves transplantation of only donor Descemet's membrane (DM) and endothelium. This procedure has a higher proportion of patients who achieve 20/20 vision, faster visual recovery, and a lower risk of endothelial rejection[9]. However, preparation of donor tissue is more challenging due to the thinner graft dimensions.

Protocol

1. Setup of Sterile Field

1. Don proper attire including cap and mask. Wear eye protection when in the processing room.
2. Prepare the processing area. When using a processing room, maintain continuous operation of the fan to create positive pressure in the room.
3. Gather all supplies. Check expiration dates, package integrity, and sterilization indicator.
4. Wash hands and don non-sterile gloves.
5. Open the sterile kits to establish a sterile field. When using a processing room, place the kits on the table and aseptically open the wraps. Do not utilize the outer 1½ inches of the wrap as part of the sterile field as this segment is considered unsterile.
6. Using aseptic technique, open and drop sterile items onto sterile field unless already part of the premade pack.
7. Move microscope so that it is above the sterile field, turn on light source of microscope, and adjust oculars as necessary.
8. Obtain corneal tissue to be stripped (in preservation media) and place next to the prepared sterile processing area. Loosen cap of viewing chamber and let it rest on top of viewing chamber base with no threads engaged. Discard non-sterile gloves.
9. Open one pair of sterile gloves and sterile gown with towel.
10. Perform surgical scrub. Put on sterile gown, then sterile gloves.
11. Open instrument boxes so that all supplies are accessible. Open moisture impermeable drape to create the work area.
12. Attach needle to a 5 cc syringe. Using a hemostat, prepare the 30 G needle by placing a bend 1 mm from the bevel, and another bend 1 mm from the base (to form an “S” shape).
13. Assemble the glass syringe for the trypan blue stain by placing the plunger into the syringe and finger tightening the plunger. Attach cannula.
14. Attach the cannula to the balanced salt solution (BSS) bottle.
15. Separate the trephine punch from the vacuum block and set aside. Center the vacuum block within the field of view of the microscope.
16. Bring the vacuum block into focus by adjusting the microscope settings.

2. Procedure for Donor Tissue Preparation

1. Lift lid of viewing chamber using foil square as a barrier. With opposite hand and using forceps, gently grasp cornea by sclera rim to remove cornea. Replace lid of viewing chamber.
2. Completely depress the suction syringe, then place cornea epithelial side down on the vacuum block and ensure that it is centered. (**Figure 1**)
3. Gently lower seating ring on to the vacuum block using the guide posts. Engage vacuum by slowly releasing piston of the suction syringe. Remove the seating ring and set aside. (**Figure 2**).
4. Perform a partial trephination to score through the Descemet’s membrane.
5. Adjust microscope zoom and focus so that the tissue is well visualized.
6. Tilt the vacuum block and use a sterile swab spear placed at the limbus to remove excess medium. (**Figure 3**)
7. Place enough stain at the limbus to stain the edge of the scored Descemet’s membrane. Allow the stain to remain on the endothelium for 60-90 sec. (**Figure 4**)
8. Remove stain:
    1. Tilt the vacuum block to pour the stain off of the cornea and onto gauze pad.
    2. Use the surgical spears to remove excess stain from the limbal area of the cornea, taking care not to touch the endothelium. (**Figure 5**)
9. Use several drops of BSS to rinse the cornea of staining solution, and sterile swab spears to remove excess BSS. (**Figure 6**)
10. Place 1-2 drops of BSS on the sclera rim to prevent tissue from drying. Repeat as necessary throughout the procedure.
11. Perform partial dissection of Descemet’s membrane:
    1. Beginning where the tubing connects to the vacuum block, using open forceps, place one tip at the edge of the score mark and gently separate Descemet’s membrane and endothelium away from the stroma. (**Figure 7**).
    2. Separate Descemet’s membrane and endothelium not more than 1 mm from the scored edge and continue dissection 360° around. (**Figure 8**).
    3. Note location of any micro tears by placing a dot on the vacuum block with the skin marker.
    NOTE: If the trephine did not penetrate completely through Descemet’s membrane, use the beveled edge of the prepared 30 G needle to complete the score through Descemet’s membrane where necessary, followed by a second application of staining as needed. If Descemet’s membrane’s adhesiveness is impeding the partial dissection, remove the endothelium outside the trephinated area as needed.
12. Use forceps to remove any endothelial tags that overlap the score mark.
13. Consider the location of previously marked micro tears and rotate the vacuum block so that the largest is at 6 o’clock. This will become the hinge of the flap.
14. Using the forceps, grasp the Descemet’s membrane at 12 o’clock. Gently separate the Descemet’s membrane from the stroma by peeling towards the hinge. Create the hinge by stopping the separation 2 mm from the score mark.
NOTE: Peripheral edges of flap will begin to scroll during separation. (**Figure 9**).
15. Gently lay the flap back in place on the stroma. Use BSS and swab spears to unscroll the endothelium and return it to its original position. (**Figure 10A-C**)
16. Remove residual fluid from between Descemet’s membrane and stroma with sterile swab spears so the flap stays in position. (**Figure 11**)
17. Use sterile swab spears to soak up any excess moisture from the sclera near the hinge. Use the skin marker to draw an arrowhead on the sclera rim pointing to the center of the hinge, remove any excess ink using a sterile swab spear. (**Figure 12**)

18. Release vacuum by depressing the piston of the syringe connected to the vacuum block. While vacuum is disengaged, use forceps to remove cornea from the vacuum block.
19. Lift lid of viewing chamber using foil square as a barrier. Place the cornea in the viewing chamber, endothelial side up, and replace lid of viewing chamber.
20. Repeat above procedure steps with mate cornea, if applicable. Use gauze to clean the vacuum block before processing the second tissue.
21. Tighten the viewing chamber lid(s) and remove tissue from the processing area.
22. Break down the sterile field by disposing of items according to Biohazardous Waste Management Plan. Remove contaminated gloves, and gown. Wash hands and don non-sterile gloves.
23. Perform post-cut slit lamp evaluation and specular microscopy of the cornea(s) and record the findings.

Representative Results

Proper separation of DM and endothelium from stroma is essential in preparing precut tissue for DMEK. Fifty donor corneas with a mean age of 65 ± 6 years (range 55-75), death to processing time of 5.9 ± 2.3 days (range 1-10) and endothelial cell density (ECD) of 2,616 ± 321 cells/mm^2 (range 2,049-3,247) were used to assess feasibility and reproducibility of the surgical preparation technique by two eye bank technicians. The success rate was 72% in the first 25 cases and increased to 80% in the second half with an overall success rate of 76%. Point biserial correlation (Rpb) was used to determine correlation with preoperative tissue factors. The donor age (Rpb = 0.18), death to processing time (Rpb = 0.07) and ECD (Rpb = -0.11) did not correlate with successful preparation of tissue. The post-preparation ECD (n = 37) of 2,676 ± 284 (range 2,041-3,205) was not significantly different from the pre-preparation ECD (p = 0.72). Unsuccessful outcomes resulted from DM tears (10%), and severe cell loss (14%). These cases were also associated with difficulty remounting tissue and incomplete staining of DM edge.

Eye-bank assessment of patient outcomes provides essential data for clinical success and adverse events associated with DMEK which may help improve the adaptation of the surgical technique in practice and utilization of eye-bank prepared tissue. Nine pre-cut DMEK tissue were provided to five surgeons for 9 patients. One case had a dislocation at 1 day and 1 week without need for rebubbling. Visual acuity at 1 day ranged from 20/400 to light perception and by 1 week improved to 20/103 (range 20/30-20/200).

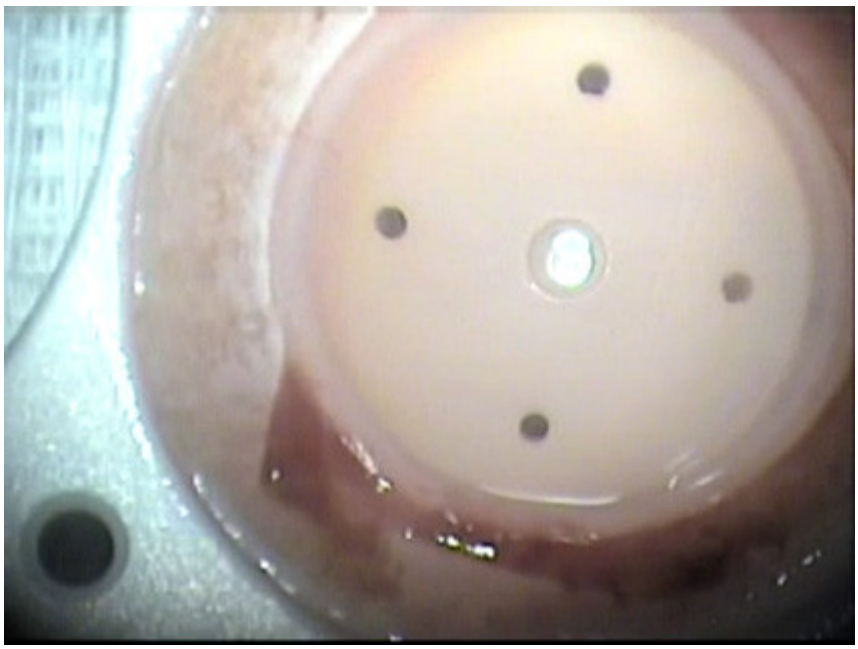

Figure 1. Tissue mounting. The corneal tissue is mounted and centered on the trephination block.

Figure 2. Tissue trephination. The vacuum ring is seated and the corneal tissue is partially trephined including Descemet's membrane.

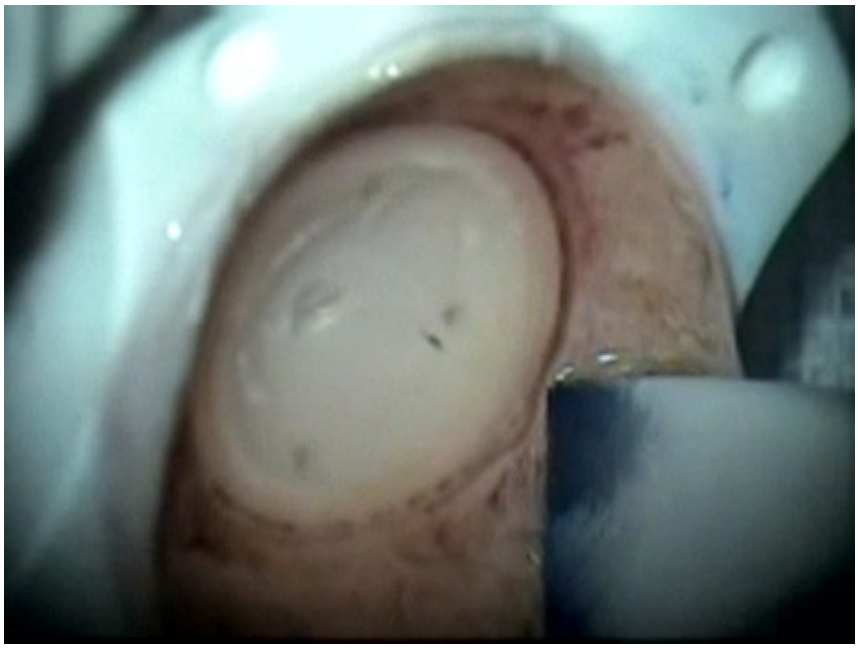

Figure 3. Storage media removal. Excess storage media is removed prior to stain application.

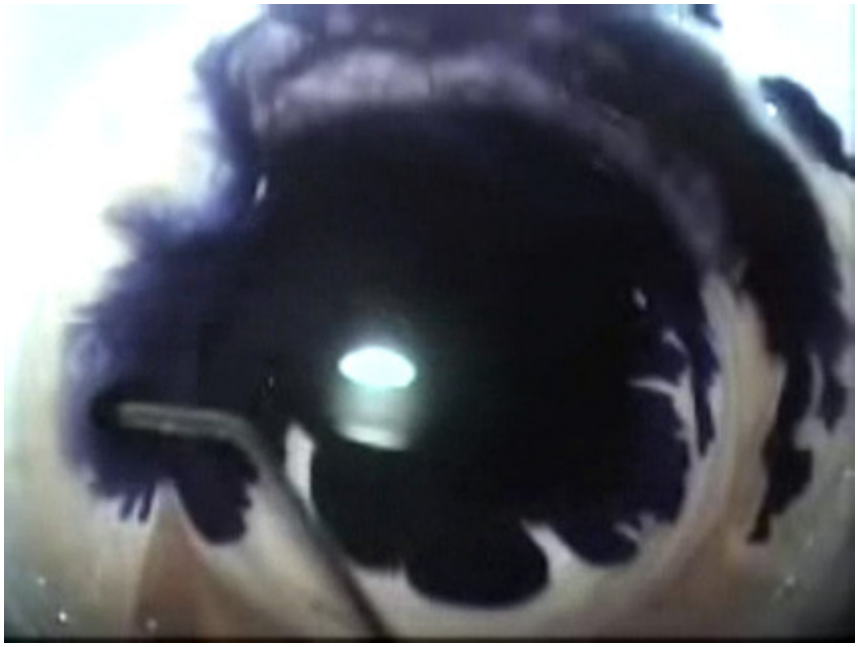

Figure 4. Trypan blue application. Apply trypan blue stain on Descemet's membrane surface with filling of the well for 60-90 sec.

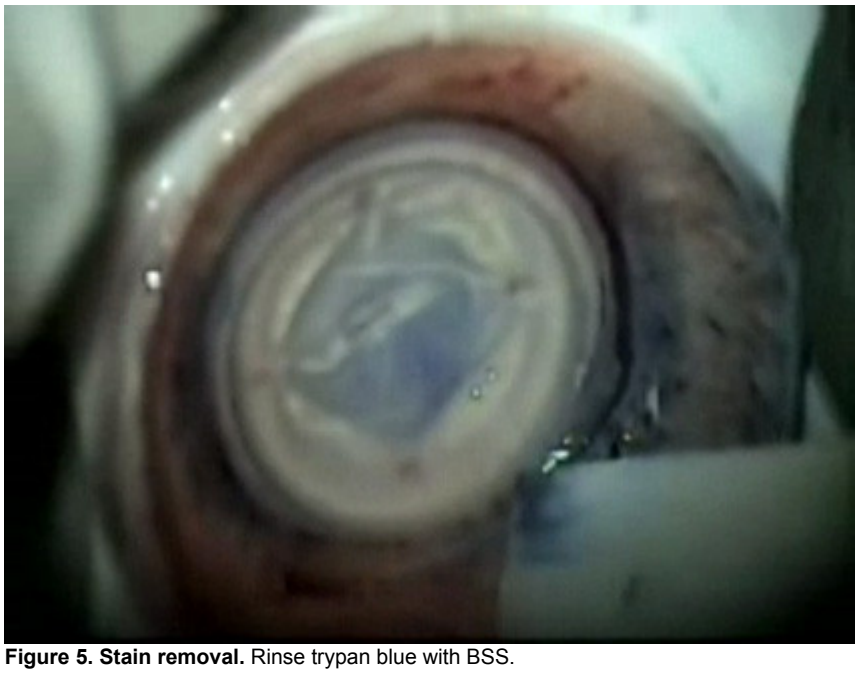

Figure 5. Stain removal. Rinse trypan blue with BSS.

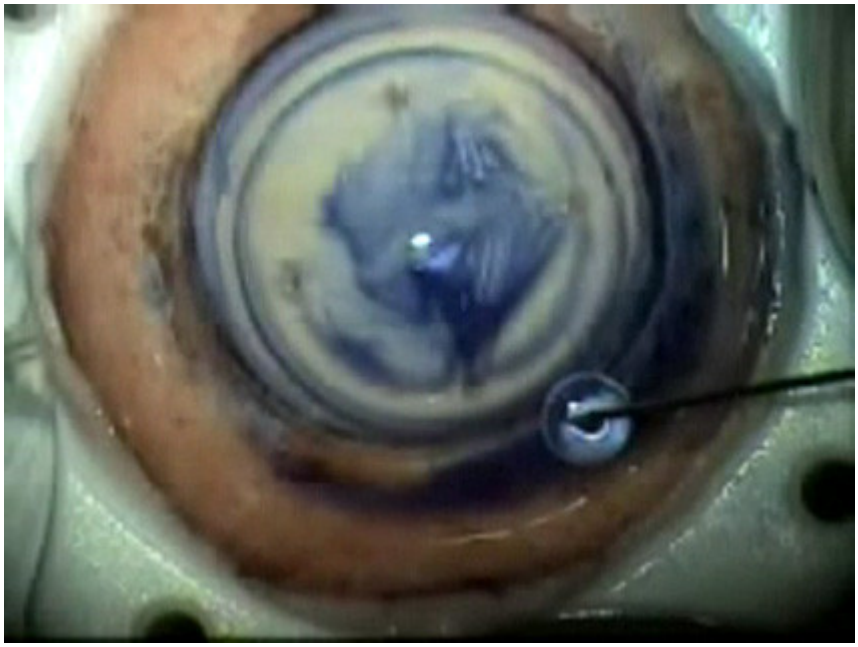

Figure 6. Stain removal. Complete removal of stain.

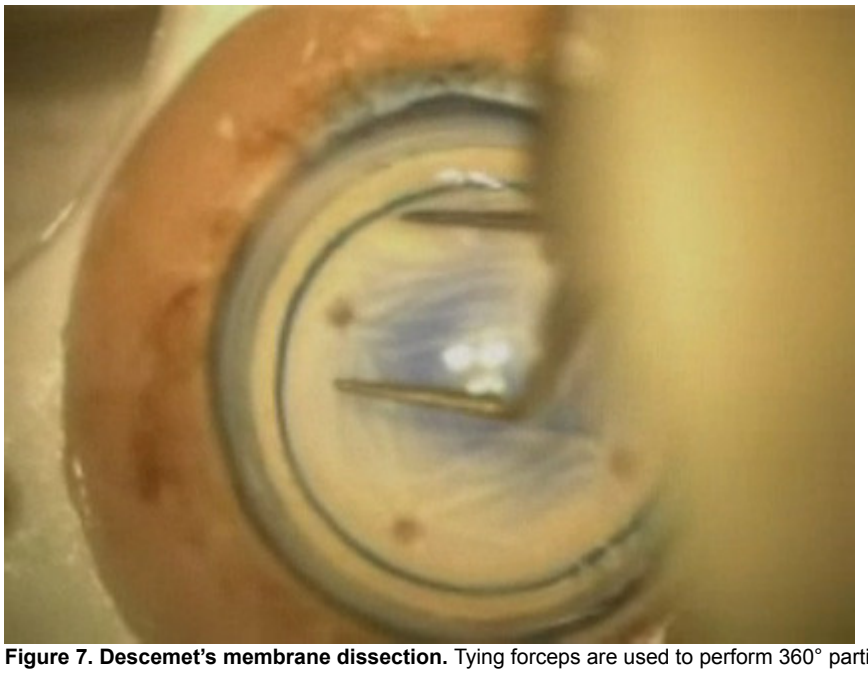

Figure 7. Descemet's membrane dissection. Tying forceps are used to perform 360° partial dissection.

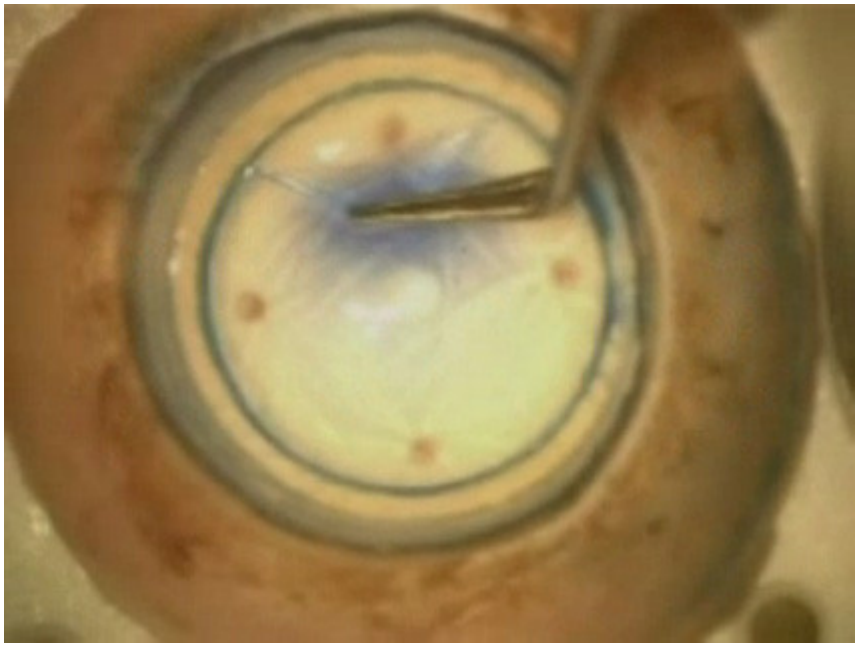

Figure 8. Descemet's membrane stripping. Begin to strip Descemet's membrane.

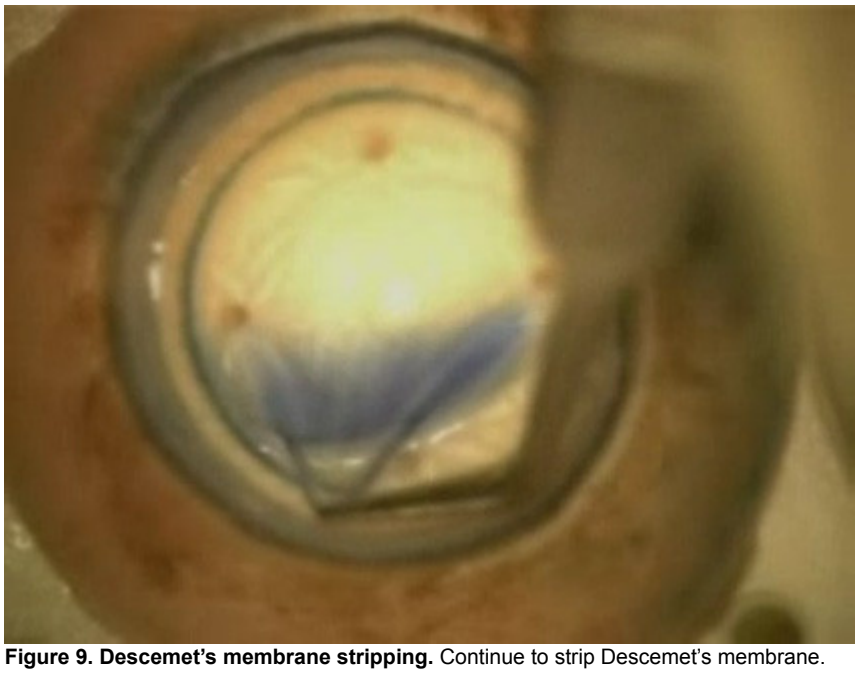

Figure 9. Descemet's membrane stripping. Continue to strip Descemet's membrane.

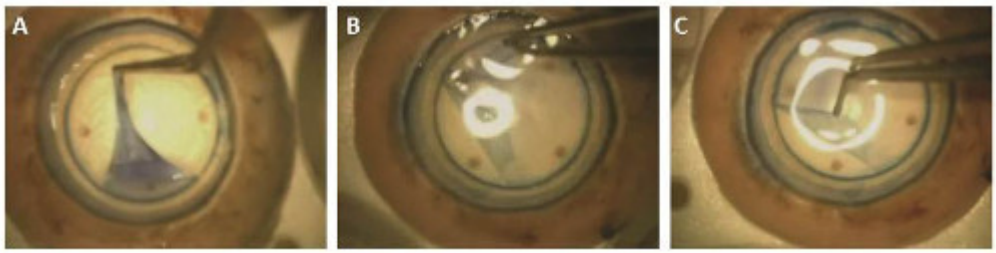

Figure 10. Reposition Descemet's membrane. (A) Return Descemet's membrane to the stromal bed, **(B)** BSS application to aid in Descemet's membrane unfurling, and **(C)** complete unfurling of Descemet's membrane to original position.

Figure 11. Balanced salt solution removal. Removal of extra BSS from beneath the flap.

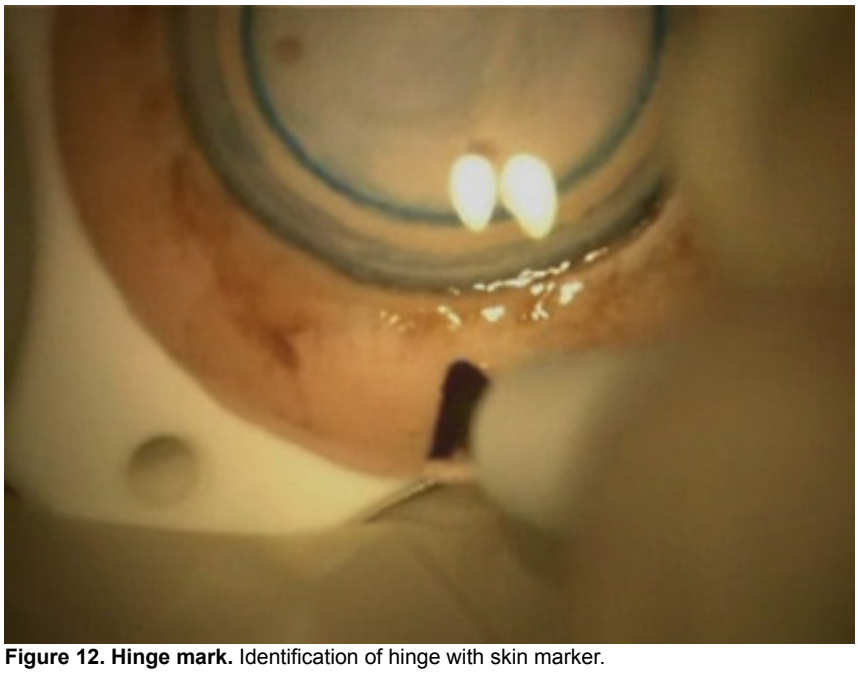

Figure 12. Hinge mark. Identification of hinge with skin marker.

Discussion

The corneal endothelial cell layer plays a critical role in maintaining corneal clarity through dehydration. Diseases of the endothelial cells can result in significant visual impairment. DMEK aims to replace the diseased endothelial cells with a donor graft consisting of Descemet's membrane and endothelial cell layer. Donor tissue preparation with an intact DM and adequate endothelial cell count are critical to the survival of the graft tissue. Tearing of DM during donor tissue preparation can result in tissue loss and wastage of donor tissue. Widespread application of DMEK has been limited by the challenges in preparing the graft tissue and mastering the complex surgical technique. With improvements in instrumentation and availability of eye-bank prepared precut tissue, this procedure has the potential to become the standard treatment for corneal endothelial diseases.

Current techniques for donor tissue preparation include use of an air-bubble to separate the DM from the stroma to facilitate the removal of graft tissue from donor cornea[10]. SCUBA (submerged cornea using backgrounds away) technique of DMEK graft preparation involves harvesting the donor tissue under Optisol or balanced salt solution (BSS), minimizing the surface tension and chances of tearing[11]. Yet another technique in

DMEK graft preparation is leaving a peripheral rim of stroma yielding a graft that is easier to the handle and insert[12]. The donor tissue may also be prepared with a custom trephine to leave hinges of intact DM while lifting the graft with BSS[13].

The overall success rate with our technique for DMEK graft preparation was limited compared to previously published techniques to 76% which may reflect that these were the first 50 tissues being prepared by two eye bank technicians[14,15]. However, the outcomes did improve in the second half of tissue prepared. In addition, our technique does not require specialized instruments, making DMEK easily accessible as well as more cost effective. The visual recovery can be faster and better with DMEK due to lack of the stromal interface[5]. Studies have also demonstrated fewer higher order aberrations on the posterior surface of the cornea than DSEK or PKP[16]. Incidence of graft rejection is significantly lower with DMEK than with other keratoplasty techniques[17]. The endothelial cell density following DMEK is comparable to Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK)[18]. DMEK offers a minimally invasive surgery maintaining the structural integrity of the eye and reducing the risk of postoperative astigmatism, wound leaks and postoperative glaucoma linked to prolonged topical steroid use.

In summary, DMEK allows replacement of diseased endothelium without removing recipient corneal stroma or additional tissue. The surgical technique is challenging in part due to the reproducibility of donor tissue preparation. Eye bank preparation for DMEK may allow for improved adaptation of DMEK similar to previous endothelial keratoplasty techniques.

Disclosures

Hassan Tausif and Dr. Navasuja Chandrasekaran have nothing to disclose. Drs. Mian, Shtein, and Woodward are medical directors for the Michigan Eye Bank. Mr. Mavin, Mr. Titus, and Ms. Johnson are employees of Midwest Eye Banks.

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