Descemet Membrane Endothelial Keratoplasty Donor Preparation: Navigating Challenges and Improving Efficiency

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Purpose: The aim of this study was to describe the challenges in Descemet membrane endothelial keratoplasty (DMEK) donor preparations and provide new strategies to achieve success.

Methods: A series of 263 consecutive DMEK preparation attempts by a novice surgeon during a corneal fellowship are described. In all cases, the Descemet membrane (DM) and the endothelium were peeled off from the donor cornea while it was submerged in corneal storage medium.

Results: The success rate of preparing DMEK tissue was 99%. Three donor preparations of 263 (1.1%) could not be completed successfully because spots of strong adherence between the DM and the stroma caused multiple horseshoe-shaped tears (HST) to form in the DM. Lamellar splitting of the DM ("partial thickness HST") preceded the formation of most HSTs. At least 1 HST occurred in 13% of donor preparations. In donor pairs (right and left corneas of 1 individual donor), if 1 cornea had any HSTs, there was a 60% chance that the contralateral cornea would have at least 1 HST. If 1 cornea had multiple HSTs, there was an 80% chance that the contralateral cornea would have at least 1 HST. Noting this trend, 3 donor corneas were returned to the eye bank unopened for other uses after their mates had multiple HSTs.

Conclusions: With appropriate techniques, DMEK donor preparation can be highly successful, even for a novice surgeon. When a donor develops multiple HSTs, we recommend not using the mate for DMEK because of a higher risk of encountering a preparation difficulty.

Key Words: DMEK, SCUBA, donor preparation, horseshoe tear, line rule

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Descemet membrane endothelial keratoplasty (DMEK), in which the donor tissue consists of the bare endothelium and Descemet membrane (DM), has several advantages compared with Descemet stripping automated endothelial keratoplasty, in which the donor also includes posterior donor stromal tissue. DMEK results in a better mean corrected distance visual acuity,[1–4] and it is associated with lower posterior surface higher order aberrations[5] and faster visual rehabilitation.[1,2,6,7] Further, the risk of occurrence of immunologic graft rejection episodes is significantly lower with a DMEK than with a Descemet stripping automated endothelial keratoplasty or with a penetrating keratoplasty.[8–10]

Adoption of DMEK has been slow in part because of early difficulties encountered with donor preparations and concern about the potential loss of donor tissue. When the DMEK technique was initially being developed, reported donor preparation failure rates ranged from 5% to 18% for various preparation methods.[11–14] Now with more experience, many centers report lower failure rates.[1,3,15] The purpose of this study is to document our strategies to optimize donor preparation success.

MATERIALS AND METHODS

A consecutive series of 263 DMEK preparations were attempted by a novice surgeon (L.R.T.) during a cornea fellowship (July 2011–July 2012) at Price Vision Group, Indianapolis, IN. Training included a 2-day DMEK course with wet laboratory and included observing an experienced surgeon prepare 8 DMEK tissues, practicing 8 preparations on research tissue in a wet laboratory, and preparing 5 DMEK tissues with a preceptor before preparing it solo. Donor tissue was prepared in an operating room by using standard sterile techniques for corneal transplants.

The donor preparation technique was modified from the Giebel 2008 SCUBA (Submerged Cornea Using Backgrounds Away) technique.[11,16] The Giebel technique enabled the donor DM to be peeled while it was submerged with good visualization and minimal glare.

Our modifications included scoring with a Y-hook, improving the view of the score edge, techniques to minimize tension during peeling, handling of excessive separation resistance, and handling of horseshoe-shaped tears (HSTs). A surgical video is available online.[17]

Scoring the DM

The donor was fixated with a toothed forceps while a peripheral break or "score" in the DM was created. A blunt Y-hook (9217E, Ambler Surgical, Exton, PA) was used instead of tying forceps to create the initial score in the DM

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because it produced a smoother break with fewer tears. The score line was positioned 0.5 to 1 mm central to the trabecular meshwork because the most peripheral DM is generally the most adherent and most likely to tear. The Y-hook was pressed firmly against the submerged corneoscleral rim to penetrate the DM and was lightly dragged along the periphery to propagate the score. (Caution was exercised not to press too hard or impale the delicate posterior stromal fibers. A blade was not used to create this score to avoid cutting into the stroma, which would make it difficult to grasp or lift the DM without also grabbing the edge of the connected stroma.) Before rotating to the next area, the rim of the outer DM was pushed outward (making it easy to peel away later). If counterpressure was needed to penetrate the DM, the centrally protruding viewing chamber pillar (present on a Krolman viewing chamber, K55-57007-23, Bausch & Lomb, Rochester, NY) was used with the tissue submerged, or the donor was placed on a cutting block (Fig. 1).[17]

Improving the View of the Score Edge

The off label use of VisionBlue (DORC International, the Netherlands) improved the view of DM breaks and DM scarring (cataract wounds). The donors were stained after the excess storage solution was dripped away so that it would not block the stain. The stain was decanted off the donor, and the tissue was resubmerged in the viewing chamber. Incomplete blue lines were investigated to verify if they represented incomplete scoring or incomplete staining. Complete blue lines were checked to verify that they represented a true score. "Snail tracks" in the endothelium from the donor harvest also stain blue as do insufficient scoring strokes that only denuded endothelial cells.[17] Such unscored zones can cause radial tears during the next step of lifting the edge of the DM. Removing the peripheral rim of the DM (Fig. 1) served 2 purposes: it improved the view of the DM edge to see if radial tears were present, and it allowed unsuccessful score zones to be discovered and rescored before radial tears developed.[17] As with a stray capsulorhexis, radial tears were "rescued" to prevent central extension during subsequent steps (Fig. 1).[16,17]

Lifting the Edge of the DM

Ing suggested lifting the edge of the DM for 360 degrees with a small blunt instrument, such as a microfinger, before grasping and peeling the edge to reduce the risk of radial tear formation (personal communication Jeffrey Ing, 2010). We used a blunt microfinger (Mastell Precision Instruments, Rapid City, SD or Moria, Antony, France) to lift the edge with a "glide technique." The tip of the microfinger was penetrated 0.5 mm under the edge of the DM, held against the stroma, and glided circumferentially while maintaining the depth of the

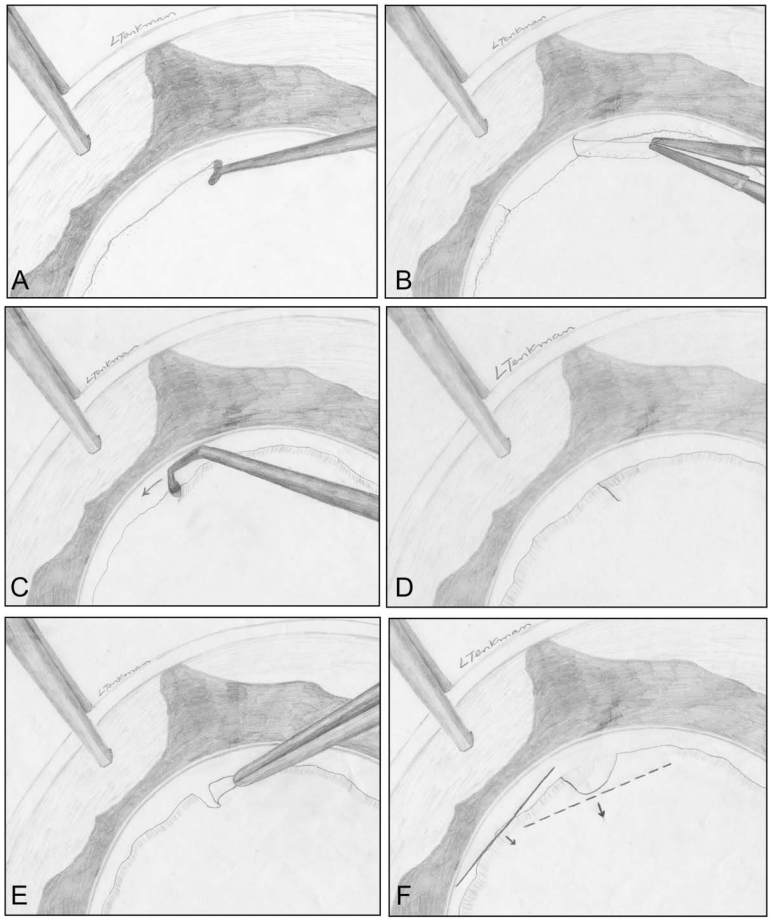

FIGURE 1. Scoring the DM, improving the view of the DM edge, and lifting the DM edge. A, Scoring with the Y-hook. B, Removing the peripheral rim of the DM. C, Lifting the DM edge with a microfinger with "the glide technique." D, Radial tear. E, "Rescuing" a radial tear. F, After rescuing a radial tear, do not lift or peel the DM starting at the "crater" because this violates the "line rule" (dotted line). Start at a peripheral location that does not violate the line rule (solid line).

penetration to lift a consistent width of the DM. The microfinger was always advanced away from fixation (not toward) to avoid bunching up or tearing the tissue. If the DM attachment was firm such that penetration was difficult, the centrally protruding viewing chamber pillar was used for counterpressure. If the DM was too adherent for gliding, the edge was lifted with centrally moving motions of the microfinger (Fig. 1).[17]

The following concept was used to guide edge lifting: "Progress downhill from peaks to craters." "Peaks" were focal areas where the score line diverged peripherally; peaks had minimal adjacent DM holding them down (minimal tension). "Craters" were the opposite (maximal tension). The lift was started peripherally at the peaks and "glided downhill" when advancing circumferentially. Rather than "gliding uphill," the surgeon skipped ahead to place the microfinger at the peak and glided backward "downhill." This concept was also applied after removing a radial tear, which creates a "crater"; the microfinger was glided back and forth to lift the peripheral DM down to the depth of the crater. The crater was lifted last.

Techniques to Minimize Tension During Peeling

Partially peeling the peripheral DM a full 360 degrees before trephination allowed any zones of especially strong adherence (that would be prone to develop tears) to be discovered before trephination. Such imperfections were excluded from the final donor. The limbus was fixated with a 0.5-mm forceps. The DM edge was grabbed with a Tubingen forceps (2457E, Ambler Surgical), exactly central to fixation. Tubingen forceps are wide and distribute tension more than do smaller standard tying forceps. To avoid tears, the Tubingen forceps were not twisted or "toed in" and "stayed low" (not elevated away from the cornea). Each pull was straight across. Prominent stress lines appeared if the pull deviated to the side. As discussed below, stress lines often preceded HSTs.

The DM was peeled by quadrants ("Corridor Method"). This technique minimized tension by minimizing the width of the peel zone (Fig. 2).[17] The more centrally a peel is taken, the wider it gets, with increasing tension on the DM. The peel edge of the first quadrant was taken about halfway to the center of the cornea. Peel 2 was started 180 degrees away and also taken halfway to the center. Peel 3 was started 90 degrees to the left or the right of peel 2. A narrow corridor of unpeeled DM remained at this point. Because the peel zone now could get no wider than this corridor, the tension was low, making it safe to peel quadrant 3 close to the center of the cornea. Peel 4 (180 degrees away) was taken halfway to the center. Efforts were made to grab a "peak," not a "crater," as the starting point of each pull. The meridian of peel 4 was

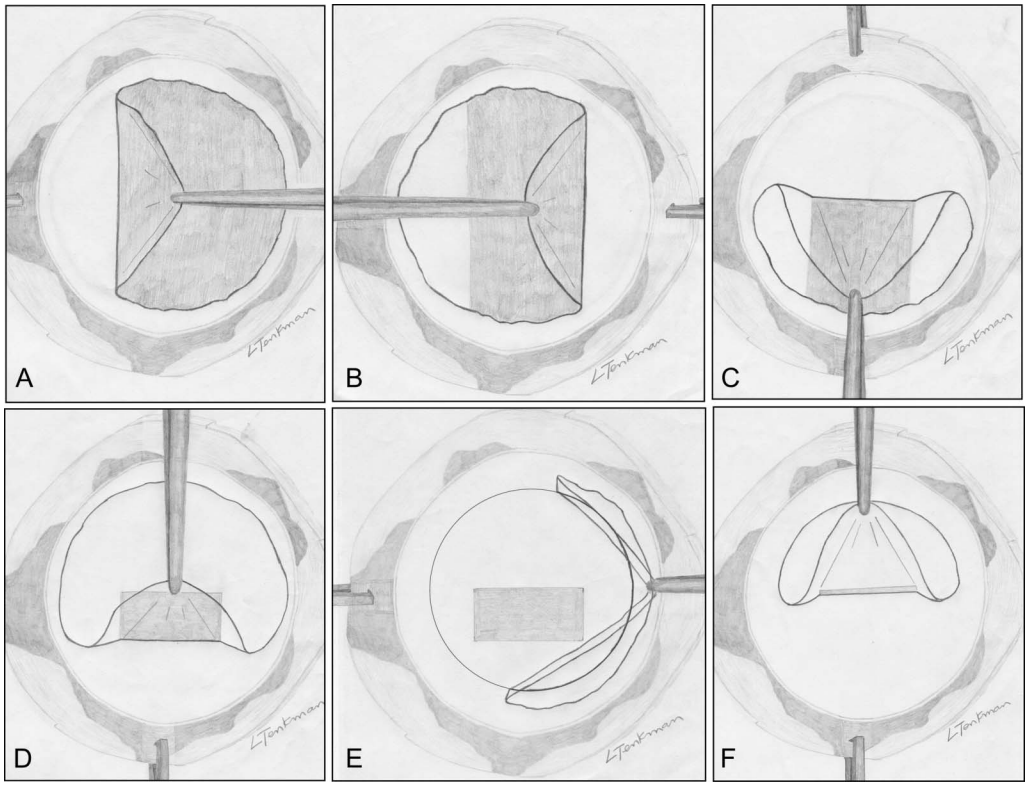

FIGURE 2. Techniques to minimize tension during peeling ("Corridor Method"). The more centrally a peel is taken, the wider it gets, and the tension on the DM increases. This technique minimizes tension by quickly creating a "narrow corridor" of the unpeeled DM to limit the peel width. Shaded areas represent the DM that has not been separated yet. A, B, Two opposing sides are peeled halfway to the middle. C, The next peel is taken all the way to the middle. D, This peel is also taken halfway to the middle. E, The donor is trephined, and the newly created peripheral rim of the DM is removed. F, The final peel is started from the same meridian as D; this way, the donor separates before the endothelium held by the Tubingen forceps risks being dragged over the contralateral sclera.

noted as the intended start site for the final peel after trephination: because pull 3 was taken close to the middle, the donor would fully separate from the stroma before the forceps advanced over the opposing sclera (thus reducing the risk of endothelial touch).

Handling of Excessive Separation Resistance

To avoid the formation of HSTs, a special technique was necessary when separation resistance was encountered. Separation resistance, which was often greater on the first 2 quadrants, could be felt as tension or seen as "smile configuration" and stress lines. When the lead edge of the peel had minimal resistance, it formed a straight line. When the right and left edges of the peel lagged behind the central portion (forming a "smile"), the resistance was high (Fig. 3). Pulling further here created prominent stress lines, which typically extended from the forceps to the right and left sides of the peel line. Stress lines from separation resistance would not go away, even when pulling perfectly straight across. Because HSTs could result from further pulling, a technique was used to reduce separation resistance and smile configuration (Fig. 3). The edge was peeled 2 mm at 45 degrees to both the sides of this site, then the peel was continued from the original site. This technique lessened tension by limiting the width of each peel.

"The Line Rule" helped the surgeon anticipate and visualize DM separation resistance (Figs. 1, 3). The surgeon imagined a horizontal line drawn perpendicular to the Tubingen vector of pull at the peel edge. If any unpeeled DM lagged behind this line ("smile configuration"), it had an increased risk of developing HSTs. Lifting or peeling of the DM was not started at sites that violated the line rule. Peripheral areas were addressed first.

Sometimes the DM was so adherent and brittle that "peel by quadrant" was not possible. In those cases, the DM was instead peeled every 1- to 2-o'clock hours to minimize the tension in each meridian. HSTs sometimes still developed in difficult donors despite such cautious handling.

Handling of HSTs

Continuing to pull after an HST developed could tear the donor in half. Interestingly, "descemetoschisis," or "lamellar splitting" of the DM, seemed to accompany most HSTs as a tongue or triangular attachment of the anterior layers of the DM on the stroma.

Because HSTs are common, appropriate management techniques were essential. First, if an HST was suspected but hard to see, focal VisionBlue staining was used. To complete the preparation in the presence of an HST, 1 strategy was to stop pulling at that site, partially peel all other quadrants, trephine, and then start the final donor peel 180 degrees away from the HST.[17] If 2 HSTs developed 180 degrees away from each another, the tongue of 1 HST had to be lifted so that the peel could be continued through that HST (Fig. 4). The centrally protruding Krolman viewing chamber pillar was used for counterpressure to lift the tongue.[17]

Trephining the DM

Trephine size was selected as a compromise between host cornea size and pathology. We used an 8-mm diameter for patients with Fuchs dystrophy, who typically have a healthy peripheral endothelium and a 9-mm diameter for pseudophakic bullous keratopathy or failed endothelial keratoplasty patients,

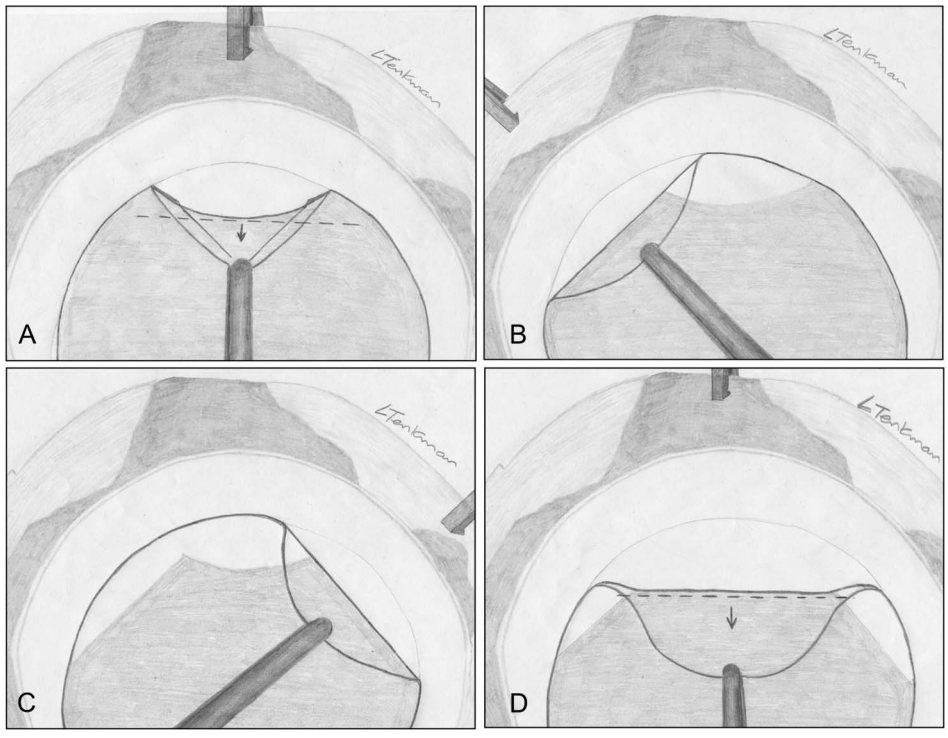

FIGURE 3. Handling of excessive separation resistance. If peeling from any quadrant demonstrates excessive separation resistance, it may be necessary to complete a given quadrant with several pulls designed to limit the width of the peel and the tension on it. A, Separation resistance can be visualized as a "smile configuration." This violates the "line rule" (the sides of the peel edge lag behind a line drawn tangential to the peel). B–D, Peel a couple of millimeters of the DM 45 degrees to both the left and to the right of the initial area before returning to it to peel further.

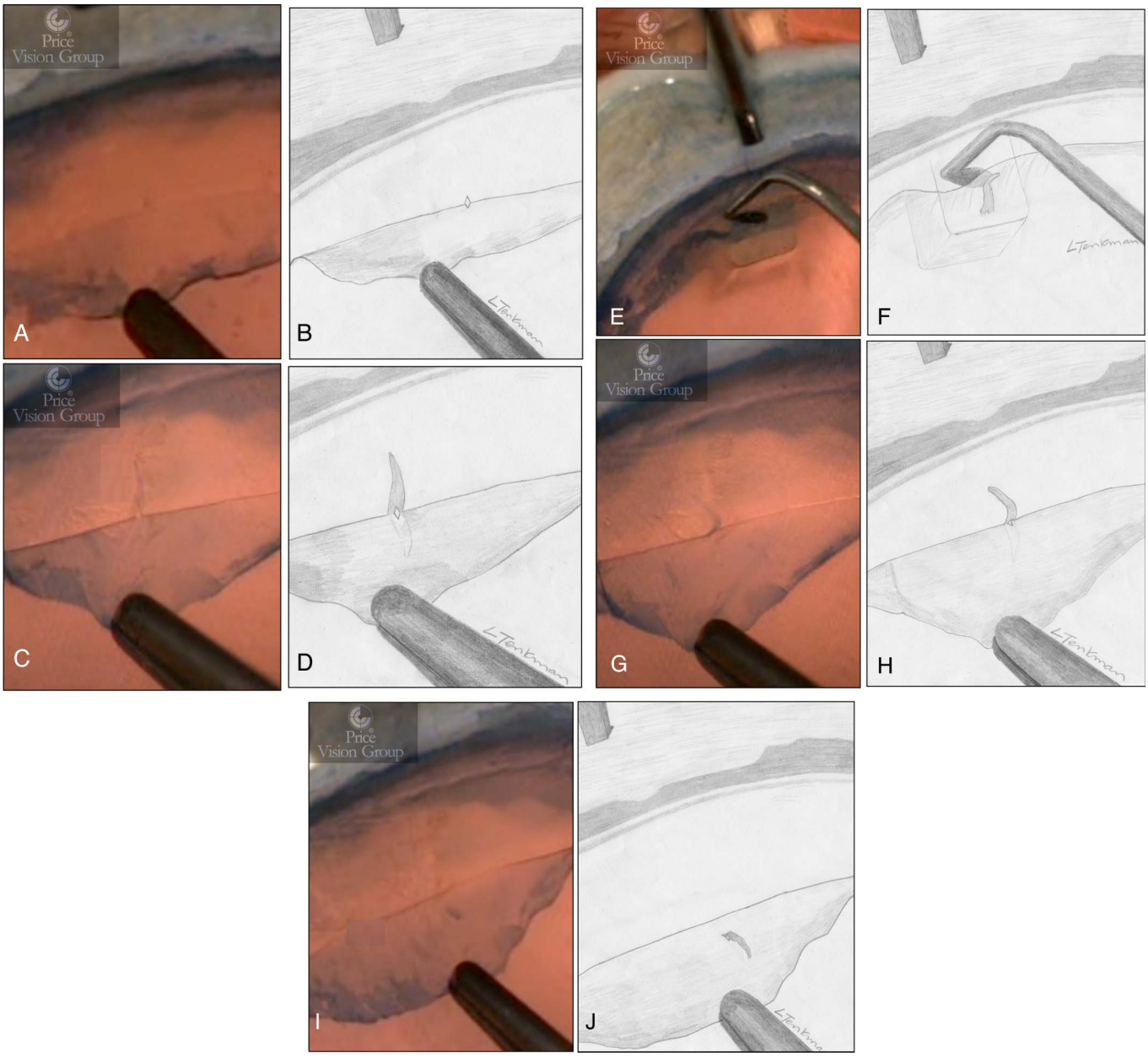

FIGURE 4. Handling of HSTs. High definition drawings are provided to illustrate what is going on in the lower definition photographs. A, B, A "full-thickness" HST is noted. C, D, VisionBlue staining reveals that a "partial thickness" HST began before the full-thickness HST appeared. This partial thickness tear is caused by "descemetoschisis" or the "lamellar splitting" of the DM. Descmetoschisis in 1 cornea predicts its likelihood of occurrence in the donor's contralateral cornea. Descmetoschisis can be noted during peeling before conversion to a full-thickness HST. With continued pulling, the descmetoschisis either breaks off or converts to a full-thickness HST. E, F, To "pull through" a full-thickness HST, the tongue must first be lifted. Use the centrally protruding pillar of the Krolman viewing chamber to get anterior to the DM to scrape the tongue off the stroma with centrally moving motions. Because lamellar splitting typically precedes the full-thickness HST, the scraping must start more peripherally than the full-thickness tear. G–J, Once the entire HST is lifted, cautiously continue peeling that quadrant. Stress lines going obliquely across an HST can cause it to rip wide open.[16] To prevent ripping, grab the DM edge exactly radial to the HST.

but if the host horizontal corneal diameter was <11.50 mm, we used a diameter not >8.5 mm (Fig. 5).

Delaying trephination until now allowed imperfections discovered during partial peeling to be excluded from the final donor graft. To trephine, the corneoscleral rim was placed on the cutting block and aligned with any imperfections outside the trephine zone. A cutting block with a marked central 8-mm area, such as the Hanna punch with a Price cutting block (17169 & 630/660–7540, Moria, Fig. 6), helped get perfect alignment if a decentered punch was needed.

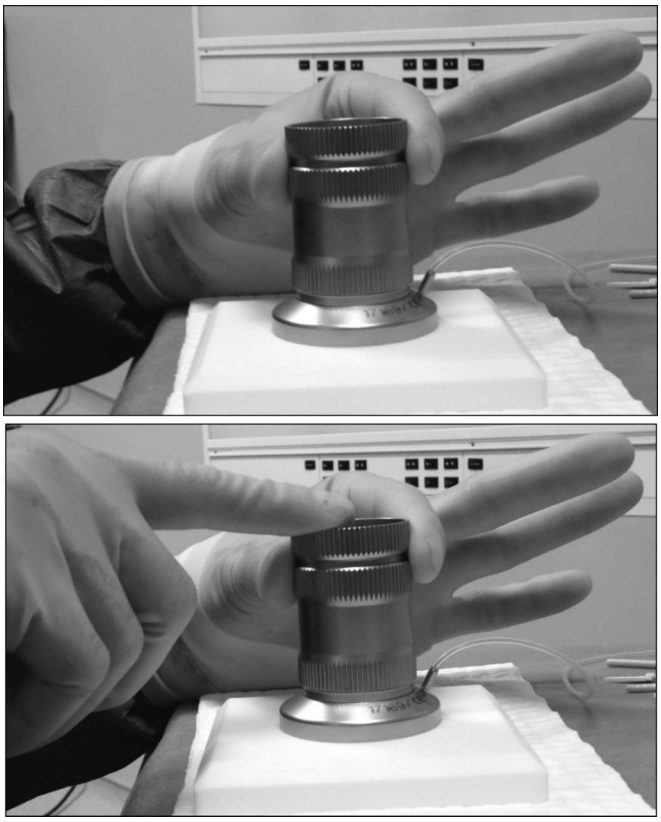

FIGURE 5. Trephining the DM. The grip demonstrated (top photograph) prevents the rotation of the trephine blade and allows the surgeon to feel when the blade has penetrated during taps (bottom photograph). It is important to stop after the gap lessens so the cornea is only penetrated but not perforated.[17]

Suction was activated on the cutting block. The DM was checked to ensure that it was not curled. If it was curled, the storage solution was wicked from under the DM with a cellulose sponge so that the DM followed the fluid peripherally to lay flat. More fluid was dripped on and soaked away as necessary. The guide cylinder was attached. The trephine punch cylinder was advanced into the guide until the blade made contact with the cornea without rotating. The cornea's thickness held the punch cylinder up so that a small gap was present between the guide and punch rims. The thumb and index finger of 1 hand were placed in this gap to hold the circumference of both rims to prevent rotation while the other hand lightly tapped the punch in all 4 quadrants. When the gap lessened, tapping was stopped so that the cornea was only penetrated but not perforated (Fig. 5).[17]

Final Peeling of the Donor

The donor was briefly restained with VisionBlue, the sclera was grasped at the meridian of peel 4, submerged, and the peripheral rim of the DM beyond the trephine marks was removed with caution to separate sites with adhesions last. The final peel was started at the same meridian where peel 4 was made, so the donor separated when the peel reached the middle (Fig. 2). When submerged, the donor formed a scroll with the endothelium on the outside. The scroll was secured in the corneoscleral rim filled with the storage solution if it was to be used at that time. It was placed in a glass vial with smooth edges filled with the storage solution if it was intended to be used another day.

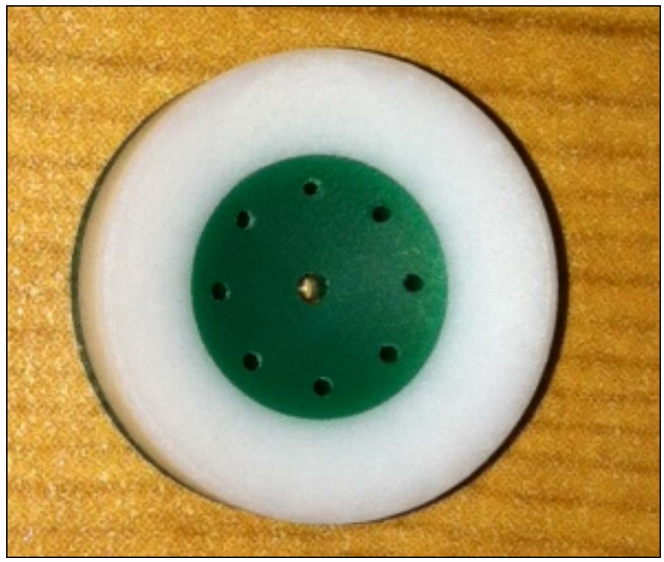

FIGURE 6. Use of a colored cutting block. This cutting block outlines in green where an 8-mm trephine blade will fall. This allows the surgeon to position imperfections outside of what will become the DMEK donor graft.

RESULTS

Of 263 attempted DMEK preparations, 260 (99%) were successful and 3 were not (the 116th, 137th, and 259th attempts). Donor 116 lost about 15% of its area because of the formation of HSTs. In the 137th and 259th cases, so many HSTs were present that the DM could not be separated from the stroma. Two other donors were missing some area, one because of HSTs and the other because of cataract wounds, but the missing area was $<5\%$, so they were used for surgery.

HSTs were present in 35 attempted preparations (13%) and resulted in preparation failure 5.7% of the time that they occurred. The number of HSTs ranged from 1 to 18 with the average "HST-positive donor" having 2.7 HSTs. If the first donor of a pair had a full-thickness HST, there was a 46% chance that the mate would have at least 1 HST (6 of 14). The odds of the mate having at least 1 HST rose to 75% if the first had multiple full-thickness HSTs (3 of 4). With increasing experience, it was possible to recognize lamellar splitting before a full-thickness HST occurred, and the surgeon would pull from the opposite side or lift the area of lamellar splitting and pull through it to prevent the development of a full-thickness HST. If "lamellar splitting recognized and arrested before progression toward full-thickness HST" was counted the same as an "HST," then the odds of the mate having at least 1 HST went up to 60% (9 of 15) if the first had any HSTs and 80% if the first had multiple HSTs (4 of 5). Often,

the mate was returned to the Eye Bank unopened if deemed to have a high risk of developing multiple HSTs.

DISCUSSION

This report demonstrates that HSTs pose frequent challenges that those using the SCUBA DMEK preparation technique should know how to handle. Although using a bad technique can cause HSTs, they also seemed to be donor specific, because the risk of formation of HSTs on a given cornea was substantially increased if an HST developed in the contralateral cornea. As reported by Schlotzer-Schrehardt et al,[18] variations in adhesive matrix proteins may explain why DM adherence is higher in some donors than it is in others.

Additional studies are needed to help predict which donors are most likely to manifest lamellar DM splitting and HSTs. For now, the best advice is if the first cornea from a pair is difficult to prepare because of excessive HSTs, the second is likely to be difficult as well, so it should be returned to the eye bank for use on another occasion.

The thickness of the DM increases with age,[19] and others and we have noticed that young donor tissue is more difficult to use for DMEK, because it curls up tightly and can be very difficult to uncurl inside the anterior chamber. Therefore, for DMEK, we only used donor tissue that was >40 years old.

In conclusion, DMEK preparation has become efficient and reliable, but one must be ready for the rare donor that is challenging and time consuming. It can be cost effective to prepare DMEK tissue a day or 2 before surgery with minimal staff to avoid excess operating room time, and preparation a day or 2 ahead does not seem to affect surgical outcomes.[20]

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