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Chapter 10

An Eye Bank DMEK Tissue Preparation Program for Corneas Stored at 4°C

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Abstract

This book chapter discusses details about establishing and maintaining a program for the preparation of DMEK tissue in an eye bank setting. Administrative considerations for eye banks are reviewed such as: staffing, cost of implementation, training, and tissue loss. Step-by-step instructions for graft preparation are provided. Finally, tissue evaluation and procedure validation are reviewed.

Keywords: cornea, endothelial keratoplasty, DMEK, eye bank

Introduction

Eye Banks play a critical role in the recovery, screening, and processing of corneal tissue for transplantation. The role of "tissue processor" has expanded in the past decade with the advent of Descemet Stripping Automated Endothelial Keratoplasty (DSAEK). In the US, DSAEK tissue preparation is commonplace with over 20,000 procedures performed in 2013 [1]. As Terry [2] and Cursiefen [3] note in separate editorials, tissue preparation for Descemet membrane endothelial keratoplasty (DMEK) surgery has been one of several barriers to adopting a surgical technique that may provide superior vision and faster healing than DSAEK [4, 5] as well as lower rejection rates [6]. The risk of a DMEK graft tearing during the preparation procedure is still significant, especially in the early learning curve [4].

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Moving this risk to the eye bank avoids costly operating room tissue loss and surgery cancellation. This chapter will give a broad overview of implementing a new procedure, DMEK tissue preparation, *in an eye bank setting.* The same advantages that eye bank tissue preparation brought to the DSAEK procedure can now be enjoyed by surgeons who wish to offer DMEK to their patients as a treatment option.

Administrative Considerations for DMEK Tissue Preparation Implementation in an Eye Bank Setting

A DMEK tissue preparation program at an eye bank requires the administrative staff to consider a number of issues. Does the eye bank have the community need to support a high quality program? Is there a suitable processing environment available to prepare the tissue? Is there adequate staffing for implementation of the procedure if the all the community needs are met? Does the eye bank have the appropriate guidance to ensure compliance with any additional training, regulatory and industry identified standards? These questions, and others, all must be asked with careful consideration to the availability of funding. In the US, funding is usually based on work performed, and thus an eye bank that is implementing a project will have to figure out how to make the investments in this program from their reserves or through fund raising before they can ever see their costs, both fixed costs in the program start-up and ongoing costs of running a tissue processing program, returned.

Meeting a Community Need

First and foremost, before an eye bank embarks on the implementation of a highly complex new service line, a needs assessment to ascertain the projected volume of surgery is warranted. Because of erratic surgical adoption patterns, it may not be unusual for an eye bank to get sporadic requests for DMEK prepared tissue from surgeons early after DMEK grafts are offered by an eye bank. Is this justification for a new eye bank program? At what point does the community need tip the balance in favor of moving forward with a new program? This is a question that must be answered locally.

Community need is closely aligned with the buy-in of the eye bank Medical Director(s) and any surgeons served by the eye bank that may be able to act as champions of a DMEK program at the eye bank. The full support of the Medical Director(s) and other EK surgeons the eye bank serves will result in better training and feedback to the eye bank about the quality of the work performed. Additionally, once the program is implemented, the eye bank will be expected to meet the surgical demand even if demand increases rapidly. The eye bank must consider training and staffing to ensure program continuity once the community surgeons have come to rely on the availability of pre-peeled DMEK grafts. Staffing disruptions or other calamities will become an adverse service issue if the eye bank cannot deliver on its new service promise to the community. A baseline community need will ensure an adequate volume of graft preparation which will in turn help to keep technical skills sharp.

Adequate procedure volume will drive revenue from the procedures performed and help offset the financial investment in the program. If funding is not based on a fee for service model as it is in the US, then the baseline community need can be used to justify the program costs to potential funding sources such as governmental organizations or philanthropic entities.

Cost Considerations

The capital investment in time, infrastructure and material is significant. This must be weighed against the community need for provision of this important step along with the resources available to the eye bank. While the costs are significant, provision of this service is of great benefit to the endothelial keratoplasty community in that tissue can be prepared in advance of the surgery allowing for quality control measures to be instituted and time saved in the operating room.

Implementation of an Appropriate Processing Environment

Published techniques for DMEK tissue preparation require the use of microscopy to visualize the thin (15μm) Descemet's membrane [7-10]. This poses several issues for the eye bank engaged in more traditional eye banking activities such as DSAEK preparation or corneoscleral disc (CSD) excision from a whole eye. Both can be performed without the aid of magnification and are typically done in this manner in a laminar flow hood. Issues to consider are the manner in which microscopy can be employed, the required processing environment from which to deploy microscopy, and the development of a novel skill set in eye bank technicians who are likely unfamiliar with working in a magnified environment. The last issue will be covered more thoroughly in a discussion regarding the training needs of a DMEK program.

Ideally, a microscope with foot pedals for changing focus and magnification will aid the dissection of the endothelium-Descemet membrane complex (EDM). Purchase of a new high-end operating microscope with superior optics will enhance visualization, but there a number of high quality operating microscopes available in the aftermarket that can save quite a bit of cost while still achieving the level of magnification and operator control desired. It is important to consider the availability of parts and service for whichever model is chosen.

Figure 1 demonstrates four examples of successfully implemented DMEK tissue processing environments. All comply with Eye Bank Association of America (EBAA) Standard E1.200 [11] and US Food and Drug Administration rule §1271.195 [12] as well as published literature on safe corneal tissue processing [13]. The trade-offs of these approaches are worth careful consideration. Working with a dissecting microscope as demonstrated in Figure 1A provides for excellent technician protection from any airborne disease transmission concerns and the environment is well controlled and is relatively easy to clean with quicker

turn-around times between procedures. However, microscopy is not foot controlled in this environment and the working area is limited.

Figure 1. A) Dissecting microscope set up in a vertical laminar ISO 5 flow hood with biosafety shield (courtesy Minnesota Lions Eye Bank). B) Operating microscope set up in a large ISO Class 5 horizontal laminar flow hood (courtesy Iowa Lions Eye Bank). C) Operating microscope set up in ISO Class 5 work zone inside at tissue processing room (courtesy Georgia Eye Bank). D) Operating microscope set up in an ISO Class 5 clean room (Lions VisionGift).

Additionally, items outside the biosafety cabinet may be in a less controlled environment if the laminar flow hood (LFH) is outside a room supplied by high efficiency particulate air (HEPA) filtered air. Figure 1B allows for the use of a full operating microscope with foot pedals. The LFH blows air over the tissue and directly on to the operating technician which is a theoretical safety risk although there has never been a report of any issues related to this type of operating environment while processing ocular tissue intended for transplant. This environment is larger to accommodate an operating microscope, so the cleaning of the work area will take slightly longer than option A, but the use of an operating microscope with foot controls and more manipulation of the magnifying head allows for a great deal of flexibility for the operator to get a comfortable view of the tissue which changes as the procedure dictates.

Option 1C is an ISO Class 5 clean zone with built-in HEPA filters which creates an even larger work area than Option 1B. Option 1D, an ISO Class 5 clean room, allows for a super clean environment for all equipment and personnel working on the procedure and provides the same allowance for an operating microscope but it is the most labor-intensive to clean after each procedure.

A clean room is also the most expensive option and least flexible with regard to moving equipment to a new location unless it is a modular design. All options have been successfully employed and should be considered in light of the eye bank's other operations, capital resources, and staff and Medical Director preferences.

Training and Staffing Considerations

Routine removal of the corneoscleral disc requires adept dexterity and care so as not to induce striae in the endothelial monolayer. This procedure has been common in eye banks for decades. However, success with the standard corneoscleral disc recovery procedure does not guarantee a good outcome for training for DMEK preparation. Care must be taken to find technicians that have steady hands, patience, and the ability to learn to "operate" in a totally new manner in which their hands are no longer visible as they peer through the oculars of a microscope. While many eye bank technicians can learn this procedure, due to the high demands of this procedure there is a higher training "fail" rate than other procedures in the eye bank.

Eye banks interested in adopting this processing technique would be wise to carefully screen prospective technicians and be prepared to abort training should a good fit not be present.

The primary skills required for this procedure are: steady hands as observed through the operating microscope, patience at all times throughout the procedure, and rigorous attention to minute details. Concerns about a technician's aptitude in any of these areas cannot be ignored. If these native skills are present and reinforced with sufficient practice, a good training outcome is likely.

Here it is worth mentioning that assistance from a DMEK champion, perhaps an eye bank Medical Director or a local physician with an interest in DMEK, will help reduce the learning curve immensely for training at least the technician in charge of training other technicians. Without the aid of someone familiar with work in a magnified environment, technician training will take longer than if this guidance is not available.

Tissue Loss/Tissue Wastage

Eye banks are granted stewardship over altruistically donated tissue, either by the next-of-kin, or by the donor herself in cases of first person authorization. This is a role that is taken very seriously by the eye banking community.

Every time tissue is handled there is risk inherent in the procedure such that it either may not be used due to a break in aseptic technique or physical damage to the tissue.

DMEK preparation, with its steep learning curve, puts the eye bank in the position of balancing the desire to utilize all anatomical gifts to their maximum benefit to honor the donors' wishes, and the chance that the tissue will be rendered unsuitable due to the complex nature of the processing that is required to provide the delicate EDM which is considered by many surgeons to provide the best care to recipients with certain ocular conditions such as Fuchs dystrophy.

Eye banks launching a new DMEK program should understand the potential impacts of tissue loss that may occur as a direct result of program implementation.

Published data from Iowa Lions Eye Bank and Lions VisionGift report a 7.7% failure rate and a 4.1% failure rate in consecutive series from both eye bank programs [14]. These data are from early in both DMEK programs and included donors with a history of diabetes.

Iowa subsequently reported a statistically significant reduction in tissue preparation failure to 2.32% [15] after the exclusion of diabetic donors for DMEK preparation. Iowa Lions Eye Bank and Lions VisionGift have similar preparation techniques. Eye banks launching a new program can use these data as training benchmarks.

Additionally, the economic impact of tissue loss can be factored in to any cost analysis performed when setting processing fees. Finally, these data can be used to help determine if the eye bank's stewardship role are adversely impacted should tissue losses become greater than anticipated. Obviously, Lions VisionGift has made the decision in close consultation with its administrative and medical leadership that provision of this service to recipients is worth the unfortunate loss of tissue. Lions VisionGift continuously strives to reduce tissue losses as part of its DMEK preparation program and those efforts have been successful. However, these efforts will continue as long as we continue to prepare DMEK grafts.

Tissue Selection

Following donor eligibility inclusion for transplantation by EBAA, FDA, and local eye bank standards, tissue is determined suitable for DMEK preparation following thorough evaluation with a slit-lamp exam and specular microscopy analysis for determining the endothelial cell density and tissue quality. Some considerations for selecting DMEK tissue include: a) donor tissue age, perhaps greater than 50, will provide a thicker DM [16] which is easier to peel and yields a graft that is easier for the surgeon to handle [17]. b) the presence of an intact DM in the graft zone free from cataract surgery scars c) endothelium with a minimum cell density of 2000 cells/mm^2 prior to DMEK processing and c) diabetes mellitus has been shown to increase the risk of graft tearing during preparation and donors with prolonged history of the disease should be excluded to reduce the risk of tearing [18].

Provided there is demand for the mated tissues, one strategy to conserve time and resources is to process both corneas during the same sitting. If tissue is selected for DMEK preparation and the graft is difficult to peel or tears excessively, it is unwise to peel the mate as mated tissues will likely display the same properties [19, 20].

There are instances when DMEK preparation can offer a second chance for the EDM to be transplanted following processing by an eye bank or incidence of prior refractive surgery. For example, if during a DSAEK preparation there was an irregular or unintentionally thick graft but the endothelium is otherwise suitable or, if the donor cornea has scars deep into posterior stroma, it is possible these tissues may be prepared successfully for DMEK. Before the advent of DMEK, this tissue was deemed unsuitable for transplantation.

Tissue allocation is performed in concert with the end-using surgeon. Graft parameters such as donor age, medical history, endothelial cell density, time interval between preparation and implantation, and minimum graft size all have surgeon biases that will need to be clearly communicated to the eye bank staff.

Procedure Overview

Our procedure has been developed with EBAA standards in mind which require evaluation of tissue after any manipulation has been performed. Once the appropriate donor tissue has been selected, our procedure is straightforward and requires minimal equipment or supplies in order to be successful.

The use of an operating or dissecting scope is required in order to achieve the magnification needed to visualize all aspects of the procedure. Foot controls allow the operator to easily magnify the field in order to clearly identify the structures of interest throughout the procedure. Once the surgical field has been established, our procedure starts by removing the tissue from the storage media and allowing the excess media to drain onto sterile gauze or similar absorbent material. We currently use a Barron Vacuum Punch for Donor Tissue (Katena, Denville, NJ) as our working surface for the corneoscleral disc (CSD) due to the punch's low height above the operating table and the support the block provides for the scleral rim. Although vacuum pressure is not necessary in the suction block in order for the procedure to be successful, it is recommended for novices to avoid tissue movement during the procedure. If desired, vacuum pressure is applied with one hand, while simultaneously placing and centering the cornea onto the suction block. Once centration has been obtained, the seating ring is applied to the tissue with pressure sufficient to ensure vacuum is maintained behind the tissue. The vacuum syringe is released followed by removal of the seating ring from the suction block assembly. The seating ring serves two purposes: one, to secure the tissue to the suction block and provide stability during the procedure, and two, to provide a uniform impression in the cornea near the limbus which can be useful at a later point. When first learning the procedure, we recommend the following few steps to assist in visualization of the endothelium/Descemet membrane complex (EDM). Removal of excess storage media is recommended at this time, followed by application of a few drops of VisionBlue (DORC, NL). Excess storage media interferes with the stain in VisionBlue to some extent, reducing the visibility provided to the tissue. The stain uptake is almost immediate, with longer times providing more stain uptake and contrast. Once sufficient time has elapsed, approximately 60 seconds, the CSD is gently rinsed with enough Balanced Salt Solution (BSS, Alcon, Ft. Worth, TX) to remove the VisionBlue from the well of the cornea.

While a cystotome is also acceptable, our preference has been to use a 13mm 30 gauge needle to score just through the EDM. Using a hemostat, the tip of the needle is grasped with the bevel of the needle facing up and the 2-3mm at the tip bent to an angle between 45° and 90°, with a second bend of the needle made in the opposite orientation 2-3 mm from the Luerlock base. For right handed operators, this is best done by holding the needle with the right hand and the hemostat with the left hand. The left hand and hemostat will rotate counter-clockwise bending the needle to the first desired angle, followed by a bend clockwise at the base.

Once the needle has been bent to the desired angle, the scoring of EDM can commence. Consider the CSD much like the face of a clock. The hand holding the needle should be at approximately 3 o'clock, with the needle being held above the cornea and the tip scoring EDM at limbus across the cornea at the 9 o'clock point. The bevel should be facing peripheral to the cornea and a very slight angle given to the needle. Ideally, the tip of the needle will follow behind the sharp edge. Placement of the score line is extremely important as this early

step can set the stage for a successful peel. When the seating ring was applied to the cornea, the shape was changed to match the contour of the suction block and seating ring resulting in a raised circle of tissue immediately central to the point of contact. With the cornea centered correctly, this raised circle is perfectly situated to guide needle placement for scoring EDM. As Price and McKee describe, consider the needle like a boat; as the needle is moved across the tissue a "wake" is created in the EDM causing tissue on either side of the needle to peel up [19]. Using the raised area created by the seating ring, one can guide the tip of the needle around the peripheral cornea, avoiding the trabecular meshwork while staying peripheral enough to ensure a graft of adequate size for surgical use.

Whether using the raised area as a guide for needle placement or not, complete disruption of EDM must be completed 360° around the periphery. Short overlapping strokes are recommended for scoring. This allows finer direction of the score line and allows the operator to stop and correct any mistakes or large areas of EDM that may unintentionally peel up during this step.

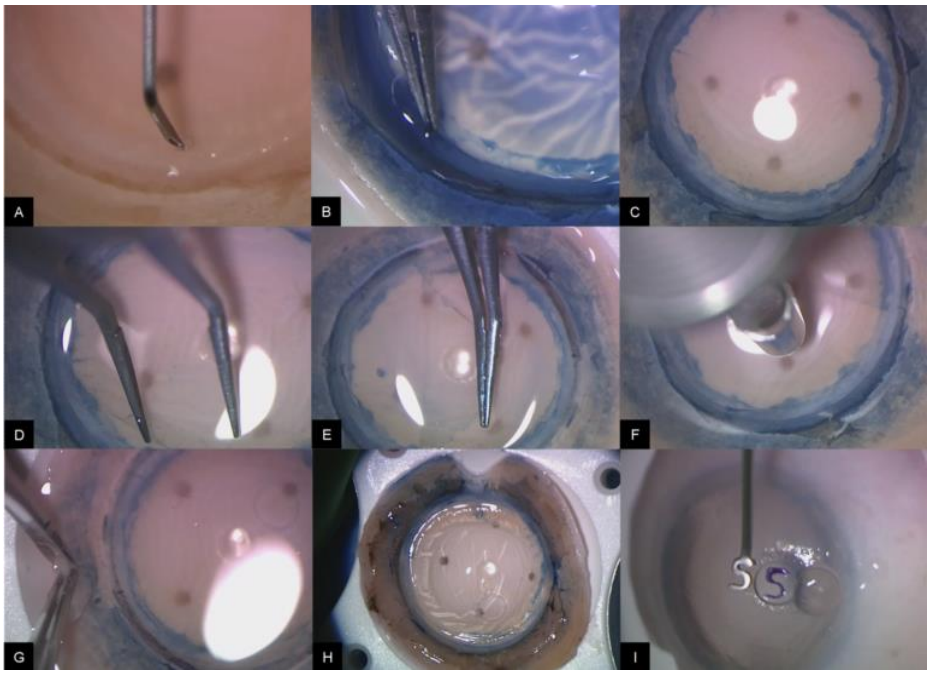

Figure 2. The endothelium/Descemet membrane complex (EDM) is scored with a bent 30g needle. B) EDM is stained with trypan blue and fragments that may pose a risk of tearing during the peel are removed. C) The EDM is rinsed free of extraneous trypan blue. D) The edge of the EDM is lifted circumferentially. E) Approximately 90% of the EDM is peeled. F) While the EDM is reflected in a pool of storage solution, a 2mm trephine is used to punch a window into the stroma. G) EDM is returned to anatomic position and a notch is removed to denote the hinge location attaching the last 10% of EDM. H) The cornea with peeled EDM and stromal window is visualized from the posterior aspect. I) The cornea is removed from the suction block and positioned epithelium up for placement of an S-stamp through the stromal portal.

Once complete, 2-3 drops of VisionBlue are added to the well of the cornea and allowed to remain in contact with the endothelium until adequate staining of the tissue has occurred. The time needed is left up to the operator's discretion, but typically takes less than 30

seconds. The tissue is gently rinsed with BSS to remove the VisionBlue, and a few drops of storage solution are applied to the tissue. Only enough storage solution is needed to keep the endothelium from drying out, but we typically apply media to fill the well of the cornea to the score line near the limbus. Applying media at this stage is extremely important in order to keep the endothelium healthy, and the media also allows the EDM to float which helps throughout the rest of the procedure.

At this point a clear demarcation line should be seen in the tissue, with denuded stroma stained blue and the untouched endothelium remaining free from stain.

The tying forceps are now used to gently separate EDM less than 1mm from the peripheral edge of score line. This is done to ensure no micro-adhesions or any areas of incomplete scoring are addressed prior to the peel. The inside edge of the forceps are particularly well suited for this task. The forceps should be held at or around the 12 o'clock position on the cornea, and with the forceps open the outer foot is swept centrally at the edge of EDM. The toe/foot of the forceps should gently scrape across the denuded stroma and catch the loose edge of EDM causing it to pull towards the center of the cornea. It is very important to avoid any tendencies to bluntly dissect EDM from the posterior stroma at this point. Rather, using a gentle sweeping and tugging motion, the forceps should be used to lift and separate EDM from the stroma. Blunt dissection can work, but generally produces small radial tears around the edge, which during peeling can extend into the graft area or tear the graft completely rendering it unusable. One to two millimeters of the toe of the forceps should be kept behind EDM, providing tension towards the center of the well. EDM will naturally peel away from the posterior stroma with this provided tension, and ideally the leading edge of where EDM separates from the stroma will be just slightly ahead of the toe of the forceps. In a sense, the forceps are really used to hook and gently pull the EDM towards the center of what would be the anterior chamber more than any other action. These gentle tugging and sweeping motions with the forceps will generally allow EDM to separate without radial tears and provide a continuous defect-free edge which helps avoid potential problems in the future steps. If at any time during this step one notices the stroma is being pressed or manipulated in an attempt to bluntly dissect EDM, stop and try a less heavy hand. The stroma is essentially left untouched during this part of the procedure. Using small sweeping/tugging motion with the forceps under EDM, continue moving 360° around until the entire peripheral EDM is free to 1mm or less central to the edge.

Should any radial tears be noticed during peripheral EDM separation, it is acceptable to eliminate the tear by grasping EDM and removing as small a section as possible immediately at the point of the tear. Doing so will create a continuous tear free edge of EDM which helps avoid potential problems or weak points during peeling. Should any difficulties be encountered while dealing with a radial tear or should the operator choose not to remove a small section, the peel can still be successful as long as care is taken when encountering that section during the peel.

Selection of an area to grasp for peeling should take into account the scleral rim on the opposite side of the cornea. Sufficient area for a scleral resection is needed to indicate the point where EDM is still attached to the stroma. If all edges of EDM and the scleral rim are equivalent, the point where EDM is peeled from is essentially up to the operator. Preference is given to any areas of EDM protruding further towards the periphery than others to allow as large an area of tissue as possible to be grasped by the forceps. The length of the foot on the tying forceps is 4mm, and while grasping the full 4mm of tissue is not practical, 2-3mm

should be easily achievable. Any existing/remaining radial tears should also be considered and ideally situated towards the end of the peel or hinge, or in the 4-8 o'clock area of the tissue.

Once the location of forceps placement for peeling has been determined, the operator must be careful when first grasping EDM. Once EDM is grasped, the forceps must move towards the opposite limbus without any initial movement left or right. At this early point of peeling, movement to either side places tension on the tissue which frequently causes large horseshoe-shaped tears and may result in a reduced graft size. The forceps should remain at the same level (approximately even with the limbus) and should not follow the natural contour of the cornea. Slow progression with peeling EDM is essential to allow any possible tears or problems to be realized immediately. Peeling EDM too quickly could result in a relatively minor issue becoming catastrophic before it is even noticed. The point of highest tension on the tissue will be when EDM is separating at the equator, so extra attention should be paid to the peripheral edges on either side of the graft at this point. Should there be a pre-existing radial tear in the periphery, peeling slowly while paying close attention to the tear until the point of separation is past (the tension is then focused further away from tear) should allow the rest of the peel to be successful. Once the forceps holding EDM reach the limbus where the hinge will be located, the operator should gently lift the graft up (towards the scope) to avoid having the endothelium contact the scleral rim or suction block base. Carefully lifting EDM will continue the peel across the cornea. Once the desired area of EDM has been peeled from the stroma, the operator should simultaneously relax their grip on the forceps while moving the forceps and EDM slightly down towards the cornea and across to the opposite limbus where peeling originated. Relaxing the grip on the forceps will separate the tying platform slightly, and should the operator move beyond the point where EDM can stretch, the graft will pull away from the forceps before tearing. Once EDM is relaxed onto the remaining preservation solution, carefully free the forceps and take a breath.

A drop or two of preservation solution placed into the well of the cornea will allow the graft to float back into a nearly normal conformation. Using carefully placed lint-free absorbent surgical spears, such as Merocel (Medtronic, Minneapolis, MN, USA), the operator can rely on capillary action to pull the graft into proper place without the operator contacting the endothelium with any instruments. If the tissue fails to return to completely normal conformation, simply apply more storage solution and repeat the process with lint-free surgical spears.

For Grafts without an S-Stamp

To indicate the point where EDM is still attached to the stroma, scissors are used to cut a "V" shaped wedge out of the scleral rim. Avoid distorting the shape of the cornea while performing cuts in the scleral rim. The "V" should point to the hinge, where EDM is still attached to the stroma. The resection needs to be large enough to clearly differentiate the area from the rest of a potentially irregular scleral rim. Once done, introduce forceps into the space between the scleral rim and the suction block, ensuring there is no point of the tissue dried onto or adherent to the suction block 360° around the scleral rim. Grasp the scleral rim near the scleral resection, and transfer to viewing chamber filled with storage solution. Gently rest the anterior stroma onto the media first, and then slowly submerge the scleral rim into the

media scleral resection side first. This ensures that the media will flow over the EDM from the hinge to the free side. Fluid moving across the prepared cornea in the opposite direction could disrupt the EDM and cause it to float away from the stroma. While not known to be unhealthy for the graft, this can cause problems in evaluation by specular microscopy. Alternatively, a small amount of fluid may be removed from the viewing chamber before placing the cornea anterior side down into the storage solution and resting it on the pedestals. The additional fluid should then be carefully added to the posterior side of the cornea, flooding it until the corneoscleral disc is fully submerged.

For Grafts with an S-Stamp

Leaving a small amount of storage solution on the EDM, grasp EDM at same point where forceps touched EDM during peeling and fold EDM over to the opposite limbus creating a "taco" out of the tissue, with endothelium facing in. Using a 2mm Elliot Trephine, create the stromal window that will allow access to Descemet membrane for application of the gentian violet-inked S-Stamp (Moria, Antony, France). Care must be taken to avoid inadvertent trephination of the graft as well. Using the Barron Donor vacuum suction block, ideal placement of the 2mm trephine is such that the trephine will overlap one of the suction holes. This allows for nearly complete trephination, while leaving a small section of the anterior stroma intact. Once the location is determined, firm pressure is applied to the trephine in order to cut through the entire stroma. Gentle rocking of the trephine is also helpful in ensuring the desired amount of trephination occurs. Once trephination has occurred, using forceps in the opposite hand, press and hold down the stroma immediately peripheral to the trephine position and gently remove the trephine from the stroma. Should a complete trephination occur, carefully remove the stromal plug from the trephine and set it aside on a lint free surface maintaining orientation for later replacement.

Using storage solution and surgical spears, carefully float EDM back into normal configuration, and remove as much fluid as possible from the stroma-EDM interface. Placing the surgical spear at the edge of EDM while tipping the suction block towards the surgical spear can help remove the fluid. Once the fluid is removed, the scleral resection will take place in the shape of a "V" with the point indicating the small hinge of EDM still attached to the stroma. The resection needs to be significant enough to distinguish it from any other possible irregularities in the scleral rim. Once completed, it is important to make sure the scleral rim is completely free from the suction block underneath the tissue. Carefully move the tips of forceps between the scleral rim and the suction block, sweeping away from the tissue and block at any point where tissue is attached.

Grasp the scleral rim at the edge, and invert the tissue onto the top, or flat part, of the seating ring so that the tissue is resting on the scleral rim. Find the stromal "window" and if the hinged plug is present, carefully find the point opposite the hinge, and flip the plug onto the peripheral stroma. The plug may want to fold back into its natural position in the stroma. Drying the epithelium on the region immediate adjacent to the plug will enhance adhesion when the plug is reflected. Should this not work, using a surgical spear to hold the plug down while resting the handle of the spear on the field next to the seating ring will also work. There will likely be a significant amount of media pooled in the stromal window which will need to be removed. Several surgical spears may be needed to remove all fluid; the anterior side of

EDM will need to be dry to allow the ink to adhere. Using a surgical skin marker with gentian violet ink (P-3, Accu-line, Hyannis, MA), rub the marker on the S stamp to transfer the ink. It is important to note that the ink has an alcohol carrier and alcohol has been shown to kill underlying endothelial cells [21]. Therefore, it is important to allow the alcohol to evaporate before applying the ink to the graft. Dry ink applied in this manner has been shown to be safe for underlying endothelium in DSAEK preparations [22]. Once Descemet membrane and ink are sufficiently dry, gently touch the S stamp to DM so the full S contacts the membrane and transfers the ink. Should an incomplete S be delivered, dry Descemet and repeat the steps until the S stamp is satisfactory. Replace the plug either by simply flipping the hinged plug back into place, or by placing the free plug back into the stromal window, maintaining correct anatomical orientation.

As described above for corneas without an S stamp, place the tissue in a corneal viewing chamber with fresh storage solution. The tissue is now ready for post-preparation evaluation.

Assessment of DMEK Grafts

Paramount to successful preparation of DMEK grafts for transplantation is the ability to determine if the graft is suitable for transplantation. While not specifically part of the formal post-preparation evaluation, trypan blue, a vital dye specifically applied to aid visualization of the scored DM, can assist in determining suitability of DMEK graft for transplantation. After staining, any areas of endothelial damage become obvious through the operating microscope (figure 3A).

Staining at this time point gives a view of endothelial quality and location of IOL scars if they are present, prior to final eye bank manipulation of the EDM. Technicians can note areas of particular concern where excessive staining in the graft zone may be incompatible with future graft adherence in the unlikely event that any are identified. These areas of concern can then be evaluated with slit lamp microscopy and rated for use or non-use according to standard evaluation protocols.

Following the DMEK preparation, the tissue is again placed in a corneal viewing chamber to facilitate the slit-lamp (Figure 3B and 3C) and specular microscopy evaluation (Figure 3D). Slit lamp and specular microscopy are performed and both must be rated acceptable prior to release for transplantation. Specular microscopy, which samples only a small area of the endothelium, must be used in conjunction with careful slit lamp examination in order to avoid overemphasizing the value of the reported endothelial cell density as measured by the device [23]. The minimum acceptable endothelial cell density after preparation is 2000 cells/mm^2 at our institute.

During the post-preparation slit lamp examination, special attention is paid to the extent of stress lines and cell drop out, if any. Retro-illumination is particularly helpful to illuminate the extent of damage that may have been induced during a processing event. A fine slit beam is used to verify that the graft is free from the overlying stroma and that there are no tears in the graft. Due to DM separation, there can be a great deal of focusing in order to visualize endothelium with a slit beam. The estimated maximum graft size, free of any tears, is recorded along with information regarding location and extent of any peripheral tears or scars

in DM outside the graft zone. Of particular interest to surgeons are any concerns about graft sizes or endothelial defects.

This information is all documented in the donor record and reported to the surgeon on a DMEK prepared graft tissue report form. Figure 5 demonstrates to the surgeon a representative diagram of the prepared tissue and is included as a reference on all tissue reports.

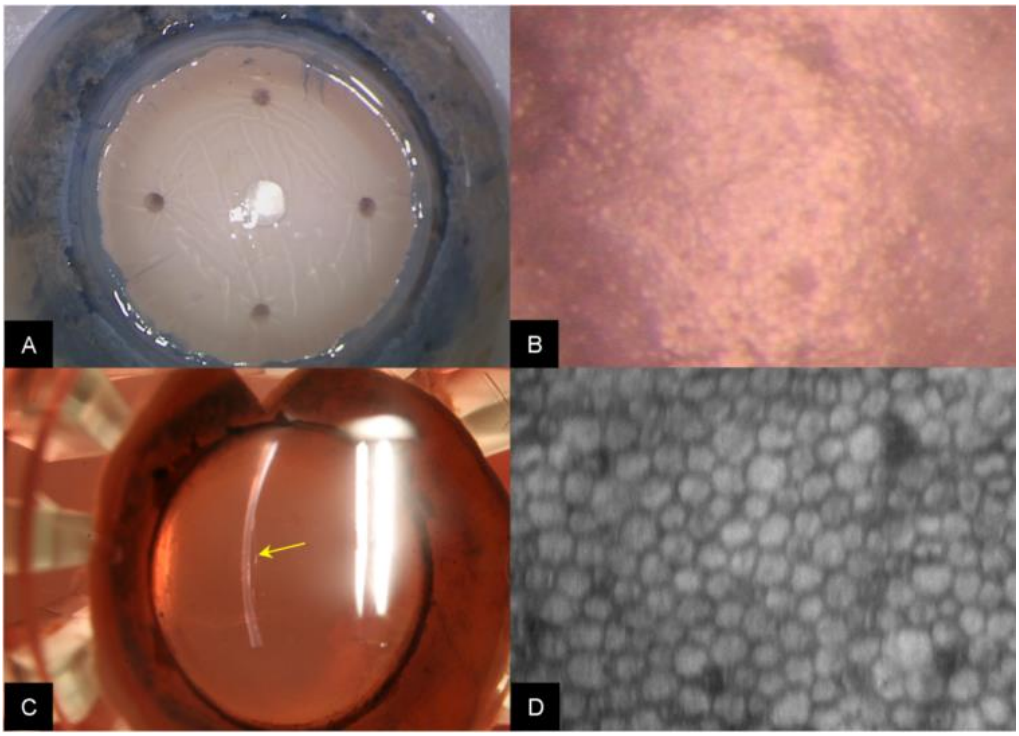

Figure 3. A) Trypan blue staining enables endothelial damage to become obvious prior to initiation of the peel. B) Slit-lamp image of specular reflection used to visualize endothelium on a DMEK prepared graft (40X). C) Slit-lamp image utilizing fine slit-beam to visualize graft continuity in near native position on a DMEK prepared graft (10X). The arrow points to the separated Descemet membrane-endothelial complex. D) Specular photomicrograph of endothelium following DMEK preparation (211 X).

Validation of Procedures

Eye bank processing of DMEK tissue saves surgeons time in the OR *and* provides the added benefit of post-processing tissue quality verification. Eye banks employing a new procedure have certain regulatory requirements for validation. In the US, the FDA is the governmental body that requires validation to ensure that a procedure does not introduce transmissible or adventitious agents to the tissue being transplanted [24]. Our approach to validating the procedure to comply with FDA regulations was to culture tissue before and after processing to ensure that no contamination occurred during processing. Each eye bank implementing DMEK procedures must work closely with their Medical Director(s) and

Regulatory Affairs/Quality Assurance personnel to ensure that validation activities are appropriately documented prior to processing tissue intended for transplant.

While the FDA, with regard to "minimally manipulated" human tissues intended for transplantation, is concerned mainly with disease transmission, the EBAA provides some standards to ensure that eye banks who do process tissue intended for endothelial keratoplasty at least check the tissue by slit lamp and specular microscopy as required in standard EBAA Medical Standard F1.000 [11]. While the EBAA requires that eye banks do this evaluation after processing, it is up to the eye bank's discretion how to ensure that these evaluations are meaningful. Lions VisionGift performed extensive vital dye staining to ensure that technician post-processing evaluations were accurately identifying endothelial damage that could render the tissue unsuitable for transplantation.

Validation of the post-processing tissue evaluation proficiency consisted of demonstrating parity between technician judgments for suitability for transplant compared to maximum endothelial cell loss (ECL) parameters established by our Medical Director. This validation process included post-preparation slit-lamp and specular microscope evaluation followed by calcein AM staining of viable cells on DMEK graft. Image J analysis was used to quantify ECL [25]. It was imperative that the technician suitability determination was within range of established allowable ECL determined by staining and analysis. The major steps for the quantification of ECL include: prepare calcein AM (eBioscience, San Diego, CA), carefully place a few drops of this mixture on the cornea positioned in trephine block and allow it to be metabolized by the cornea endothelium. Next, trephine DMEK graft then gently transfer to a glass slide. A viscoelastic gel (USIOL, Lexington KY) is helpful to spread and flatten graft onto a slide which permits photomicroscopy with an inverted fluorescent microscope (Alltion (Wuzhou) Co, China). Finally, a photomontage is constructed of the entire graft which allows for cell loss analysis with Image J via its trainable segmentation plugin.

In order to arrive at a range of acceptable cell loss to guide technicians in their tissue evaluations, we assembled an album of DMEK graft photomontages with varying degrees of ECL, each with the corresponding Image J with trainable segmentation demonstrating live versus dead zones (see Figure 4).

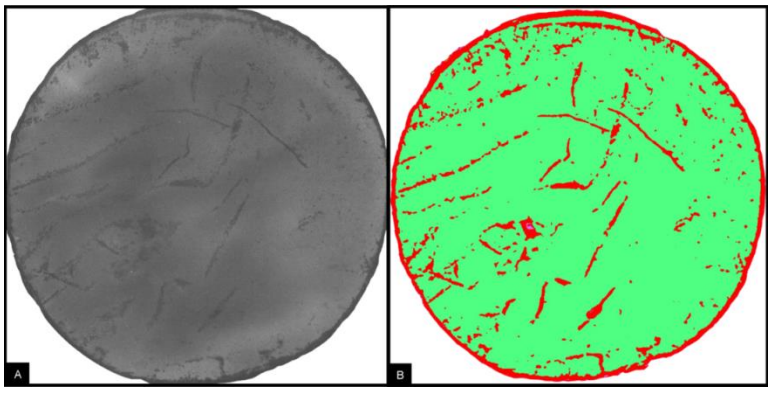

Figure 4. A) Greyscale photomontage of a DMEK graft stained with calcein AM. B) Endothelial Cell Loss determined using trainable segmentation with Image J.

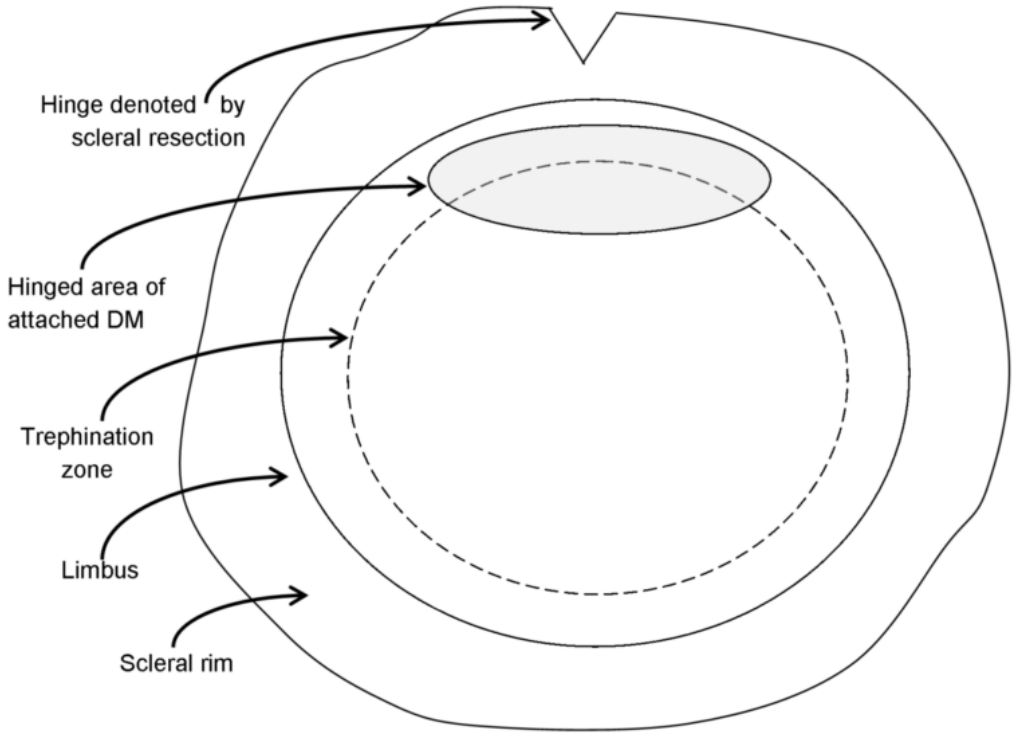

Figure 5. Diagram of eye bank prepared DMEK graft provided to end-using surgeon.

The actual calculated percent cell loss was masked for this step. The Medical Director evaluated each image of DMEK grafts and categorized each tissue as being acceptable for transplant or unacceptable based upon overall extent of ECL and specific patterns of ECL. For example, a large focal area of cell loss near periphery would be unacceptable but if similar ECL is diffusely scattered over entire graft, it could be considered acceptable.

Finally, by revealing the percentage of ECL as determined by segmentation analysis, the range of acceptable ECL following DMEK preparation became evident. This ECL data together with the technician evaluations to determine suitability offer a validated procedure for post-processing evaluation.

Conclusion

Eye banks can safely prepare DMEK grafts with an acceptable level of tissue loss. This allows for careful quality checks after tissue preparation, saves time in the OR, and transfers the risks associated with tissue loss to the eye bank. While tissue loss is a sentinel event in an eye bank, at least a replacement graft can be acquired and prepared in time to avoid surgery cancellation. In order to successfully implement an eye bank DMEK tissue preparation program, careful consideration and incorporation of the guidance in this chapter will help to attenuate the steep learning curve that DMEK tissue preparation poses. At this stage in the DMEK era of endothelial keratoplasty, eye banks do not have to go through this process in a vacuum.

There are many good programs from which to seek mentors for assistance with implementation of new programs. Training and assistance from an established program can save an eye bank a great deal of time and resources if such an arrangement can be made. Finally, we leave you with three words that will help ensure success prior to provision of the first graft for DMEK in your eye bank: “practice, practice, practice”.

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