

Comparative evaluation of four Descemet membrane endothelial keratoplasty graft preparation techniques



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Objective: To compare subjective and objective outcomes of 4 different Descemet membrane endothelial keratoplasty (DMEK) peeling techniques performed by novice surgeons at different stages in their surgical career.

Design: An ex vivo prospective study.

Methods: In the first round, 2 DMEK peeling techniques were pitched against each other: the peripheral scoring and Sinskey dissection technique with the peripheral scoring and microhoe dissection and the peripheral blunt microhoe dissection against the scleral spurectomy and microhoe dissection. Three surgeons with different operative experience performed the peeling. Outcome measures included graft peeling time, surgeon's peeling difficulty grading (on a scale of 1–10, 1 being the easiest and 10 the hardest), number of radial and circumferential tears before and after trephination, and tissue loss. The 2 techniques that performed the best from the first round proceeded to the final round to identify the best overall technique.

Results: In total, 90 tissues (45 pairs) were peeled by 3 surgeons. Following the first-round results, the peripheral scoring and Sinskey dissection and peripheral blunt microhoe dissection proceeded to the final round. There were no significant differences between the groups in terms of peeling times, subjective feeling of difficulty, post-trephination tears, and peeling success rates ($P > 0.05$ for all). However, the peripheral scoring and Sinskey dissection technique had significantly fewer pretrephination radial tears (1.3 ± 1.3 vs 6.1 ± 5.2 , $P = 0.007$) and circumferential tears (0.6 ± 0.9 vs 1.8 ± 2.1 , $P = 0.02$).

Conclusions: This study demonstrates that the learning curve can be overcome quickly with appropriate DMEK peeling techniques. The peripheral scoring and Sinskey dissection peeling technique allows efficient peeling with fewer related tears.

Objectif: Comparer les résultats subjectifs et objectifs de 4 techniques de pelage dans la kératoplastie endothéliale de la membrane de Descemet (DMEK) réalisées par des chirurgiens débutants à différentes étapes de leur carrière chirurgicale.

Nature: Étude prospective ex vivo.

Méthodes: Lors d'un premier tour, on a comparé 4 techniques de pelage, en opposant 2 techniques entre elles: la dissection périphérique réalisée à l'aide du crochet de Sinskey contre la dissection périphérique réalisée à l'aide d'un *microhœ*, d'une part, et la dissection périphérique réalisée à l'aide d'un *microhœ* émoussé contre la résection de l'éperon scléral réalisée à l'aide d'un *microhœ*, d'autre part. Trois chirurgiens de niveaux d'expérience différents ont réalisé le pelage. Au nombre des paramètres de mesure, mentionnons le temps de pelage du greffon, le degré de difficulté à réaliser le pelage ressenti par le chirurgien (sur une échelle de 1 à 10, 1 correspondant au degré le moins élevé de difficulté et 10, au degré le plus élevé), le nombre de déchirures radiales et circonférentielles avant et après la trépanation et la perte de tissu. Les deux techniques qui ont donné les meilleurs résultats lors du premier tour ont été comparées dans le cadre d'un dernier tour afin d'identifier la meilleure technique globale.

Résultats: Au total, 3 chirurgiens ont réalisé le pelage de 90 tissus (45 paires). À la suite des résultats du premier tour, la dissection périphérique réalisée à l'aide du crochet de Sinskey et la dissection périphérique réalisée avec un *microhœ* émoussé ont été retenues pour le dernier tour. On n'a pas enregistré de différence significative entre les groupes quant au temps de pelage, au degré subjectif de difficulté, aux déchirures post-trépanation et au taux de réussite du pelage ($p > 0,05$ pour l'ensemble des mesures). Cela dit, la dissection périphérique réalisée à l'aide du crochet de Sinskey a entraîné significativement moins de déchirures radiales ($1,3 \pm 1,3$ vs $6,1 \pm 5,2$; $p = 0,007$) et circonférentielles ($0,6 \pm 0,9$ vs $1,8 \pm 2,1$; $p = 0,02$) avant la trépanation.

Conclusions: Notre étude permet de constater que la courbe d'apprentissage peut être aplanie rapidement grâce à l'utilisation de techniques de pelage appropriées dans la DMEK. La technique reposant sur la dissection périphérique réalisée à l'aide du crochet de Sinskey donne lieu à un pelage efficace qui s'accompagne d'un moins grand nombre de déchirures.

Descemet membrane endothelial keratoplasty (DMEK) is the most anatomically exact surgical means of replacing diseased corneal endothelium commonly found in endothelial dystrophy, pseudophakic bullous keratopathy, and iridocorneal endothelial syndrome.^{1–3} The advantages of this technique include reduced incidence of rejection, improved

visual outcomes, and accelerated rate of recovery when compared with other techniques such as Descemet's stripping automated endothelial keratoplasty or penetrating keratoplasty.¹ Accordingly, the use of this procedure for patients with endothelial compromise has increased in popularity since its emergence in 2009.¹

Despite the many benefits of DMEK, a challenge of this technique is the learning curve for both the tissue preparation and transplantation.¹ A number of eye banks now provide prestripped and preloaded DMEK donor corneal tissue, which can facilitate surgery.^{4,5} However, this increases the cost of the tissue preparation; moreover, not all centres have access to or can afford prestripped DMEK tissue for endothelial transplantation.

To obtain successful and functional grafts, various donor cornea preparation techniques are used by eye bank technicians and cornea surgeons. The aim of this paper is to compare 4 techniques for DMEK donor tissue preparation currently used by corneal surgeons at the University of Toronto. The tissues were prepared by investigators at different stages of their training, all with no prior experience in DMEK peeling.

Methods

Ethics approval

The work described has received approval from the University Health Network Research Ethics Board (Toronto Western Hospital, Toronto) and was conducted in compliance with the tenets of the Declaration of Helsinki. All donors or their family consented to donating their corneas for research purposes.

In this prospective ex vivo study, we evaluated the learning curve of 3 investigators at different levels of training (a medical student, an ophthalmology resident, and a cornea fellow) in performing DMEK graft preparation using 4 different techniques in a wet laboratory setting. Ninety paired disqualified donor corneoscleral rims of 45 donors were received from the Eye Bank of Canada, Ontario Division. Demographic data were collected, including donor age, sex, time of death, cause of death, time from death to harvesting, time of tissue in preservation culture, and medical history. All donor tissues used were stored in corneal storage solution (Optisol; Bausch & Lomb, Rochester, NY) and kept refrigerated at 4°C until the stripping process was performed.

Peeling techniques

Peripheral scoring and Sinskey dissection. After placing the donor corneoscleral rim on a Barron donor corneal punch (Barron Precision Instruments LLC, Grand Blanc, Mich.) with the endothelium facing up, the endothelium was stained with Trypan blue (VisionBlue; DORC, Zuidland, the Netherlands) for 20 seconds. The excess stain was then absorbed using a Weck-cell (Beaver Visitec International, Waltham, Mass.). Then a 15-degree blade was used in a tangential motion to score the outer 1–2 mm of the peripheral Descemet membrane (DM) from the stroma, which represents the most adherent part at the Schwalbe line. This was followed by submerging the corneoscleral graft in balanced saline solution (BSS). A Sinskey hook was then used to dissect the DM from the underlying stroma peripherally along the incised edge all the way around. After 1–2 mm of the DM was freed from its insertion peripherally, a fine nontoothed forceps was used to separate the DM from the underlying stroma. This peeling process continued until approximately 50% of the DM had been peeled, leaving half of it still attached to the underlying stroma. The tissue was then replaced in its original position with the help of BSS irrigation and was then dried. VisionBlue was applied again for 20 seconds to facilitate visualization of the DM. The central 8 mm was then incised with an 8 mm Barron donor trephine (Katena Products Inc, Parsippany-Troy Hills, NJ). The corneoscleral rim was then refilled with BSS. A fine nontoothed forceps was used to remove the remaining DM beyond the 8 mm trephined margin. The same forceps was then used to peel the remaining adherent part of the 8 mm DM graft until it was completely separated and formed a double scroll (Fig. 1).

Peripheral scoring and microhoe dissection. This technique is similar to the peripheral scoring and Sinskey dissection (PSSD) technique with some modifications. Following 360-degree scoring with a 15-degree blade, instead of using a Sinskey hook to dissect the DM, a Rootman/Goldich modified Sloane microhoe (Katena Products Inc) was used to per-

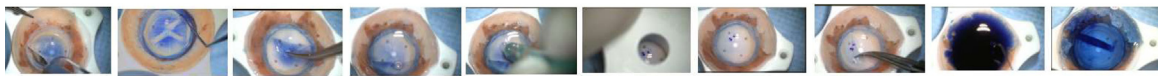


Fig. 1—Screenshots of the various stages of the peripheral scoring and Sinskey dissection (PSSD) technique.



Fig. 2—Screenshots of the various stages of the peripheral scoring and microhoe dissection (PSMD) technique.



Fig. 3—Screenshots of the various stages of the peripheral blunt microhoe dissection (PBMD) technique.

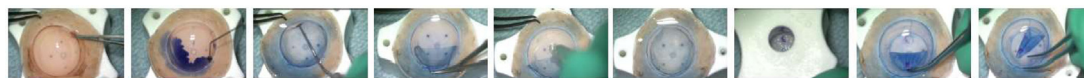


Fig. 4—Screenshots of the various stages of the scleral spurectomy and microhoe dissection (SSMD) technique.

form the 360-degree blunt dissection to free up the peripheral 1–2 mm of the DM from the underlying stroma (Fig. 2).

Peripheral blunt microhoe dissection (PBMD). The donor corneoscleral rim was placed on a Barron donor corneal punch (Barron Precision Instruments LLC) with the endothelium facing up and stained with Trypan blue (VisionBlue; DORC) for 20 seconds. The excess stain was absorbed using a Weck-cell (Beaver Visitec International). The corneoscleral rim was filled with BSS and the Rootman/Goldich modified Sloane microhoe to perform a 360-degree blunt dissection within the nonpigmented trabecular meshwork (posterior to the Schwalbe line). This dissection was continued centrally to disinsert the Schwalbe line and 1–2 mm anteriorly to separate the DM from the underlying stroma to allow for grasping of the DM. Caution was exerted so as not to create a false plane of dissection in the stroma. A fine nontoothed forceps was used to separate the DM until 50% was separated. The rest of the peeling process was the same as PSSD (Fig. 3).

Scleral spurectomy and microhoe dissection (SSMD). In this technique, the donor corneoscleral rim was placed on a Barron donor corneal punch with the endothelium facing up. A 0.12 forceps (Katena Products Inc) was then used to perform a scleral spurectomy by grasping an area of the scleral spur and peeling it for 360 degrees. This helps create a cleavage plane by releasing the peripheral Descemet adhesion while also providing a clearer visualization unobstructed by iris material. The rest of the peeling process is similar to the peripheral blunt microhoe dissection (PBMD) technique (Fig. 4).

Flow of comparison between techniques

Because of similarities between the PSSD and peripheral scoring and microhoe dissection (PSMD) techniques, a head-to-head comparison between them was initially performed using matched eyes from the same donor (pool 1). Similarly, because of similarities between the scleral spurectomy and

microhoe dissection (SSMD) and PBMD techniques, a head-to-head comparison between them was initially performed as well using matched eyes from the same donor (pool 2). The superior technique from pools 1 and 2 would then be compared head-to-head using matched eyes from the same donor (pool 3). A flow diagram depicting the sequence of peeling and comparisons is provided in Fig. 5.

Sequence of peeling and surgeon fatigue

Each surgeon received a demonstration on how to perform each technique by an author who was well versed in that technique ($n > 200$). To avoid fatigue, each surgeon performed each technique no more than once per day. To avoid learning-curve bias, each surgeon performed each of the 4 peeling techniques once in a random sequence.

Peeling data collection

All techniques were video recorded at high quality and high magnification for evaluation of surgical time, number of pretrephination radial and circumferential tears, number of post-trephination radial and circumferential tears, and whether the tissue was usable at the end. Radial tears were defined as a single linear break in the DM perpendicular to the dissected peripheral rim of the DM. All videos were reviewed by 2 independent authors (E.C. and M.M.); in cases of disagreement, resolution was achieved by mutual discussion.

Peeling difficulty

Surgeons were asked to rate the difficulty of the peeling using a Likert-type scale (1–10) immediately following each peel, with 10 representing an impossible peel and 1 representing a simple, straightforward peel.

Overall score

For each category (i.e., peeling time, subjective feeling, pretrephination radial tears, pretrephination circumferential tears, post-trephination radial tears, post-trephination circumferential tears, and peeling success), a point was awarded to the group that had a superior outcome for that pair of eyes. The overall points were tallied and deemed the overall score. In cases of equivalence, no points were awarded.

Statistical techniques

Data were recorded in Microsoft Excel (2019; Microsoft Inc, Redmond, Wash.) and analyzed using Minitab 19 (Minitab LLC, State College, Pa.). For comparison of continuous variables of paired eyes, the paired *t*-test was used, whereas the McNemar test was used for categorical

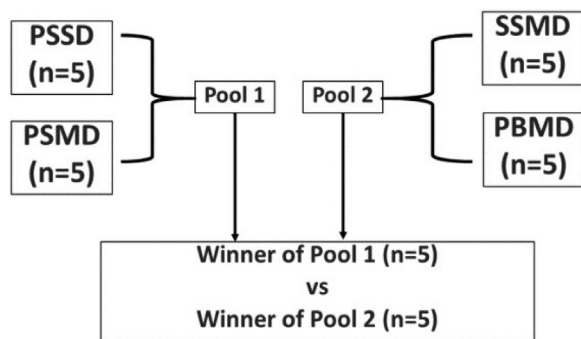


Fig. 5—Flow of sequence of surgical techniques for each surgeon.

Table 1—Comparison of outcomes of PSSD and PSMD (pool 1)

| Parameter | PSSD (n = 15) | PSMD (n = 15) | P value* |
|---|---------------|---------------|----------|
| Peeling time (mean ± SD), min | 15.7 ± 5.4 | 15.8 ± 5.8 | 0.94 |
| Subjective feeling of difficulty (1–10) | 3.9 ± 3.1 | 4.6 ± 2.6 | 0.25 |
| Pretréphination | | | |
| Radial tears (mean ± SD), n | 1.7 ± 2.0 | 2.9 ± 2.9 | 0.07 |
| Circumferential tears (mean ± SD), n | 0.6 ± 0.9 | 1.4 ± 1.8 | 0.08 |
| Post-tréphination | | | |
| Radial tears (mean ± SD), n | 0.2 ± 0.8 | 0.7 ± 1.6 | 0.29 |
| Circumferential tears (mean ± SD), n | 0.3 ± 0.8 | 0.5 ± 1.1 | 0.55 |
| Peeling success, % | 93.3 | 80.0 | 0.49 |
| Overall score (mean ± SD), points | 2.5 ± 1.6 | 1.1 ± 1.3 | 0.049 |

PSSD = peripheral scoring and Sinskey dissection; PSMD = peripheral scoring and microhoe dissection

*Paired t-test for continuous variables and McNemar test for categorical variables.

Table 3—Comparison of outcomes for PSSD and PBMD (final pool)

| Parameter | PSSD (n = 15) | PBMD (n = 15) | P value* |
|--------------------------------------|---------------|---------------|----------|
| Peeling time (mean ± SD), min | 11.7 ± 5.3 | 9.6 ± 3.5 | 0.14 |
| Subjective feeling (1–10) | 3.8 ± 2.2 | 4.6 ± 2.4 | 0.17 |
| Pretréphination | | | |
| Radial tears (mean ± SD), n | 1.3 ± 1.3 | 6.1 ± 5.2 | 0.007 |
| Circumferential tears (mean ± SD), n | 0.6 ± 0.9 | 1.8 ± 2.1 | 0.02 |
| Posttréphination | | | |
| Radial tears (mean ± SD), n | 0.4 ± 1.1 | 0.2 ± 0.6 | 0.49 |
| Circumferential tears (mean ± SD), n | 0.5 ± 0.9 | 0.5 ± 1.2 | 0.84 |
| Peeling success, % | 86.7 | 80.0 | 1.00 |
| Overall score (mean ± SD), points | 2.3 ± 2.1 | 1.1 ± 1.1 | 0.11 |

PSSD = peripheral scoring and Sinskey dissection; PBMD = peripheral blunt microhoe dissection

*Paired t-test for continuous variables and McNemar test for categorical variables.

variables. For comparison of the two final techniques, a mixed-effects model was used. The threshold for statistical significance was defined as $P < 0.05$.

Results

Overall, 90 eyes of 45 donors that were peeled were included in this study. The average age of the donors was 61.3 ± 11.3 years, and 47% were female. The average time from death to harvesting was 3 hours and 55 ± 21 minutes, and the mean time from harvesting to peeling was 15 hours and 43 ± 25 minutes. On initial assessment, prior to peeling, there were no signs of gross damage to the tissue.

Comparison between peeling techniques

Table 1 provides a paired comparison of PSSD versus PSMD (pool 1). There were no significant differences between the groups in terms of peeling times, subjective feeling of difficulty, pretrephination tears, post-trephination tears, and peeling success rates ($P > 0.05$ for all). The PSSD technique had a significantly better overall score (2.5 ± 1.6 vs 1.1 ± 1.3 , $P = 0.049$) and therefore was selected to proceed to the final pool.

Table 2 provides a paired comparison of PBMD versus SSMD (pool 2). There were no significant differences between the groups in terms of peeling times, subjective

feeling of difficulty, pretrephination tears, post-trephination circumferential tears, and peeling success rates ($P > 0.05$ for all). The PBMD technique had significantly fewer post-trephination radial tears (0.2 ± 0.6 vs 0.9 ± 1.2 , $P = 0.02$) and a significantly better overall score (2.3 ± 0.9 vs 1.3 ± 0.6 , $P = 0.002$) and therefore was selected to proceed to the final pool.

Table 3 provides a paired comparison of PBMD versus PSSD (final pool). There were no significant differences between the groups in terms of peeling times, subjective feeling of difficulty, post-trephination tears, peeling success rates, and overall scores ($P > 0.05$ for all). The PSSD technique had significantly fewer pretrephination radial tears (1.3 ± 1.3 vs 6.1 ± 5.2 , $P = 0.007$) and circumferential tears (0.6 ± 0.9 vs 1.8 ± 2.1 , $P = 0.02$).

To compare PSSD with PBMD using all 30 eyes peeled for each technique, a mixed-effects model was used (Table 4). Briefly, there were no significant differences between the 2 techniques in terms of peeling time, post-trephination tears, and peeling success rates ($P > 0.05$ for all). The PSSD technique had significantly fewer pretrephination radial tears (mean difference, -4.37 , $P < 0.001$) and circumferential tears (mean difference, -1.19 , $P = 0.003$).

Discussion

The ideal qualities of a harvesting technique may include an efficient, cost-effective, and reliable technique that minimizes endothelial cell loss and avoids inadvertent tears during harvesting.⁶ Thus, a prerequisite for a successful endothelial transplant is a reproducible and minimally atraumatic technique to harvest the DM–endothelial complex. Our study is the largest known to date that compares 4 common donor endothelial peeling techniques and may provide a reference for technique comparison and selection of donor graft peeling.

It is interesting to note that the significant differences between the techniques all emanate at the pretrephination stage. This was expected because the most critical aspect is to detach the DM from its strongest adhesions in the stroma.

Table 2—Comparison of outcomes for SSMD and PBMD (pool 2)

| Parameter | PBMD (n = 15) | SSMD (n = 15) | P value* |
|--------------------------------------|---------------|---------------|----------|
| Peeling time (mean ± SD), min | 17.5 ± 8.7 | 18.8 ± 8.5 | 0.67 |
| Subjective feeling (1–10) | 5.3 ± 1.6 | 5.1 ± 1.9 | 0.63 |
| Pretréphination | | | |
| Radial tears (mean ± SD), n | 5.8 ± 5.7 | 4.3 ± 3.4 | 0.24 |
| Circumferential tears (mean ± SD), n | 1.7 ± 2.1 | 1.9 ± 2.1 | 0.81 |
| Posttréphination | | | |
| Radial tears (mean ± SD), n | 0.2 ± 0.6 | 0.9 ± 1.2 | 0.02 |
| Circumferential tears (mean ± SD), n | 0.4 ± 0.7 | 0.3 ± 0.6 | 0.75 |
| Peeling success, % | 93.3 | 80.0 | 0.63 |
| Overall score (mean ± SD), points | 2.3 ± 0.9 | 1.3 ± 0.6 | 0.002 |

PBMD = peripheral blunt microhoe dissection; SSMD = scleral spurectomy and microhoe dissection

*Paired t-test for continuous variables and McNemar test for categorical variables.

Table 4—Mixed-effects model of PSSD versus PBMD

| Outcome | PSSD (n = 30) | PBMD (n = 30) | Difference of means | Standard error of difference | P value |
|---------------------------|---------------|---------------|---------------------|------------------------------|---------|
| Time peeling, min | 14.0 | 13.05 | 56.9 | 82.1 | 0.50 |
| Subjective feeling (1–10) | 3.94 | 5.01 | –1.06 | 0.40 | 0.01 |
| Pretrephination | | | | | |
| Radial tears, n | 1.57 | 5.94 | –4.37 | 1.04 | <0.001 |
| Circumferential tears, n | 0.56 | 1.75 | –1.19 | 0.36 | 0.003 |
| Posttrephination | | | | | |
| Radial tears, n | 0.27 | 0.17 | 0.10 | 0.20 | 0.61 |
| Circumferential tears, n | 0.44 | 0.48 | –0.04 | 0.24 | 0.87 |
| Peeling success, % | 90.2% | 86.5% | 3.8% | 8.0% | 0.64 |

PSSD = peripheral scoring and Sinskey dissection; PBMD = peripheral blunt microhoe dissection

Note: Values represent adjusted means. Random factors were donor tissue identification number, and fixed factors were technique (PSSD or PBMD), surgeon (medical student, resident, fellow), and phase (phase 1/2 or 3).

Although the PSSD and PSMD techniques are similar, the use of the finer pointed Sinskey hook may allow for less traumatic separation of the anterior DM by releasing more adhesions. Angling the Sinskey hook on its side, with the pointed end snugly parallel to the stromal wall, ensures that the peripheral adhesions are efficiently broken. While the blunt, broader-based Rootman/Goldich modified Sloane microhoe provides excellent purchase for initiating and finding the appropriate anatomic dissection plane, it is not suitable after peripheral scoring because it does not easily separate the depths of the DM–stromal plane because of its thickness. The microhoe is suitable for releasing at least 1 mm of the scored tissue, but using it to peel further into the centre risks tenting the DM and inducing a tear. For this reason, the PSSD technique was chosen as the more successful technique between the 2 peripheral scoring techniques.

When comparing the 2 peripheral blunt DMEK peeling techniques, the PBMD triumphed over the SSMD. Popularized by Melles, the peripheral blunt dissection allows gentle separation just posterior to the Schwalbe line.⁷ In the PBMD technique, we start at the trabecular meshwork. After creating an initial wedge and flap of tissue, the microhoe can be slid into the pocket created and be followed circumferentially 360 degrees following the plane. Although the scleral spurectomy allows a clear operative view by removing residual uveal tissue, it can cause the operator to enter into a plane that is too deep. Furthermore, some corneoscleral rims have minimal or no uveal tissue, which makes the starting point of the peripheral blunt dissection challenging.

When comparing the 2 finalist techniques—PSSD and PBMD—it is surprising that the time for peripheral scoring with a 15-degree blade was not faster. However, the PSSD technique requires a 2-step process of first ensuring that the tear does not run out and remains within the confines acceptable for an 8 mm partial trephination followed by Sinskey dissection. The PBMD technique, however, requires a simple 360-degree dissection, and once the plane is commenced, the microhoe can be moved in a circumferential manner to release the DM flap. It is thus understandable why subjective confidence scores were higher with the PBMD technique because the peripheral blunt dissection

can be performed in a more controlled manner with a greater diameter of tissue available, and hence the margin of error is superior. However, with the PSSD being scored more anteriorly and hence in an area of fewer adhesions, it leads to an easier peel and consequently fewer pretrephination radial and circumferential tears.

Previous studies have explored the various head-to-head comparisons of DMEK stripping techniques. Parekh et al.⁸ described a 5-way comparison of techniques for stripping donor tissue using a Sinskey hook, epithelial spatula, punch, donor trephine, peripheral endothelium scoring, pneumatic dissection, and submerged hydroseparation. The authors found that in reference to efficiency and acceptable endothelial cell loss, the preferred methods included manual peeling techniques via the Sinskey hook and donor trephine. Similarly, evaluation of manual peeling techniques versus the liquid bubble technique indicated that manual techniques provided more consistent results and created less damage to the graft.⁹ Contrarily, Birbal et al.¹⁰ indicated that no-touch techniques such as pneumatic and hydrodissection minimize tissue manipulation, resulting in less graft damage and cell loss compared with manual peeling techniques. Yoeruek et al.⁶ compared air and manual forceps dissection, which yielded no significant differences in apoptotic cell death or loss of endothelial cells. Evaluation of manual peeling techniques, however, found that the standard submerged cornea using backgrounds away (SCUBA) method may be preferable in regard to time for tissue preparation as well as tears when compared with the newer Muraine technique.¹¹ Sella et al.¹² compared the PBMD technique with the modified SCUBA (mSCUBA) technique. They concluded that the SCUBA technique had a shorter learning curve with shorter peeling time and fewer complications than PBMD. Finally, in a comparison of big-bubble methods for DMEK tissue preparation, Ruzza et al.¹³ found that using either air or liquid as the separation medium provided successful results; however, the liquid bubble method resulted in improved yield, tissue diameter, and endothelial cell integrity.

Although we compared several peeling methods commonly used at our centre, there are other peeling techniques described in the literature, such as the DMEK kite technique described by Chandra Bala.¹⁴ and the yogurt

technique described by Tzamalīs et al.¹⁵ In the DMEK kite technique, a new punch was developed, the Bala Asymmetric Vacuum Cornea Punch (BPI, Brand Blanc, Mich.), which is similar to the Baron punch with some modifications. It has a diameter of 7.5 mm and a pedicle that is 3.0 mm long and 1.2 mm wide at the tip. There is also an asymmetric notch on the cutting edge of the punch that helps in the orientation of the graft in the eye. This peeling technique is similar to the PBMD technique described earlier. Briefly, after placing the donor tissue on the block, Trypan blue was used to stain the endothelial surface, and the periphery is bluntly dissected for 270 degrees using the Rootman/Goldich DMEK dissector. Periodic restaining with Trypan blue was used to visualize the adhesions between the DM and the stroma. The periphery is freed until 1–2 mm anterior to Schwalbe line. Then 70%–80% of the DM–endothelial complex was peeled using non-toothed forceps. The punch was then applied, making sure to include the trabecular meshwork in the tip of the pedicle. This DMEK kite technique is thought to have several advantages. First, it helps in graft orientation by 4 different methods: inking the graft pedicle tip at one corner, using the circumferential scleral fibres on the pedicle (which indicate the nonendothelial surface), the orientation notch, and the Veldman Venn technique. Moreover, instead of injecting the graft into the anterior chamber, the graft is dragged using the pedicle. This is thought to help in preventing retropupillary placement of the graft, avoiding touching the graft, and enabling graft placement in hazy eyes. The pedicle also is thought to hold the graft in place during the process of gas injection. The author described a short learning curve when the manipulation time significantly decreased after the fifth case, with a significant decrease in endothelial cell loss.

In contrast, the yogurt technique is characterized by using a specific guarded punch circular blade missing 1 clock hour, which will create a hinge mimicking the opening of a yogurt container. When using this technique, the donor corneoscleral tissue is placed on the cutting block and then stabilized using vacuum pressure. Trypan blue is then applied for 20 seconds and then washed with BSS. The BSS is then dried, and the modified guarded punch blade is applied, leaving a hinged area of 1 clock hour. The DM is peeled off from the stroma at the hinge using a blunt instrument. This peeled area is restained with Trypan blue and then dried using Weck-cel. Part of the hinge is then cut using a sharp instrument, and a small orthogonal area is left to be grasped later for peeling. The authors described this technique as quick (>9.1 and 6.1 minutes on average), and they did not have a significant difference in the failure rate or tissue loss among study participants (senior surgeon, independent surgeon, and fellow). They also described no significant loss of endothelial cells before and after peeling.

The main limitation of our study is that while it is the largest study to date comparing different DMEK peeling

techniques, we were not able to demonstrate any significant differences between the different levels of expertise because of the relatively low sample size. Any future study comparing the outcomes of peeling among the different experience groups would require a larger sample size. Second, another important metric to evaluate the success of each peeling technique is to assess the endothelial cell density after graft harvesting. This was evaluated by Muraine et al.¹⁶, who found a 4.1% loss of endothelial cells after DMEK dissection. However, this is an important limitation that requires further evaluation because some techniques may lead to greater endothelial cell loss than others. Parekh et al.¹⁷ investigated the learning curve of DMEK graft preparation in an eye bank. They confirmed a learning curve involved but found with practice and a standardized DMEK peeling technique that endothelial cell loss and tissue wastage could be reduced. Another potentially confounding error is the consistency and quality of the corneoscleral tissue rims. Influencing factors such as donor age, temperature, post mortem time, storage time, and incubation period in organ culture are all potential sources that could affect peeling abilities.^{18,19} We attempted to eliminate these factors by using paired eyes, but there still might be slight variations even between these tissues.

Our study suggests that the PSSD technique for DMEK graft preparation is easier to master with a greater chance of donor graft preparation success compared with the PBMD technique. Larger-scale studies are required to evaluate peeling success in a real-world setting, with emphasis on endothelial cell count, graft attachment, and graft survival. Further evaluation of the learning curve of the different expertise levels will further help new adopters in choosing a successful and comfortable technique. We believe that the techniques described in this paper will help surgeons and eye bankers to select the best option for DMEK graft preparation.

References

1. Trindade BLC, Eliazar GC. Descemet membrane endothelial keratoplasty (DMEK): an update on safety, efficacy and patient selection. *Clin Ophthalmol* 2019;13:1549–57.
2. Donaghy CL, Vislissel JM, Greiner MA. An introduction to corneal transplantation [Internet]. EyeRounds.org [2015 May 21]; Available from: <http://EyeRounds.org/tutorials/cornea-transplant-intro/> [accessed December 27, 2020]
3. Dapena I, Ham L, Melles GR. Endothelial keratoplasty: DSEK/DSAEK or DMEK—the thinner the better? *Curr Opin Ophthalmol* 2009;20:299–307.
4. Newman LR, DeMill DL, Zeidenweber DA, et al. Preloaded Descemet membrane endothelial keratoplasty donor tissue: surgical technique and early clinical results. *Cornea* 2018;37:981–6.
5. Borovik AM, Perez M, Lifshitz T, et al. Peripheral blunt dissection: using a microhoe-facilitated method for Descemet membrane endothelial keratoplasty donor tissue preparation. *Cornea* 2017;36:1270–3.

6. Yoeruek E, Bayyoud T, Hofmann J, Szurman P, Bartz-Schmidt K-U. Comparison of pneumatic dissection and forceps dissection in Descemet membrane endothelial keratoplasty: histological and ultrastructural findings. *Cornea* 2012;31:920–5.
7. Melles GRJ, Ong TS, Ververs B, van der Wees J. Descemet membrane endothelial keratoplasty (DMEK). *Cornea* 2006;25:987–90.
8. Parekh M, Borroni D, Ruzza A, et al. A comparative study on different Descemet membrane endothelial keratoplasty graft preparation techniques. *Acta Ophthalmol* 2018;96:e718–26.
9. Bhogal M, Balda MS, Matter K, Allan BD. Global cell-by-cell evaluation of endothelial viability after two methods of graft preparation in Descemet membrane endothelial keratoplasty. *Br J Ophthalmol* 2016;100:572–8.
10. Birbal RS, Sikder S, Lie JT, Groeneveld-van Beek EA, Oellerich S, Melles GRJ. Donor tissue preparation for Descemet membrane endothelial keratoplasty: an updated review. *Cornea* 2018;37:128–35.
11. Brissette A, Conlon R, Teichman JC, Yeung S, Ziai S, Baig K. Evaluation of a new technique for preparation of endothelial grafts for Descemet membrane endothelial keratoplasty. *Cornea* 2015;34:557–9.
12. Sella R, Einan-Lifshitz A, Sorkin N, Chan CC, Afshari NA, Rootman DS. Learning curve of two common Descemet membrane endothelial keratoplasty graft preparation techniques. *Can J Ophthalmol* 2019;54:467–72.
13. Ruzza A, Parekh M, Salvalaio G, et al. Bubble technique for Descemet membrane endothelial keratoplasty tissue preparation in an eye bank: air or liquid? *Acta Ophthalmol* 2015;93:e129–34.
14. Bala C. Pedicle Descemet membrane endothelial keratoplasty performed using a new corneal punch. *J Cataract Refract Surg* 2020;46:953–60.
15. Tzamalīs A, Vinciguerra R, Romano V, et al. The “yogurt” technique for Descemet membrane endothelial keratoplasty graft preparation: a novel, quick and safe method for both inexperienced and senior surgeons. *Cornea* 2020;39:1190–5.
16. Muraine M, Gueudry J, He Z, et al. Novel technique for the preparation of corneal grafts for descemet membrane endothelial keratoplasty. *Am J Ophthalmol* 2013;156:851–9.
17. Parekh M, Ruzza A, Romano V, et al. Descemet membrane endothelial keratoplasty learning curve for graft preparation in an eye bank using 645 donor corneas. *Cornea* 2018;37:767–71.
18. Redbrake C, Salla S, Frantz A, et al. Metabolic changes of the human donor cornea during organ-culture. *Acta Ophthalmol Scand* 1999;77:266–72.
19. Komuro A, Hodge DO, Gores GJ, et al. Cell death during corneal storage at 4°C. *Invest Ophthalmol Vis Sci* 1999;40:2827–32.

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