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The "Yogurt" Technique for Descemet Membrane Endothelial Keratoplasty Graft Preparation: A Novel Quick and Safe Method for Both Inexperienced and Senior Surgeons

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Purpose: To describe and evaluate the efficacy and safety of a novel technique to prepare Descemet membrane endothelial keratoplasty (DMEK) donor grafts using a newly designed partial-thickness hinge punch.

Methods: The novel punch has a circular guarded blade missing 1 clock hour, creating an uncut hinge on the donor cornea. In addition, 2 straight cuts are made by the punch perpendicular to the edge of trephination toward the trabecular meshwork in the hinge area. After the donor corneoscleral rim is positioned endothelial side up, a partial-thickness trephination is performed avoiding any rotational movements. Descemet membrane is lifted from Schwalbe line in the hinge area, and DMEK graft is peeled after desired marking without further preparation.

Results: Three surgeons of different experience levels on DMEK (senior/independent/fellow) initially applied the new technique in 18 research corneas, divided into equal groups. Two failures in graft preparation were noted, defined as radial tears extending $\geq$0.5 mm. The mean preparation time was 6.21 $\pm$ 1.45 minutes. No statistically significant differences were noted in success rate, duration, and endothelial cell loss (ECL) between surgeons ($P > 0.05$). ECL was evaluated as an average of 5 readings on randomly selected graft areas, not including graft periphery. Fifteen additional research corneas were stripped by 1 single user in an eye bank setting. No tissue loss was recorded, whereas ECL and mortality rate remained unaffected after preparation ($P = 0.64$ and $P = 0.72$, respectively).

Conclusions: This new DMEK graft preparation technique, simulating the opening of a yogurt cup, seems to be a safe and an efficient method, providing shorter preparation time and low failure rates independent of surgeon's experience level.

Key Words: Descemet membrane endothelial keratoplasty, DMEK graft preparation, preparation technique

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Fuchs endothelial corneal dystrophy and pseudophakic corneal edema have lately become the main indicators for corneal transplantation, especially after the establishment of phacoemulsification as the gold standard in cataract surgery.[1,2] The management of corneal endothelial failure has changed substantially in the past years, moving from penetrating keratoplasty (PK) to more elective lamellar procedures.[3] Endothelial keratoplasty (EK) is now the standard of care because it offers better outcomes and faster visual recovery than PK.[4] According to the Eye Bank Association of America, the number of PK procedures performed in the United States has significantly decreased from 2005 to 2015, whereas EK has been the most commonly performed corneal transplantation and continues to increase.[5]

Since its introduction by Melles et al in 2006, Descemet membrane endothelial keratoplasty (DMEK), although technically more challenging than other EK procedures, has gained in popularity becoming the first-line treatment of many corneal surgeons in the management of corneal endothelial dysfunction.[6,7] According to recent surveys, one of the most common barriers among corneal surgeons to uptake EK is anxiety related to tissue preparation.[8] The first step for successful surgery is to master a donor tissue–harvesting technique that can consistently provide an intact graft to transplant.[9]

It has lately been established that endothelial graft preparation techniques are diverse and feature different strengths and weaknesses.[10,11] Although many techniques have been proposed so far, there is no consensus on a so-called standardized method. The most commonly used techniques include pneumatic dissection,[12] no-touch technique,[13,14] submerged hydroseparation technique,[15] liquid separation technique,[16] and few more that have been thoroughly evaluated earlier.[10] Although the stripping method has been the most widely adopted,[10,17] it can sometimes be difficult, particularly when Descemet membrane (DM) is strongly adherent to the underlying stroma, making it occasionally challenging to identify a cleavage plane to start DM stripping. This phenomenon, more commonly seen in younger donors, could be attributed to variation in the intermediary "Bowman-like" zone of randomly arranged collagen fibers at DM–stroma interface, which have been shown to serve as an anchoring function between DM and posterior stroma.[18,19] To our knowledge, although most methods applied worldwide have fair to excellent success rates, there is currently no corneal trephine system that overcomes all difficulties. Therefore, novel techniques and modalities might be of great value to further increase safety and efficacy in DMEK graft preparation.

The aim of this study was to present a novel method for DMEK graft preparation, evaluating its safety and efficacy when used by surgeons of different experience levels. The new method is called the "Yogurt technique" because it resembles the opening of a yogurt cup using a newly designed corneal punch.

MATERIALS AND METHODS

Description of the Technique (Yogurt Technique) and the Hinge-Guarded Punch

See Video 1 (Supplemental Digital Content 1, http://links.lww.com/ICO/B46) for a description of the Yogurt technique.

- The donor corneoscleral disc is grasped carefully with toothed forceps from the scleral rim, and it is positioned endothelial side up on the cutting block of the device.
- It is important that the donor disc is properly centered on the cutting block, ensuring that the limbus is equally distanced from the peripheral markings of the cutting block 360 degrees (Fig. 1A).
- Vacuum is applied by means of a spring-loaded syringe attached to the cutting block (applying negative pressure) to secure position and stabilization of the corneoscleral disc.
- Trypan blue solution 0.4% (Sigma-Aldrich, Deisenhofen, Germany) is applied on the endothelial side and left for 20 seconds in place to stain the endothelium/DM and facilitate better visualization of the procedure.
- Trypan blue solution is rinsed off with a balanced salt solution (BSS; Alcon Laboratories, Fort Worth, TX) and using a triangle ophthalmic sponge along the periphery avoiding any contact to the endothelium.
- A partial-thickness trephination with the 100-μm guarded punch blade is performed avoiding any rotational movements (Fig. 1B).
- The above-described DMEK punch has a circular guarded blade missing 1 clock hour, creating an uncut hinge on the donor cornea (Fig. 1C).
- After partial-thickness trephination, the donor disc is stained again for 20 seconds with trypan blue solution 0.4% and then rinsed off with BSS.
- The uncut hinge of approximately 40 degrees arc is being identified and brought opposite to the surgeon's field at the 12-clock hour.
- In addition, during punching, 2 straight cuts are made almost perpendicular to the edge of the circular cross section toward the trabecular meshwork in the hinge area. The perpendicular cuts are also of partial thickness by means of a guarded 100-μm blade.
- A nonsharp, pointed instrument (eg, Sinskey hook) is used to identify the end of DM at the level of Schwalbe line in the uncut hinge area (Fig. 1D).
- DM with overlying endothelium is peeled off from the underlying corneal stroma using a curved spatula or a crescent blade (Fig. 2A).
- DM peeling is performed carefully beyond both angles of the hinge (the 2 ends of the circular cross-section), taking care to avoid inducing any tears to the graft (Fig. 2B).
- The peeled edge is placed back using BSS, and thereafter, the graft is stained again with trypan blue and rinsed off.
- The detached hinge is being cut with a blade to leave only an orthogonal triangle part that will act as marking when the graft is placed in the recipient's eye, allowing identification of correct graft orientation. The hypotenuse of the orthogonal triangle created lies clockwise to the right (90 degrees) angle, so that when inserted in the anterior chamber and unfolded, it should appear anticlockwise as the endothelial side should be facing downward (Fig. 2C).
- The DMEK graft is grasped with forceps (tying, jewelers, or other DMEK forceps) from the triangle marking and further stripped in a single-peel technique (Fig. 2D).

Experimental Evaluation of the Novel Technique

The new technique was initially evaluated on corneas provided by Manchester Eye Bank (Manchester Eye Tissue Repository; NHS, Manchester, United Kingdom) not suitable for transplantation because of low endothelial cell density (ECD, <2200 cells/mm^2). Three surgeons with different levels of experience applied the new technique using 18 donor corneas, divided into equal groups (6 each). A senior surgeon ($\geq$200 DMEK procedures performed), an independent surgeon ($\geq$50 but $<$200 DMEK procedures performed), and a corneal fellow ($<$50 DMEK procedures performed) participated in the study. The donor tissues were randomly assigned to surgeons. Fifteen additional research corneas were stripped by 1 single user (A.T.) in the setting of an eye bank, and all tissues were evaluated before and after preparation by a masked cell biologist to assess endothelial cell mortality and ECD.

To calculate mortality rate and ECD after preparation, all grafts were stripped until almost 90% from the donor underlying stroma, and then, they were placed back with

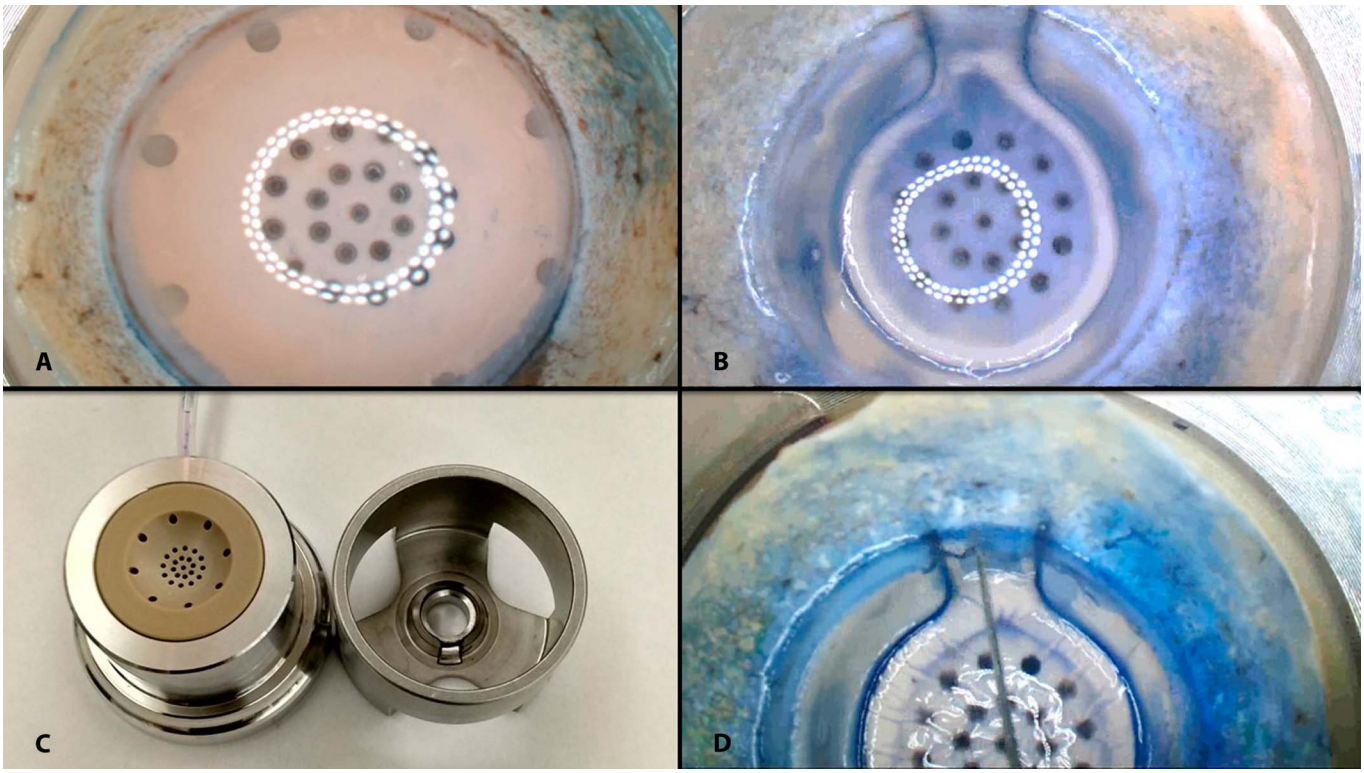

FIGURE 1. A, Corneal graft centered on the cutting block endothelial side up. B, Configuration of cutting pattern after partial-thickness trephination with the guarded hinge punch. C, Novel DMEK hinge punch featuring a circular guarded blade missing 1 clock hour, creating an uncut hinge on the donor cornea and perpendicular cuts to the periphery. D, Identification of DM anatomical end at the level of Schwalbe line in the uncut hinge area with a Sinskey hook. (The full color version of this figure is available online at www.corneajrnl.com).

BSS to facilitate examination under the optical microscope by the masked cell biologist. The diameter of the circular blades used in the study was 8 mm in all cases.

All graft preparations were recorded and further analyzed. The following measures were used to evaluate the safety and efficacy of the new technique:

- Tissue loss: *Absolute success* was defined as preparation without any radial tears, *relative success* as preparation with 1 radial tear less than 0.5 mm in length, and *failure* as multiple peripheral tears or a single large one extending more than 0.5 mm.
- Preparation time: The time of each preparation (in minutes and seconds) was recorded by an independent rater, and, thereafter, it was confirmed by viewing the video recordings. The timer was started on placement of the donor tissue on the cutting block, and the end point of the preparation was defined as the full reattachment of the 90% stripped DMEK graft to the underlying donor stroma.
- Endothelial cell loss (ECL): Corneal grafts were evaluated by a masked cell biologist before and after preparation. All tissues were initially stained using trypan blue (0.4%) for 20 seconds and washed with phosphate-buffered saline (PBS; Sigma-Aldrich). The corneas were placed in a sterile Petri dish containing a hypotonic sucrose solution (to increase definition of cell borders) with the epithelium uppermost and examined at ×100 magnification of an inverted microscope (Primovert; Zeiss, Switzerland). The tissues after trypan blue staining were washed with PBS before Hoechst, ethidium homodimer, and calcein AM staining. Approximately 4 μL of Hoechst 33342 (Thermo Fisher Scientific, Rochester, NY), 4 μL of ethidium homodimer EthD-1, and 2 μL of calcein AM (Live/Dead viability/cytotoxicity kit; Thermo Fisher Scientific) were mixed in 1 mL of PBS. From the final solution, 300 μL was directly added on the endothelium of the DMEK tissue resting on the cornea and incubated at room temperature in the dark for 30 minutes. Both the ECD and cell death were counted before and after DM stripping. Mortality rate and ECD were measured by manually counting the cells using a 10 × 10 reticule fixed inside the eyepiece of an inverted optical microscope (×100 magnification, Primovert; Zeiss). Five readings were made on randomly selected graft areas, and the average was recorded. The damage induced by trephination to the graft edges was not evaluated.

RESULTS

The novel DMEK graft preparation technique was initially applied in 18 research corneas by 3 surgeons of different experience levels (6 cases each). The average age of donors was 69.7 ± 9.2 years (range = 58–79 years), and

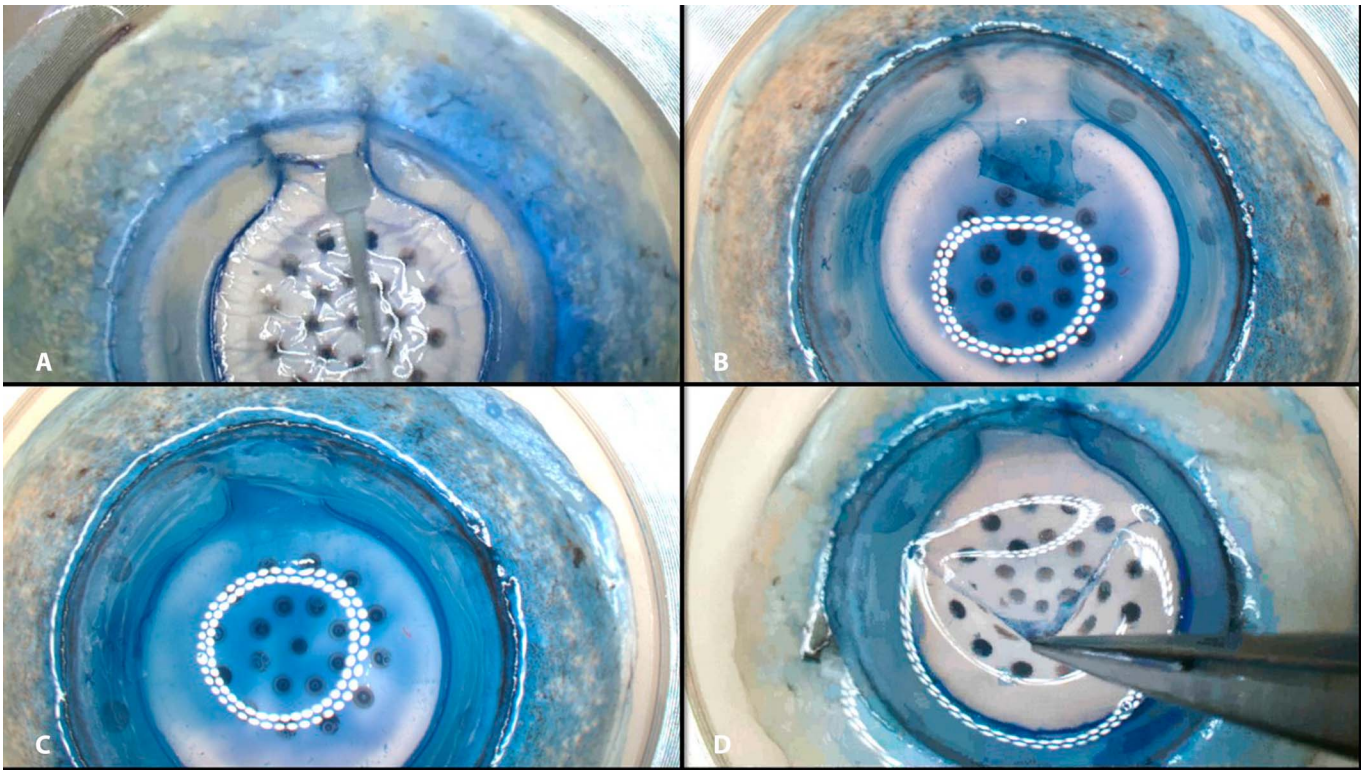

FIGURE 2. A, Peeling of DM with overlying endothelium in the hinge area using a crescent blade. B, DM peeled beyond the angles of the hinge. C, Orthogonal triangle marking. D, DMEK graft stripped using tying forceps in a single-peel technique. (The full color version of this figure is available online at www.corneajrnl.com).

the mean endothelial cell count was 1892.4 ± 156.3 cells/mm^2. Five donors (27.8%) had a recorded history of diabetes mellitus, whereas 11 (61.1%) suffered from arterial hypertension. No differences were noted in any of the donor tissue characteristics between surgeons ($P > 0.05$). Further details on donor tissues are provided in Table 1. All surgeons had no previous exposure to the above-described technique and were asked to perform it according to the above-mentioned instructions.

Tissue loss was recorded in 1 case (independent surgeon), whereby a radial tear measuring half of the diameter of the DMEK graft was noticed during stripping. One more case was considered as failure showing a 1.5-mm radial tear (corneal fellow). Success rate of graft preparation was subsequently 88.9% (16/18 cases). Absolute success was noted in 15 cases (83.3%), whereas in 1 case (5.6%), a small peripheral radial tear (0.4 mm) was induced, and preparation was finished without further complications. No statistically significant difference was found between surgeons regarding tissue loss (χ^2 test, $P = 0.56$, contingency coefficient = 0.378).

The time needed for graft preparation ranged between 3.2 and 9.1 minutes, yielding an average of 6.21 ± 1.45 minutes, not differing significantly between surgeons of different experience levels (analysis of variance, $P = 0.39$). Further details on graft preparation by each surgeon are provided in Table 2. The mean preparation time using

TABLE 1. Donor Tissue Characteristics and Comparison Between Surgeons

	Total	Senior Surgeon	Independent Surgeon	Corneal Fellow	*P*
Age (yrs)	69.7 ± 9.2	68.3 ± 8.6	70.7 ± 9.6	70.2 ± 9.7	0.89*
Sex	10 M/8 F	3 M/3 F	4 M/2 F	3 M/3 F	0.79†
Death to preservation time (h)	12.1 ± 2.3	11.8 ± 2.4	12.1 ± 1.9	12.4 ± 2.7	0.94*
Death to preparation time (d)	12.9 ± 4.6	13.1 ± 5.4	13.2 ± 4.9	12.4 ± 4.3	0.61*
ECD (cells/mm^2)	1892.4 ± 156.3	1912.6 ± 141.2	1881.2 ± 167.5	1883.4 ± 149.7	0.93*
Diabetes mellitus	5/18	2/6	1/6	2/6	0.76†
Arterial hypertension	11/18	4/6	4/6	3/6	0.79†

*Assessed with analysis of variance test.
†Assessed with χ^2 test.
F, female; M, male.

TABLE 2. Success Rates (With Percentages in Parentheses), Preparation Time (in Minutes), and ECL (%) After DMEK Graft Preparation

	Total	Senior Surgeon	Independent Surgeon	Corneal Fellow	*P*
Absolute success	15/18 (83.3)	5/6 (83.3)	5/6 (83.3)	5/6 (83.3)	0.56*
Relative success	1/18 (5.6)	1/6 (16.7)	0/6 (0)	0/6 (0)	
Failure	2/18 (11.1)	0/6 (0)	1/6 (16.7)	1/6 (16.7)	
Preparation time	6.21 ± 1.45	5.53 ± 1.45	6.58 ± 1.24	6.51 ± 1.62	0.39†
ECL	2.67 ± 1.98	2.49 ± 1.81	2.74 ± 2.31	2.78 ± 2.19	0.92†

*Assessed with χ^2 test.
†Assessed with analysis of variance test.

the novel technique was plotted against the preparation time that the same surgeons needed to perform 12 successful DMEK graft preparations (4 each) using the scoring method. The analysis showed that the Yogurt technique resulted in a quicker preparation not only in cases of absolute success but also in cases with small radial tears ($P < 0.001$ and $P = 0.02$, respectively).

The novel method was additionally evaluated in another eye bank setting (Veneto Eye Bank, Mestre, Italy). DMEK graft preparation was performed by a single user (A.T.) in 15 research corneas. The donor mean age and ECD was 71.6 ± 5.4 years and 1723.3 ± 182.3 cells/mm^2, respectively. No tissue loss or failure was noticed because absolute success was recorded in 14 of 15 cases (93.3%) and a minor radial tear (<0.5 mm, relative success) in 1 case (6.7%). Graft preparation time varied between 4.17 and 11.09 minutes with a mean of 5.86 ± 2.28 minutes. Cell mortality did not show any statistically significant difference before and after preparation (3.81% ± 3.8% vs. 4.57% ± 5.2%, respectively, $P = 0.72$, Student *t* test). ECD did not show any significant decrease after preparation (ECD = 1684.4 ± 260.7, $P = 0.64$), whereas cell loss yielded an average of 2.31% ± 4.3%.

DISCUSSION

DMEK has been a breakthrough in the management of corneal endothelial pathologies in the past decade, offering a quick rehabilitation of vision and a superior final visual outcome in comparison with previous surgical techniques.[1–7] A safe and efficient preparation method of donor grafts is a strong prerequisite for successful surgery, and in a recent survey, half of corneal surgeons participating in a DMEK wet laboratory expressed that anxiety related to tissue preparation is one of the major perceived barriers to uptake DMEK.[8]

Many techniques for DMEK graft preparation and modifications of them have been proposed so far, yielding varying results for safety and efficacy.[9–11] In a recent updated review, Birbal et al[10] reviewed 25 techniques described in the literature from 2006 to 2018. In most techniques, the time needed for graft preparation was not reported, failure rates varied between 0% and 17%, and ECL (only reported in a few studies) was mostly less than 10%.

Although several authors have already tried to propose a standardized technique for DMEK graft preparation,[9,12–14,17] there is still a great variation in techniques used by corneal surgeons and eye bank technicians all over the world. The main reason for this disparity is probably the fact that every user tends to adopt the technique that is most convenient and doable in their setting. Therefore, ease of use, a short learning curve, and a reasonable cost of instruments/disposables are important factors in the uptake of a new technique.

One of the most popular standardized protocols uses the application of a partial-thickness trephination and a cleavage hook to identify the cleavage plane and to de-adhere the periphery of the graft from the underlying stroma before peeling off DM. This might occasionally be challenging due to strong adherence of DM to the underlying stroma after punching. In contrary, DM with the overlying endothelium can be very easily peeled off from Schwalbe line because this is the anatomical end point of it. This has already been proposed as a no-touch technique to peel DM from Schwalbe line or the trabecular meshwork at 360 degrees.[9] However, this preparation method requires time and increases the risk of unintentional damage to the graft because it is grasped at many points with forceps.

The novel technique described in this study has been designed to overcome some of the aforementioned main difficulties and, importantly, is reproducible. We have named the new method Yogurt technique because DMEK graft preparation resembles the opening of a yogurt cup. The technique depends on a specially designed punch that generates a circular graft but leaves a hinge at the limbus. Thus, a graft can be punched from the donor cornea, and Descemet–endothelium complex can be peeled by grabbing the tab generated by the uncut hinge that connects to the limbus. In that way, the problem of identifying a cleavage plane to peel the graft after punching is overcome because DM is stripped easily from its natural end that lies toward Schwalbe line.

Furthermore, this technique is much faster in comparison with previously applied methods because it does not demand circumferential (360 degrees) detachment or peeling of DM edge.[10,20] Although all radial tears recorded in our cohort were not associated with leaving the graft edges unpeeled before striping the graft, one could always try to do so if they feel it provides extra safety to the preparation process. Moreover, it has a very low learning curve because it does not require special skills and can be easily performed by both experienced and inexperienced surgeons/users.

Marking of the graft is helpful for corneal surgeons performing DMEK because graft orientation is often difficult to identify and a graft placed upside down can definitely lead to surgical failure and the need for reoperation.[2] Various methods have been suggested for marking of the graft.[7,8,10]

Most use direct marking such as cutting of an inner triangle or other asymmetric marking removing part of the graft periphery within the graft leading to ECL. This problem is overcome with the new technique because marking and manipulation of the graft is only performed through an orthogonal triangle tissue that is outside the circular DMEK graft. The technique has already been applied to real surgery conditions, leading to no further complications or increase of graft detachment rate.

Finally, cost-efficacy is crucial for a new method to be established. One further advantage of DMEK over previous lamellar keratoplasties such as Descemet stripping automated EK is the fact that it does not require expensive equipment, such as a microkeratome, to prepare the endothelial graft. The newly described technique might be less expensive compared with some other methods that use 2 corneal punches instead of 1. However, one should always keep in mind that a designated punch might have additional costs.

Furthermore, when only 1 punch is used, this minimizes the option for the operator to start peeling from another point if a radial tear occurs and re-punch in an area devoid of tears or damage. The new DMEK punch does not limit the location of the graft to the central cornea, and grafts could also be offset to avoid specific areas. One just needs to make sure there is enough space for the hinge formation toward the periphery and the trabecular meshwork.

The results of our study show that this technique has a good safety and efficacy profile and might provide another option for surgeons and eye bank technicians when choosing a suitable method for DMEK graft preparation. However, it has several limitations that should be kept in mind.

Above all, ECL and mortality rate in this study have been evaluated in each case only as an average of 5 measurements on randomly selected graft areas, instead of examining the whole graft, as it has already been described in other cohorts.[21] This analysis might be prone to bias, and it probably underestimates the amount of ECL because we did not consider damage induced to the periphery of the graft. In fact, cell death caused by the punch alone at the edge of the trephination likely contributes to a significant proportion of the overall damage to the total graft. Therefore, the rates of ECL and mortality reported in this study should be considered carefully and only as a measure to compare between different type of users in a clinically oriented study and would not be appropriate to conclude on the real extent of endothelial cell damage in a laboratory study. Consequently, DMEK graft preparation time should also be taken into consideration thoughtfully, when ECL is not properly measured.

Furthermore, variations in donor tissue characteristics, and especially the proportion of diabetic tissues, might affect success rates in DMEK preparation.[22,23] However, in our cohort, no significant differences were noted between surgeons in any of these features, strengthening our results.

In conclusions, the novel Yogurt technique by means of a guarded hinge punch, resembling the opening of a yogurt cup, seems to be an easy, quick, efficient, and safe method to prepare DMEK grafts independently of surgeon's experience level. Further prospective studies are needed to evaluate its efficacy in real-time surgical conditions comparing it with other preparation techniques.

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