Advances in endothelial keratoplasty

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Part I

Donor Tissue Preparation

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Chapter 2

Donor Tissue Preparation for Descemet Membrane Endothelial Keratoplasty: An Updated Review

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ABSTRACT

Purpose: To provide an overview of the current literature on donor tissue preparation for Descemet membrane endothelial keratoplasty (DMEK).

Methods: A comprehensive database search without date restrictions was performed in Pubmed and in The Cochrane Library in May, 2017. Keywords included *Descemet membrane endothelial keratoplasty, corneal transplantation, graft, harvest, dissection, preparation, endothelial cell and endothelial cell density.* Articles aiming to describe or evaluate a technique for DMEK graft preparation were considered eligible and were included in this review.

Results: A graft dissection technique that provides consistent tissue qualities and a low risk of preparation failure is essential for surgeons and eye banks preparing DMEK tissue. Various techniques have been described aiming to facilitate DMEK graft dissection, including manual dissection, pneumatic dissection, and hydrodissection. All show a trend toward a no-touch technique, e.g. without direct physical tissue manipulation during tissue harvesting, as a potential ideal approach to minimize graft damage.

Conclusion: An overview of the current harvesting techniques available for DMEK may benefit corneal surgeons as well as eye banks in choosing the best approach for each specific user.

INTRODUCTION

In the past decade, endothelial keratoplasty (EK) has rapidly replaced penetrating keratoplasty (PK) for the management of corneal endothelial disorders.[1] Descemet membrane endothelial keratoplasty (DMEK), the latest refinement of EK, allowed for further evolution of the field by enabling selective replacement of bare Descemet membrane (DM) with its endothelial layer.[2] Providing near-perfect restoration of the corneal anatomy, DMEK yielded faster visual rehabilitation,[3-6] improved visual outcome,[3-5] and lower graft rejection rates compared with earlier types of EK.[7-10]

Although DMEK is gaining widespread acceptance and numbers are showing a continued increase of DMEK procedures to 1522 in 2013, 2865 in 2014 and 4694 in 2015 in the United States alone,[11] the procedure is more challenging in preparing and handling the delicate donor graft.[12] Uptake of the procedure may be facilitated by proper training and choosing the most feasible harvesting technique that yields reproducible graft qualities. Since several techniques have been described for DMEK graft preparation, this review aims to provide an overview of the current literature regarding donor tissue dissecting techniques for DMEK and to provide corneal surgeons and eye banks with a useful reference for technique comparison and selection in a given setting.

MATERIALS AND METHODS

A comprehensive database search without date restrictions was performed in PubMed and The Cochrane Library in May 2017. Keywords included *Descemet membrane endothelial keratoplasty, corneal transplantation, graft, harvest, dissection*, *preparation, endothelial cell and endothelial cell density.* Search results were limited to studies published in English, studies on human corneas and full text available. Title and/or abstract of all records were screened for relevance. Articles aiming to describe or evaluate a technique for DMEK graft preparation were considered eligible and were included in this review, which resulted in inclusion of 50 articles on this topic.

RESULTS

Surgical technique

Current and evolving techniques to procure donor tissue for DMEK show a trend toward a no-touch technique, in which there is no direct physical graft handling, as a potential ideal approach to minimize endothelial cell loss.[12] Harvesting techniques may broadly be classified into those based on manual peeling and those aiming to achieve detachment of DM by either injecting air or liquid between DM and the posterior stroma (i.e., the pre-DM plane).

Manual dissection

Manual dissection entails carefully peeling away DM with its endothelial layer from the underlying stroma. Melles et al.[13] pioneered this technique describing superficial trephination of the posterior stroma and stripping of DM with the corneoscleral rim submerged in a balanced salt solution (BSS). Zhu et al.[14] described applying 4 incisions through DM to facilitate stripping. In 2008, Lie et al.[15] introduced the basis for the current technique. After mounting the donor corneoscleral rim on a custom-made fixation device with the endothelial side up, DM was cut anterior to the trabecular meshwork and pushed centrally. Grasping the outer edge of the graft, DM was loosened over 180 degrees and stripped for two-thirds. By submerging the rim in BSS, superficial trephination and complete stripping of DM were facilitated, after which the isolated graft spontaneously formed a roll with the endothelial layer facing outward. Groeneveld-van Beek et al.[16] modified the technique into the standardized 'no-touch' technique, in which DM with the adjacent trabecular meshwork is loosened over 360 degrees rather than over 180 degrees and trephined on a soft contact lens instead of on the anterior cornea.

Giebel and Price reported on the submerged cornea using backgrounds away (SCUBA) technique, which involved manually harvesting DM with the rim submerged in Optisol or BSS.[17,18] Tenkman et al.[19] used a blunt Y-hook rather than tying forceps to score the DM. After circumferentially lifting the scored edge of DM with a microfinger and grasping it with Tubingen forceps, DM was partially peeled in 4 quadrants with the central area still attached (i.e. the Corridor method). Repositioning of the graft on the underlying stroma preceded superficial trephination and complete peeling of the tissue.

Kruse et al.[20] used a razor blade to eliminate tissue outside an 8-mm marked zone to prevent tearing on the inside of the demarcated line and two forceps

instead of one for stripping, aiming to minimize formation of folds and surface tension affecting the endothelial cells. In 2013, Yoeruek et al.[21] described the use of two untoothed curvilinear forceps instead of one traditional forceps to facilitate stripping. Sikder et al.[22] reported on removing most of the donor stroma overlying DM with a microkeratome and removing the residual stroma with a Barraquer sweep-assisted lamellar dissection.

Tausif et al.[23] described a technique in which partial trephination of DM was followed by trypan blue-staining of the scored edge and a partial circumferential dissection of DM. Previously marked microtears were positioned at 6 o'clock representing the hinge of the flap. Using tying forceps and starting at 12 o'clock, DM was dissected by peeling toward the hinge. Peeling was stopped at 2 mm from the score mark, after which the center of the hinge was marked with a skin marker.

Pneumatic dissection

Pneumatic dissection entails injecting air into the deep stroma to obtain detachment of DM at the level of DM or pre-DM (Dua's layer), a concept which was first described by Anwar and Teichmann for anterior lamellar keratoplasty.[24] Modifications in the technique have allowed its use for DMEK. In 2005, Ignacio et al.[25] described mounting a corneoscleral rim on an artificial anterior chamber to apply negative pressure using air before trephining DM inside the Schwalbe line. Subsequently, positive pressure was applied to separate the peripheral part of DM from the central part. The graft was undermined from the underlying stroma using a blunt spatula. Venzano et al.[26] reported on trypan blue-staining of the endothelium to visualize needle positioning before introducing a big bubble. Zarei-Ghanavati et al.[27] described injecting air into a cornea with the endothelial side up rather than with the epithelial side up, followed by aspiration of the previously injected air to facilitate collapse of the big bubble; this technique was referred to as 'the Reverse big-bubble technique'.[28]

In 2010, Busin et al.[29] described microkeratome-assisted removal of two-thirds of the anterior stroma prior to air-injection. The air bubble was left inflated until the time of surgery. Another extension of pneumatic dissection 'DMEK with a stromal rim' (DMEK-S) was described by Studeny et al.[30] After introducing an air bubble into the pre-DM plane and removing 80% of the stroma, a circle of 6 mm in diameter was demarcated and the letter S was written on the stromal

rim. The bubble was then entered with scissors, and the remaining central part of the stroma was eliminated, resulting in a graft with a stromal rim.

In 2014, Agarwal et al.[31] reported on a pneumatic dissection technique for 'Pre-Descemet endothelial keratoplasty' (PDEK), a form of DMEK in which pre-DM layer, Descemet membrane and endothelium are transplanted. Air was introduced into a cornea with the endothelial side up to establish an air bubble between pre-DM and stroma or pre-DM and DM. Immediately after a big bubble was achieved, the bubble was marked with a trephine and trypan blue was injected to improve visualization of the graft. Corneal scissors were used to ensure complete detachment of the graft along the circumference of the trephination.

Hydrodissection

Hydrodissection entails applying a pressurized fine stream of liquid, either culture medium or BSS, into the pre-DM plane to obtain dissection of DM. For the procedure to be successful, a proper injection site and correct intensity of the applied pressure are essential.

In 2013, Muraine et al.[32] described a technique in which superficial trephination of DM was performed over 330 degrees rather than over 360 degrees. Using a spatula or Troutman forceps, the peripheral endothelium was detached on either side of the uncut 30 degrees to create a liftable flap and enter the pre-DM plane with a needle; culture medium or BSS was injected to obtain detachment of DM.

Salvalaio et al.[33] reported on the 'standardized submerged hydroseparation technique' (SubHys-technique), which involved introducing a bevel-up needle into a cornea submerged in organ culture until the bevel was completely inserted. Approximately 0.3 mL organ culture was injected to separate DM from the stroma. Additional culture medium was injected with increased pressure aiming to establish a bubble >10 mm in diameter. Thereupon, the cornea was mounted onto an artificial anterior chamber with the epithelial side up to trephine and excise the anterior cornea. The residual peripheral stroma was removed using microscissors. In 2016, Szurman et al.[34] described a 'no-touch' liquid bubble technique. After creating a sharp incision under the iris base, the Schlemm canal was entered to loosen the zone of high adherence by tangential dissection with a blunt spatula. Detachment of DM was obtained by injecting a vital dye into the pre-DM plane and simultaneous blocking of

reflux with a surgical pad. After corneal trephination, the donor tissue could be lifted from the stromal side using a spatula with a smooth, rounded olive tip extremity (i.e. olive spatula) to facilitate donor harvesting.

Anatomical analysis

The accuracy of mechanical lamellar dissection may be analysed using hematoxylin and eosin (H&E)-staining or periodic acid-Schiff (PAS)-staining with subsequent light microscopy analysis at respectively, x200 and x400 magnification or transmission electron microscopy and immunohistochemistry.[35,36]

McKee et al.[35] showed the superiority of PAS-staining over H&E-staining in revealing a sharp distinction between DM and stroma. Using the former method, McKee et al. and Ruzza et al. described residual stroma in all grafts harvested with pneumatic dissection, whereas very low to no residual stroma was reported for grafts prepared with hydrodissection.[35,37] These results may suggest that pneumatic dissection yields a very thin Descemet stripping endothelial keratoplasty (DSEK) graft rather than a DMEK graft.

In 2013, Schlötzer-Schrehardt et al.[36] analyzed 343 grafts and 7 whole corneoscleral rims after respectively, successful and unsuccessful manual peeling of DM (bimanual submerged technique). Transmission electron microscopy and immunohistochemistry revealed that failure to separate DM from the underlying stroma (2%) was due to the presence of ultrastructural peg-like linkages and increased adhesive glycoproteins along the Descemet membrane and stromal interface resulting in extremely strong adhesion of DM to the stroma. No stromal residues were observed after successful tissue preparation. In another study, Sikder et al.[22] used anterior segment optical coherence tomography for imaging of the donor graft, thereby revealing residual stroma underlying the graft.

Graft quality

Descemet grafts may be prepared by the surgeon in the operation room before surgery or one day in advance[17,20] or may be pre-dissected in an eye bank for up to 1 to 2 weeks before surgery.[15,16]

In contrast to surgeon-cut tissue, pre-dissected tissue allows for postprocessing evaluation of the donor graft, providing corneal surgeons with accurate information about the graft before surgery. In vitro assessment of endothelial quality is performed before and after graft preparation, using either light

Table 1. Overview donor tissue harvesting techniques with in vitro outcomes for Descemet membrane endothelial keratoplasty (DMEK).

	Study	Year	Harvesting technique	Number of grafts	Preparation time (min)	Mean graft size (mm)	Average ECD (cells/mm^2)		EC loss due to tissue preparation (%)	Tissue loss due to failed preparation (%)
							Before EDM stripping	After EDM stripping		
Manual dissection	Melles et al.[13]	2002	-	5	N/A	9.0	N/A	N/A	3 (±1)	N/A
	Zhu et al.[14]	2006	-	48	3-5	6-7	N/A	N/A	<3	17
	Lie et al.[15]	2008	Standardized traditional technique	10	N/A	9.5	2701 (±302)	2719 (±322)	4-7	0
	Yoeruek et al.[21] Standard forceps	2013	-	8	11.0#	8.5	N/A	N/A	7 (±3)^	0
	Curvilinear forceps			8	6.4#	8.5	N/A	N/A	3 (±2)^	0
	Giebel and Price et al.[17, 18]	2009	SCUBA technique	72	N/A	8.5-9.0	N/A	N/A	N/A	8
	Kruse et al.[20]	2011	Bimanual submerged technique	80	From 90 to <30	8.0	N/A	2600 (±252)	N/A	1
	Schlötzer-Schrehardt et al.[36]	2013		350	N/A	N/A	N/A	2553(±229)	N/A	2
	Sikder et al.[22]	2011	Microkeratome and Barraquer sweep assisted dissection	N/A	N/A	≥8.5	N/A	N/A	N/A	N/A
	Groeneveld-van Beek et al.[16]	2013	Standardized ('no-touch') technique	62	N/A	9.5	2539 (±120)	2519 (±125)	<5	0
	Livny et al.[39]	-		1075	N/A	N/A	N/A	N/A	N/A	3
	Tenkman et al.[19]	2014	-	263	N/A	8.0 (FED) 9.0 (PPBK or failed EK) <8.5 (Host corneal dm <11.5)	N/A	N/A	N/A	1
	Tausif et al.[23]	2014	-	50	N/A	N/A	2616 (±321)	2676 (±284)	N/A	24

Table 1. Overview donor tissue harvesting techniques with in vitro outcomes for Descemet membrane endothelial keratoplasty (DMEK). (*continued*)

	Study	Year	Harvesting technique	Number of grafts	Preparation time (min)	Mean graft size (mm)	Average ECD (cells/mm^2)		EC loss due to tissue preparation (%)	Tissue loss due to failed preparation (%)
							Before EDM stripping	After EDM stripping		
Pneumatic dissection	Ignacio et al.[25]	2005	-	7	N/A	9.0	N/A	2095 (±704)	8 (±7)	0
	Venzano et al.[26]	2010	Anwar big-bubble technique	16	N/A	Group A N/A. Group B N/A. Group C 7.3	N/A	N/A	Group A: 83 (±10) Group B: 15 (±11) Group C: 3 (±3)	11
	Zarei-Ghanavati et al.[28]	2010	Reverse big-bubble technique	1	N/A	8.5	N/A	N/A	N/A	N/A
	Busin et al.[29]	2010	-	20	N/A	8.1	N/A	N/A	4 (±4) after 7 days	5
	Studeny et al.[30]	2010		20	20-30	8.0	N/A	2888 (±265)	N/A	N/A
	Krabcova et al.[40]*	2011	DMEK-S	12	N/A	N/A	2875 (±222)	2725 (±156)	5	N/A
	Krabcova et al.[41]**	2012		10	N/A	N/A	2249 (±147)	2131 (±157)	5	N/A
	Agarwal et al.[31]	2014	PDEK	5	N/A	7.6	N/A	N/A	N/A	0
	Altaan et al.[42]	2015		10	N/A	8-8.5	996 (±284)	943(±273)	5 (±4)	0
Hydrodissection	Muraine et al.[32]	2013	Liquid bubble technique	12	N/A	N/A	2765 (±256)	2651 (±305) after 3 days	4	4
	Salvalaio et al.[33]	2014	SubHys-technique	30	N/A	11.0	1920 (±223)	N/A	27 after 7 days	0
	Parekh et al.[43]	2014		54	N/A	10.8	2024 (±229)	2018 (±221)	11	0
	Szurman et al.[34]	2016	No-touch liquid bubble technique	86	<3	8.3	N/A	N/A	N/A	1

min= minutes; mm= millimeters; ECD= endothelial cell density; mm^2= square millimeter; EDM= Descemet membrane with endothelial monolayer; EC= endothelial cell; SCUBA= Submerged cornea using backgrounds away; FED= Fuchs endothelial dystrophy; PPBK= Pseudophakic bullous keratopathy; EK= endothelial keratoplasty; dm= diameter; DMEK-S= Descemet membrane endothelial keratoplasty with a stromal rim; PDEK= Pre-Descemet's endothelial keratoplasty; SubHys= Standardized submerged hydro-separation; N/A= not available.

Data are expressed as mean (SD), absolute numbers, or percentages (SD).
Group A = Tissue stored with an inflated air-bubble
Group B = Air-bubble deflated immediately after detachment of the Descemet membrane was obtained
Group C = Descemet membrane with endothelial monolayer was trephined from the endothelial side after deflating the air-bubble

P-value = 0.01
^ *P*-value= 0.04
* First row: endothelial cell density >2500 cells/mm^2; second row: endothelial cell density 2200-2500.
** Endothelial quality assessments were available for a total of 57 grafts.

microscopy for organ-cultured tissues or specular microscopy for cold stored tissues.[38] Endothelial cell morphology and viability are mainly evaluated using provoked swelling with 1.8% sucrose and staining with trypan blue 0.04% to visualize the cell borders and accentuate cells with damaged cell membranes and denuded areas of DM.[15,16] The endothelial cell density and viability of the corneoscleral rim are assessed using either an inverted light microscope or specular microscopy, and digital photographs are acquired. Endothelial cell density is calculated by manual counting using the fixed frame method or using a special image analysis program. After preparation of the DM graft, the roll is evaluated using the same method.

In vitro preparation outcomes

Evaluating the outcomes of the different tissue dissection methods (Table 1), manual peeling is observed to result in the least endothelial cell loss and tissue wastage compared with other dissection methods. Although it seems as if hydrodissection yields the largest mean graft size, the opposite is true. Although the maximum graft size is dependant on the size of the achieved air- of liquid bubble in, respectively, pneumatic dissection or hydrodissection, manual dissection allows the user to obtain the whole DM surface diameter.

Clinical endothelial outcomes

Independent of the applied dissection technique, clinical studies on the endothelial outcome after DMEK reveal a sharp decline of the endothelial cell density within the first 6 months postoperatively, followed by a stable decrease thereafter (Table 2).

DISCUSSION

In this study, we intended to provide an overview of the current and evolving graft dissecting techniques to provide corneal surgeons and eye banks with a useful reference for technique comparison and selection in a given setting. This information may assist them to adopt DMEK as a preferred surgical technique in the management of corneal endothelial disorders. In particular, minimization of endothelial cell loss seems critical to attain reproducibility of graft quality.

Using the standardized ‘no-touch’ technique,[16] preservation of the trabecular meshwork allows for complete stripping of DM and facilitates further handling

Table 2. Clinical endothelial outcome of DMEK harvesting techniques.

	Study	Year	Harvesting technique	Main indications for surgery	Number of grafts	Tissue loss due to failed preparation (%)	Preoperative ECD (cells/mm^2)	Number of grafts	ECD at 6m FU (cells/mm^2)	Mean ECD decrease at 6m FU (%)
Manual dissection	Ham et al.[44]	2009	Standardized traditional technique and/or Standardized ('no-touch') technique	FED	50	N/A	2618 (±201)	35	1876 (±522)	30
	Ham et al.[45]	2009		FED/ BK	26	N/A	2620 (±210)	26	1850 (±540)	29
	Ham et al.[46]	2010		FED/ BK	71	N/A	2630 (±200)	58	1870 (±520)	29
	Parker et al.[47]	2011		FED/ BK	225	N/A	2570	186	1710	34
	Dirisamer et al.[48]	2011		FED/ BK	200	N/A	2560 (±186)	173	1690 (±520)	34
	Baydoun et al.[49]	2012		FED/ BK	300	N/A	N/A	254	N/A	35
	Baydoun et al.[50]	2015		FED/ BK	352	N/A	2533 (±216)	327	1626 (±507)	35
	Peraza Nieves et al.[51]	2017		FED/ BK	500	N/A	2530 (±210)	447	1600 (±490)	37
	Price et al.[17]	2009	SCUBA technique	FED/ BK	72	17	N/A	38	N/A	32
	Guerra et al.[3]	2011		FED/ BK	136	First 40 cases 13%; Subsequent 96 cases:0%	2980 (±252)	124	N/A	31 (3m)
	Feng et al.[52]	2014		FED/ BK	673	N/A	2924	N/A	2147	27
	Deng et al.[53]	2015		FED/ BK	40	N/A	2878 (±212)	27	N/A	23
	Gorovoy et al.[54]	2015		N/A	125	N/A	2740 (±210)	125	2210 (±550) (1y)	19 (1y)
	Kruse et al.[20]	2011	Bimanual submerged technique	FED/ BK	80	1	2600 (±252)	74	1526 (±341) (1m)	N/A
	Laaser et al.[55]*	2011		FED/ BK	37	N/A	2647 (±236)	22	1587 (±373)	N/A
					45	N/A	2515 (±249)	25	1457 (±285)	N/A
	Tourtas et al.[6]	2012		FED/ BK	38	N/A	2575 (±260)	38	1520 (±299)	41
	Schlögl et al.[56]	2016		FED/ BK	97	N/A	2602 (±243)	97	N/A	42 (1m)
	Studeny et al.[30]	2010	DMEK-S	FED	18	N/A	2888 (±265)	18	1608 (±503) (1y)	44 (1y)
	Studeny et al.[57]	2013		FED/ BK	71	6	2907 (±51)	71	1273 (±82) (1m)	44

Table 2. Clinical endothelial outcome of DMEK harvesting techniques. (*continued*)

	Study	Year	Harvesting technique	Main indications for surgery	Number of grafts	Tissue loss due to failed preparation (%)	Preoperative ECD (cells/mm^2)	Number of grafts	ECD at 6m FU (cells/mm^2)	Mean ECD decrease at 6m FU (%)
Pneumatic dissection	Agarwal et al.[31]	2014	PDEK	BK	5	0	N/A	5	1551 (±65) (1m)	N/A
Hydrodis-section	Muraine et al.[32]	2013	Liquid bubble technique	FED	50	4 (replaced with back-up grafts)	2656 (±28)	47	1658 (±43)	N/A

ECD= Endothelial cell density; mm^2= square millimeter; FED= Fuchs endothelial dystrophy; BK= bullous keratopathy; FU= Follow up; d=day; m= month; y= year; PDEK = Pre-Descemet endothelial keratoplasty; N/A= not available;

Data are expressed as mean (SD), absolute numbers, or as percentages (SD).

* First row: patients who had received a graft prepared from a corneoscleral button that had been stored in Optisol-GS at 4C; second row: patients that had received a graft from a corneoscleral button that had been stored in Dulbecco modified eagle medium containing streptomycin and penicillin as well as fetal calf serum or in CorneaMax medium containing streptomycin and penicillin as well as fetal calf serum at 34 C.

of the graft. As the whole DM surface is peeled employing this method, it allows the user to obtain the maximum possible graft size, whereas other techniques might be limited by the size of the achieved bubble in this particular aspect. In addition, by trephining the donor DM on a soft contact lens instead of on the anterior cornea, endothelial cell damage in the trephination area is minimized and the anterior cornea is left intact, rendering it eligible for anterior lamellar keratoplasties. Donor tissue preparation using this technique does not require special or expensive equipment and can be performed at minimal cost, which makes it readily accessible to most corneal surgeons in contrast to techniques that require the use of a microkeratome for partial dissection of the graft.[22,29]

The additional stromal ring in DMEK-S[30] facilitates further handling of the graft, allows for marking of the anteroposterior orientation of the lamellae and minimizes the risk of upside-down grafting, thus avoiding unnecessary technical DMEK failure. The use of a vital dye in the 'no-touch' liquid bubble technique[34] improves homogenous staining of the stromal side of DM and leads to better orientation of the lamellae intraoperatively, thereby saving valuable surgical time as staining has already been performed.

Comparative studies (in vitro) on the feasibility and success of the different dissection methods are scarce and report on small sample sizes.[37,58-60] Manual dissection was shown to result in less tissue wastage compared with pneumatic dissection[58] and hydrodissection[59] and to yield a significantly higher area percentage covered by viable cells compared with hydrodissection (P=0.04) (Table 3).[60] Comparing pneumatic dissection to hydrodissection, the latter method was observed to have higher yield, to require fewer injections to achieve detachment, to require a lower pressure to facilitate big-bubble formation, and to yield a larger maximum graft size.[37] As for the various storage methods, graft storage as a free-floating roll was demonstrated to be superior to partial peeling (90%) and liquid bubble techniques in retaining viable endothelial cells during graft storage.[61]

Studies reporting the clinical endothelial outcome after DMEK for grafts prepared by the different techniques are predominantly available for manually dissected grafts. However, independent of the applied graft preparation technique, an endothelial cell density decrease of 19 to 44% at the 6 month follow-up compared with preoperative values was observed (Table 2), which was then usually followed by a slower decrease. Several explanations have been offered for the endothelial cell density decline within the first post-

Table 3. Comparative studies on dissection methods for DMEK.

Study	Year of Publication	Number of Grafts	Manual Dissection	Pneumatic Dissection	Hydrodissection	P-value
Yoeruek et al.[58]	2012	32				
Graft preparation time			19.7 min	8.8 min	-	<0.00
Graft preparation failure			6.3%	12.5%	-	N/A
Mean ECD decrease			9.9%	8.6%	-	0.55
Apoptotic cells			0.4 (±0.3) %	0.4 (±0.3) %	-	0.91
Ruzza et al.[37]	2015	20				
Graft preparation failure			-	20%	0%	N/A
No. injections required			-	1.9 (±1.1)	1.4 (±0.7)	0.33
Mean quantity required			-	1.1 (±1.3) ml	0.8 (±0.5) ml	0.41
Mean diameter bubble			-	9.1 (±1.7) mm	9.8 (±1.8) mm	0.44
Maximum diameter graft			-	10 mm	11 mm	N/A
Determined percentage of endothelial cell death			-	8.9 (±12.4) %	6.3 (±9.6) %	0.63
Brissette et al.[59*]	2015	20				
Graft preparation time Surgeon Fellow			301 (±85) s 523 (±58) s	-	359 (±83) s 543 (±44) s	0.46 0.33 0.24
Graft tears			0		5	<0.05
Bhogal et al.[60]	2016	16				
Area covered by viable cells			87.7 (±1.4) %	-	75.5 (±5.6) %	0.04

ECD= Endothelial cell density; N/A= not available; ml= millilitre; mm= millimetre. min= minutes; s= seconds.
Data are expressed as mean (SD), as absolute numbers or as percentages (SD).
* Endothelial cell viability was not evaluated on a validated scale.

operative months, including the use of different devices to evaluate the graft in vitro in the eye bank and *in vivo* postoperatively. Several research groups demonstrated a systematic overestimation of the actual pool of viable endothelial cells supplied by eye banks by assessing pan-corneal endothelial cell viability.[38,62,63] Early postoperative cell loss was attributed to failing to recognize areas deprived of endothelial cells and/ or non-viable cells, failing to take into account auxiliary cell loss between the initial cell count in the eye bank and the surgery, including very small endothelial cell samples (50-300) and failing to take account of the 3-dimensional aspect of the graft.[62,63] Limitations of this review are the small sample sizes of the available in vitro studies and that evaluation of endothelial cell density and viability was not uniformly assessed in most of the included studies (Table 1). This is also due to the fact that there is a paucity of methods to accurately evaluate and quantify endothelial cell viability without damaging the graft. Limited availability of adequate data highlights the importance of re-evaluating current practice. Further studies

of larger sample size and long-term follow up are warranted to ensure that potential donor grafts are fully utilized.

DMEK graft dissection techniques are diverse and feature different strengths and weaknesses. Although a single technique does not need to be universally adopted, it is imperative for those preparing DMEK tissue to know the different techniques available and use the best technique for each specific user.

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