Original Article

An approach to reduce Descemet's membrane scrolling: Relevance to Descemet's membrane endothelial keratoplasty (DMEK)

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Purpose: We aimed to determine whether Descemet's membrane (DM) scrolling occurs primarily along the vertical or horizontal axis and establish whether oval trephination along the axis of least scrolling can reduce the grade of the scroll. **Methods:** The longest limbus-to-limbus axis on 28 sclerocorneal discs was taken as the horizontal axis. The horizontal (n = 7) or (right angles to it) vertical (n = 6) axis was marked on DM before peeling it off. The direction and grade of scrolling was observed. Narrow strips (3–4 mm wide) were then cut along the two axes (n = 4 each) and the scrolling pattern was observed. Ellipses (7 × 9 mm) of DM were punched along the two axes (n = 6 each) and the scrolls graded. Immunofluorescent staining for elastin on horizontal and vertical tissue sections from three DM samples was performed. The intensity and thickness of elastin staining were measured. **Results:** Twenty-four (85.72%) DM samples showed scrolling along the horizontal axis, none showed scrolling along the vertical axis, and four (14.28%) samples showed a spiral scroll, regardless of which axis was marked (grade 3.7 and 3.6). Vertically oval discs showed significantly reduced scrolling (grade 1.2) compared to horizontally oval discs (grade 3.5). Narrow strips of DM showed a similar scrolling pattern. Immunohistology showed no difference in any of the parameters examined along the two axes or from the center to the periphery. **Conclusion:** DM scrolls primarily along the horizontal axis. Vertically oval DM samples show minimal scrolling, which can be an advantage in DMEK. Differential scrolling is not determined by the distribution of elastin.

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Endothelial keratoplasty (EK) is the standard procedure for corneal endothelial pathologies that compromise vision and are often associated with persistent corneal edema. Descemet's stripping endothelial keratoplasty (DSEK), Descemet's membrane endothelial keratoplasty (DMEK) and pre-Descemet's endothelial keratoplasty (PDEK) are three established EK procedures, of which DMEK is the gold standard procedure.[1-4] Unlike DSEK, DMEK and PDEK capitalize on the scrolling characteristics of the Descemet's membrane (DM), which allows the EK tissue to form narrow scrolls and be transferred to the recipient eye through narrow wounds (≤2.5 mm). This confers the distinct advantages of a strong wound often not requiring a suture and minimally induced astigmatism. Another distinct characteristic of the scrolling of EK tissue is that it always scrolls with the endothelial cells (ECs) outside. Surgeons have long used this fact to determine the correct orientation of the tissue intraoperatively. The use of the an "S" or "F" mark on the DM side further enhances the ability to correctly orientate the tissue.[5] It has been shown that it is primarily the DM that scrolls, and that it is the DM that confers the scrolling attribute to the PDEK tissue.[6]

It is well known that DM with ECs obtained from younger donors produces tighter scrolls, though the clinical outcomes in experienced hands are similar with young and old donors.[7-9] Various maneuvers involving tapping, stroking, and injecting jets of fluid are described to unscroll the EK tissue in the recipient eye. The time and maneuvers required to unscroll donor EK tissue often determine EC loss during DMEK and PDEK. The tighter the scroll and, consequently, greater the duration and maneuvers required to unscroll it, the more cell loss is likely to happen.[10,11]

In this study, we explored the meridional scrolling characteristic of DM in the vertical and horizontal meridians.

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We report that the DM scrolls almost exclusively along the horizontal meridian (taken as the longest anterior diameter, approximately 3 to 9 o'clock). Based on this observation, we examined the behavior of vertically oval and horizontally oval trephined DMEK tissue and have proposed a protocol for DMEK tissue preparation to reduce the tightness of the scroll, which, in turn, could make it easier to unscroll during DMEK surgery. The study also investigated whether meridional scrolling was determined by the distribution of elastin along the vertical and horizontal meridians.

Methods

The research was conducted in compliance with the local use of human tissue regulations and approvals. A total of 28 human cadaveric sclerocorneal discs (21 research-grade and seven transplant grade-tissues [DM discarded from corneas used for deep anterior lamellar keratoplasty]) were used in this study. The donor age ranged from 45 to 92, with the mean age being 69 years. There were six female and 22 male donors. Causes of death included cancer, stroke, ruptured aneurysm, ischemic heart disease, sepsis, and multiorgan failure. All sclerocorneal discs were stored in organ culture in Eagle's minimum essential medium with 2% fetal bovine serum for 3–4 weeks (transplant-grade tissue) and up to 12 weeks (research-grade tissue).

Determining horizontal and vertical meridians

On the basis that the visible white-to-white diameter is longest in the horizontal meridian, the horizontal meridian was determined by marking the furthest points, that is, the longest diameter, on the anterior surface of the cornea, just inside the limbus of the sclerocorneal disc [Figs. 1 and 2]. This was validated by the following procedure. A clockface was imprinted on the back of a 150-ml gallipot with a central 15-mm hole, as illustrated in Fig. 2. Eight sclerocorneal discs were removed from the culture medium, rinsed in saline, and mounted on a Barron artificial anterior chamber (Corza Medical, Parsippany, NJ, USA) and filled with fluid to a pressure of 18–20 mmHg. The clockface device was placed on the mounted sclerocorneal disc, such that the limbus and the adjacent sclera were visible through the central hole. The longest axis of the sclerocorneal disc, from clock hour to clock hour, was read by two independent observers and recorded individually in a blinded manner. The interobserver agreement on the longest axis was determined using intraclass correlation coefficient (ICC) with Statistical Package for the Social Sciences (SPSS) Version 28 (IBM SPSS Statistics for Windows, Armonk, NY, USA) and was interpreted as follows: (1) <0.50 = poor reliability, (2) 0.5–0.75 = moderate reliability, (3) 0.75–0.90 = good reliability, and (4) >0.9 = excellent reliability.[11] The results were reported as mean with 95% confidence interval (CI).

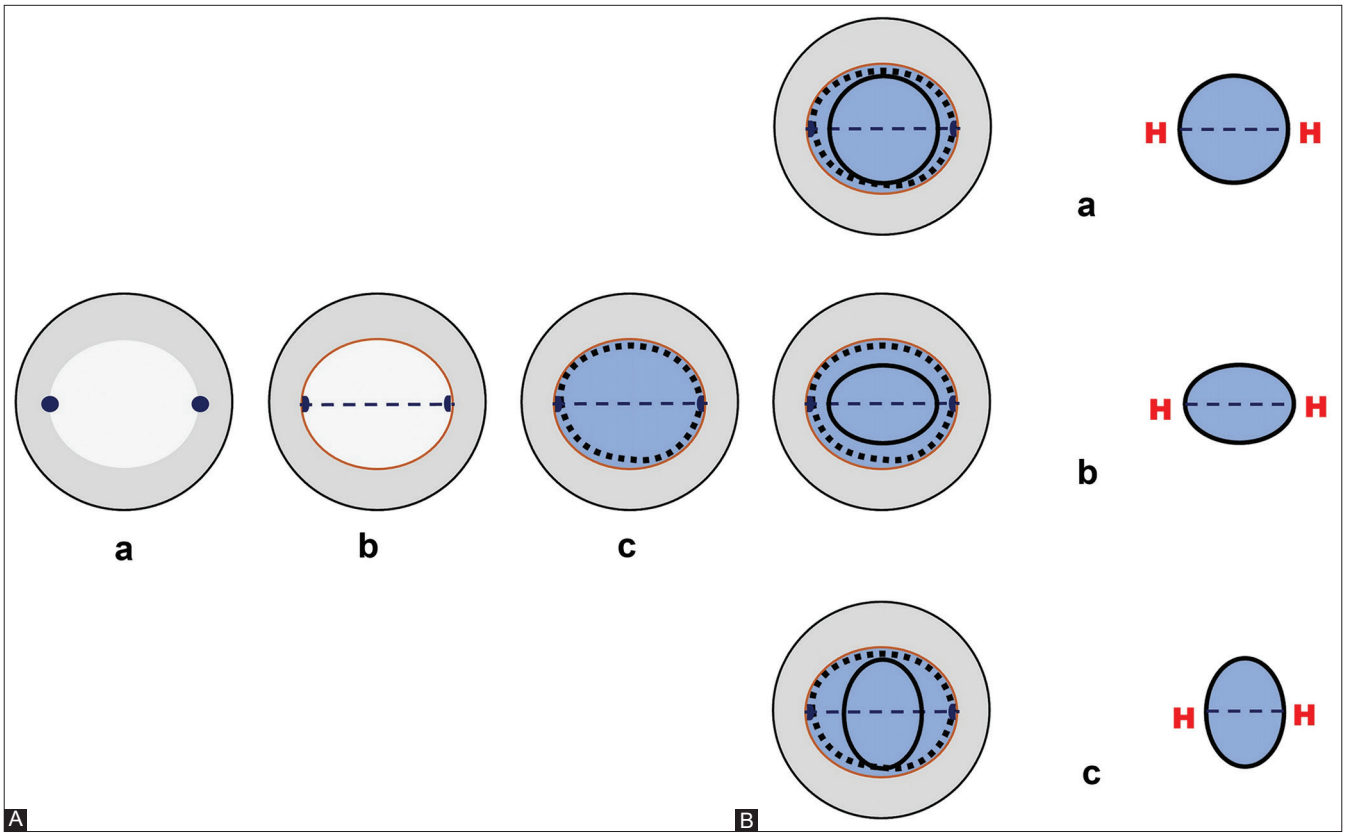

Figure 1: Schematic representation of DM tissue preparation as for DMEK. A. (a) Edges of horizontal axis (longer, 3 to 9 o'clock) on the anterior surface (epithelial side) of sclerocorneal disc were marked with dots using a tissue pen. (b) The disc was turned around and a line was drawn on DM connecting the two dots. The brown elliptical line represents the pigmented trabecular meshwork at the corneal periphery. (c) DM and ECs were stained with VisionBlue® and DM scored along the circumference (dotted line). B. (a) Circular trephination of an 8-mm disc of DM. (b) Horizontally oval trephination of DM. (c) Vertically oval trephination of DM. The oval trephine was 9 mm (major axis) ×7 mm (minor axis). DM = Descemet's membrane, DMEK = Descemet's membrane endothelial keratoplasty, ECs = endothelial cells, H–H = horizontal axis. In 'B' a, b and c, the area of trephination and the trephined tissue are depicted in the left and right diagram of each pair respectively

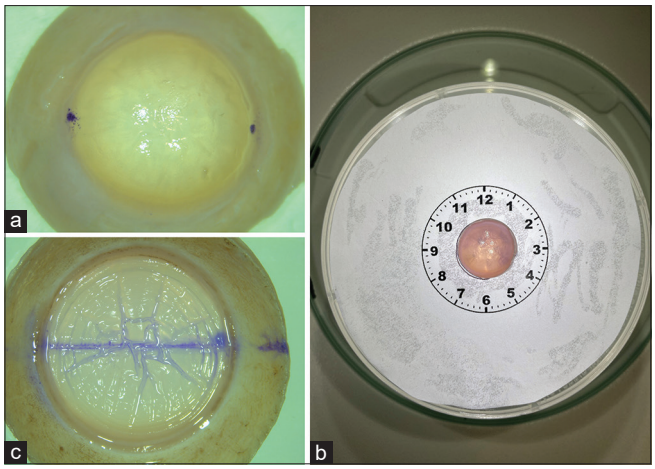

Figure 2: Marking of the longest (horizontal, 3 to 9 o'clock) axis of the cornea. (a) Two blue dots on the epithelial surface of the limbus represent the 3 and 9 o' clock positions. (b) A clockface with a central aperture was placed on a sclerocorneal disc mounted on the artificial anterior chamber. The observers recorded the clock hours of the longest axis independently to assess agreement. (c) The sclerocorneal disc was turned around and a horizontal blue line was drawn on DM from dot to dot, to represent the horizontal axis, before peeling DM. DM = Descemet's membrane

For subsequent experiments, the long axis was marked as described above, with a dot just inside the limbus in the clear cornea, at either end of the longest axis. The disc was then dismounted from the artificial anterior chamber, dried by draining saline by touching the edge against an absorbent tissue, and placed in a Petri dish with the concave endothelial side up. The long axis was further marked gently by joining the two dots with a curved inked metal strip, leaving a blue line on the endothelium from dot to dot (seven samples) [Figs. 1 and 2]. For the vertical axis, the endothelium was marked with a blue line at right angles to the two dots (six samples). The long axis was taken as the horizontal meridian and the axis at right angles to this as the vertical meridian.

DM peeling

The endothelial surface of the disc was stained with vision blue (VisionBlue; DORC, Zuidland, the Netherlands) for 2 min and washed with balanced salt solution (BSS). DM was scored circumferentially for 360° approximately 2 mm central to the visible trabecular meshwork. It was stained again with vision blue for 2 min and washed. The central edge of the scored DM was undermined and lifted with the blunt tip of a Birks tying forceps (Malosa Medical, Elland, UK). After freeing the entire circumference of the scored DM, it was held with the forceps at one edge and gently peeled off either completely (eight samples) or two-thirds of the way, laid back, and trephined with an 8-mm circular punch (Katena, Denville, NJ, USA) and peeled off (five samples).

The peeled DM was placed in a Petri dish filled with BSS and allowed to scroll. The direction of scrolling was noted in relation to the marked horizontal or vertical meridian. The tightness of the scrolls was graded from 0 to 4 as previously described.[6,12] Images were taken with a stereo microscope (model #S6D; Leica Microsystems, Milton Keynes, UK).

Cutting strips of DM

A central horizontal or vertical strip, 3–4 mm wide, was cut along the horizontal and vertical meridians, respectively (four samples each), by unfolding horizontally scrolled DM on an absorbent tissue paper placed in a Petri dish and pressing down along the length of DM in the appropriate direction, with a surgical blade. The strips were reimmersed in BSS, and the scrolling direction and grade was determined.

Oval trephination

A purpose-made prototype of a 7 × 9 mm oval trephine with a total surface area of 49.5 mm^2 (e. janach®, Italy) was constructed. This compares to the surface area of 50.3 mm^2 of an 8-mm circular trephine. Another 15 samples of DM were prepared as for DMEK and marked along the horizontal axis (3 to 9 o'clock). Twelve samples that scrolled along the horizontal meridian were selected. DM tissue was opened flat, with the endothelial side up, on the posterior surface of the sclerocorneal button and trephined with the long axis of the oval trephine aligned horizontally (six samples) and vertically (six samples) [Fig. 1]. The oval DM discs were placed in BSS and allowed to scroll. The direction and grade of scrolling was determined.

Immunofluorescence staining and semi-quantitative analysis

Tissue samples were placed on a low-binding Petri dish with EC upside and gently unscrolled or flattened by grasping the edges with two pairs of Birks forceps. Two cross cuts (one along the horizontal axis and another along the vertical) were made with a sharp blade and tissue samples were placed in a square mold with optimal cutting temperature compound (OCT) and snap frozen on dry ice, as previously described.[13] The discs of DM with EC were processed as shown in Fig. 1 and sectioned for localization and estimation of elastin (from the center to periphery) along the horizontal (n = 3) and vertical (n = 3) axes using immunofluorescence staining.

Ten-micrometer-thick sections of OCT embedded DM + EC were fixed with 4% paraformaldehyde for 20 min, followed by blocking for 1 h with 5% normal donkey serum (made in 1× phosphate-buffered saline [PBS] containing 0.05% Triton-X100 [PBST]). The sections were incubated with polyclonal rabbit anti-human primary antibody against elastin (5 µg/mL final concentration; Abcam, Cambridge, UK) overnight at 4°C. The sections were washed and incubated with donkey anti-rabbit IgG Alexafluor 488 conjugate secondary antibody (ThermoFisher Scientific, Dartford, UK) for 1 h at room temperature. After washing, the slides were mounted in ProLong antifade mounting compound containing DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) (ThermoFisher Scientific, UK) and photomicrographs were captured using a fluorescent microscope (model # DM IL LED Flou, Leica Microsystems).

The relative fluorescence intensity and thickness of elastin staining band and full thickness of DM + EC sections were estimated using ImageJ software (NIH, Bethesda, MD, USA). Student's t-test (horizontal versus vertical measurements) was performed using Prism software (version 9.0; GraphPad, San Deigo, CA, USA).

Results

Interobserver variation on determining the longest (horizontal) axis: The two observers who determined the longest axis or the horizontal meridian of the cornea demonstrated an

excellent interobserver agreement (n = 8 corneas; ICC = 0.98 [95% CI: 0.92–1.00], P < 0.001). The longest axis most likely, but not necessarily corresponded to the true horizontal meridian of the cornea.

Scrolling direction of whole samples and 8-mm discs of DM [Table 1]: Twelve of the 13 samples showed scrolling of DM in the horizontal meridian. All samples in which the horizontal axis was marked and five of the six in which the vertical axis was marked showed scrolling in the horizontal meridian [Fig. 3]. One sample that was marked in the vertical axis showed a spiral scroll. The majority of the samples, regardless of age, showed grade 3–4 scrolling. None of the samples scrolled in the vertical meridian. Mean grade of the scrolls is given in Table 1.

Scrolling direction of strips of DM [Table 2]: All four horizontal strips prepared from horizontally scrolled DM showed grade 3–4 scrolling in the original horizontal meridian. All four vertical strips prepared from horizontally scrolled DM showed a gentle spiral twist (grade 1). None of the strips scrolled in the vertical meridian [Fig. 4].

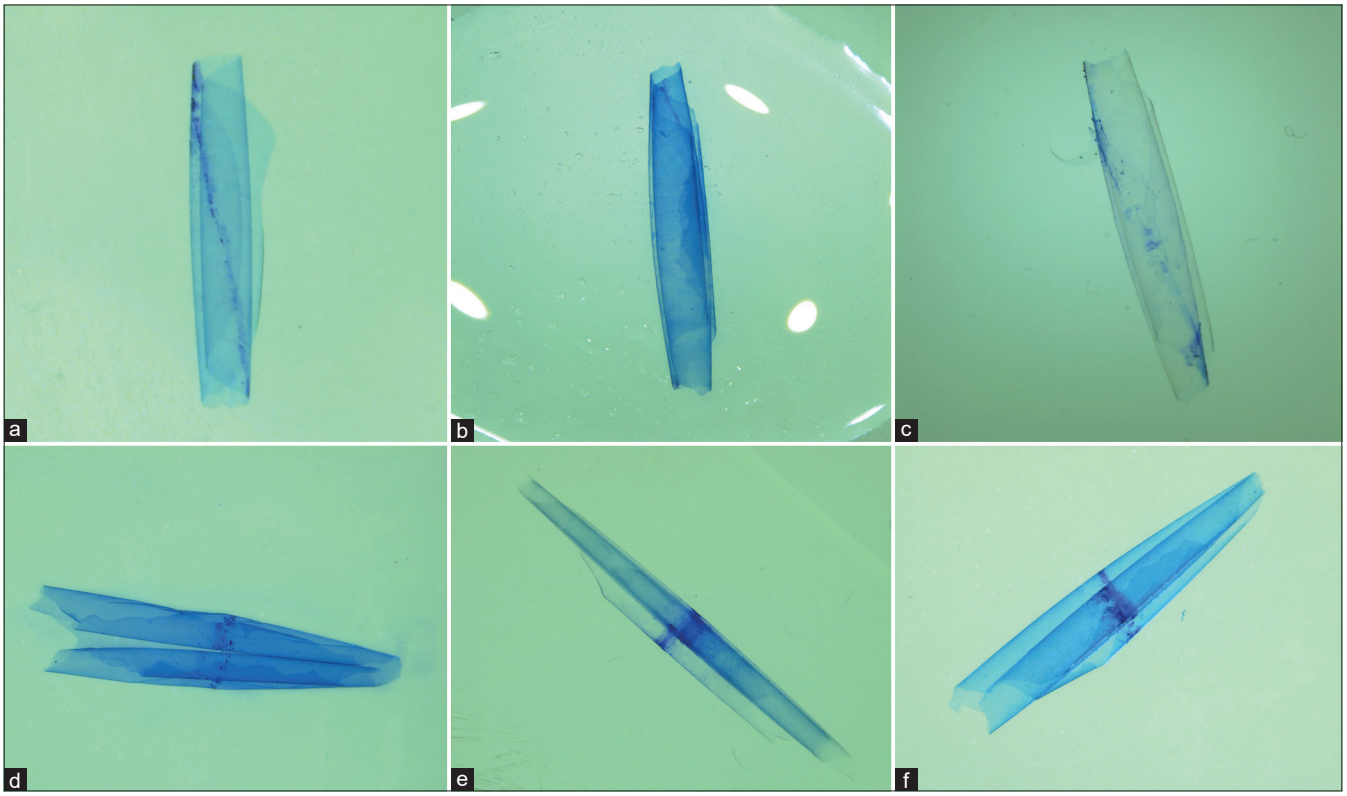

Figure 3: Scrolling of DM marked along the vertical and horizontal axes. (a–c) Three representative samples of DM showing horizontal scrolling of samples marked along the vertical axis. (d–f) Three representative samples of DM showing horizontal scrolling of samples marked along the horizontal axis. DM = Descemet's membrane

Table 1: Direction of scrolling of DM samples

Sample	Meridian marked	Direction of scrolling				
		Horizontal	Average grade	Spiral	Grade	Vertical
Whole samples n=13[4][a]	Horizontal, n=7[4]	7 [4]	3.7	0	-	0
	Vertical, n=6	5	3.6	1	3	0

Number in square brackets[4] represents DM obtained from transplant-grade tissue, removed from donors during deep anterior lamellar keratoplasty. [a]Of the nine research-grade DM samples, five were trephined with an 8-mm circular trephine, before peeling DM from the donor cornea to give an 8-mm disc of DM. DM=Descemet's membrane

Table 2: Direction of scrolling of Descemet's membrane strips

Sample	Direction of scrolling				
	Horizontal	Average grade	Spiral	Average grade	Vertical
Horizontal strips (n=4)	4	3.5	0	-	0
Vertical strips (n=4)	0	-	4	1.5	0

Scrolling direction and grade of oval trephined DM [Table 3]: Of the 15 samples of DM prepared as for DMEK and marked along the horizontal axis, 12 scrolled along the horizontal meridian and three showed spiral scrolls [Fig. 5]. The spiral scrolling samples were excluded and the remaining 12 were divided into two groups of six matched pairs. Two pairs with grade 3 scrolls and four pairs with grade 4 scrolls were trephined with an oval trephine, either horizontally or vertically, such that each pair had one vertical and one horizontal oval trephined DM sample. The horizontally oval trephined samples scrolled back to the original grade of scrolling, but the vertically oval trephined samples showed only grade 1 scrolling [Fig. 6].

Of the total of 28 DM samples, 24 (85.72%) showed scrolling along the horizontal axis, four (14.28%) showed a spiral/oblique scroll, and none showed scrolling along the vertical axis.

Immunofluorescent staining for elastin: Sections along the horizontal and vertical axes showed continuous anterior distribution of elastin as a narrow, but intensely staining band [Fig. 7]. The fluorescence intensity of elastin band was consistent and showed no significant difference along the horizontal (20.72 ± 1.22 [arbitrary units]) and vertical (19.10 ± 1.98 [arbitrary units]) axes or from the center to the periphery [Fig. 8]. The thickness of the anterior elastin staining band remained unchanged throughout the horizontal (1.94 ± 1.15 μm) and vertical (1.81 ± 0.21 μm) axes [Fig. 9]. Similarly, no significant differences were seen in the thickness of DM along the horizontal (11.37 ± 0.91 μm) and vertical (11.82 ± 1.34 μm) axes [Fig. 10].

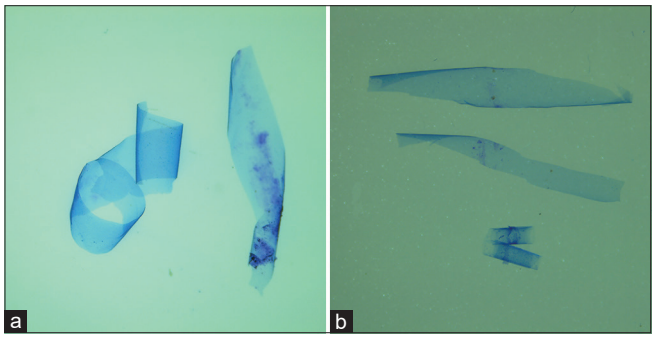

Figure 4: Scrolling of strips of DM cut along the vertical and horizontal axes. (a) Horizontal and vertical strips from a sample where the vertical axis was marked. The horizontal strip shows the classical scrolling (grade 3) along the horizontal axis, while the vertical strip only shows a gentle spiral twist. (b) Horizontal and vertical strips from a sample where the horizontal axis was marked. The horizontal strip shows the classical scrolling (grade 4) along the horizontal axis, while the vertical strips only show a gentle spiral twist with no scrolling. DM = Descemet's membrane

Discussion

With the advent of DMEK, the unique scrolling characteristic of DM, with the ECs outside, has assumed considerable clinical significance. It provides the surgeon with the clue to guide unscrolling with correct orientation of DM, with EC facing the iris. DM also accords the scrolling characteristic to PDEK tissue.[6] The elasticity of DM and the "swollen ECs" were offered as explanations for this unique characteristic. Later, it was shown that the distribution of elastin as a discrete layer (band) on the anterior surface of DM was responsible for the unidirectional scrolling, as it was seen even after removal of EC with dispase treatment and could be reversed by treating the tissue with the enzyme elastase.[13]

It has been demonstrated that the thickness of DM and grade of the scroll change with age. DMs from older donors are thicker and scroll less than those from younger donors where DM is thinner. Age and thickness are probably two independent factors.[9,10,14,15] Though there may be a correlation between the grade of scroll and the amount of elastin in the anterior part of DM, there is no evidence to support this. The various manipulations and maneuvers that have been described and deployed to unscroll DM in DMEK and PDEK are likely to contribute to EC loss during these EK procedures.[10,11,16] Placement of an F or S mark on the anterior surface of DM helps with orientation, but none of the procedures described reduce the grade of the scroll. Attempts at limiting, modifying, or reversing the tightness

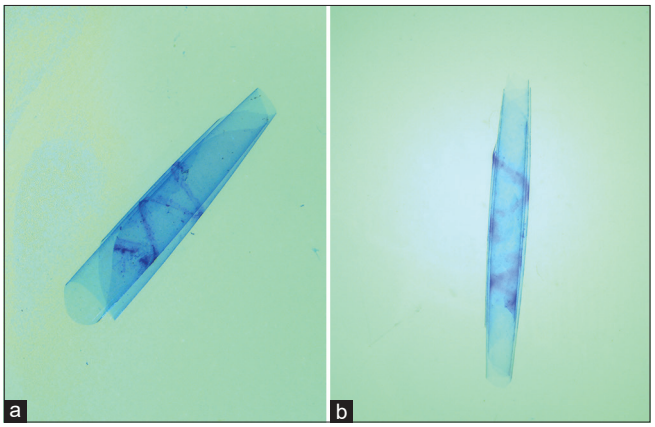

Figure 5: Spiral scrolling of DM. (a and b) Two samples of DM where the horizontal axis was marked with a blue ink mark, which shows a spiral configuration. This indicates that the samples did not scroll along the marked horizontal axis. DM = Descemet's membrane

Table 3: Direction and grade of scrolling of DM tissue prepared with oval trephination

Sample	Meridian marked	Direction of scrolling				
		Horizontal	Average grade	Spiral	Average grade	Vertical
Whole samples *n*=15[3]	Horizontal, *n*=15[3]	12 [2]	3.6	[a]3 [1]	3–4	0
Horizontal oval trephination	Horizontal, *n*=6	6	3.5	0	0	0
Vertical oval trephination	Horizontal, *n*=6	6	1.2	0	0	0

Number in square brackets[3] represents DM obtained from transplant grade tissue, removed from donors during deep anterior lamellar keratoplasty. [a]These three samples were excluded from the horizontal and vertical oval trephination in the rows below. DM=Descemet's membrane

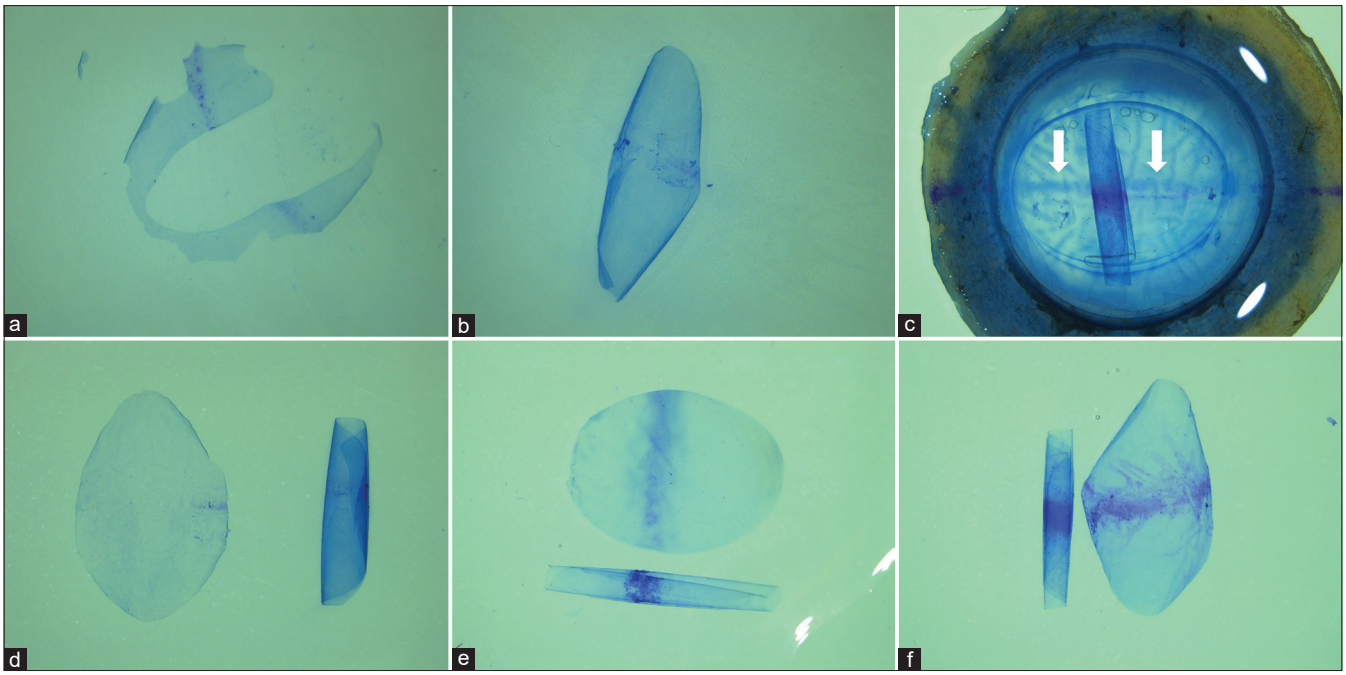

Figure 6: Scrolling of DM following oval trephination along the horizontal and vertical axes. (a) The rim of discarded DM after oval trephination showing the shape of the trephine cut. (b) The oval DM disc, trephined at right angles to the horizontal axis marked with a blue line, showing a grade 1.5 scroll. (c) The oval trephine mark on the stromal bed is seen. The imprint of the blue ink mark is visible on the stroma, along the long axis (horizontal, 3 to 9 o'clock). The horizontally oval trephined DM has scrolled along the long axis (grade 4). (d–f) Three pairs of vertically oval and horizontally oval trephined DM. The horizontally oval trephined DM shows a grade 3 scroll (d) and grade 4 scrolls (e, f). The vertically oval trephined DM samples show scrolling grade of 1–1.5. DM = Descemet's membrane

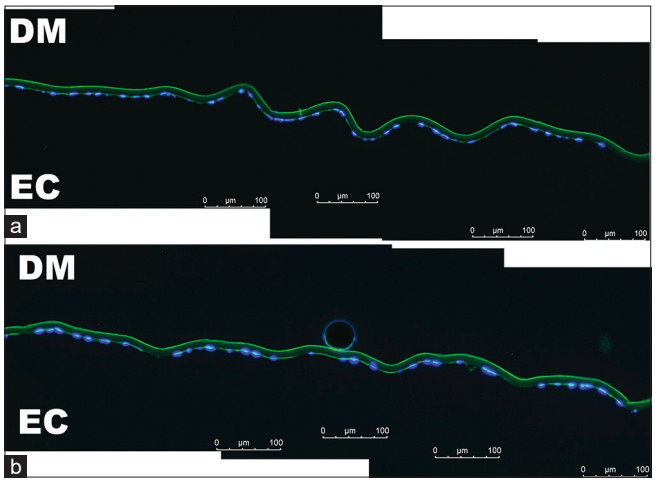

Figure 7: Immunofluorescent staining of elastin (rabbit polyclonal anti-human elastin antibody) in DM along the horizontal and vertical axes. The green immunofluorescent bands of elastin on the anterior surface of DM are illustrated in the horizontal (a) and vertical (b) sections of the center of DM. The images represent montages of parallel sections that were cut along the horizontal and vertical axes from the center (left) to the periphery (right). The nuclei of ECs are stained blue with a nuclear stain (DAPI). DM = anterior surface of Descemet's membrane, EC = endothelial cell

and configuration (single or double) of the DM scroll have been made with enzyme treatment,[17] polymer coating,[18] or mechanical means.[14,15] Thus, scrolling of DM is both a friend and a foe. The scroll allows introduction of the tissue into the anterior chamber through a narrow entry wound, but unscrolling of DM in the anterior chamber poses technical challenges and can lead to EC loss.

In 2021, Wacker *et al*.[19] studied the scrolling patterns of over 200 donors and established that the DMEK graft scrolled predominantly vertical to the donor's cornea. The eye bank technician marked the rim of the donor buttons during trephination and recorded the position relative to the donor's axis. The authors categorized three scrolling axes, vertical (0°–30° and 150°–180°), oblique (>30°–60° and 120°–<150°), and horizontal (>60°–<120°). We attempted to narrow the axes further by defining the longest diameter, on a clock face (3 to 9 o'clock) of the anterior oval-shaped sclerocorneal perimeter, as the horizontal meridian. The axis at right angles to this (12 to 6 o'clock) constituted the vertical meridian in our description. Scrolling along this meridian would form a vertical disposed scroll lying along the vertical meridian, which was termed a vertical scroll by Wacker *et al*.[19] They concluded that DMEK grafts have a natural and stable scrolling tendency at the vertical axis of donor's cornea, which is the same as what we found, but by using an ink line to mark the axes, we demonstrated that the scrolling was often along the 3 to 9 o'clock axis (scroll lying along the vertical axis of the cornea) as indicated by tight circle formed by the ink line, as illustrated in Fig. 3. More importantly, in our experiments, no sample scrolled along the vertical axis (12 to 9 o'clock axis). This was quite evident when we examined strips of DM cut along the vertical axis. Compared to the horizontally cut strips, which formed neat scrolls, the vertical strips only showed a spiral twist or a corkscrew configuration. This scrolling pattern was observed regardless of whether it was the horizontal or the vertical axis

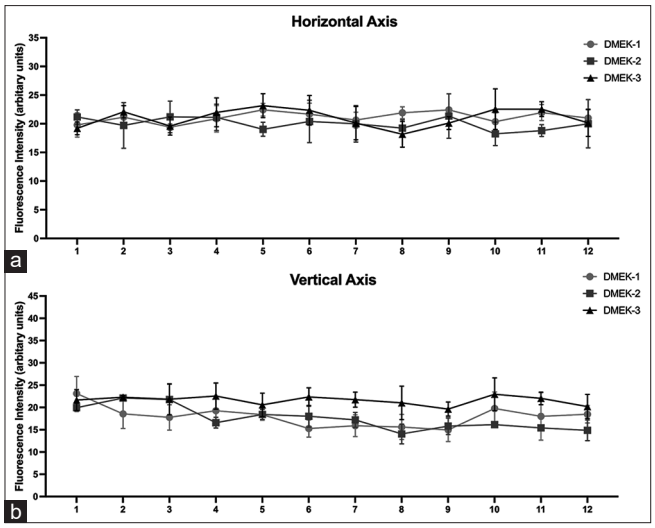

Figure 8: Fluorescence intensity of the anterior staining band of elastin in DM sections along the (a) horizontal and (b) vertical axes. Line graphs plotted for 12 nodes of RFU. Each node (100 μm long) represents the average of 10 serial semi-quantitative measurements in reference to the background of the staining band from the center to the periphery along the horizontal axis (top) and the vertical axis (bottom) for duplicate serial sections from each of *n* = 3 DMEK samples. Error bars represent SD of 10 serial measurements along each 100 μm node from the center to the periphery of two parallel sections from each of *n* = 3 DMEK samples. Student's *t*-test was performed using the Prism software (v9.0) between the mean of RFU from 12 nodes along the horizontal and vertical axes, respectively (three samples of DM are labeled DMEK 1, 2, and 3). There was no significant difference in the fluorescence intensity in the different axes or from the center to the periphery in the samples studied. DM = Descemet's membrane, DMEK = Descemet's membrane endothelial keratoplasty, RFU = relative fluorescence units, SD = standard deviation

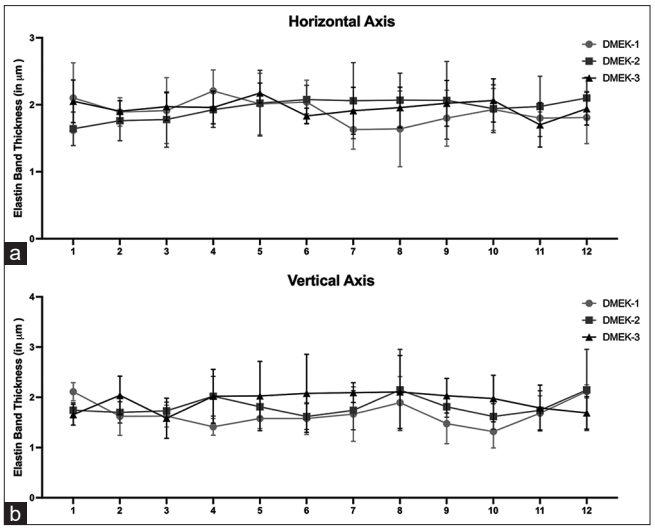

Figure 9: Thickness of the anterior staining band of elastin in DM sections along the (a) horizontal and (b) vertical axes. Line graphs plotted for 12 nodes of thickness measurements of the elastin staining band in the anterior part of DM. Each node (100 μm long) represents the average of 10 serial measurements in reference to the scale bar, from the center to the periphery along the horizontal axis (top) and the vertical axis (bottom) for duplicate serial sections from each of *n* = 3 DMEK samples. Error bars represent SD of 10 serial measurements along each 100-μm node from the center to the periphery of two parallel sections from each of *n* = 3 DMEK samples. Student's *t*-test was performed using the Prism software (v9.0) between the mean thickness values (in μm) from 12 nodes along the horizontal and vertical axes, respectively (three samples of DM are labeled DMEK 1, 2, and 3). There was no significant difference in the thickness of the elastin staining band in the different axes or from the center to the periphery in the samples studied. DM = Descemet's membrane, DMEK = Descemet's membrane endothelial keratoplasty, SD = standard deviation

that was marked with the ink, showing that the ink mark did not influence the direction of scrolling.

On the basis of the above information, we hypothesized that oval-shaped DM tissue with a greater vertical element (12 to 6 o'clock) would scroll less than oval-shaped DM tissue with a greater horizontal element (3 to 9 o'clock). Our study validated this hypothesis, with the grade of scroll dropping almost by 50% in the vertically oval trephined tissue. One can postulate that it would be easier to unscroll vertically oval trephined DMEK tissue in the anterior chamber, thus obviating some of the intraoperative challenges and possibly limiting EC loss. This will have to be tested with real-life experience and cell counts.

The posterior corneal curvature is ellipsoid, with the vertical meridian being more curved than the horizontal, resulting in with-the-rule astigmatism. This could imply that orientation of the oval DM graft would need to be aligned to that of the recipient cornea and could influence graft attachment or graft detachment rates. The oval shape of the donor tissue would make alignment easier, however, it has been shown that nonalignment of the axes of circular donor grafts and recipient beds did not have any significant impact on graft detachment.[20] The surface area of the oval trephined tissue is about 0.8 mm^2 less that of a corresponding 8-mm circular trephine. Theoretically, this would have little impact on the total number of ECs transplanted, but potentially oval trephines with different major and minor axes can be constructed, just as circular trephines with different diameters are currently in use.

What might be the explanation for this preferential scrolling along the horizontal axis? Based on previous reports showing that the distribution of elastin on the anterior surface of DM contributes to its unique scrolling pattern, with ECs outside,[13] we considered the possibility that there might be differential distribution of elastin along one meridian compared to the other. We studied this by examining the thickness and intensity of fluorescent staining of the elastin band in the vertical and horizontal axes. We were not able to show any significant difference in either of these parameters. Treatment of scrolled DM with elastase enzyme results in unscrolling of the tissue,[13] indicating that the presence and distribution of elastin determines the preferential scrolling of DM with EC outside. It does not explain the preferential (horizontal) meridional scrolling of DM. Further exploration of the orientation and architecture of the collagen matrix of DM may provide an explanation. A minority of the DM samples scrolled in an oblique meridian, as clearly illustrated by the spiral configuration of the ink line. This would suggest the existence of natural variation in the tissue architecture might be an explanation for preferential scrolling along the horizontal axis. Though there was good agreement between the observers, an error in the marking of the horizontal axis would also result in oblique or spiral scrolling.

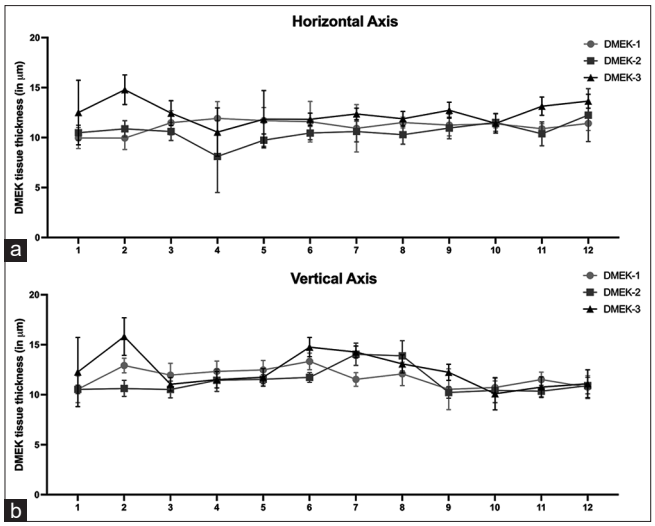

Figure 10: Full-thickness measurements of DM in sections along the (a) horizontal and (b) vertical axes. Line graphs plotted for 12 nodes of thickness measurements. Each node (100 μm long) represents the average of 10 serial measurements in reference to the scale bar, of DM from the center to the periphery along the horizontal axis (top) and the vertical axis (bottom) for duplicate serial sections from each of $n = 3$ DMEK samples. Error bars represent SD of 10 serial measurements along each 100 μm node. Student's *t*-test was performed using the Prism software (v9.0) between the mean of full thickness (in μm) of parallel sections from 12 nodes along the horizontal and vertical axes, respectively (three samples of DM are labeled DMEK 1, 2, and 3). There was no significant difference in the thickness of DM in the different axes or from the center to the periphery in the samples studied. DM = Descemet's membrane, DMEK = Descemet's membrane endothelial keratoplasty, SD = standard deviation

One limitation of our study was that only older donor samples were studied. The mean age was 69 years, though it did include one patient aged 39 years and two patients aged 45 years. Nevertheless, the results do suggest that use of an oval trephine to prepare vertically oval DMEK tissue could provide a simple answer to ease some of the technical challenges and possible associated EC loss related to donor tissue unscrolling in DMEK. It might also enable use of younger donor tissue, where the above issues are more pronounced.

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Conflicts of interest

There are no conflicts of interest.

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