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

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**Abstract**

**Background/Objectives:** 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.

**Subjects/Methods:** The longest limbus-to-limbus axis on 28 sclero-corneal discs was taken as the horizontal axis. The horizontal (n=7) or (right angles to it) vertical (n=6) axis was marked on the DM before peeling it off. The direction and grade of scrolling was observed. Narrow strips (3-4mm wide) were then cut along the two axes (n=4 each) and the scrolling pattern observed. Ellipses (7x9mm) 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 3 DM samples was performed. The intensity and thickness of elastin staining were measured.

**Results:** 24 (85.72%) DM samples showed scrolling along the horizontal axis, none along the vertical axis, and 4 (14.28%) 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 center to periphery.

**Conclusion:** DM scrolls primarily along the horizontal axis. Vertically oval DM samples show minimal scrolling, which can be an advantage in DMEK. The differential scrolling is not determined by the distribution of elastin.

**Introduction**

Endothelial keratoplasty (EK) is the standard procedure for corneal endothelial pathologies that compromise vision, 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 minimal induced astigmatism. Another distinct characteristic of the scrolling of EK tissue is that they always scroll with the EC 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 PDEK tissue.\(^6\)
It is well known that DM with endothelial cells (EC) obtained from younger donors produce tighter scrolls though clinical outcomes in experienced hands are similar with young and old donors. Various maneuvers involving tapping, stroking and injecting jets of fluid, are described to unscroll EK tissue in the recipient eye. The time and maneuvers required to unscroll donor EK tissue often determine endothelial cell loss during DMEK and PDEK. The tighter the scroll and consequently the greater the duration and maneuvers required to unscroll it, the more cell loss is likely.

In this study we explored the meridional scrolling characteristic of DM in the vertical and horizontal meridians. 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 behaviour of vertically oval and horizontally oval trephined DMEK tissue and propose 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 the meridional scrolling was determined by the distribution of elastin along the vertical and horizontal meridians.

**Material And Methods**

All research was conducted under the local Human Tissue Authority license and approved by the Midlands Research Ethics Committee. Donor sclero-corneal discs were obtained under an approved Material Transfer Agreement from the Transplant Tissues and Eye Bank Services, National Health Service Blood and Transplant, Liverpool, UK. A total of 28 human cadaveric sclero-corneal discs (21 research-grade and 7 transplant-grade [DM discarded from corneas used for deep anterior lamellar keratoplasty]) were used in this study. The donor age ranged from 45–92 with a mean age of 69 years. There were 6 female and 22 male donors. Causes of death included cancer, stroke, ruptured aneurysm, ischaemic heart disease, sepsis and multiorgan failure. All sclero-corneal discs were stored in organ culture in Eagle’s minimum essential medium with 2% fetal bovine serum for 3 to 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 i.e the longest diameter, on the anterior surface of the cornea, just inside the limbus of the sclero-corneal 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 the figure (Fig. 2). Eight sclero-corneal discs were removed from the culture medium rinsed in saline and mounted on a Barron artificial anterior chamber (Corza medical, Parsippany, New Jersey) and filled with fluid to a pressure of 18–20 mm of Hg. The clockface device was placed on the mounted sclero-corneal disc such that the limbus and adjacent sclera was visible through the central hole. The longest axis of the sclero-corneal disc, from clock hour to clock hour, was read by two independent observers and recorded individually in a blinded manner. The inter-observer agreement on the longest axis was determined using intraclass correlation coefficient (ICC) with SPSS Version 28 (IBM
SPSS Statistics for Windows, Armonk, NY, USA), and was interpreted as follows: (1) $< 0.50 = \text{poor reliability}$; (2) $0.5 - 0.75 = \text{moderate reliability}$; (3) $0.75 - 0.90 = \text{good reliability}$; and (4) $> 0.9 = \text{excellent reliability}$. The results were reported in mean with 95% confidence interval (CI).

For subsequent experiments, the long axis was marked as described above, with a dot, just inside the limbus in clear cornea, at either end of the longest axis. The disc was then dismounted from the artificial anterior chamber, dried by draining the 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 (7 samples) (Figs. 1 and 2). For the vertical axis, the endothelium was marked with a blue line at right angles to the two dots (6 samples). The long axis was taken as the horizontal meridian and the axis at right angles to this, as the vertical meridian.

**Descemet’s membrane (DM) peeling.**

The endothelial surface of the disc was stained with vision blue (VisionBlue®, Zuidland, Netherlands) for two minutes and washed with balanced salt solution (BSS). The DM was scored circumferentially for $360^\circ$ approximately 2 mm central to the visible trabecular meshwork. The DM was stained again with vision blue for two minutes 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 (8 samples) or two thirds of the way, laid back and trephined with an 8 mm circular punch (Katena, Denville, NJ) and peeled off (5 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. Images were taken with a stereo microscope (model #S6D, Leica Microsystems, Milton Keynes, UK).

**Cutting Strips of Descemet’s membrane.**

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

**Oval Trephination.**

A purpose-made prototype of a 7 x 9 mm oval trephine, with a total surface area of 49.5 square mm (e. janach®, Italy) was constructed. This compares to the surface area of 50.3 square mm 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, endothelial side up, on the posterior surface of the sclero-corneal button, and trephined with the long axis of the oval trephine aligned horizontally (6 samples) and vertically (6 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 un-scrolled 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. The discs of DM with EC were processed as shown in Fig. 1 and sectioned for localization and estimation of elastin (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 minutes followed by blocking for 1 hour with 5% normal donkey serum (made in 1x 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, UK) overnight at 4°C. The sections were washed and incubated with donkey anti-rabbit IgG Alexafluor 488 conjugate secondary antibody (ThermoFisher Scientific, UK) for 1 hour at room temperature. After washing, slides were mounted in Prolong antifade mounting compound containing DAPI (ThermoFisher Scientific, UK) and photomicrographs were captured using a fluorescent microscope (model # DM IL LED Flou, Leica Microsystems, Milton Keynes, UK).

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

**Results**

**Inter observer 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 inter-observer 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 8mm discs of Descemet's membrane, (Table 1).** Twelve of the 13 samples, showed scrolling of the 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 (Figure 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 to 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 Descemet’s membrane, (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 strips scrolled in the vertical meridian, (Figure 4).

Scrolling direction and grade of oval trephined Descemet’s membrane (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 3 showed spiral scrolls. (Figure 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 4 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 (Figure 6).

Of the total of 28 DM samples, 24 (85.72%) showed scrolling along the horizontal axis, 4 (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 (Figure 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 (Figure 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 (Figure 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 (Figure 10).

Discussion

With the advent of DMEK, the unique scrolling characteristic of the DM, with the endothelial cells outside, has assumed considerable clinical significance. It provides the surgeon with the clue to guide the unscrolling with correct orientation of the DM, with the EC facing the iris. The DM also accords the scrolling characteristic to PDEK tissue. The elasticity of the DM and the ‘swollen endothelial cells’ 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 the DM was responsible for the unidirectional scrolling, as it was seen even after removal the EC with dispase treatment and could be reversed by treating the tissue with the enzyme elastase.

It has been demonstrated that the thickness of the DM and grade of the scroll change with age. DM from older donors are thicker and scroll less than those from younger donors where the DM is thinner. Age and thickness are probably two independent factors. Though there may be a correlation between the grade of scroll and amount of elastin in the anterior part of the DM, there is no evidence to support this.
The various manipulations and maneuvers that have been described and deployed to unscroll the DM in DMEK and PDEK are likely to contribute to EC loss during these EK procedures.\textsuperscript{10, 11, 16} Placement of an F or S mark on the anterior surface of the DM helps with orientation, but none of the procedures described reduce the grade of the scroll. Attempts at limiting, modifying or reversing the tightness and configuration (single or double) of the DM scroll have been made with enzyme treatment,\textsuperscript{17} polymer coating,\textsuperscript{18} or mechanical means.\textsuperscript{14, 15} Thus, the scrolling of the 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 its unscrolling in the anterior chamber poses technical challenges and can lead to endothelial cell loss.

In 2021 Wacker et al\textsuperscript{19} studied the scrolling patterns of over two hundred 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.” They categorized three scrolling axes, (0 to 30 degrees and 150 to 180 degrees), oblique (> 30 to 60 degrees and 120 to < 150 degrees), and horizontal (> 60 to < 120 degrees). 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 descriptions. 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.\textsuperscript{19} They concluded that DMEK grafts have a natural and stable scrolling tendency at vertical axis of donor's cornea., which the same as what we found but by using the 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 (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 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 endothelial cell 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 non alignment of the axes of circular donor grafts and recipient beds did not have any
significant impact on graft detachment.\textsuperscript{20} The surface area of the oval trephined tissue is about 0.8 square mm less that that of a corresponding 8mm circular trephine. Theoretically this would have little impact on the total number of endothelial cells 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 the DM contributes to its unique scrolling pattern, with the endothelial cells outside,\textsuperscript{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\textsuperscript{13} indicating that the presence and distribution of elastin determines the preferential scrolling of DM with the EC outside, it does not explain the preferential (horizontal) meridional scrolling of the DM. Further exploration of the orientation and architecture of the collagen matrix of the 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 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.

One limitation of our study was that only older donor samples were studied. The mean age was 69 years, though it did include one aged 39 years and two 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 endothelial cell loss related to donor tissue unscrolling, in DMEK. It might also enable use of younger donor tissue, where the above issues are more pronounced.

\textbf{Declarations}

\textbf{Acknowledgements}

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\textbf{Conflict of interest}

HS Dua is a consultant to Arctic vision and Thea and has shares in Glaxosmithkline and NuVision biotherapies. None of these are in any way related to the content of this manuscript.

None of the other authors have any conflict of interest declaration to make.

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### Tables

#### Table 1. Direction of scrolling of Descemet’s membrane samples

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<thead>
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<th>Sample</th>
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<th>Direction of scrolling</th>
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</thead>
<tbody>
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<td>Whole samples</td>
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<tr>
<td>*n =13 [4]</td>
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<td></td>
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<tr>
<td>Horizontal</td>
<td>Average Grade</td>
<td>Spiral Grade</td>
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</tr>
<tr>
<td>Vertical, n=6</td>
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</table>

Number in square brackets [4] represent Descemet’s membrane (DM) obtained from transplant grade tissue, removed from donors during deep anterior lamellar keratoplasty.

*Of the 9 research grade DM samples, 5 were trephined with an 8mm circular trephine, before peeling the DM from the donor cornea to give an 8 mm disc of DM.

#### Table 2. Direction of scrolling of Descemet’s membrane strips
Table 3. Direction and grade of scrolling of Descemet’s membrane tissue prepared with oval trephination

<table>
<thead>
<tr>
<th>Sample</th>
<th>Meridian marked</th>
<th>Direction of scrolling</th>
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<td></td>
<td>Horizontal</td>
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<td>Horizontal oval trephination</td>
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</tr>
<tr>
<td>Vertical oval trephination</td>
<td>Horizontal n=6</td>
<td>6</td>
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</table>

Number in square brackets [3] represent Descemet’s membrane (DM) obtained from transplant grade tissue, removed from donors during deep anterior lamellar keratoplasty.

§These three samples were excluded from the horizontal and vertical oval trephination in the rows below.

Figures
Figure 1

Schematic representation of Descemet's membrane (DM) tissue preparation as for Descemet's membrane endothelial keratoplasty (DMEK).

A. (a) Edges of horizontal axis (longer, 3 o'clock to 9 o'clock) on the anterior surface (epithelial side) of sclero-corneal disc is marked with dots using a tissue pen. (b) The disc is turned around and a line was drawn on the DM connecting the two dots. The brown elliptical line represents the pigmented trabecular meshwork at the corneal periphery. (c) The DM and endothelial cells (EC) were marked with Vision blue® and the 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 the DM. The oval trephine was 9 mm (major axis) x 7 mm (minor axis). H-H = Horizontal axis.
Marking of the longest (horizontal, 3 o'clock 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 sclero-corneal disc mounted on artificial anterior chamber. The observers recorded the clock hours of the longest axis independently, to assess agreement. C. The sclero-corneal disc was turned around and a horizontal blue line was drawn on the Descemet's membrane from dot to dot, to represent the horizontal axis, prior to peeling the DM.
Figure 3

Scrolling of the Descemet's membrane (DM) marked along the vertical and horizontal axes.

A, B, C. Three representative samples of DM showing horizontal scrolling of samples marked along the vertical axis. D, E, F. Three representative samples of DM showing horizontal scrolling of samples marked along the horizontal axis.
Figure 4

Scrolling of strips of Descemet's membrane (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.
Figure 5

Spiral scrolling of Descemet's membrane (DM).

A, 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.
Figure 6

Scrolling of Descemet's membrane (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, E, 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 to 1.5.
Figure 7

Immunofluorescent staining of elastin (rabbit polyclonal anti-human elastin antibody) in Descemet's membrane (DM) along the horizontal and vertical axes.

The green immunofluorescent bands of elastin on the anterior surface of the DM are illustrated in the horizontal (A) and vertical (B) sections of the center of the DM. The images represent montages of parallel sections that were cut along horizontal and vertical axes from center (left) to periphery (right). DM = anterior surface of Descemet's membrane. EC = the endothelial surface. The nuclei of the EC are stained blue with a nuclear stain (DAPI).
Figure 8

Fluorescence intensity of the anterior staining band of elastin in Descemet's membrane sections along the A. horizontal and B. vertical axes.

Line graphs plotted for 12 nodes of relative fluorescence intensity. Each node (100 µm long) represents the average of 10 serial semi-quantitative measurements in reference to the background of the staining band from center to 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 standard deviation (SD) of 10 serial measurements along each 100 µm node from center to 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 relative fluorescence units (RFU) from 12 nodes along the horizontal and vertical axes, respectively.
(Three samples of DM are labelled 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.

Figure 9

Thickness of the anterior staining band of elastin in Descemet's membrane 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 the DM. Each node (100 µm long) represents the average of 10 serial measurements in reference to the scale bar, from center to 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 standard deviation (SD)
of 10 serial measurements along each 100 µm node from center to 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 (in µm) from 12 nodes along the horizontal and vertical axes, respectively. (Three samples of DM are labelled 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.

Figure 10

Full thickness measurements of the Descemet’s membrane 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 the DM from center to 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 standard deviation (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 labelled DMEK 1, 2 and 3). There was no significant difference in the thickness of the DM in the different axes or from the center to the periphery in the samples studied.