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Title: Elastin content and distribution in endothelial keratoplasty tissue determines direction of scrolling.

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Corresponding Author: Professor Harminder S. Dua, MD, PhD

Corresponding Author's Institution: Queen's Medical Centre, Nottingham, UK

First Author: Imran Mohammed, PhD

Order of Authors: Imran Mohammed, PhD; Andrew R Ross, MD, MSc (Joint first author); John O Britton, BMedSci; Dalia G Said, MD, FRCS; Harminder S. Dua, MD, PhD

Abstract:

Purpose: Descemets membrane endothelial keratoplasty (DMEK) and pre-Descemets endothelial keratoplasty (PDEK) tissues always scroll with the endothelial cells (EC) outside. We designed a study to understand the reason for this behaviour.

Design: Experimental study.

Methods: Elastin content in Descemets membrane (DM), pre-Descemets layer (PDL), central and peripheral stroma, sclera and trabecular meshwork were measured by the Fastin elastin assay kit. Distribution of elastin in DM, PDL and anterior lens capsule (ALC) were examined by immunohistology. The effect of recombinant elastase enzyme and the effect of complete removal of EC and epithelial cells on the scrolling of DM and ALC respectively, were studied.

Results: PDL showed the highest elastin content among the different tissues studied. Elastin localized as a distinct anterior band in the DM and was uniformly distributed in the PDL demarcating the latter from corneal stroma. Enzymatic treatment of DM with elastase reversed scrolling and corresponded with degradation or disappearance of elastin. Removal of EC did not affect the direction of scrolling. ALC behaved in the same manner with regard to distribution of elastin, scrolling and removal of epithelial cells.

Conclusions: This pattern of elastin distribution in DM explains why DMEK and PDEK tissues always scroll with the EC outside. This behavior is not influenced by the EC. High elastin content and uniform distribution in the PDL suggest a structural difference from the posterior stroma.

Title: Elastin content and distribution in endothelial keratoplasty tissue determines direction of scrolling.

Authors: Imran Mohammed, PhD, Andrew R. Ross, MD, MSc, John O. Britton, BMedSci, Dalia G. Said, MD, FRCS, Harminder S. Dua, MD, PhD.

Affiliations: Larry A Donoso laboratory for eye research, Academic Ophthalmology, Division of Clinical Neuroscience, University of Nottingham, Nottingham, UK.

Corresponding author:

Professor Harminder Singh Dua,

Chair and Professor of Ophthalmology

Academic Ophthalmology

Queens Medical Centre, Derby Road, Nottingham

NG7 2UH

Email: harminder.dua@nottingham.ac.uk

Tel: 00 44 115 9249924 ext. 65354

Fax: 00 44-115-9709963

Short title: Elastin in endothelial keratoplasty tissue

Introduction:

Lamellar keratoplasty has brought several advantages and introduced new challenges in corneal transplantation procedures.[1] Endothelial keratoplasty (EK) for pathologies related to the corneal endothelial cells (EC), provides anatomical or near-anatomical replacement of the diseased tissue, and maintains strength and integrity of the globe and generally does not induce astigmatism. It is also associated with a definite reduced risk of transplant rejection.[2, 3] The popular EK procedure is Descemets stripping endothelial keratoplasty (DSEK). DSEK tissue can be prepared manually or with an automated keratome but always has a variable thickness of posterior stroma[4] in addition to the Descemets membrane (DM) and EC. This tissue remains flat because of its volume and requires a relatively larger incision for its insertion. Descemets membrane EK (DMEK)[5] and pre-Descemets EK (PDEK)[6] are relatively recent additions to EK procedures. The former uses only DM and endothelium for transplantation, while the latter includes an additional 15 to 20 microns of the pre-Descemets layer (Dua's layer, PDL) in addition to the DM. The PDL has also been termed the Dua-Fine layer by the American Association of Ocular Oncologists and Pathologists. Both DMEK[7] and PDEK[6] provide better visual outcomes compared to other procedures and both tissues have a similar characteristic in that they form a scroll, always with the EC on the outside. This fact is critical to determine the correct side of the tissue that should be apposed to the recipient stroma in EK. It has been shown that DMEK scrolls the most, PDL the least and PDEK tissue moderately.[8] While the natural scrolling of the tissue allows insertion through a very small incision, its un-scrolling in the eye prior to attachment poses a significant challenge, which can lead to loss of endothelial cells and consequent risk of failure of the graft.[9]

To develop a consistent and effective method to un-scroll the donor tissue in the eye, it is important to understand why these tissues scroll with the EC outside. This question has eluded a definitive answer though two explanations have been proposed without supporting evidence: swollen EC cause the tissue to scroll with the DM inside and/or the elastin content of the DM and PDL. The latter assertion does not attempt to explain why the tissues always roll with the endothelium outside.[10-14] Through a series of experiments, we have been able to provide evidence to show that the PDL contains a high concentration of elastin and that the direction of scrolling is determined by the content and distribution of elastin in the DM irrespective of the presence or absence of EC.

Methods:

An ex-vivo experimental study on 31 human eye bank sclera-corneal discs consented for research was designed. The discs were stored in Eagle's organ culture medium for 3 to 5 weeks. The cause of death was infection (n= 5), cardiac (n= 7), cancer (n= 6), neurological (n = 4) and others (n = 9). The donor age ranged from 66-82 with a mean age of 72 years. All discs were obtained from the National Health Service Blood and Transplant, Manchester Eye Bank, UK.

Fourteen anterior lens capsules (ALC) were collected from patients undergoing routine phacoemulsification after obtaining patients' consent. The age of these patients ranged from 72-80 years. Patients with pseudo-exfoliation syndrome or posterior synechiae were excluded. Trypan blue dye (VisionBlue®, DORC, Zuidland, Netherlands) was used intraoperatively to stain the capsules. After capsulorhexis, the darker blue epithelial surface was placed on a petri dish and an 'F' mark was made on the anterior surface of the capsule.

Elastin assay in human cornea, limbus, trabecular meshwork and sclera.

Eight sclera-corneal discs were used. After applying the PDEK clamp (e.janach®, Como, Italy)[15] air was injected in the corneal stroma with a 30 gauge hypodermic needle bent to an angle of 135 degrees. The needle was advanced, bevel facing the endothelium, from the peripheral scleral rim towards the central 5 mm zone of the cornea and air was injected to create type-1 big bubble.[16] The DM was carefully peeled off, the bubble deflated and the PDL was excised. A 3 mm skin biopsy punch (Stiefel, Middlesex, UK) was used to trephine the central 3 mm of corneal stroma. 3 x 3 mm blocks of the peripheral cornea and sclera were excised. The trabecular meshwork (TM) was dissected and removed. All samples, namely DM+EC, PDL, central and peripheral corneal stroma, sclera and TM; were placed in pre-weighed and labelled Eppendorf tubes. The presence of TM was confirmed by histological examination of paraffin-embedded hematoxylin and eosin stained sections.
Elastin content was measured in different tissue samples by the Fastin^{TM} Elastin assay kit (Biocolor Life Sciences, UK). Wet-tissue was weighed and hydrolysed in 0.25M oxalic acid by boiling at 95^{o}C for 60 minutes. The soluble alpha-elastin was collected by centrifugation and precipitated with equal volume of elastin precipitating reagent. To generate the standard curve, we precipitated a known amount of recombinant alpha-elastin (6.25, 12.5, 25 and 100 µg). The precipitates were further incubated with 1 ml dye reagent for 90 minutes on a mechanical shaker to generate elastin-dye complex. This complex was then recovered and solubilized with 250 µl of dye-dissociation reagent. Alpha-elastin content was measured at 513 nm wavelength using a microplate reader (Clariostar, BMG labtech, UK) and quantitated by extrapolation against the standard curve. Elastin content was expressed as µg per mg wet-tissue weight.

Immunostaining of elastin to determine elastin content and distribution of DM, PDL and ALC

Eight sclera-corneal discs were used. A PDEK clamp was applied to create a type-1 big bubble in all samples and the DM and PDL were excised in four samples as described above. In two samples the DM was peeled from only half of the tissue and in another two the DM and PDL were left attached (PDEK tissue). Tissue samples were placed on small blocks of fresh cucumber and gently un-scrolled by grasping the edges with two pairs of Birks forceps (Malosa Medical, Elland, UK). Another corresponding piece of cucumber was placed on the flattened tissue and the 'sandwich' placed in aluminum foil cups filled with optimal cutting temperature compound (OCT) and frozen at -80^{o}C.[17]
Ten to twelve micrometer thick sections of OCT embedded PDL, DM and PDL+DM and four whole corneas 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 0.3% Triton-X100 (PBST)). The sections were incubated with polyclonal rabbit anti-human primary antibody against elastin (5 μg/mL final concentration, Abcam, UK) or normal rabbit IgG as control (5 μg/mL final concentration, Santa Cruz Biotechnology, Germany). The sections were washed with PBST 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 fluorescent mounting compound (Dako, UK), examined under fluorescent microscope (B51X Olympus, Japan) and photomicrographs taken. Four ALCs were similarly immunostained.

Elastin digestion with elastase to confirm role of elastin in tissue scrolling:

Seven sclera-corneal discs were used. The DM+EC was stained with VisionBlue®. The peripheral 4 mm of the DM, where the DM is more adherent, was carefully dissected using mini-crescent knife (Mani®, Tochigi, Japan) and the DM was peeled off the stroma for half the diameter, placed back and the central 8 mm was punched using an 8 mm trephine (Katena, Denville, NJ). The DM+EC disc was then completely peeled off, placed in balanced salt solution (BSS) and allowed to scroll. The scroll was imaged and transferred to a solution of equal amounts of elastase (Promega, UK) and tris-buffered saline (TBS) (100 µl each) maintained at 37°C. The membrane was checked for spontaneous un-scrolling at half-hourly intervals. Two samples were left in 200 µl of TBS as controls. Once the DM un-scrolled, the tissue was washed, mounted on fresh cucumber, and prepared for immunostaining as discussed previously. Four ALCs underwent similar process of elastin digestion using elastase followed by immunostaining for elastin. Two capsules were left in 200 µl of TBS as controls.

Endothelial and epithelial cell removal with dispase to determine their role in tissue scrolling:

Four sclera-corneal discs were used. The discs were scanned with phase contrast microscope (Leica Microsystems CMS GmbH DM1000 LED, Wetzlar, Germany) at 10x and endothelial cells photographed. Each disc was placed in 500 µl of dispase (STEMCELL Technologies UK Ltd., Cambridge, UK) 1 U/mL for 16 hours at 4°C. Dispase was then removed by rinsing with PBS 5 times and scanned with phase contrast microscope to confirm the absence of endothelial cells. An 'F' mark was made on the posterior surface of the denuded DM, which was then peeled off as previously described. The DM was placed in BSS and checked for scrolling.

Four ALCs underwent similar process to remove the epithelium using dispase. Using phase contrast microscope, the capsules were scanned before and after dispase. The capsules were then placed in balanced salt solution and checked for rolling.

Results:

Elastin assay/ Quantification of Elastin:

Isolated PDL tissue showed significantly higher amount of elastin content compared to tissue specimen from other parts of the cornea, sclera and TM (figure 1). The average elastin content as measured in μg/mg wet-tissue weight (n=8 for each group) was 37.2 ± 2.75 for PDL, 21.4 ± 3.81 for DM and 31.8 ± 7.70 for TM. Other tissues namely central stroma, corneal periphery and sclera showed low levels of elastin (<10 μg/mg wet-tissue weight).

Immunofluorescence staining of elastin:

Elastin was homogenously present throughout the isolated PDL sections (figure 2 top left). It was predominantly present on the anterior side (PDL side) of isolated DM sections as a distinct band measuring around 10% of the thickness of the DM (figure 2 top right). Similarly, in the PDEK tissue (combined PDL and DM) (figure 2 second row), homogenous elastin staining was seen throughout the PDL and along the anterior part of the DM, which was closely applied to the PDL. Control samples (negative controls) (figure 2 third row) showed no staining. In full thickness corneal tissue sections, elastin staining was localized to the PDL and DM only, with the corneal stroma showing minimal staining resembling the negative controls (figure 2 fourth row). The staining pattern was similar to that seen in PDEK tissue described above.

ALC samples showed a band of elastin staining, relatively anterior in position (figure 2 bottom left). The band measured around 35% of the thickness of the capsule and exhibited less dense staining compared to that of DM.

Elastin digestion with elastase:

In the five DM samples treated with elastase, the DM showed gradual un-scrolling (figure 3 top). Un-scrolling started as early as 1.5 hours and complete tissue un-scrolling (figure 3 second row left) was noted after a mean of 4 hours and 36 minutes (SD ± 1.39). Immunostaining of these samples showed degradation, fragmentation (figure 3 second row right) or complete disappearance of the anterior elastin band. The two control DM samples treated with tris-buffered saline (TBS) showed no change in scrolling pattern (figure 3 third row left). Immunostaining of these samples showed an intact anterior elastin band in DM as previously described (figure 3 third row right).

ALCs treated with elastase showed un-scrolling at a mean of 2 hours and 30 minutes (SD ± 0.65) (figure 3 fourth row). Immunostaining of the capsules showed disappearance of the elastin band (figure 3 bottom left). The two control samples that were incubated in TBS showed no change in scrolling or staining pattern.

Endothelial and epithelial cell removal with dispase:

The four DM samples treated with dispase showed complete disappearance of the endothelial cells when examined with phase contrast microscopy (figure 4 top). All denuded DM samples scrolled with posterior (endothelial) surface outside. This was easy to ascertain by observing the 'F' mark (figure 4 bottom). In other words, removal of EC did not change the direction of scrolling of DM.

The four ALC samples scrolled with the epithelium outside (figure 5 left) as ascertained by the location of the 'F' mark inside the scroll. After treatment with dispase the scrolling pattern remained unchanged despite complete denudation of epithelial cells (figure 5 right).

Discussion:

The un-scrolling of DMEK and PDEK tissue is an important surgical step in EK. It poses a challenge to surgeons performing these procedures and can affect the visual outcome.[9] During the procedure, it is crucial to confirm that the tissue un-scrolls in the right direction i.e. the endothelium away of the recipient cornea. When excessive manipulation is required to achieve this, the risk of endothelial cell loss is increased and can affect graft survival.[18]

Despite the critical nature of this issue, the reason for the consistent directional scrolling of these tissues has never been fully understood. Two suggestions have been put forth; the elasticity of these tissues attributed to their elastin content[10-14] and the swollen endothelial cells, which direct the tissue to scroll in that way.[10]

There are several reports on the elastin content in human corneas but its specific localisation has proved difficult despite use of different histological stains.[11, 19, 20] Lewis et al[12] used tannic acid-based staining and pointed to the presence of elastin, in high concentration, in the posterior cornea. White et al[11] and Lewis et al[12] using two different staining protocols and en-face serial scanning electron microscopy pointed to the presence of complex elastic fibres, in high concentration in the PDL. They also showed that the elastin was lost in keratoconus. The presence of elastin would confer elasticity to the tissues and explain the scrolling of DM but not the consistent directional nature of the scrolling i.e. with the endothelium on the outside of the scroll. Moreover, despite the increased concentration of elastin in the PDL, as shown in this study, it is known to scroll less than the DM.[8] This too requires an explanation.

Quantification of the elastin content in the tissues studied showed that DM, PDL and TM have the highest concentration of elastin compared to the rest of the cornea and sclera. Further, the distribution of elastin in the DM was distinct, with a concentrated presence, as a densely staining band, in the anterior part of DM and a more diffuse distribution, seen as faint staining, throughout the tissue. The distribution of elastin in the PDL was more generalised without a predilection for any specific part of this tissue.

The presence of the anterior dense band of elastin in DM would confer increased elasticity to the anterior surface of the DM relative to the posterior part causing it to scroll with the endothelial cells out. In contrast, the even distribution of elastin throughout the PDL causes the PDL to scroll much less despite its higher elastin content. PDEK tissue on the other hand, which is a composite of DM+EC+PDL also scrolls with the endothelium outside but much less than DM alone.[8] This indicates that most of the scrolling of PDEK tissue is induced by the DM and the splinting action of the PDL causes it to scroll less than DM alone (figure 6). Further

confirmation of the role of elastin in the scrolling of DM came from the elastase digestion experiments. Spontaneous and complete un-scrolling of the DM was observed with elastase digestion. The variability in the time taken for this to occur probably relates to the difference in elastin content, which in turn could be affected by the age of the tissue donors and time in storage.

Intuitively one would assume that the presence of the EC would play a role in determining the direction of scrolling, as has been suggested.[10] However, by removing all EC by dispase treatment, prior to stripping the DM, we demonstrated that the presence or absence of EC did not influence the direction of scrolling. If the endothelial cells play a role in DM (DMEK tissue) scrolling, tissue with lower EC densities would be expected to unfold easily compared to those with higher densities. Heinzelmann et al[18] have however shown the opposite i.e. DMEK tissue from donors with higher endothelial densities tend to unfold easily. This observation and the finding of our study that the DM scrolls in the same direction even after removal of all EC, contradict the notion that endothelial cells play a role in the directional scrolling of DM and support the role of the differential distribution of elastin in this regard.

The relatively high elastin content in the PDL relative to other parts of the cornea is a novel finding that adds to the structural difference of this layer of the cornea. Embryologically, the DM is secreted by the EC formed by the first wave of neural crest cells, and it is suggested that the PDL represents the posterior condensation of the acellular primary stroma as it is pushed out by the expanding secondary stroma produced by the keratocytes (third wave of invading neural crest cells).[21, 22] There is also evidence to suggest that this posterior most part of the stroma is influenced by the endothelium with regard to its collagen and hyaluronic acid content.[23-26] Although Schlotzer-Schrehardt et al[27] suggested that PDL is not a distinct layer but a part of the posterior stroma; the above data and its high elastin content, which demarcates the PDL from the posterior stroma demonstrated herein, add further evidence to support the distinct nature of the PDL. Moreover, the content and distribution of elastin demonstrated in this study can also explain the impervious nature of the PDL.[16, 28, 29] When air is injected in the corneal stroma to separate the PDL and DM in the operation called deep anterior lamellar keratoplasty it permeates the entire stroma till it reaches and cleaves the tissues in a plane between deep stroma and PDL. Air does not pass through the PDL, which expands into the anterior chamber of the eye as a type-1 big bubble. Rarely, air passes through peripheral fenestrations in the PDL[15, 28] and cleaves the DM from the PDL as a type-2 big bubble, which also expands into the anterior chamber. Intraoperatively, when the air is released, and the anterior stroma is excised, both PDL and DM bounce back to their original position, shape and dimension, all of which can be explained by the elastin content and elasticity of these layers.

The anatomical link between the posterior stroma/PDL and the TM has been known sometime[30] and recently reinforced.[31] In this study we found that the PDL and TM had the highest concentration of elastin among the different tissues studied but the difference between these two tissues was not statistically significant. This data further strengthens the link between PDL and TM with potential implications for glaucoma.

The crystalline lens capsule with its layer of epithelial cells has similarities to the DM+EC. The elasticity of the ALC is an important attribute in the physiology of accommodation allowing the lens to change shape to focus both parallel and divergent rays of light.[32] Furthermore, it is known that the ALC rolls with the epithelial cells outside, which has also been noted during cataract surgery.[33] We therefore used it as a viable positive control to examine whether the observations made with DM could be repeated with the ALC. Though the distribution of elastin was slightly different from DM, it was present as a broader, diffuse band towards the anterior half of the ALC. Like the DM, the ALC too consistently scrolled with the epithelial cells outside, in vitro, which was maintained when the epithelial cells were removed by dispase treatment; and un-scrolled when treated with elastase. Intuitively, based on the natural curvature of the ALC, one would expect it to scroll inwards, with the epithelial cells inside but like the DM it behaved in the opposite manner. This suggests that biologically, modified basement membranes have a similar structure and behaviour.

The effect of donor age, type of storage medium, duration in storage medium and pre-existing conditions like diabetes could affect the grade of scrolling and difficulty in unscrolling. These variables were not examined in this study though the age range of the donors was similar to what is normally used in DMEK surgery[34] and none of the donors were diabetic.

This study provides an explanation for the consistent scrolling pattern of the DM, which is of considerable clinical significance. It also suggests that controlled digestion of the elastin prior to insertion of the DM+EC composite in DMEK and PDEK might make unfolding easier in the eye. However, this latter aspect would need extensive evaluation to ascertain the viability and density of EC in relation to elastase treatment. The study also provides further evidence on the structure of the PDL and DM.

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Figure captions:

Figure 1 Elastin content in the tissues studied.

Elastin was quantified in different tissues of cornea-scleral discs using Fastin Elastin assay kit. Pre-Descemets layer (PDL) showed significantly increased amount of elastin compared to Descemet membrane (DM), central stroma, cornea periphery, trabecular meshwork (TM) and sclera. Data is normalized as μg per mg wet tissue weight and presented as mean value ± standard deviation (SD) for n=8 in each group. Statistical significance was set at $p \leq 0.05$ (Student t-test). There was no statisticaly significant difference in elastin content between TM and PDL.

Figure 2 Immunofluroescent staining of the different tissues studied (representative samples are shown).

(Top left) Pre-Descemets layer (PDL) showing homogenous staining for elastin. (Top right) Descemets membrane (DM) showing a dense band of elastin staining anteriorly. The rest of DM shows faint diffuse staining. (Second row left) Elastin staining in pre-Descemets endothelial keratoplasty (PDEK) (Endothelial cells+DM+PDL) tissue. The staining seen is a combination of that illustrated for the individual layers in the previous two figures, i.e. homogenous elastin staining is seen in PDL with an anterior band in DM. (Second row right) PDEK tissue for a part of which PDL has been removed up to the white arrow. Elastin staining of the PDL and DM are clearly visible as described above. (Third row) Negative controls of PDL and DM respectively. Nonspecific rabbit IgG was used as primary antibody. (Fourth row left) Section of full thickness of cornea showing the difference in elastin staining between stroma anterior to PDL and DM. The PDL and anterior band of the DM show more intense staining compared to the stroma. Arrows point to endothelial cells. (Fourth row right) Negative control of full thickness section of human corneal tissue. (Bottom left) Anterior lens capsule showing elastin staining as a broad band located anteriorly in the section. Arrows point to epithelial cells. Scale bar is represented in microns (50 μm = 400x).

Figure 3 Elastase digestion of Descemets membrane (DM) and anterior lens capsule (ALC).

(Top left) DM before incubation with elastase, showing the usual scrolling pattern. (Top right) DM showing gradual un-scrolling after 3.5 hours of incubation in elastase. (Second row left) Complete tissue un-scrolling after 5 hours. (Second row right) Photomicrograph of immunofluorescent stained DM in ‘second row left’. The elastin band is fragmented (black arrows). (Third row left) DM control sample after 12 hours of incubation in tris buffered saline (control) showing that the classic scrolling pattern has not changed. (Third row right) Photomicrograph of immunofluorescent stained DM in ‘third row left’ showing that the anterior band of elastin in the DM is preserved. (Fourth row left) ALC before incubation with elastase showing the normal scrolling pattern. (Fourth row right) ALC showing complete un-scrolling after 2.5 hours of elastase digestion. (Bottom left) Photomicrograph of imunofluorescent stained ALC

in 'fourth row right' showing complete disappearance of elastin staining**.** The DM and ALC were stained with trypan blue.

Figure 4 Descemets membrane (DM) scrolling after removal of endothelial cells (EC) with dispase.

(Top left) Phase contrast photomicrograph of DM showing hexagonal corneal EC. (Top right) Total disappearance of corneal EC seen in 'A' is shown after dispase treatment for 16 hours at 4°C. (Bottom left) Illustrates the F mark placed on the endothelial side of cornea-scleral disc before dissection of DM. (Bottom right) DM showed no change in scrolling pattern, which was determined by the visualizatoin of the F mark on the outside i.e. on the surface from where the EC were removed. The DM was stained with trypan blue.

Figure 5 Anterior lens capsule (ALC) scrolling after removal of lens epithelium with dispase.

(Top left) Scrolling of ALC is seen. (Top right) ALC showed no change in the scrolling pattern after removal of epithelial cells by treatment with dispase (the white mark is an artefact caused by light reflection). (Bottom left) Phase contrast photomicrograph showing lens epithelial cells on ALC. (Bottom right) Total disappearance of lens epithelium was noted after dispase treatment for 16 hours at 4°C. The ALC samples were stained with trypan blue.

Figure 6 A schematic diagram showing the role of Descemets membrane (DM) and pre-Descemets layer (PDL) in the scrolling of endothelial keratoplasty (EK) tissue.

(Top left) The uniform distribution pattern of elastin in the PDL causes it to exhibit minimal scrolling. (Top right) The differential distribution pattern of elastin in DM with an anteriorly located band would cause the tissue to scroll towards its anterior surface (direction of the black arrows) even when the endothelial cells are removed. (Bottom left) In DMEK tissue (DM + endothelium), the differential elastin distribution pattern in DM causes the tissue scroll the most. (Bottom right) In pre-Descemets membrane EK tissue (PDL + DM + endothelium), the PDL acts as a splint counteracting the scrolling effect of anterior elastin band in DM, resulting in reduced scrolling.

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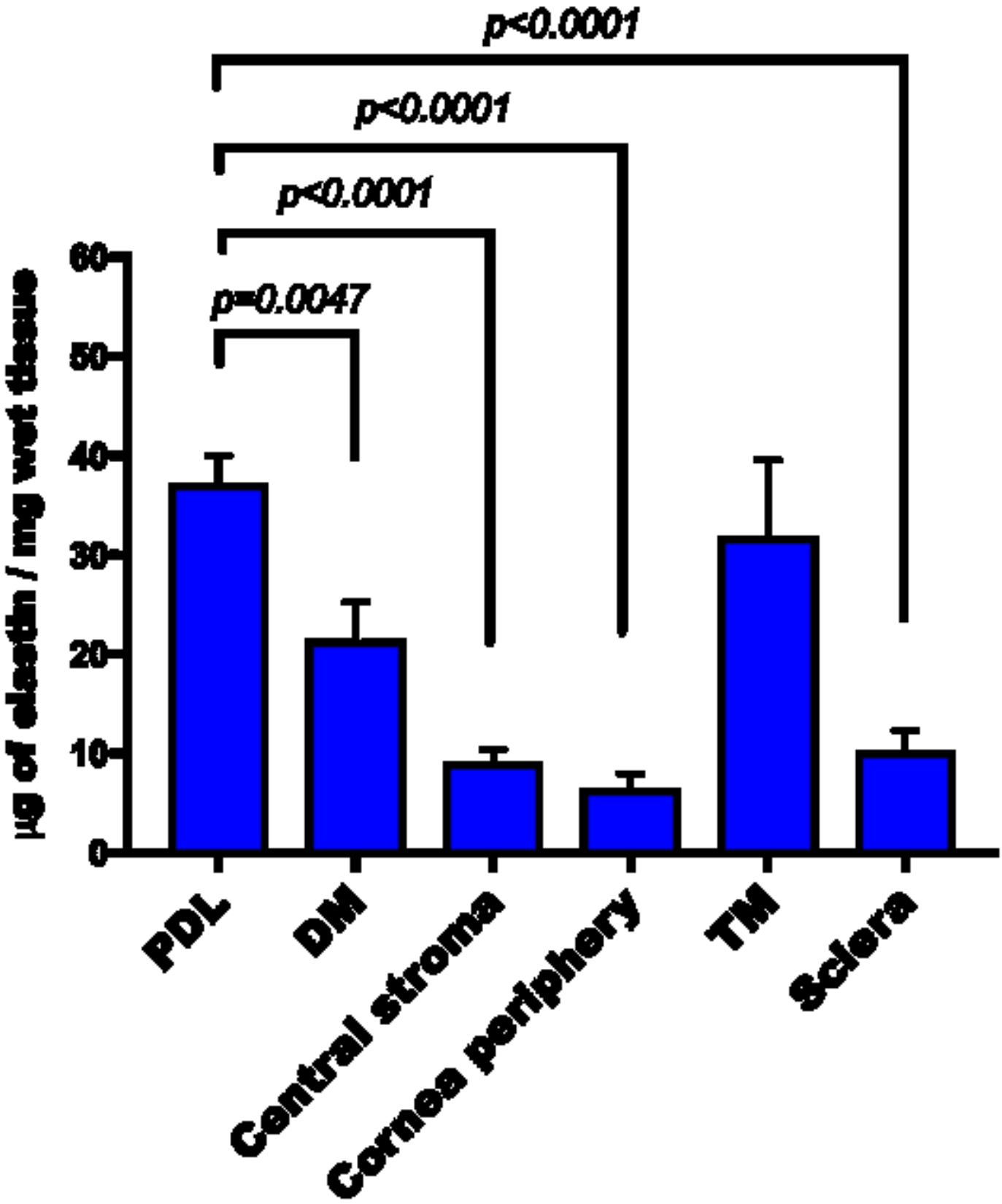

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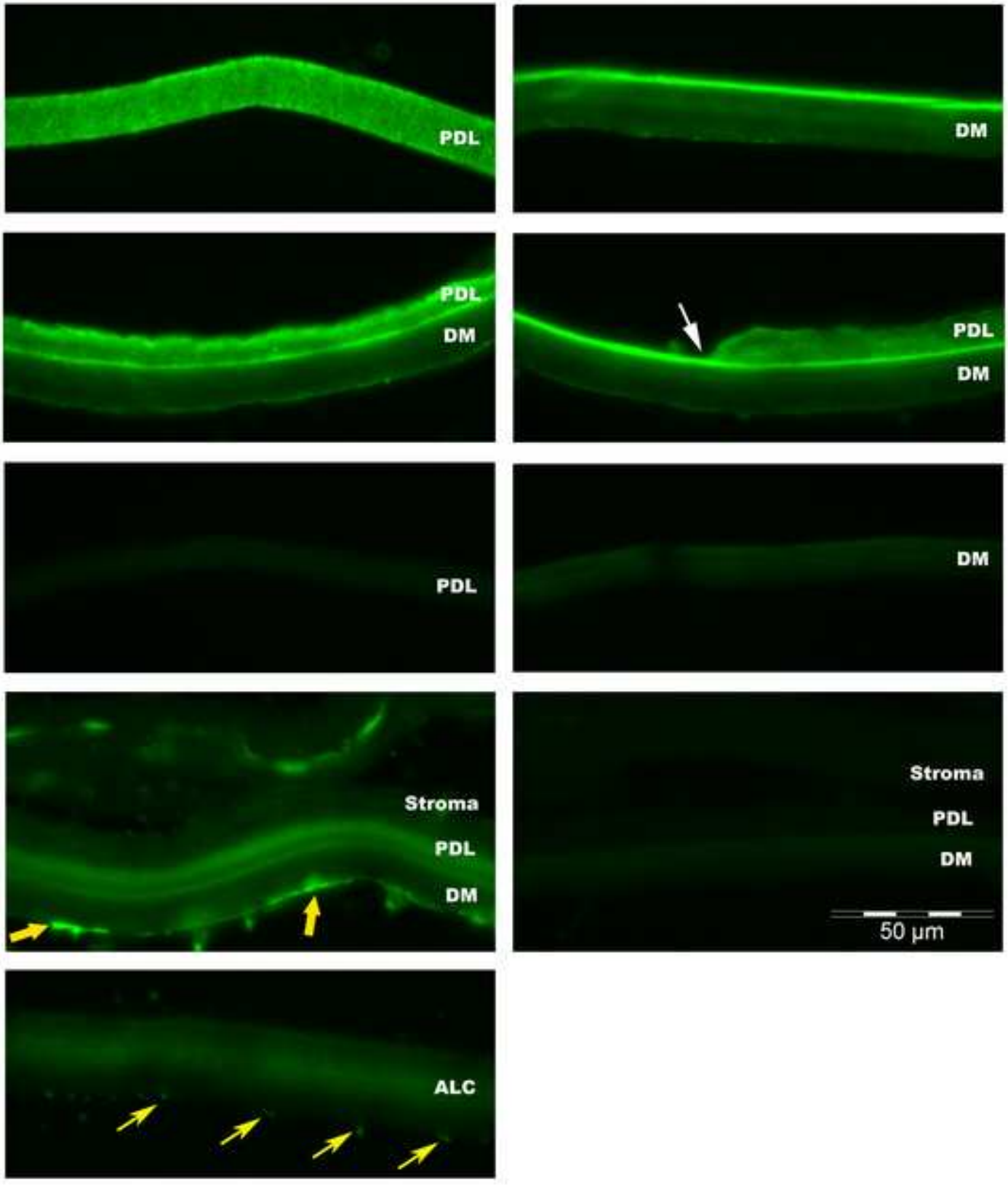

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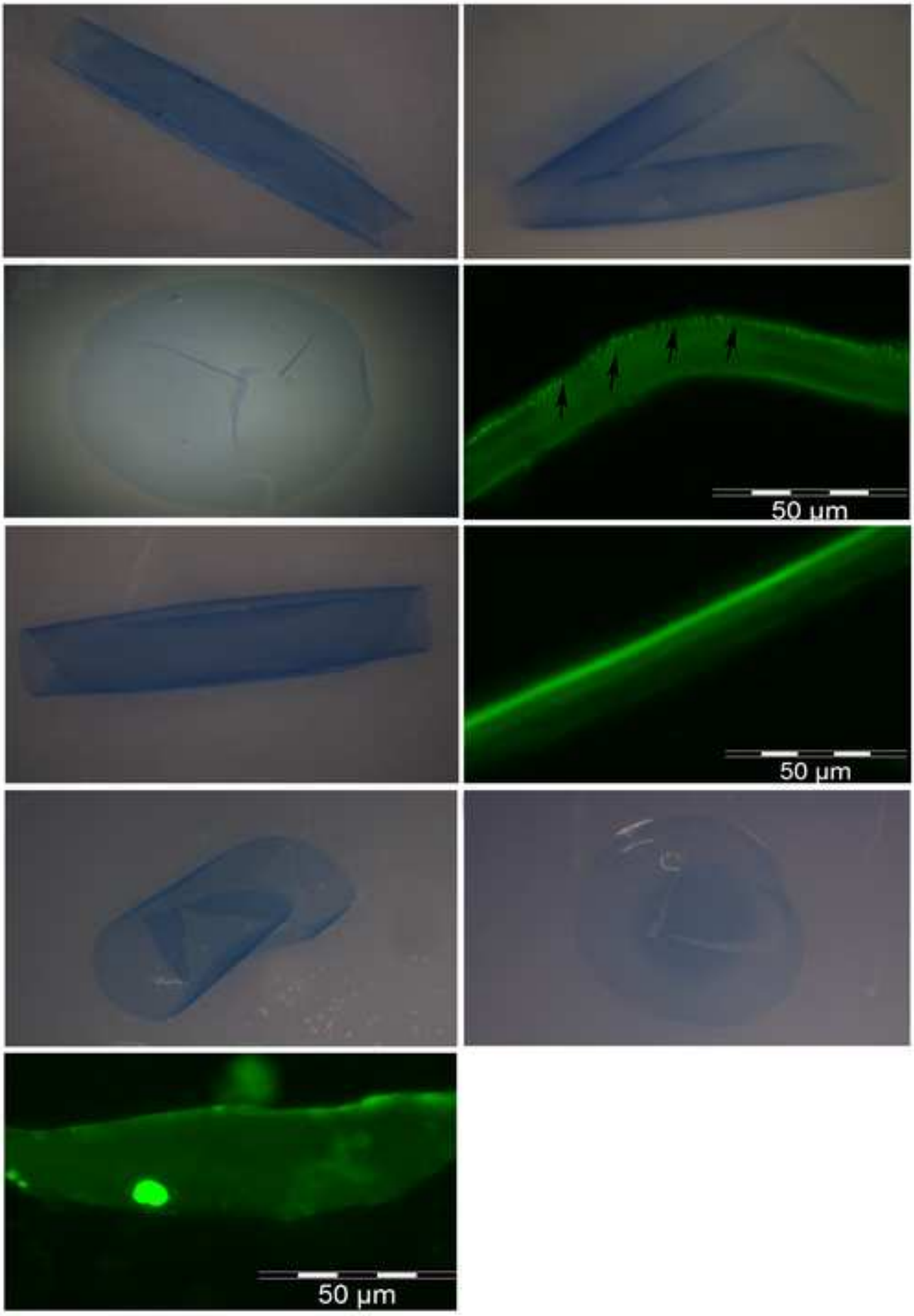

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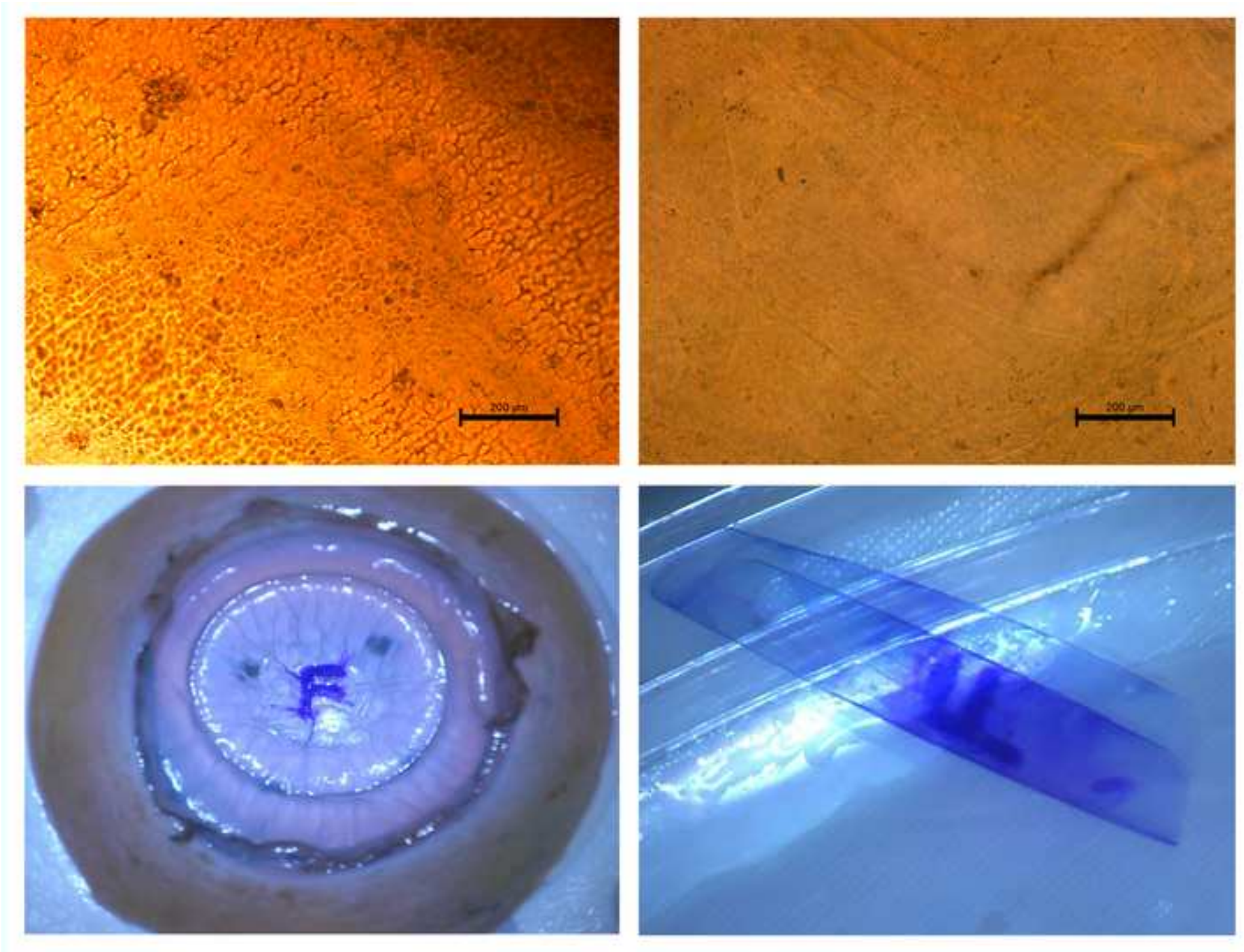

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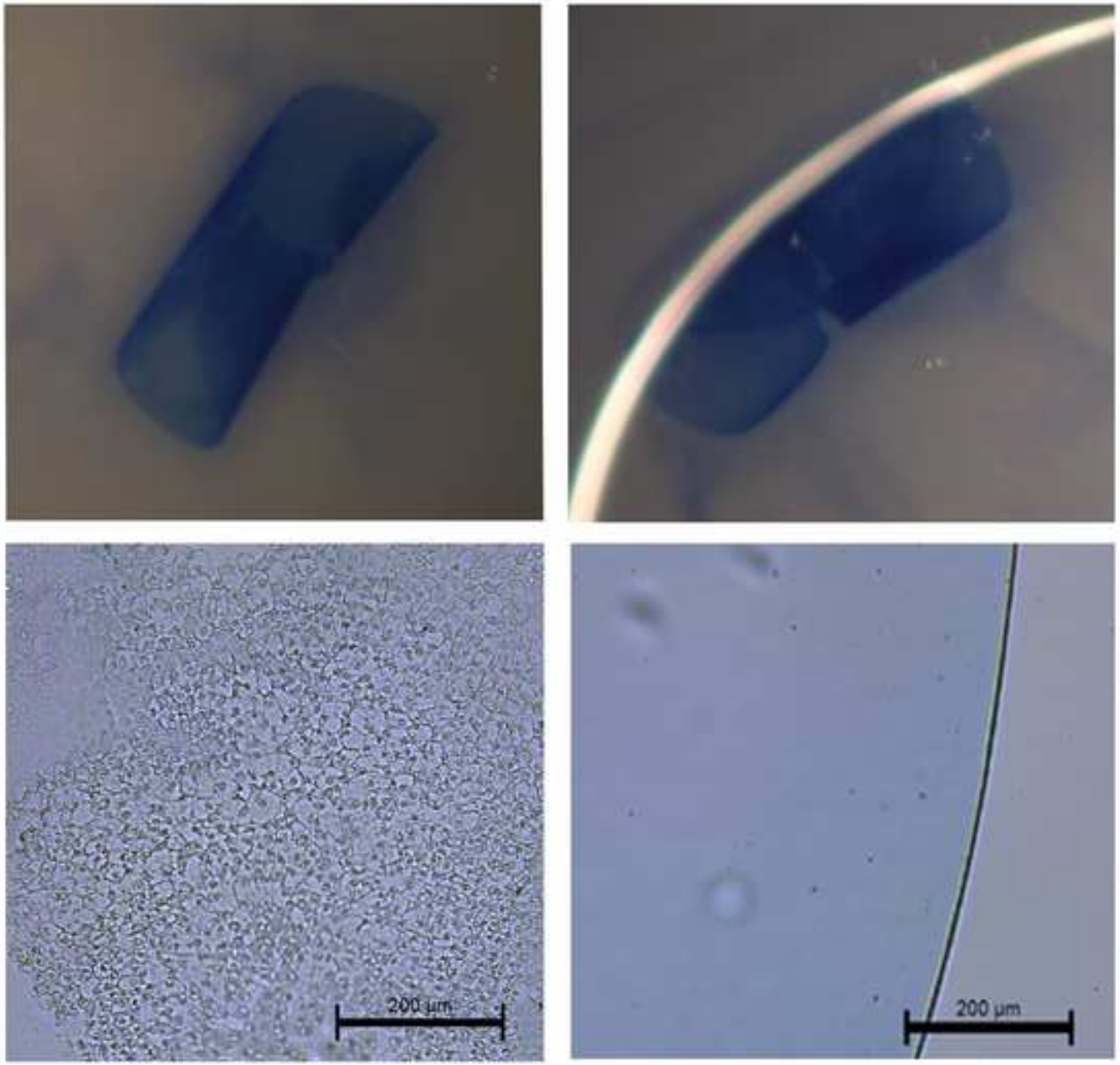

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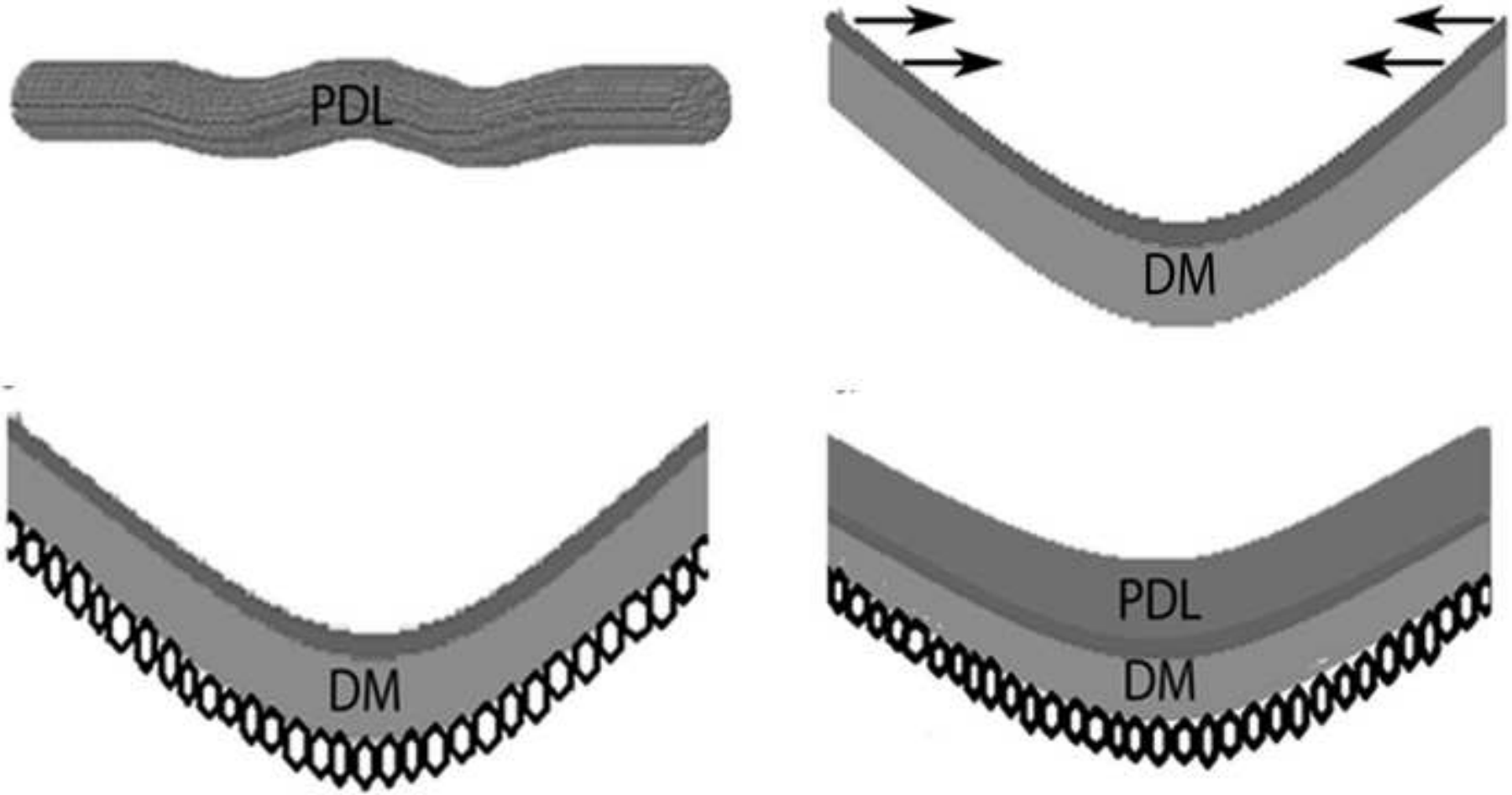

***Table of Contents Statement**

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Elastin is evenly distributed through the pre-Descemets layer (PDL) but concentrated as a band in the anterior part of the Descemets membrane. This explains why endothelial keratoplasty tissues always scroll with the endothelium outside. PDL contains more elastin than any other part of the cornea.

Highlights:

- Endothelial keratoplasty (EK) grafts scroll with endothelial cells (EC) outside.
- Scrolling depends on elastin presence in anterior part of Descemets membrane (DM).
- Digestion of elastin in DM resulted in spontaneous un-scrolling of EK grafts.
- Denudation of EC did not influence the scrolling pattern of EK grafts.