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The pre-Descemet's layer (Dua's layer, also known as the Dua-Fine layer and the pre-posterior limiting lamina layer): Discovery, characterisation, clinical and surgical applications, and the controversy

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ABSTRACT

The pre-Descemet's layer/Dua's layer, also termed the Dua-Fine layer and the pre-posterior limiting lamina layer, lies anterior to the Descemet's membrane in the cornea, is 10 μm (range 6–16) thick, made predominantly of type I and some type VI collagen with abundant elastin, more than any other layer of the cornea. It has high tensile strength (bursting pressure up to 700 mm of Hg), is impervious to air and almost acellular. At the periphery it demonstrates fenestrations and ramifies to become the core of the trabecular meshwork, with implications for intraocular pressure and glaucoma. It has been demonstrated in some species of animals.

The layer has assumed considerable importance in anterior and posterior lamellar corneal transplant surgery by improving our understanding of the behaviour of corneal tissue during these procedures, improved techniques and made the surgery safer with better outcomes. It has led to the innovation of new surgical procedures namely, pre-Descemet's endothelial keratoplasty, suture management of acute hydrops, DALK-triple and Fogla's mini DALK.

The discovery and knowledge of the layer has introduced paradigm shifts in our age old concepts of Descemet's membrane detachment, acute corneal hydrops in keratoconus and Descemetoceles, with impact on management approaches. It has been shown to contribute to the pathology and clinical signs observed in corneal infections and some corneal dystrophies. Early evidence suggests that it may have a role in the pathogenesis of keratoconus in relation to its elastin content. Its contribution to corneal biomechanics and glaucoma are subjects of current investigations.

Prologue

"There are basically two kinds of people in this world. Those who accomplish something and those that claim to. The first group is less crowded." Mark Twain ….and there are those who self-assume the role of sorting people into these two "kinds".

"The reception of an original contribution to knowledge can be divided into three phases. First, it is ridiculed as untrue, impossible, or useless. Second, people then acknowledge that there may be something to the idea, but declare it would never be of any practical use. Third and finally, when the discovery has received general recognition, people say that the idea is not original and had been anticipated by others." W.I.B. Beveridge ….and the final phase when the naysayers demonstrate their acceptance by saying no more; in that silence, Science and the world move on.

"In the meantime we should all remember the admonition from Ecclesiastes 1:9: "There is nothing new under the sun."" (Jester et al., 2013)

"… but the Sun is constantly shedding new light on old objects" (Dua et al., 2014c)

"Science is self-correcting not self-congratulating" (Schwab, 2013) … and much too often non-self vilifying. Scientific progress occurs in

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Abbreviations

AAOOP	American Association of Ophthalmic Oncologists and Pathologists
ACH	Acute corneal hydrops
ASCRS	American Society of Cataract and Refractive Surgery
aSMA	Alpha-smooth muscle actin
AS-OCT	Anterior segment optical coherence tomography
BB	Big bubble
CCT	Central corneal thickness
CD	Cluster of differentiation
CH	Corneal hysteresis
COECSA	College of Ophthalmologists of Eastern, Central and Southern Africa
DALK	Deep anterior lamellar keratoplasty
DAPI	4′,6-diamidino-2-phenylindole
dDALK	Descemetic deep anterior lamellar keratoplasty
DL	Dua's layer
DLEK	Deep lamellar endothelial keratoplasty
DLKP	Deep lamellar keratoplasty
DM	Descemet's membrane
DMD	Descemet's membrane detachment
DMEK	Descemet's membrane endothelial keratoplasty
DSAEK	Descemet's stripping automated endothelial keratoplasty
DSEK	Descemet's stripping endothelial keratoplasty
EC	Endothelial cells
ECM	Extracellular matrix
EK	Endothelial keratoplasty
GCD	Granular corneal dystrophy
IK	Infectious keratitis
IOP	Intraocular pressure
ISBB	Intra-stromal big bubble
MCD	Macular corneal dystrophy
OCT	Optical coherence tomography
OHT	Ocular hypertension
PDCD	Pre-Descemet's corneal dystrophy
pdDALK	pre-Descemetic deep anterior lamellar keratoplasty
PDEK	Pre-Descemet's endothelial keratoplasty
PDL	Pre-Descemet's layer
PK	Penetrating keratoplasty
POAG	Primary open angle glaucoma
PRDX3	Peroxiredoxin 3
STALK	Subtotal anterior lamellar keratoplasty
STS	Steroid sulfatase
TALK	Total anterior lamellar keratoplasty
TEM	Transmission electron microscopy
TGFb1	Transforming growth factor beta 1
TM	Trabecular meshwork

a series of small truths, some lesser truths, some greater truths, which build on or stride over previous truths. Progress is often retarded by the denial of some truths but the greater truth eventually emerges by asserting what is and discarding what is not.

"Today is the Yesterday of Tomorrow" What is today state of the art, may tomorrow be relegated to the dustbin of history" (Dua HS in Keeler et al., 2013)

1. Introduction

Over one hundred and fifteen years ago, the year 1905 is marked as the year that heralded the beginning of modern corneal transplantation surgery, when the Austrian physician, Eduard Konrad Zirm performed the first successful human lamellar corneal transplant. However, neither the concept nor the operation were novel at that time. Several attempts at transplanting corneas in animals and transplanting animal corneal tissue in humans were made over, at least the preceding one hundred years (Crawford et al., 2013). The next significant landmark was achieved twenty five years later, in 1931, when Russian (Ukrainian) ophthalmologist Vladimir Filatov successfully performed full thickness corneal transplants in humans (Galst and Maryshev, 2009). Over the decades since, the number of corneal transplants has steadily grown to around two hundred thousand a year worldwide but falling far short of the demand by a ratio of 1:70 (Gain et al., 2016). Growth in corneal transplant surgery was complemented by innovations and inventions in techniques, immunosuppression and anaesthesia, microscopes, sutures, and instrumentation that promoted and facilitated improved outcomes. Despite the above, major problems of immunological rejection and failure, suture related complications including induced astigmatism and a weak graft-host junction, often leading to globe rupture with loss of contents following even minor trauma; have dogged full thickness corneal transplants through the century. Immune mediated rejection remains the most common cause of corneal graft failure (Borderie et al., 2011; Gunasekaran et al., 2014; Keane et al., 2014; Reinhart et al., 2011). Surprisingly, many answers to these problems came from innovations in surgical techniques, more than from understanding of immune mechanisms, immunomodulation and immunosuppression.

The indications for corneal transplantation can be broadly divided into two types. One, where the corneal stroma is affected, primarily by scarring, dystrophies or ectasia; but the Descemet's membrane (DM) and endothelium are normal; and two, where the DM and/or the endothelium are affected as in endothelial dystrophies or following surgical trauma, but the stroma is normal. For the former indications, deep anterior lamellar keratoplasty (DALK), wherein the host DM and endothelium are retained and the affected stroma and epithelium are replaced, is the preferred option. DALK eliminates the risk of immune mediated endothelial rejection and consequent graft failure, but the problems related to sutures, induced astigmatism, and weak graft-host junction remain, though the grafted eye is stronger compared to full thickness or penetrating keratoplasty (PK) (Zarei-Ghanavati et al., 2010). For indications related to endothelial diseases, endothelial keratoplasty (EK), wherein the normal or relatively normal host stromal tissue and epithelium are retained whilst the DM and endothelium are replaced, is the preferred option. This eliminates suture related problems, induced astigmatism and leads to rapid visual recovery. Endothelial rejection is not eliminated but the risk is considerably reduced compared to PK (Faraj et al., 2012; Lee et al., 2009). For these indications, modern lamellar corneal grafts or selective corneal transplantation has largely replaced PK. For indications where the disease affects all layers or is associated with heavy vascularisation, PK remains the viable option.

2. Discovery of the Pre-Descemet's layer/Dua's layer (PDL/DL)

After Filatov's exposition of PK it became the most popular technique for all indications. However, its disadvantages of risk of rejection, suture related problems, induced astigmatism, weak graft-host junction and raised intraocular pressure (IOP) more than can be accounted by the use of steroids alone, were always a concern (Singh et al., 2019; Stechschulte and Azar, 2000). The open-sky nature of the surgery with risk of expulsive choroidal hemorrhage, loss of intraocular contents and infection leading to endophthalmitis, compounded the risks. The incentive to refine anterior lamellar keratoplasty, in particular the closed globe 'extra-ocular' nature of the surgery, led to improvements in techniques aimed towards removing more and more of the corneal

stroma, the principle driver being 'the deeper the better' with the Holy grail being the removal of all tissue anterior to the DM (Anwar, 1972; Barraquer, 1972; Ehrlich et al., 1988; Melles et al., 1999; Morrison and Swan, 1982; Polack, 1971; Rich et al., 1988).

Though various mechanical techniques claimed superiority and took the lamellar dissection plane deeper in to the stroma, it was the Big Bubble (BB) technique, developed by Anwar (Anwar and Teichmann, 2002a) that consistently 'bared' the DM, and in effect delivered the Holy grail. This rapidly became the most used technique and made DALK popular, though still with considerable surgical challenges and a steep learning curve. Injection of viscoelastic into the stroma, to reach the same plane of dissection was presented as and alternative approach to achieve the same end (Güell and Aristizábal-Montes, 2014; Manche et al., 1999; Shimmura et al., 2005). Viscoelastic injection in the stroma can lead to an intrastromal BB formation, a feature that is not seen with air injection (Ross et al., 2018). It was the firm belief of surgeons who performed BB DALK that, when a BB was achieved either by air or viscoelastic injection, the DM was laid bare, and the technique was dubbed the 'Descemet's baring technique' (Al-Torbak et al., 2006; Alio et al., 2002; Amayem and Anwar, 2000; Anwar, 1974; Anwar and Teichmann, 2002a, 2002b; Archila, 1984; Fontana and Tassinari, 2007; Manche et al., 1999; Zarei-Ghanavati et al., 2010).

2.1. DALK surgical concepts in the early days

The principles of DALK surgery, despite several variations and adaptations, are essentially the same. The standard technique for BB DALK consisted of central deep lamellar trephination of the desired diameter, injection of air through a needle or canula into the deep stroma of the central cornea inside the trephination until a BB is formed. Two types of BB were described; one with a white margin, which indicated that a BB had formed (Anwar and Teichmann, 2002a) and one with a clear margin, or a double bubble (usually one complete and one partial), which were attributed to air splitting the DM between the banded and non-banded layers (Anwar, 2007; Hirano et al., 2002; Sharma et al., 2013). Once a BB was achieved, the anterior stroma was excised and a donor corneal button, from which the DM and endothelium were removed, was sutured in place, thus retaining the host's DM and endothelium. In a few cases where the operation was unsuccessful due to an intraoperative complication, the excised 'DM' was examined by histology and shown to have a lining of "residual stroma" in some but not all cases. The same observation was made by creating a BB in eye bank eyes. It was proposed that a layer of "residual stroma" could be found covering the DM in BB DALK (Jafarinasab et al., 2010; McKee et al., 2011, 2012b; Yoeruek et al., 2012). However, prior to the demonstration of "residual stroma", in 2007 we had demonstrated early evidence of the existence of a distinct layer in the deep stroma anterior to the DM and its relevance to BB DALK, and termed it the "pre-Descemet's stromal layer" (see below, Section 3). The detailed techniques of modern DALK and the science behind the surgery can be found in the chapter on 'Deep Anterior Lamellar Keratoplasty (DALK): Science and Surgery. In Albert and Jakobiec's Principles and Practice of Ophthalmology' (Dua and Said, 2021).

2.2. Clues pointing to the presence of the pre-Descemet's layer/Dua's layer

In the early 2000 when we started performing DALK by the BB technique we made certain observations that did not add up to the prevailing beliefs and concepts, and came across certain clues that pointed to the existence of a distinct additional layer in the deepest stroma that offered a plane of cleavage anterior to it, which was different from the plane along which the DM separated. Several surgeons who had performed DALK by the BB technique, as they later recalled and confirmed, had encountered the same observations (Dua et al., 2014b).

The DM that was bared, in the 'Descemet's baring technique' did not

feel and behave like the DM encountered in other procedures. It was stronger, more resilient and could withstand and bounce back after being subjected to force or pressure applied by a swab or blunt instrument (Fig. 1A). Peeling the DM from the donor button was relatively easy, offering a smooth plane of cleavage with little resistance, without any physical connections extending between the DM and the stroma
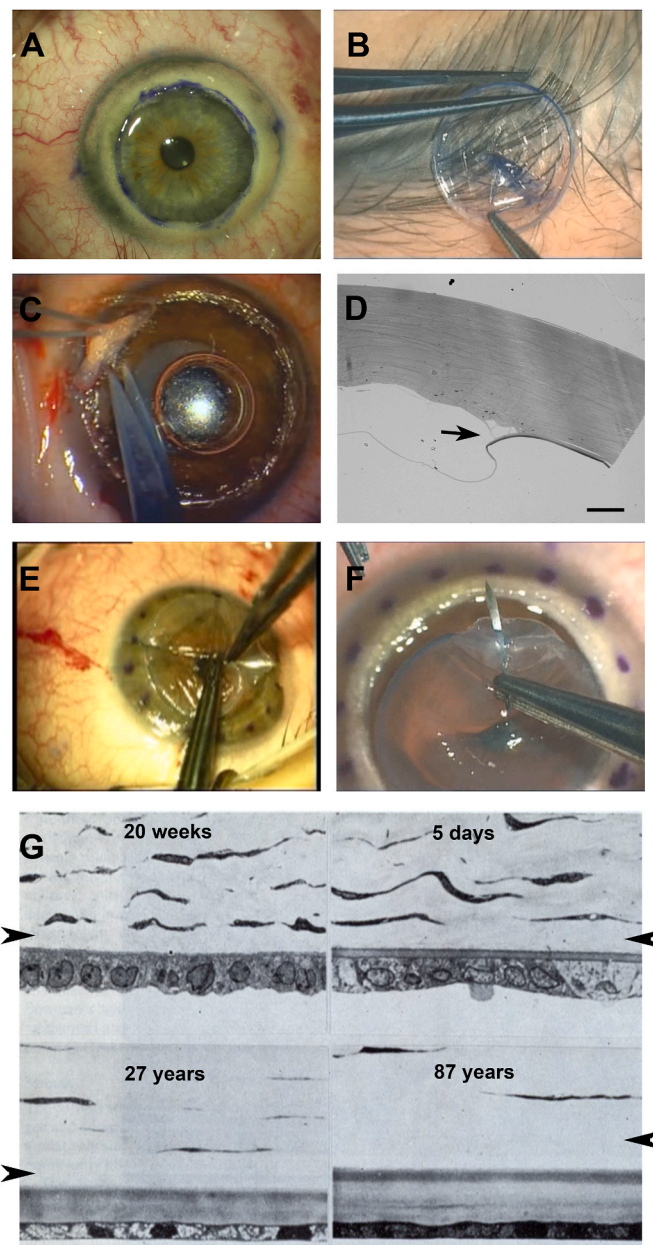

Fig. 1. Clues pointing to the presence of the pre-Descemet's layer (Dua's layer) (PDL/DL). (A) The anterior surface of the PDL/DL in a patient undergoing deep anterior lamellar keratoplasty (DALK). (B) Descemet's membrane (DM) peels off easily from the donor cornea. (C) Peeling the posterior stroma off, what was thought to be the DM (PDL/DL) reveals collagen strands that have to be cut or severed. (D) Light microscopic demonstration of the collagen strands (arrow) at the edge of the big bubble. (E) Suture needle pass through deep stroma in penetrating keratoplasty reveals the edge of the DM/PDL. (F) An edge is still visible when DM is removed from the donor DALK button. (G) Image reproduced with permission from the author, Prof John Marshall (Marshall and Grindle 1978). The thickness of the DM in eyes of different ages was shown to increase with age. The images also show the PDL/DL (arrowheads) to be present posterior to the last row of keratocytes and increases in thickness with age. This was not identified or commented on by the authors.

([Fig. 1B). Often during BB DALK, the air bubble fell short of the circular margin of the trephination either in part of all around. This also happened if the trephination was eccentric. In such instances the anterior stroma had to be physically dissected from the 'bare DM', and close to the periphery fine strands could be seen extending from the stroma to the anterior surface of the 'bare DM'. These had to be severed by blunt dissection or cut with fine tipped scissors. The clue here was that no strands of tissue were encountered when separating the DM from the stroma, why were they seen when separating the stroma from the DM (Fig. 1C and D)?

In PK, when passing sutures through the donor button, a distinct sharp edge is seen posterior to the needle, as it emerges just anterior to the DM. This edge had largely been attributed to the edge of the trephined DM. However, when suturing the donor button in DALK, from which the DM had been peeled off, a 'distinct edge' was still visible, suggesting that there was probably another layer with a sharp edge covering the posterior surface of the donor button [Fig. 1E and F]. We and others had noted that DALK conferred better security and structural integrity in response to trauma than PK. This was attributed to the retained DM in DALK but to us it was counter intuitive to assign this strength to the relatively fragile, retained DM (Dua et al., 2014b; Fontana and Tassinari, 2007; Zarei-Ghanavati et al., 2010).

Histological sections of the cornea, both related to our various research projects and those published in the literature showed an acellular zone between the last (posterior) row of keratocytes and the anterior margin of the DM. The thickness of this zone or layer, appeared to be age related (Marshall and Grindle, 1978) (Fig. 1G).

3. The evidence

3.1. Hypothesis

Based on the above clues and observations we postulated that "there exists a distinct layer in the posterior stroma that is different from the DM. In BB DALK the plane of cleavage is less often between stroma and DM and more often between the stroma and the pre-Descemet's Layer (PDL)." We presented our early results on the "pre-Descemet's stromal layer" at the annual congress of The Royal College of Ophthalmologists UK, in May 2007, Symposium - Evolving Techniques in Corneal Surgery – Layer by Layer, and further evidence was presented at the Societa Italiana Cellule Staminali e Superficie Oculare, VI CONGRESSO S.I.C.S. S.O. Lecce, June 14–16, 2007 (Fig. 2A–C).

A review of the literature at that time revealed two studies that attempted to define the plane of separation in DALK. One study was on human eye bank eyes, where viscoelastic was used to separate DM from posterior stroma in DALK (Melles et al., 2000). In the abstract the authors reported that "with light microscopy, dissection depth was located at the level of DM" and concluded that using visco-dissection, a lamellar keratoplasty can be performed quickly, with the donor-to-recipient interface just above the recipient DM, i.e., with a nearly perfect anatomical replacement of all corneal stroma." However, in the manuscript a histological illustration is presented, where at x450 magnification, "some residual stromal strands are visible over Descemet's membrane". Another study (Hirano et al., 2002) where DALK was performed by removal of the bulk of the anterior stroma by physical
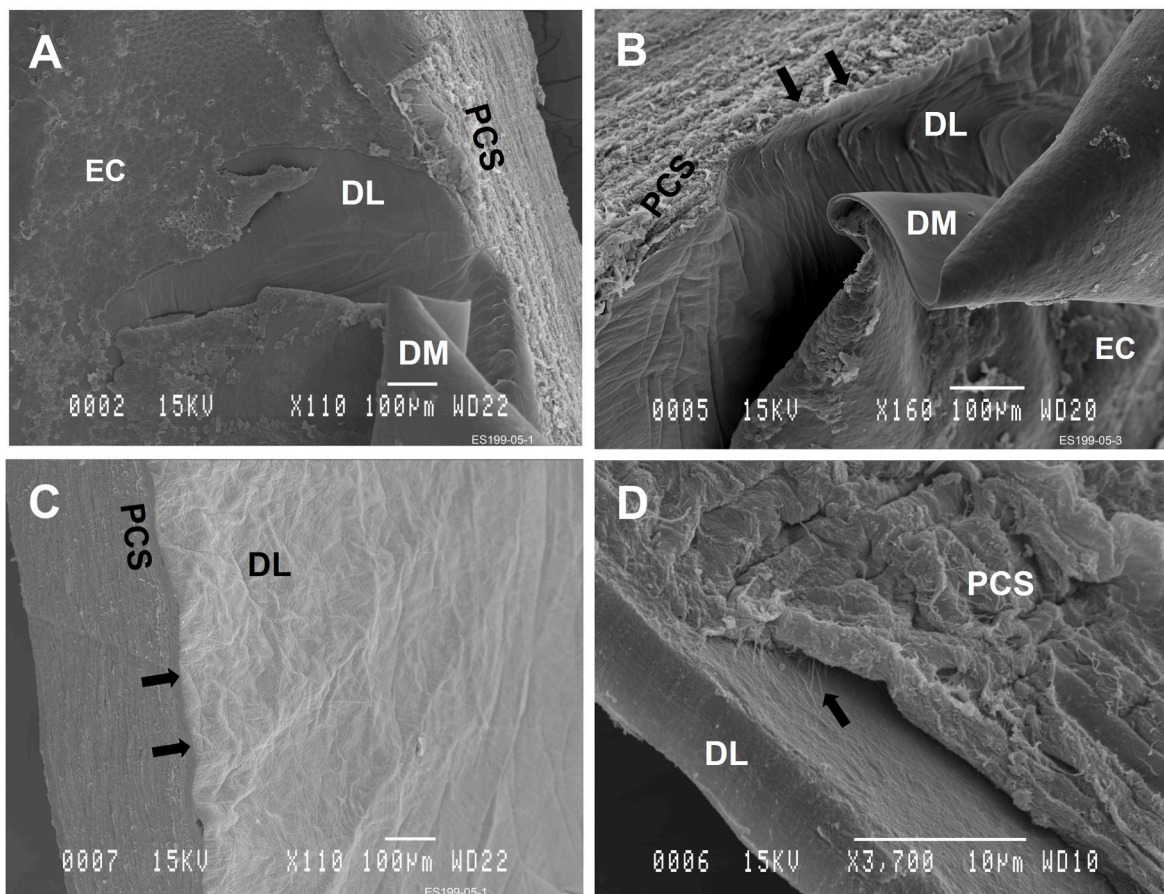

Fig. 2. Scanning electron microscopy (SEM) evidence of the pre-Descemet's layer (Dua's layer) (PDL/DL) obtained and presented in 2005. (A) SEM showing the smooth surface of the PDL/DL from where the DM has been peeled off. (B) Arrows point the edge of the PDL/DL. (C) SEM of whole cornea showing the PDL/DL after removing the DM covering. Arrows point to the edge of the PDL/DL. (D) SEM illustrating the compact nature of the PDL/DL. Arrow points to the strands of collagen that extend from the posterior stroma to the anterior surface of the PDL/DL. DM = Descemet's membrane. DL = Dua's layer. EC = endothelial cells. PCS = posterior corneal stroma. The text and numbers on the figure are technical details with the year 2005 written on them.

dissection, followed by fluid hydration and physical dissection of the deep tissue, revealed the banded part of the DM attached to the deep stroma in 2 of 4 cases. They concluded that "Separation of the deep stromal tissue from Descemet's membrane may occur within the Descemet's membrane, and the separation is probably between the anterior banded and the posterior non-banded layer of Descemet's membrane in some cases during deep lamellar keratoplasty (DLKP).

Following our presentations on the 'pre-Descemet's stromal layer' in 2007, from 2010 onwards, further reports on the presence of "residual stroma" in many but not all cases of DALK were reported. Jafarinasab et al. studied three keratoconus corneas that underwent DALK with a successful BB but were converted to PK due to DM tears. They reported the presence of a thin layer of stroma, of variable thickness in all cases (Jafarinasab et al., 2010). Other studies on the 'rupture-pressure' of the tissue left after BB DALK with a 'white-band' and on the dissection plane in clear margin BB, demonstrated the presence of residual stroma that accorded strength and integrity to the eye after BB DALK (McKee et al., 2012a, 2013). Contemporaneously with this study, Yoeruek et al., while studying the characteristics of DM removed by the forceps peeling technique or by the BB technique, did not show any residual stroma in any eye with either technique where useable DM was obtained (Yoeruek et al., 2012).

3.2. The pre-Descemet's layer/Dua's layer in relation to types and anatomy of big bubbles

To test the hypothesis presented in 3.1. we conducted a series of experiments on human eye bank donor whole globes and sclero-corneal discs to simulate DALK *ex vivo*, by the BB technique and demonstrated the presence of a distinct layer, termed the pre-Descemet's layer (Dua's layer) (PDL/DL). (Dua et al., 2013).

The experiments showed that regardless of method of insertion of the needle and air injection in the stroma, from the epithelial surface directly or after an initial trephination; or from the endothelial surface, three types of BB were achieved. Injection from the endothelial surface under the operating microscope enabled the direct visualisation and recording of the dynamics of BB formation (Dua et al., 2018).

Injected air followed a consistent pattern, emerging from the tip of the needle in the stroma, moving as one or several, fine or broad, radial tracks towards the corneo-scleral junction (Fig. 3A). Upon reaching the limbus, the movement of air occurred in a clockwise and anti-clockwise circumferential direction as 'white bands', which met to complete the circle. The width of this band was between 1 and 1.5 mm (Fig. 3B–D). Thereafter, air moved centripetally to completely fill the stroma. The peripheral cornea is more compact and thicker than the central cornea, and the fibres are more tightly knit. The force required to separate the stromal lamellae at the periphery is more than at the centre (Smolek and McCarey, 1990). The collagen fibres at the periphery are tangentially oriented to form a 'circular annulus' and probably create an anatomical
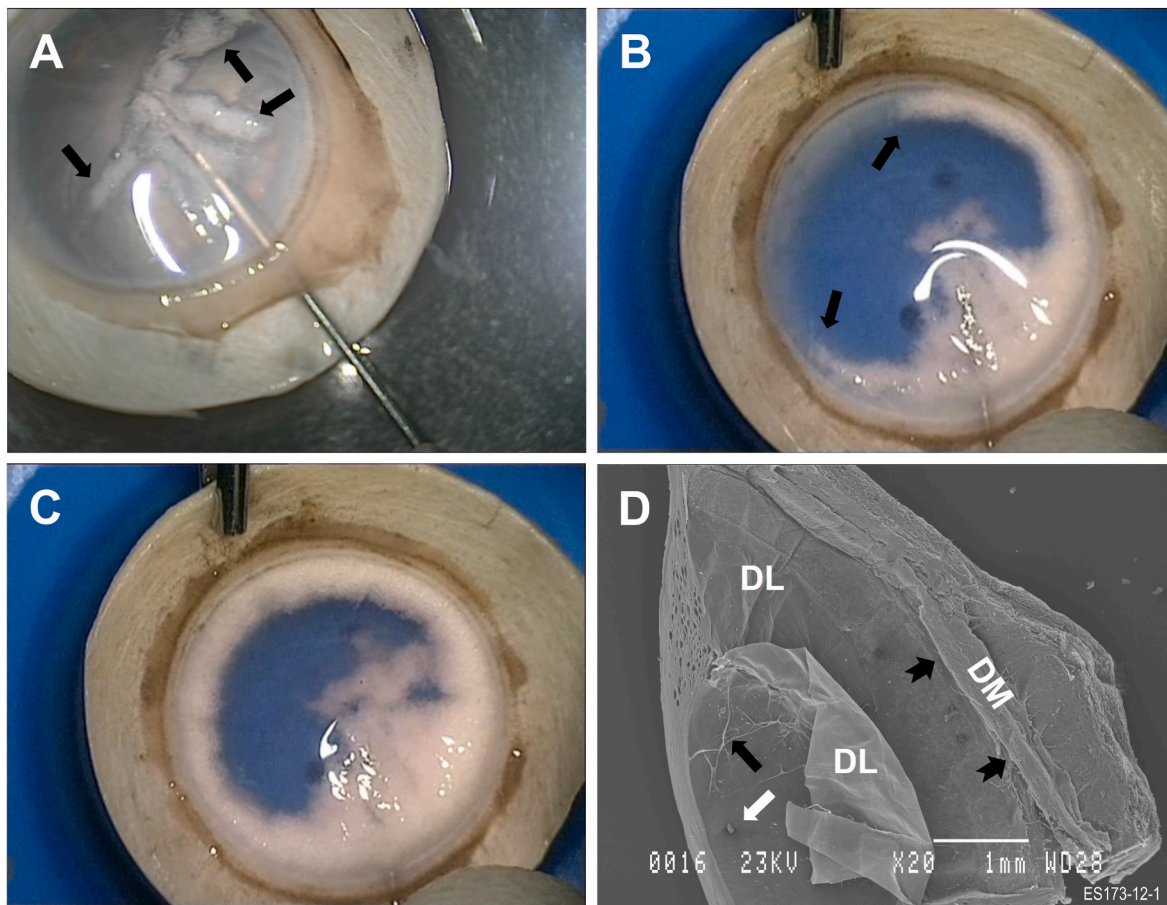

Fig. 3. Movement of air in the corneal stroma during big bubble (BB) formation. (A) Three radial tracks of air are seen (arrows) emerging at the needle tip and extending towards the limbus. (B) Clockwise and counter clockwise circumferential movement of air (arrows). (C) A complete white band (1–1.5 mm) is formed along the circumference. (D) Scanning electron micrograph of a quadrant of the cornea. The sector of the circumferential band is seen, which corresponds to the white band in 'C'. The PDL/DL of the BB is seen to extend right to the periphery beyond the margin of the BB. Arrowheads point to the cut edge of the DM. The collagen strands extending between deep stroma and the anterior surface of the PDL/DL are seen (black arrow). The white arrow points to a broken and recoiled collagen strand that appears as a 'blob' on the deep stroma. DM = Descemet's membrane. DL = Dua's layer.

landmark as they transition from orthogonal to the tangential orientation in the annulus adding to the compactness and tighter attachment of fibres at the periphery (Abahussin et al., 2009; Aghamohammadzadeh et al., 2004; Newton and Meek, 1998). The PDL/DL is firmly attached to the posterior stroma along the 'circumcorneal annulus'. This architecture of the peripheral cornea most likely determines the circular movement of the band of air and its inner anatomical landmark determines the diameter of the type 1 BB (Fig. 3C and D). This periphery of the cornea, corresponding to the width of the band of air and the firm attachment of the PDL/DL is also the flatter part of the cornea giving it its prolate shape. Evidence to support this was provided by performing topography in donor sclero-corneal discs, mounted on an artificial chamber, filled with balanced salt solution to maintain a pressure of 20 mmHg. The width of the peripheral flattening was thus determined before injecting air. The circular movement of air was shown to corresponded to the width of the flat corneal periphery (Holland S and Dua HS, personal observations). The PDL/DL contributes to the firm peripheral annulus, which provides the foundation on which the optical dome of the cornea sits.

3.3. Dynamics of BB formation

One type of BB was formed by the appearance of multiple small pockets of air at the posterior surface of the corneal stroma, which coalesced to form a central bubble that expanded centrifugally to a diameter of 8–9 mm (average 8.5 mm) (Fig. 4A and B). This was the commonest type and presented as a well-circumscribed, central dome shaped elevation. The perimeter of the BB corresponded to the central margin of the circumferentially moving 'white band' of air. The anterior wall of the BB was the emphysematous corneal stroma and the posterior wall was made of the PDL/DL and DM with endothelial cells (EC). This type of BB remained intact and did not deflate when the DM was peeled off (Fig. 4C and D). This clearly indicated that the PDL/DL was impervious to air. The posterior surface of the PDL/DL presented a rough scruffy appearance (Fig. 4D). This was termed the type 1 BB. Though the BB diameter was limited to 8–9 mm, traction on the PDL/DL demonstrated that stress lines or striae extended right up to the limbus, suggesting that the layer did not end at the margin of the BB but covered the entire posterior surface of the cornea. This was also evident on scanning electron microscopy (Fig. 4E and F). When the DM with the EC was peeled off the sclero-corneal discs prior to injection of air, a 'type 1 BB' could still be created in every sample indicating that the DM was not

essential to the creation of the BB and that 'impervious-to-air' characteristic of the PDL/DL was not afforded by the attached DM (Dua et al., 2013) (Fig. 4G and H).

Less commonly, after or during the movement of air in the stroma, as described above, a shiny, glistening BB appeared at the periphery of the sclero-corneal disc and expanded to cover the entire posterior surface of the cornea as a larger dome shaped elevation. On occasions two or rarely three such BB appeared at the periphery and then coalesced to form a large single BB (Fig. 5A–C). Attempts to peel the DM off this BB resulted in the immediate collapse of the BB indicating that the posterior wall was made of DM and EC without the PDL/DL, which remained attached to the posterior stroma as the anterior was of the BB. This was termed the type 2 BB (Fig. 5D).

On occasions both types 1 and 2 occurred simultaneously, these were termed mixed BB and later also referred to as type 3 BB in some reports. With mixed BB the type 1 component was usually complete and the type 2 component was partial or complete, usually the former, which presented at the periphery of the type 1 BB or astride it (Fig. 5E and F). Until this demonstration, mixed BB or double bubbles were attributed to a split between the banded and non-banded zones of the DM (Anwar, 2007; Hirano et al., 2002; Sharma et al., 2013). As with the type 2 BB, the type 2 component of mixed BB usually started at the periphery but on rare occasions, were formed by air escaping from the periphery of the type 1 component.

During the formation of all types of BB, tiny air bubbles could be seen escaping from the periphery of the discs, at the limbus. These bubbles emerge peripheral to the attachment of the DM and during the DALK procedure, find their way into the anterior chamber, a common observation in DALK (Fig. 6A and B). The pattern of air movement in the stroma and the formation of the different types of BB occurred regardless of the direction of the bevel of the needle, anterior, posterior of sideways; and irrespective of the depth, mid or deep stroma (Dua et al., 2018). Observations on the patterns formed by the earliest emergence of air in the stroma from the needle tip revealed either a diffuse whitening of the stroma or the formation of discrete lines, like cracked glass, which then went on to progress to the radial tracks described above. The more compact the cornea (deturgesced with addition of dextran to the storage medium) the more likely was the 'cracked glass' appearance. This observation has also been previously reported to be related to the pattern of microbial migration in infectious crystalline keratopathy (Butler et al., 2001). A key factor in the creation of a type 1 BB was the intra tissue pressure of the air injected. A certain critical pressure was
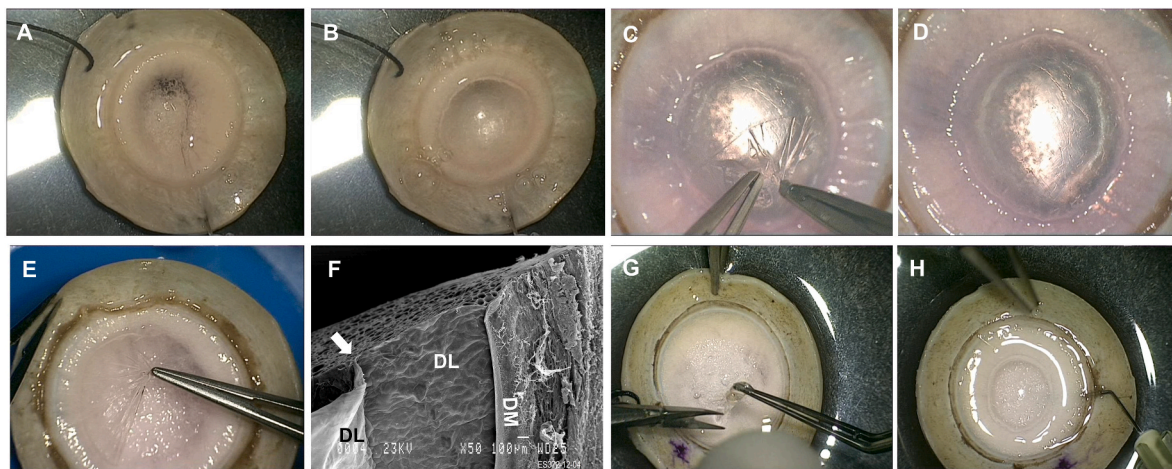

Fig. 4. Ex-vivo characteristics of a type 1 big bubble (BB). (A) The BB is starting to form in the center of the sclero-corneal disc with the collection of small air bubbles under the PDL/DL. (B) A complete type 1 BB has formed. (C) The Descemet's membrane (DM) is partially peeled off the PDL/DL in the posterior wall of the BB. (D) The DM has been completely peeled off without deflating the BB. (E) Tugging the PDL/DL reveals the tension lines extending to the periphery of the disc. (F) Scanning electron micrograph illustrates the PDL/DL extending to the periphery beyond the margin of the BB. (G) The DM has been peeled off and excised from a sclero-corneal disc. (H) A type 1 BB is produced in the same disc 'G'. DM = Descemet's membrane. DL = Dua's layer.
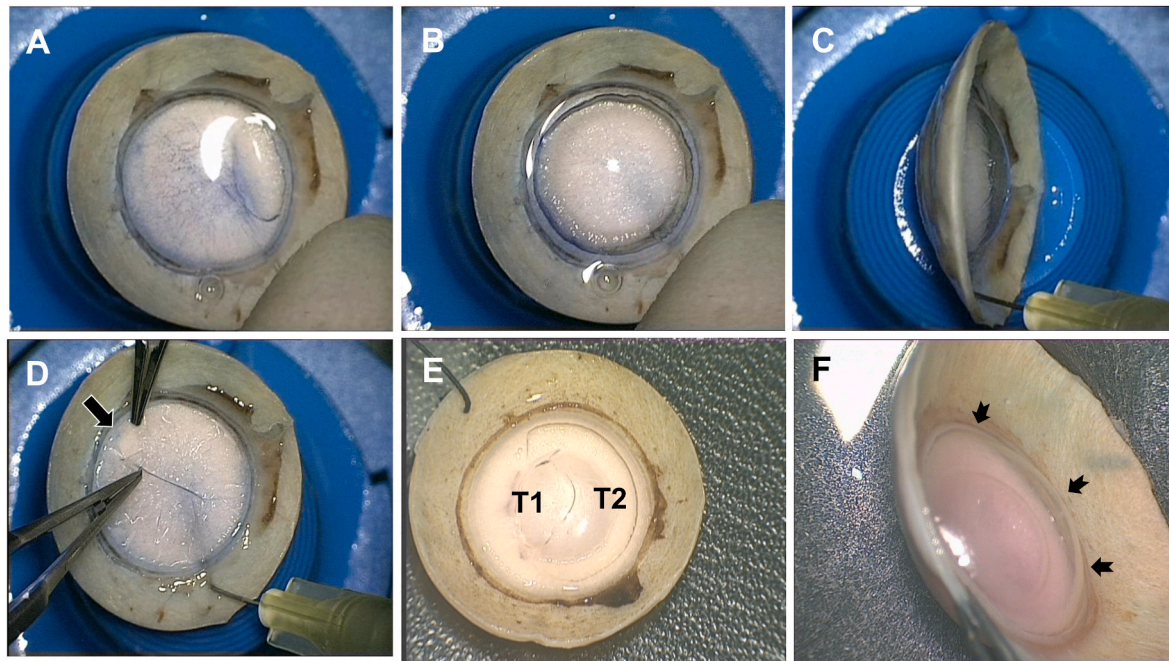
Fig. 5. Ex-vivo characteristics of type 2 and mixed big bubbles (BB). (A) A type 2 BB is seen starting at the periphery of the sclero-corneal disc. (B) A complete type 2 BB seen en face. (C) Profile view of a complete type 2 BB. (D) Peeling of the wall of the type 2 BB (DM) results in deflation and collapse of the BB. Arrow points to the hole in the DM at the commencement of peeling. (E) Mixed BB with a complete type 1 component (T1) and a partial type 2 component (T2). (F) Mixed BB with complete T1 and T2 components. Arrowheads point to the surface of the T2 component.
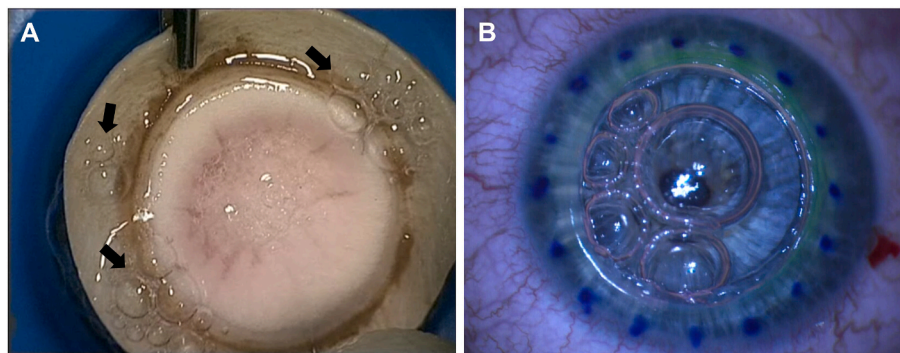

Fig. 6. Escaping air bubbles ex-vivo and in-vivo. (A) Air bubbles are seen to escape (arrows) from the periphery of the sclero-corneal disc on air injection in the corneal stroma. (B) In vivo, these bubbles escape into the anterior chamber during deep anterior lamellar keratoplasty and are seen through the exposed PDL/DL.

required to induce separation of the PDL/DL from the posterior stroma. The more the air that escaped at the periphery, the less was the intra tissue pressure attained and the greater was the force required to inject air rapidly into the stroma to compensate for the pressure loss. Striking a balance was important because when the pressure was too high the BB rapidly appeared and burst, often with an audible popping sound. Attempts to expand the size of a type 1 BB by forcing separation of the PDL/DL from the deep stroma by increasing the injection pressure or by advancing the needle tip into the cavity of the BB demonstrated that the PDL separated for a maximum of around 9 mm. Further injection resulted in the bursting of the BB. In a study where all escape of air was blocked by a specially designed clamp, AlTaan et al. (2018) measured and reported that the maximum intra tissue pressure required to create a BB was 721.9±162.08 mmHg. The mean bursting pressure of a type 1 BB was 499.91±139.88 mmHg, while that of a type2 BB was 110.78 ±18.30. The mean pressure inside the type 1 BB at its full size was 76.20 ±27.37 mmHg. The maximum volume of air required to create a type 1 BB was 0.54 ml in the situation where there was no escape of air using the clamp. However, this meant that complete stromal emphysema

occurred before the bubble formed. This was not always the case as at times air found a more direct track to the PDL/DL interface, when the volume required was less. The volume of a type1 BB was consistently 0.1 ml.

Further evidence emerges from the characterisation, clinico-pathological and surgical implications and applications of the layer as described in the sections below.

4. Characterisation – histology and immunohistology

4.1. Histology

Histological examination demonstrated that the cleavage of the PDL/DL from the posterior stroma occurred beyond the last row of kerato-cytes. Occasionally a keratocyte/keratocyte process could be seen on the anterior surface of the PDL/DL but rarely within it (Fig. 7A). The layer itself was acellular though some have reported keratocytes close to the DM (Jester et al., 2013; Schlötzer-Schrehardt et al., 2015). Using ultra high resolution optical coherence tomography (OCT) imaging in the
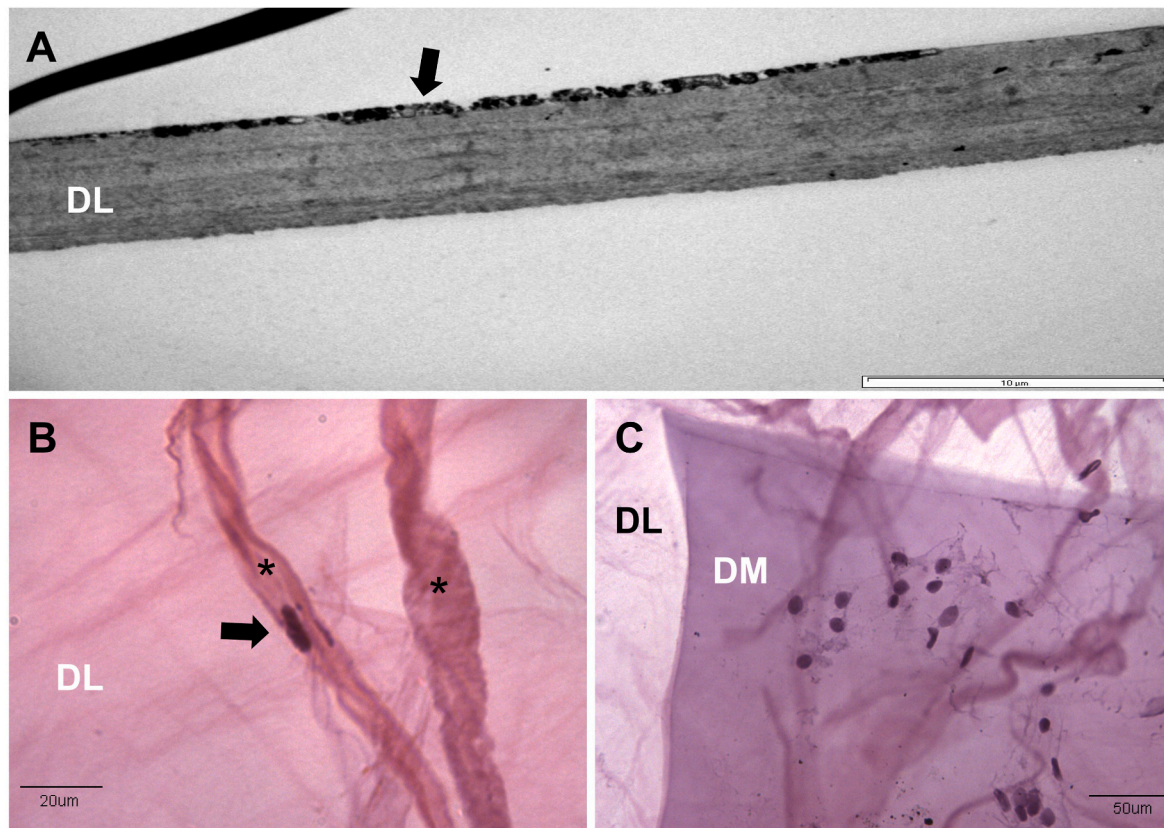
Fig. 7. Transmission electron microscopy (TEM) and whole mount staining of the pre-Descemet's layer/Dua's layer (PDL/DL). (A) TEM of isolated PDL/DL showing a keratocyte or keratocyte process on the anterior surface (arrow) but no keratocytes are seen in the layer. (B) Whole mount (approximately 1.5 mm square) of the PDL/DL with two strands of collagen (*). A single keratocyte (arrow) is seen in one strand but none in the layer. (C) Whole mount of the PDL/DL with DM attached to part of it. Nuclei of endothelial cells are seen on the DM and occasional keratocyte in the underlying strands. No nuclei are visible in the PDL/DL. DM = Descemet's membrane. DL = Dua's layer. Hematoxylin and Eosin stain.

human cornea, Bizheva et al. demonstrated the PDL/DL in the living human eye and localised it to the area posterior to the last row of keratocytes and anterior to the DM (Bizheva et al., 2016). Similarly, using micro-OCT, Chen et al. demonstrated the PDL/DL in porcine eyes, which appeared as a thin high scattering layer just anterior to the DM, with no nuclei in the image illustrated (Chen et al., 2017).

In human eyes, the mean thickness of the PDL/DL measured 10.15 ±3.6 μm, ranging from 6.3 to 15.83 μm. In comparison the DM measured a mean of 10.97±2.36 μm, with a range of 7.8–13.98 μm. The PDL/DL was composed of 5–8 thin and compact lamellae of predominantly type 1 collagen bundles arranged in transverse, longitudinal, and oblique directions. The relative compactness of the PDL/DL was illustrated by the fact the posterior corneal stroma immediately anterior to the PDL/DL, over a corresponding thickness, was made of 3–5 lamellae. Ultrastructural characteristics of the PDL/DL are given in Table 1 (Dua et al., 2013). Histology confirmed that the posterior wall of the type 1 BB was made of the PDL/DL and DM while that of the type 2 BB was made of DM only. Transmission electron microscopy (TEM) of DM obtained from type 1, type 2 and mixed BB never showed a split between the banded and non banded zones. Recoiled broken strands of collagen fibres, occasionally containing a keratocyte, could be seen as tiny microscopic lumps or lines on the anterior surface of the PDL/DL marking the plane of its cleavage from the posterior stroma (Fig. 3D). Closer to the perimeter of a type 1 BB, intact strands, bridging the space between the posterior stroma and the anterior surface of the PDL/DL could be seen (Fig. 1C and D, Figs. 2D and 3D). Some of these strands demonstrated keratocytes (Fig. 7B and C).

Besides collagen, which is the predominant component, the corneal stroma also has a well-defined network of elastin fibres

Table 1

Ultrastructural characteristics of the PDL/DL compared to the adjacent posterior corneal stroma.

Characteristic	Pre-Descemet's layer/Dua's layer	Posterior corneal stroma (of corresponding thickness)
TEM		
Lamellae	5–8	3–5
Fibril thickness*	21.7±2.43 nm	24.2±2.68 nm
Inter fibrillar distance§	9.64±7.74 nm	10.09±7.91 nm
Long spaced collagen	Present	Absent
SEM		
Collagen fibrils	Regular, parallel arrangement	Coarse, crisscross pattern with gaps created by the passage of air

TEM = Transmission electron microscopy. SEM = scanning electron microscopy. *Difference was statistically significant ($p < 0.001$). §Interfibrillar distance was measured from the centre of one fibril to the centre of the adjacent fibril. nm = nano meters.

(Asejczyk-Widlicka et al., 2007; M'ilroy, 1906). Using en-face serial scanning electron microscopy Lewis et al. (2016) and White et al. (2017) have demonstrated an annulus of an elastic fibre system in the sclero-corneal limbus. The PDL/DL has also been shown to have a high elastin content, more than the corneal stroma, by electron microscopy (Lewis et al., 2016; White et al., 2017).

4.2. Immunohistology

Using markers for different types of collagen, proteoglycans and

keratocytes (CD34) a similar composition was demonstrated in the corneal stroma and the PDL/DL. They were primarily composed of collagen I with some collagen V. However, the presence of collagens IV and VI was more pronounced in the PDL/DL especially along the anterior and posterior surfaces. The intensity of staining with markers for the proteoglycans lumican, mimecan, and decorin was similar to that in the corneal stroma. The cell adhesion protein CD34, is regarded as a marker for keratocytes (Joseph et al., 2003; Perrella et al., 2007). No cells were detected with this marker in the PDL/DL substantiating it acellular nature. The thinner fibrils of collagen in the PDL/DL compared to the posterior stroma and the similar interfibrillar distance, measured from the centre of one fibril to the centre of the adjacent fibril, indicates that there is more space, filled with proteoglycans, in the PDL/DL, which could contribute to its characteristic of being impervious to the passage of air.

With the increased popularity of lamellar corneal surgery, both DALK and EK, new observations came to light and posed new questions. In DALK, with the type 1 BB, the PDL/DL stretches posteriorly, but when the air is released, it comes back to its original position, suggesting inherent elasticity in the tissue. Equally, when a type 1 BB is created in patients with advanced keratoconus undergoing DALK, the PDL/DL does not fully recover its original configuration and post-operatively, concentric striae of redundant tissue can be seen in the layer (Fig. 8). This suggests that there is loss of elasticity in these patients. In EK, both pre-Descemet's endothelial keratoplasty (PDEK) and Descemet's membrane endothelial keratoplasty (DMEK), the donor tissue always scrolls with the endothelial cells on the outside. This unidirectional scrolling of PDEK and DMEK donor tissue is critical to the proper orientation of the tissue in the recipient eye (Agarwal et al., 2014)(Price et al., 2017). This was attributed to the "elastin content of the tissue" and to "swollen endothelial cells" (Lewis et al., 2016; Marty et al., 2016; Moshirfar et al., 2013).

DMEK tissue, made of DM and EC scrolls the most, PDEK tissue (PDL/DL + DM and EC) less so and the PDL/DL without DM and EC, scrolls the least (Dua et al., 2016) (Fig. 9.) Measurement of the elastin content of the PDL/DL, DM, central and peripheral cornea, sclera and showed that the PDL/DL had the highest concentration of elastin (37.2 ±2.75 μg/mg of wet tissue weight) which was similar to that in the trabecular meshwork (TM) (31.8±7.7) but significantly more than in the DM (21.4 ±3.81) (Mohammed et al., 2018). Despite the highest content of elastin in the PDL/DL, it scrolled the least. The mere presence of elastin therefore does not explain the unidirectional scrolling.

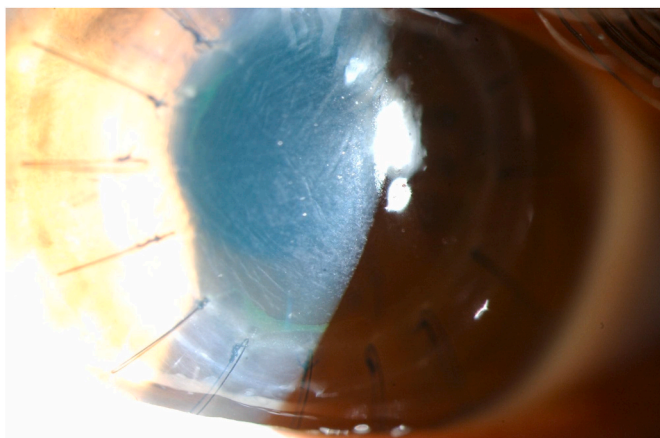Immunohistological staining of the various tissues for elastin revealed that elastin was distributed uniformly through the entire thickness of the PDL/DL, whereas it was concentrated as a densely staining band on the anterior surface of the DM (most likely corresponding to the banded zone) compared to the rest of the thickness of the DM (Fig. 10A–D) (see also Fig. 16A) The elasticity of this band would cause the DM to scroll with the anterior surface inside and the EC outside. This was confirmed by treating the DM scroll with elastase enzyme, which resulted in the spontaneous un-scrolling of the DM. In the same study, it was shown that removal of EC from the DM did not affect the scrolling of the DM. The immunohistological distribution of elastin in the PDL/DL and DM was identical in whole cornea sections and isolated samples of the DM and PDL/DL (Mohammed et al., 2018). These results provided four definite pieces of information. 1. The unidirectional scrolling is related to the selective distribution of elastin and not just its presence. The physiological role of this preferential distribution of elastin in the DM would be to keep the DM firmly apposed to the posterior surface of the PDL/DL despite the absence of any direct physical attachments. 2. Swollen endothelial cells did not contribute to the unidirectional scrolling, 3. Evidence of the structural uniqueness of the PDL/DL, which has the highest content of elastin compared to the rest of the cornea. The similar content of elastin in the PDL/DL and the TM added weight to the notion that the TM is a continuation of the PDL/DL as explained below (Fig. 10D). 4. The scrolling of PDEK tissue is largely an attribute of the attached DM.

4.3. The pre-Descemet's layer/Dua's layer and the trabecular meshwork

The PDL/DL in the central 8–9 mm of the cornea separates as the wall of the type 1 BB and in the peripheral 1–1.5 mm of the cornea it is firmly attached to the stroma but how does it terminate at the extreme periphery? A study to answer this question has revealed that the collagen fibres of the PDL/DL fan out and continue as the beams of the TM (Fig. 11A). Scanning electron microscopy of the posterior surface of the peripheral cornea after peeling off the DM has revealed that the collagen fibres of the PDL/DL fan out, divide and subdivide and continue imperceptibly as the beams of the TM (Dua et al., 2014a) (Fig. 11B and C). This is supported by similarities in the structural and cellular constituents of the PDL/DL and TM. Both have long spacing collagen/Collagen VI in abundance and both have significant amounts of elastin, more than any other part of the cornea. The elastin content of the PDL/DL and TM was not statistically different (Mohammed et al., 2018) (Fig. 10D). TM cells, that were CD34 -ve, were seen to populate the peripheral 350 to 500 μm of the PLD/DL, resting on basement membrane showing expression of laminin, within the stroma of the peripheral PDL/DL, with which the cells make macular adherens type attachments (Fig. 11D).

The cornea is a viscoelastic substance which exhibits elasticity, viscosity and hysteresis. These attributes together with thickness are linked to intraocular pressure and glaucoma. Central corneal thickness (CCT) is an important risk factor for the progression of ocular hypertension (OHT) to primary open angle glaucoma (POAG) (Jonas et al., 2005). CCT is considered to be the most important predictor of progression to POAG (Herndon et al., 2004). There is evidence to suggest that CCT could be an independent risk factor for glaucoma as thin corneas could indicate structural weakness in the peripapillary sclera, favouring optic nerve damage (Gatzioufas et al., 2013; Ghanem and Mokbel, 2010; Kotecha, 2007; Mangouritsas et al., 2009). Corneal hysteresis (CH) is considered to be an even greater risk factor than CCT, associated with progressive visual field loss. Low CH combined with high intraocular pressure are associated with a higher risk of rapid visual field progression (Deol et al., 2015; Medeiros et al., 2009). This clear association between the cornea and glaucoma/IOP may have a more direct relationship in the light of the demonstration that the TM is a direct continuation of the PDL/DL. Structural and consequent biomechanical alteration in the PDL/DL could favourably or adversely affect the TM and influence IOP.

The question that begged an answer was: If the PDL/DL is impervious

Fig. 8. Laxity of the pre-Descemet's layer/Dua's layer in advanced keratoconus. Slit lamp diffuse illumination view of the cornea after deep anterior lamellar keratoplasty in advanced keratoconus. Concentric wrinkles of the PDL/DL are seen the grafted cornea.
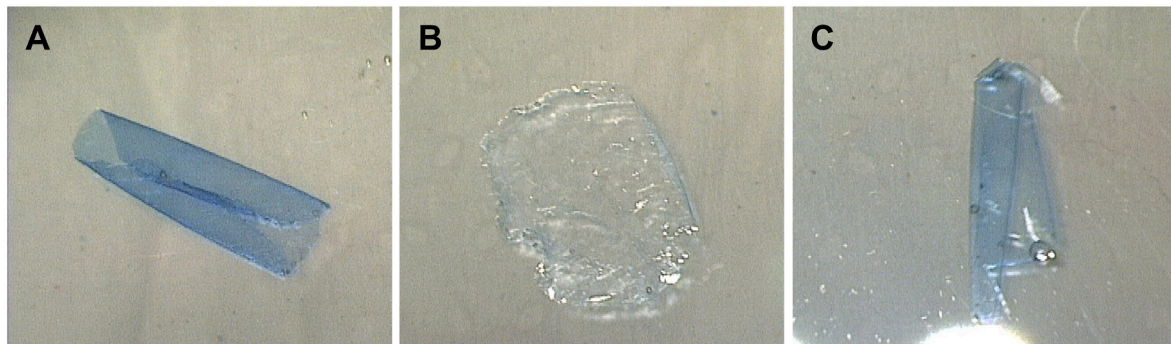
Fig. 9. Scrolling characteristics of endothelial keratoplasty (EK) tissue. (A) Pre-Descemet's endothelial keratoplasty tissue showing a grade 2–3 scroll. (B) The pre-Descemet's layer/Dua's layer (PDL/DL) and the Descemet's membrane with endothelial cells (DM + EC) of the tissue in 'A' are separated. The PDL/DL shows grade 0 scrolling. (C) The DM + EC shows grade 4 scrolling.
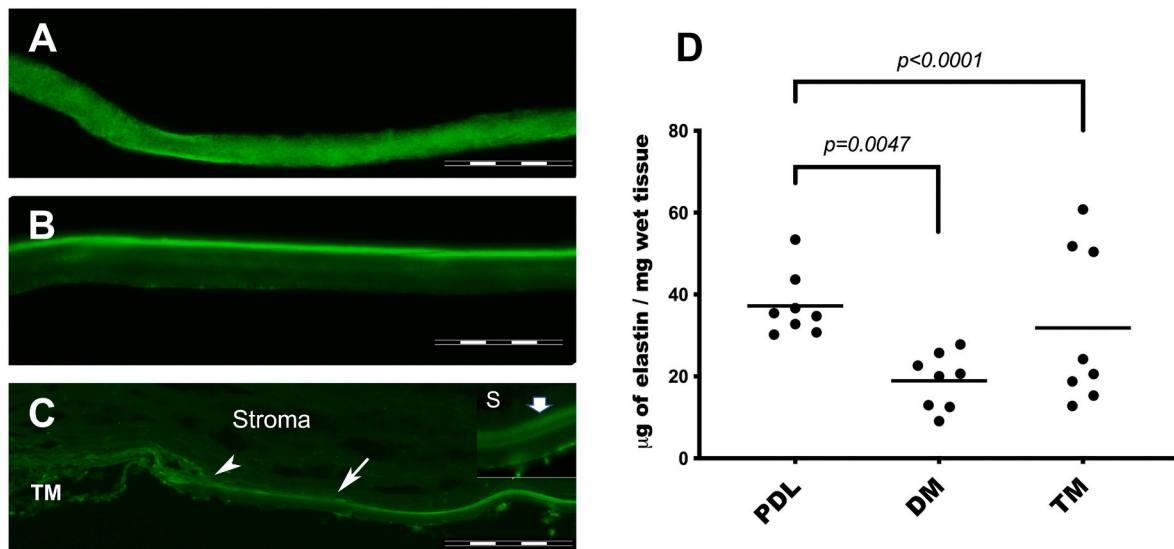

Fig. 10. Elastin content of the pre-Descemet's layer/Dua's layer (PDL/DL), Descemet's membrane (DM) and Trabecular meshwork (TM) demonstrated by immunohistology. Positive elastin stains green. No autofluorescence was observed in any tissue sample. (A) Elastin is uniformly distributed thorough the PDL/DL. Bar = 50 μm. (B) Elastin is present as a dense band on the anterior surface of the DM. Bar = 50 μm. (C) Elastin is uniformly present in the beams of the TM. Bar = 100 μm. Arrowhead shows the start of the TM. Inset shows the magnified view of the PDL/DL and DM. The PDL/DL (broad arrow) is clearly visible as a separate layer, separated from the DM by a dark, non-staining line, the interfacial matrix. The stroma (S) shows none or minimal background staining. (D) The PDL/DL has the highest elastin, which is not statistically different from that in the TM. The DM has statistically different less elastin than the PDL/DL.

10 to 12 mm-thick sections of OCT embedded tissues and fixed with 4% paraformaldehyde were immunostained using polyclonal rabbit anti-human primary antibody against elastin (5 mg/ml final concentration; Abcam, Cambridge, UK); donkey anti-rabbit IgG Alexa Fluor 488 conjugate secondary antibody (Thermofisher Scientific, Loughborough, UK) and examined under fluorescent microscope (B51X; Olympus, Tokyo, Japan) Elastin (Mohammed et al., 2018) content was determined by the Fastin Elastin assay kit (Biocolor Life Sciences, Carrickfergus, UK). Measured weights of wet tissue were hydrolyzed in 0.25M oxalic acid by boiling at 95 °C for 60 min. Solubilized alpha-elastin was recovered by centrifugation and precipitation and quantified at 513 nm wavelength against a standard curve (Mohammed et al., 2018).

to air, how does air escape to the plane between the posterior surface of the PDL/DL and the anterior surface of the DM to create a type 2 BB? The observation that a type 2 BB started at the periphery led to the answer and provided further insights into the microanatomy of the peripheral cornea (Fig. 5A). The point of commencement of a type 2 BB was marked, the DM peeled off at that site and the area was examined by scanning electron microscopy. This revealed the presence of 15–20 fenestrations, present singly or in clusters, that were randomly distributed along the perimeter of the PLD/DL at the site where the fibres started to fan out to become the beams of the TM (Fig. 12A and B). These were also present on the peripheral side of the attachment of the DM (Fig. 12C). The diameter of the fenestrations ranged from 5 to 60 μm with a mean diameter of 20.3 ± 13.84 μm (Dua et al., 2018).

Air injected in the stroma finds its way through the stroma to the fenestrations located peripheral to the attachment of the DM and escapes into the anterior chamber (as seen in DALK) (Fig. 6). Air that reaches the anterior surface of PDL/DL and spreads along the cleavage plane between it and the posterior stroma can also access the fenestration(s) located peripheral to the attachment of the DM and escape into the anterior chamber. Air escaping through fenestrations located central to the attachment of the DM escapes to the plane anterior to the DM, separating it from the posterior surface of the PLD/DL to form a type 2 BB. In *ex vivo* experiments air bubbles are seen escaping randomly along the sclero-corneal junction, corresponding to the air that *in vivo* might enter the anterior chamber and air escaping central to the attachment of the DM forms a type 2 BB and explains why the type 2 BB usually starts at the periphery and how it accesses the plane posterior to the PDL/DL despite it being impervious to air.
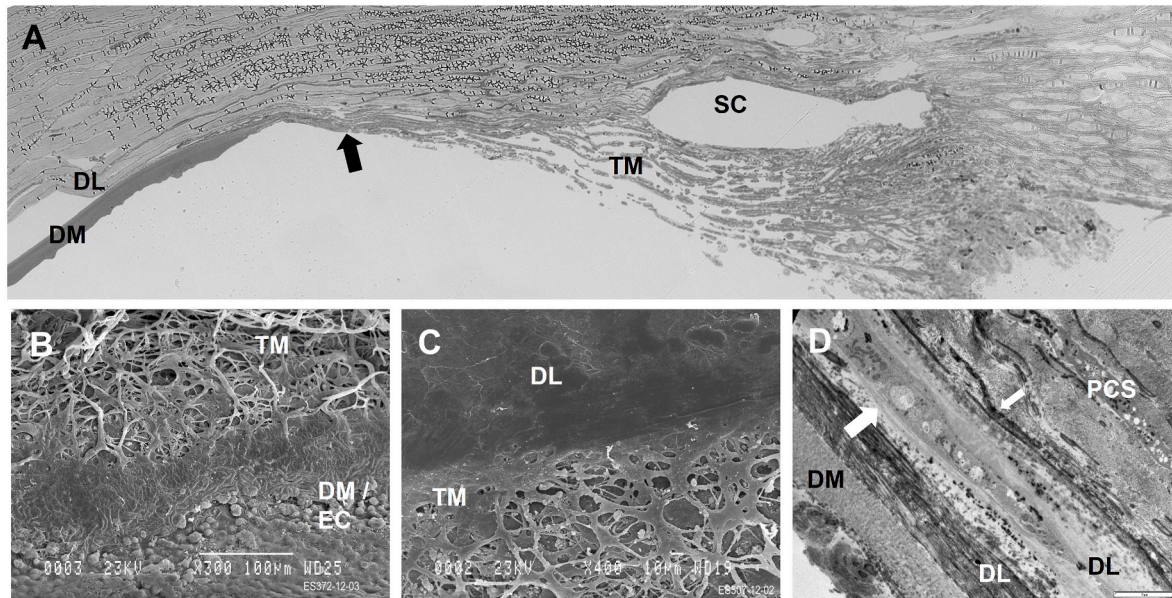
Fig. 11. The trabecular meshwork (TM) is a continuation of the pre-Descemet's layer/Dua's layer (PDL/DL). (A) Arrow points to the termination of the Descemet's membrane (DM). The PDL/DL can be seen to extend beyond that and the lamellae fan out to become the beams of the TM. (B) Scanning electron micrograph (SEM) of the TM, DM with EC and the transition zone. (C) SEM of the PDL/DL and TM after peeling off the DM. The PDL/DL can be seen to merge seamlessly with the beams of the TM. (D) A large trabecular cell is seen in the PDL/DL (thick arrow). The thin arrow points to a keratocyte lying on the anterior surface of the PDL/DL. DL = Dua's layer. DM = Descemet's membrane, TM = Trabecular meshwork, SC = Schlemm's canal, EC = endothelial cells, PCS = posterior corneal stroma.
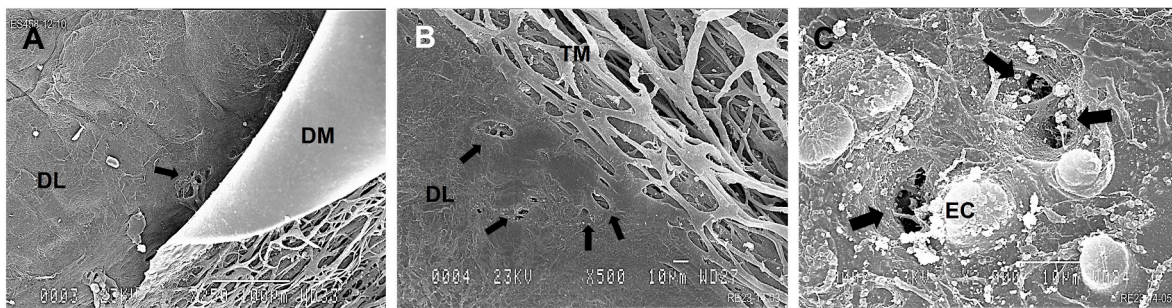

Fig. 12. Peripheral fenestrations. A. (A) cluster of fenestrations (arrow) is seen at the extreme periphery of the pre-Descemet's layer/Dua's layer (PDL/DL). (B) Multiple fenestrations at the periphery of the PDL/DL (arrows). The DM has been peeled off. (C) Fenestrations at the periphery of the DM (arrows) through which air can escape into the anterior chamber. DM = Descemet's membrane. DL = Dua's layer. EC = endothelial cells.

5. Embryology

There is an embryological basis for the development of the PDL/DL. The surface ectoderm, following the separation of the lens vesicle, is made of an anterior layer of squamous epithelium and a posterior layer of cuboidal epithelium. This initiates the development of the cornea. The cuboidal epithelium secretes glycoaminoglycans and collagen fibrils that occupy the space between the epithelium and the lens vesicle to form the 'primary stroma' of the cornea. The primary stroma is acellular (Bron et al., 1998; Cai et al., 1994; Dodson and Hay, 1971; Hay and Revel, 1969; O'Rahilly, 1983). Three waves of neural crest cells migrate from the rim of the developing optic cup. The first wave migrates between the lens vesicle and the primary stroma to form the endothelium. The second wave initiates the development of the iris and the pupillary membrane. The third wave, at around 7 weeks of gestation, migrates into the primary stroma and forms the precursors of the keratocytes. The stromal keratocytes secrete the definitive stroma, which pushes and compresses the primary stroma anterior to it, towards the surface epithelium and is believed to form the basis of the Bowman's layer. A similar fate of the posterior part of the primary stroma would form the basis of the development of the acellular PDL/DL. There is a suggestion

that the compressed posterior primary stroma forms the DM but this is disputed by others (M'ilroy, 1906) who provided evidence that the DM is produced by the secretion of membrane units by the developing endothelium (Bron et al., 1998). Both, the Bowman's layer and the PDL/DL, retain connective tissue elements from the primary stroma produced by the epithelium, but the PDL/DL is also influenced by the endothelium (Hayashi et al., 1988)(Linsenmayer et al., 1990)(Quantock and Young, 2008)(Toole and Trelstad, 1971).

In a recent study on developing human corneas from weeks 7–17, Feneck EM et al. (Feneck et al., 2020) demonstrated the acellular primary collagenous stroma, the presence of elastic fibres in the posterior peripheral corneal stroma (from week 12) and novel extensions from the endothelial cells into the primary stroma. They proposed that the endothelial cell extensions assist in the migration of the mesenchymal cells that populate the primary stroma. This further supports the hypothesis that the PDL/DL would be influenced by the endothelium that could confer characteristics that are different from the bulk of the corneal stroma. Macular corneal dystrophy (MCD) is another example of the likely close association of the DM and EC with the PDL/DL. The opacities affect the stroma and DM and endothelium (Lin et al., 2016; Zhang et al., 2019) but DM and or PDL/DL can be affected without

stroma involvement suggesting that MCD may be a stromal-endothelial dystrophy (Shi et al., 2017).

A synthesis of all the evidence above in the context of the definition of a 'layer' suggests that the PDL/DL is a distinct layer, though some aspects, especially the claim about the absence/paucity of keratocytes in the layer, have been contested. A medical dictionary definition of a layer is "A sheet of one substance lying on another and distinguished from it by a difference in texture or colour or by not being continuous with it." (Farlex, 2012). The PDL/DL fulfils the above criteria in that it offers a distinct plane of cleavage from the overlying stroma, which is exploited in lamellar keratoplasty and is spontaneously manifest in posterior corneal pathology (see below). The constituent number of lamellae, their thickness, fibril morphology in terms of diameter and interfibrillar distance, and type of collagen in the PDL/DL are different compared to the similar thickness of stroma immediately anterior to it with the fibril diameter reaching statistical difference (Table 1). Other differences are that the PDL/DL is impervious to air, hence lifts up along the plane of cleavage to form a type 1 BB, and when ablated by photo therapeutic keratotomy, a type 1 BB cannot be created. It contains more elastin than any other part of the cornea. The absence of keratocytes as described by us and corroborated by others, is another important distinction. However, the latter claim has been disputed by two researchers, (see section 8.3). Further studies are required to quantify the number of keratocytes in the PDL/DL and corresponding area of the posterior stroma. However, on the basis of differences mentioned above, the differences are sufficient for it to be recognised as a distinct layer, by several researchers and the AAOOP.

6. Clinico-pathological applications

Since the discovery of the PDL/DL and understanding its relevance to lamellar corneal surgery, its manifestations in clinical pathology began to emerge and inform our understanding of a number of conditions, some in ways that were missed for almost a hundred years.

6.1. Descemet's membrane detachment

The concept of Descemet's membrane detachment (DMD) was first introduced in 1928 by Bernard Samuels who wrote a thesis on the subject and recorded that Ernst Fuchs had observed three cases of iatrogenic DMD following iridectomy using a surgical keratome (Samuels, 1928). Since its first description, DMD has been reported to occur either spontaneously or following trauma, cataract surgery, or corneal surgeries (Dai et al., 2021; Gorski et al., 2016; Singhal et al., 2020). Despite the increased reports of DMD in the literature, the understanding of DMD has remained incomplete for nearly a century until the discovery of the PDL/DL. Over the past few decades, a number of classifications have been proposed to standardise the reporting and to guide the management of DMD, though none have recognised or considered the anatomical importance and implication of the involvement of the PDL/DL in DMD (Jacob et al., 2015; Mackool and Holtz, 1977).

In 2016, Dua and Said demonstrated the first clinical evidence and implication of PDL/DL in the pathology of posterior cornea (Dua and Said, 2016). This report highlighted a case of persistent corneal oedema following cataract surgery due to the result of a combined detachment of PDL/DL and DM in which the detached DM had torn. The DMD failed to improve despite repeated intracameral air injections. In the following years, continual accumulation of clinical evidence has further substantiated the involvement of the PDL/DL in DMD, which led to the proposal of a new classification of DMD (Dua et al., 2020). Based on the varying involvement of PDL/DL and DM, Dua et al. have classified DMD into three types corresponding exactly to the three types of BB induced by air injection as seen in DALK and its *ex vivo* simulation. Type 1 DMD (where PDL/DL and DM are detached together from the posterior stroma as in the type 1 BB); type 2 DMD (where DM is detached from the posterior surface of the PDL/DL as in a type 2BB); and type 3 DMD (like a mixed BB, which is made of a type 1 and type 2 DMD component) where the PDL/DL and DM are detached from the posterior cornea and also from each other (Fig. 13A–D). Depending on the presence or absence of a tear in DM and/or PDL/DL, DMD can be divided into either rhegmatogenous or non-rhegmatogenous, respectively (Table 2).

The new classification as described above was established on evidence gained from slit lamp, anterior segment optical coherence tomography (AS-OCT) of several patients of which five had histopathology confirmation. The type 1 DMD appears as a straight taut line, like the chord of a circle, on slit lamp and AS-OCT examination. On histology it bears out the description of the PDL/DL as a multilaminar layer of collagen, which is largely acellular. Keratocytes were seen on the anterior surface of the PDL/DL. In a couple of cases the stroma of the detached PDL/DL was populated with keratocyte derived cells but did not show inflammatory cells. This demonstrates that in long standing type 1 DMD, keratocytes can migrate into the PDL/DL, change into myofibroblasts/fibroblasts and induce scarring and contracture of the detached PDL/DL and DM (Fig. 13 E, F).

Clinically the characteristics and extent of DMD are best visualized with AS-OCT. In type 1 DMD, the detached PDL/DL with DM is often seen as a hyper-reflective straight line, which is relatively thicker than the DM seen in a type 2 DMD. A detached DM in a type 2 DMD and as part of a mixed DMD, is seen as a relatively thin, undulating, double contour hyper-reflective line. The two lines represent the change in refractive index between the PDL/DL and the DM anteriorly, and the DM and the aqueous posteriorly. The thickness of the DM appears as a dark space between the two hyper-reflective lines. With a type 1 DMD, when the PDL/DL is apposed to the DM the double contour line is not clearly visible. However, when the DM is detached from the PDL/DL as in a mixed DMD, this characteristic OCT configuration of the DM is readily seen.

DMD usually covers an area wherein the types can be different from the periphery towards the centre, especially when one or both layers are torn (rhegmatogenous). A torn type 2 DMD shows the classic scrolling of the edges along the tear, however, the amount of scrolling is much less with a torn type 1 DMD. Much of the scrolling visible at the edge of a torn type 1 DMD is induced by the DM. When the DM and PDL/DL are separated at the edge of the tear, the PDL/DL scrolls little while the corresponding DM scrolls more. In a rhegmatogenous type1 DMD, both PDL/DL and DM are torn, as often seen in acute hydrops, but an exclusive tear in the PDL/DL without a tear in the DM, has never been observed.

The differences in the morphological characteristics and the underlying pathogenesis of the different types of DMD will have implications in the management (Dua et al., 2020) (Table 2). On the basis of current knowledge of the involvement of the PDL/DL in DMD the paradigms will have to be changed. Descemetopexy or pneumodescemetopexy (intracameral injection of air/gas to reattach DMD) is currently the "gold standard" for managing DMD, though many other approaches such as mechanical tamponade, suture fixation, Descemetotomy, interface drainage, and keratoplasty have been described (Singhal et al., 2020). Occasionally, reattachment of DMD, mainly in cases with small and peripheral detachment, can occur spontaneously (Singhal et al., 2020). The 'air/gas injection' option is most likely to succeed in type 2 DMD. Besides the type, the duration of DMD, especially with regard to the occurrence of scarring/contracture of the PDL/DL, will also be a factor. The longer the duration, the more likely is the occurrence of scarring especially with type 1 DMD where the PDL/DL is populated with keratocyte derived cells. When the surface area of detached PDL/DL becomes less than that of the posterior cornea to which it has to be apposed, it is less likely to stay attached. The PDL/DL is quite elastic but when fibrosed its elasticity will be lost and a controlled Descemetotomy would be a useful option to consider. In the light of the evidence on the contributory role of PDL/DL in DMD, future studies on causation and management will help improve treatment outcomes.
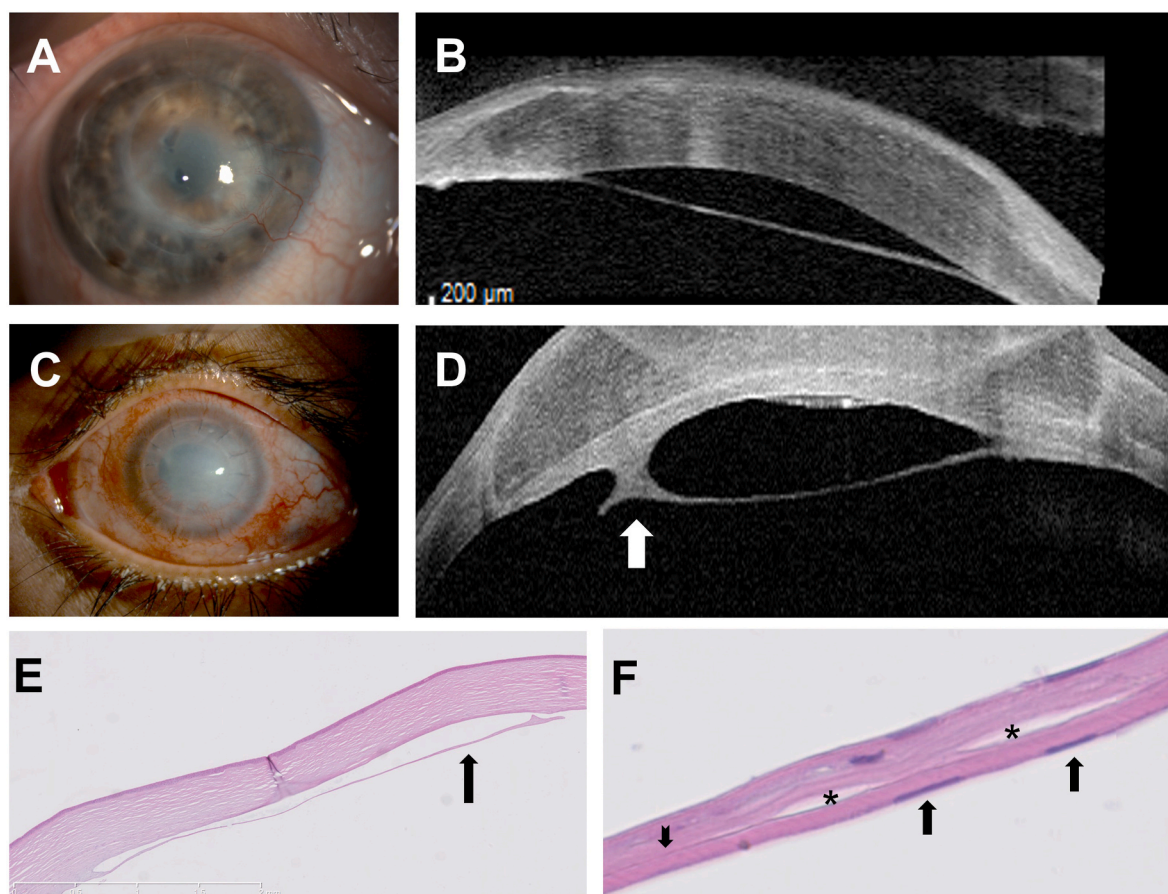
Fig. 13. The pre-Descemet's layer/Dua's layer (PDL/DL) in Descemet's membrane detachment (DMD). (A) Chronic corneal oedema in an eccentric penetrating corneal graft. (B) A type 1 DMD (combined detachment of the PDL/DL and DM) seen on optical coherence tomography (OCT) as a straight line, like the cord of a circle. (C) Persistent corneal oedema in a failed penetrating corneal graft. (D) OCT revealed a type 1 DMD in the eye shown in 'C'. A broad strand of collagen is seen to extend from the posterior stroma to the anterior surface of the PDL/DL (arrow). (E) Histology of the cornea shown in 'C' removed for a repeat corneal graft. The DMD is clearly visible (arrow). (F) Histology clearly shows the PDL/DL with keratocyte derived cells on and in the layer. Endothelial cells (arrows) are seen lining the DM. The cleavage plane between the PDL/DL and DM where they are separated (*) and apposed (arrowhead) is demonstrated.

Table 2
Clinical classification of Descemet's membrane detachment (DMD) with implications for management.

Level 1	Level 2	Level 3	Level 4
Type 1	Planar (<1 mm)	Non- Rhegmatogenous	Early (<2 weeks)
	Non planer (>1 mm)	Rhegmatogenous DM + PDL/DL	Late
Type 2	Planar (<1 mm)	Non- Rhegmatogenous	Early (<2 weeks)
	Non planer (>1 mm)	Rhegmatogenous DM	Late
Mixed	Planar (<1 mm)	Non- Rhegmatogenous	Early (<2 weeks)
	Non planer (>1 mm)	Rhegmatogenous DM	
		Rhegmatogenous DM + PDL/DL	Late

DMD can heal spontaneously when endothelial cells migrate across the gap on the posterior surface of the exposed PDL/DL or stroma and secrete a new basement membrane.
Type 2 DMD are easier to re-attach with air or gas tamponade compared to type 1 or mixed DMD.
Planar detachments are easier to re-attach than non-planar ones.
Rhegmatogeneous DMD are technically more challenging to re-attach and may require sutures or endothelial keratoplasty if unsuccessful.
Type 1 and Mixed (Type 1 component) ones become fibrosed with time (Late DMD) by the migration of keratocytes in the stroma of the PDL/DL and become difficult, if not impossible to re-attach.

6.2. Acute corneal hydrops

Acute corneal hydrops (ACH) is an uncommon but well-recognised complication that occurs in eyes with corneal ectatic disorders, particularly keratoconus and, to a lesser extent, pellucid marginal degeneration and keratoglobus (Bhandari and Ganesh, 2015; Gaskin et al., 2014). Patients with ACH are often affected by significant visual impairment, pain, and photophobia. ACH most commonly occurs following a minor trauma or eye rubbing in an eye with severe corneal ectasia (Tuft et al., 1994), though spontaneous occurrence or following corneal cross-linking has been reported (Gharebaghi et al., 2009; Said et al., 2013; Ting et al., 2019a; Tuft et al., 1994).

ACH has always been attributed to a tear in the DM, which then leads to a sudden efflux of the aqueous humour into the corneal stroma with resultant corneal oedema. A characteristic feature is the formation of fluid lacunae/lakes in the stroma that are well delineated by AS-OCT. In a case of ACH with acute haemops in an eye with advanced keratoconus struck with a cricket ball, red blood cells were demonstrated in the fluid lacunae indicating that the movement of fluid is a physical event rather than an imbibition or diffusion (Said et al., 2013). On the basis of our observations of AS-OCT images of ACH, the discovery of the PDL/DL and the knowledge that despite often noting DMD post-surgery, acute hydrops was not an associated feature, we proposed in our first publication on the PDL/DL that ACH was secondary to a tear in both DM and the PDL/DL (Dua et al., 2013) (Fig. 14A–D). Further evidence for this came from surgery undertaken to treat keratoconus by Bowman's layer
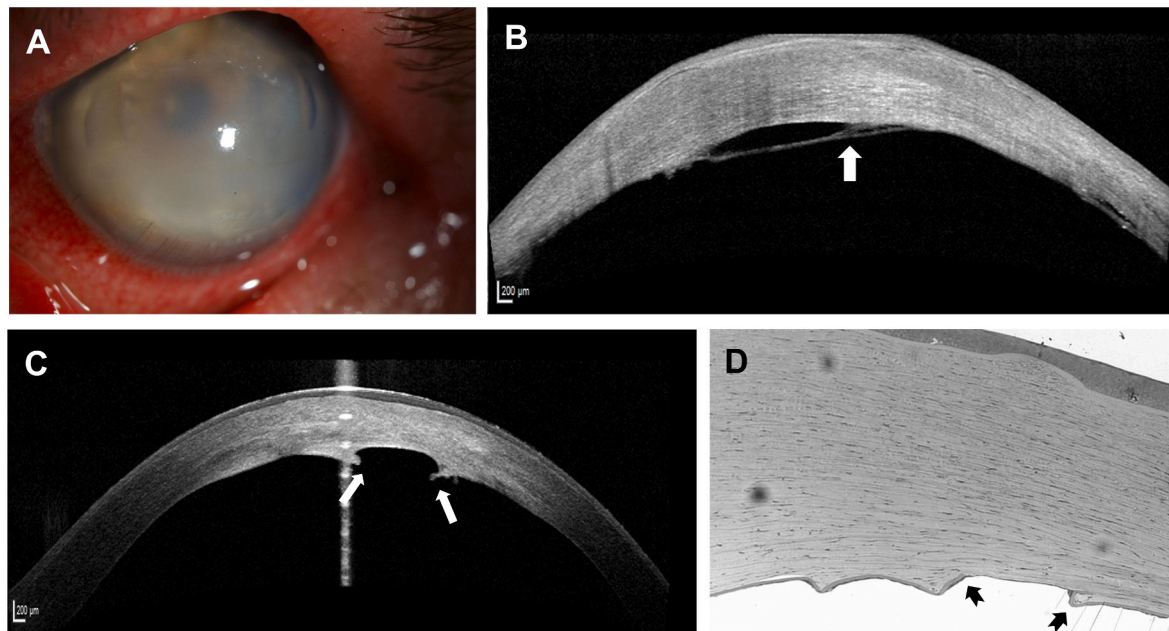
Fig. 14. The pre-Descemet's layer/Dua's layer (PDL/DL) in acute hydrops. (A) Acute hydrops in a patient with features of keratoconus and pellucid marginal degeneration. Two arcuate incisions were previously made in the horizonal meridian. (B) Optical coherence tomogram (OCT) showing a mixed Descemet's membrane detachment (DMD). This has features of a type1 DMD up to the arrow, where the PDL/DL separates from the DM. (C) OCT of the cornea shows a tear in the DM and PDL/DL. The edges of the tear are rolled and scarred (arrows). (D) Histology of a cornea removed at penetrating keratoplasty following resolution of acute hydrops in keratoconus. The edges of the tear in the PDL/DL and DM remain as scarred rolls (arrowheads). The gap in between was lined by endothelial cells migrating on the posterior stroma.

transplant (Parker et al., 2019a). Post-operatively, some patients with keratoconus had only DMD (type 2) but did not develop ACH. On the contrary, when there was inadvertent perforation posteriorly, from the stroma through PDL/DL and DM, during preparation of the anterior intra-lamellar pocket for the Bowman's layer transplant, ACH developed. It led the authors to question "Are DM detachments the root cause of corneal hydrops in keratoconic eyes?", which was the title of their paper (Parker et al., 2019a).

Following on from our initial observations and proposal that both DM and PDL/DL tears were needed for ACH to occur, we had performed a simple experiment on non-keratoconic human donor sclero-corneal discs. A posterior approximate 80–100 μm deep 3 mm incision was made through the DM and PDL/DL and posterior stroma, the discs were mounted on an artificial anterior chamber and the pressure raised from 15 to 60 mm of Hg. ACH could not be induced. This, substantiated by the observations that full thickness traumatic lacerations/surgical incisions of normal corneas are not associated with ACH, led us to conclude that ACH required a simultaneous break in both PDL/DL and DM in the context of the abnormal collagen and proteoglycan matrix of keratoconus (ectatic corneas) (Dua et al., 2013, 2015a; Ting et al., 2019). When we presented this to Dr. Melles' group by way of a letter to the journal (Dua et al., 2015a, 2013; Ting et al., 2019b), they wholeheartedly agreed (Parker et al., 2019b). This constitutes a paradigm shift in our understanding of the pathogenesis of ACH, dispelling the ages held notion that it is due to a tear in the DM.

ACH is usually self-limiting with a mean healing time of two to four months (Gaskin et al., 2014). Pneumodescemetopexy with intracameral injections of either air or gas (e.g. SF6, C2F6 or C3F8) is a popular intervention (Miyata et al., 2002; Panda et al., 2007; Ting and Srinivasan, 2014), wherein the air/gas serves as an internal tamponade to impede further influx of aqueous into the corneal stroma as well as to re-attach the PDL/DL and DM. In 2015, Yahia Cherif et al. (Yahia Chérif et al., 2015) reported a successful innovative technique of managing ACH using a combination of approximating the torn edges of the PDL/DL without doing the same with the DM tear, and intracameral

injection of air (see section 7 on surgical applications). They inferred from their success, evidence of the existence of the PDL/DL and its role in the pathophysiology and management of ACH. Regardless of how the ACH resolves, the corresponding healed area of the PDL/DL, though functionally restores endothelial cell function and corneal clearing, is physically never back to its normal self. This is demonstrated by the fact that an attempt to undertake DALK by air injection in an eye with previous acute hydrops invariably results in a tear at the healed site and escape of air into the anterior chamber without formation of a type 1 BB. The same is noted in donor eyes with previous cataract surgery where an attempt to produce a type 1 BB *ex vivo* results in rapid air escape through the incision site (main wound or side-ports) (Fig. 15A). With peripheral wounds in the PDL/DL a BB can form but can be restricted by the scarring that occurs at the wound site (Fig. 15B).

6.3. Keratoconus

Keratoconus is the most common corneal ectatic disorder with an estimated prevalence of 1:375 to 1:2000 (Najmi et al., 2019; Romero-Jiménez et al., 2010). It is characterised by progressive corneal thinning with increased myopia and irregular astigmatism. The pathogenesis of keratoconus remains to be fully elucidated, though environmental, host, and genetic factors, have been shown to play integral roles. In early and form fruste keratoconus subtle changes are noted in the posterior cornea, which manifest clinically as posterior elevation, posterior curvature and thickness changes (Andreanos et al., 2017; Kamiya et al., 2021). The anterior approximately 40% of the corneal stroma is biomechanically the strongest region, whereas the posterior 60% of the stroma is at least 50% weaker according to tensile strength studies in human donor corneas (Esporcatte et al., 2020).

Emerging evidence suggests that the PDL/DL may contribute to the pathogenesis of keratoconus, which would be consistent with the observation that ACH in keratoconus, the most dramatic consequence of progressive keratoconus, manifests in the PDL/DL and posterior stroma as described above in section 6.2. The high and evenly distributed
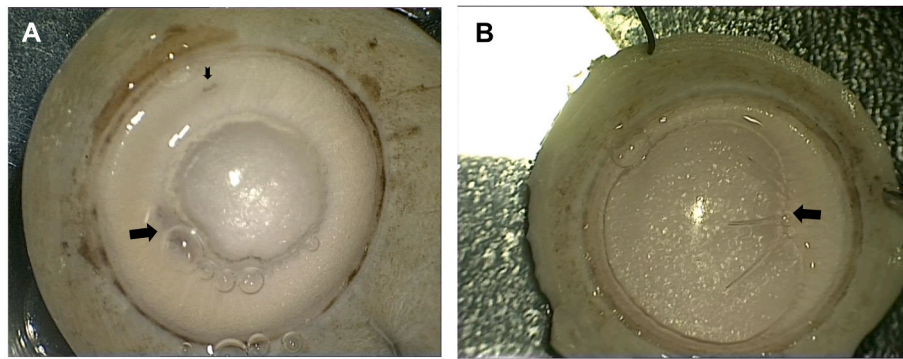
Fig. 15. Escape of air through wounds involving the pre-Descemet's layer/Dua's layer (PDL/DL). (A) Air bubbles are seen to escape from the internal edge of a cataract wound (arrow) in a donor sclero-corneal disc. Air can also escape from the side-port wounds (arrowhead). A type 1 big bubble (BB) has formed. (B) A type 2 BB has stopped at the site of a cataract wound (arrow) where the Descemet's membrane is scarred down. Stress lines are seen radiating form the site of the scar.
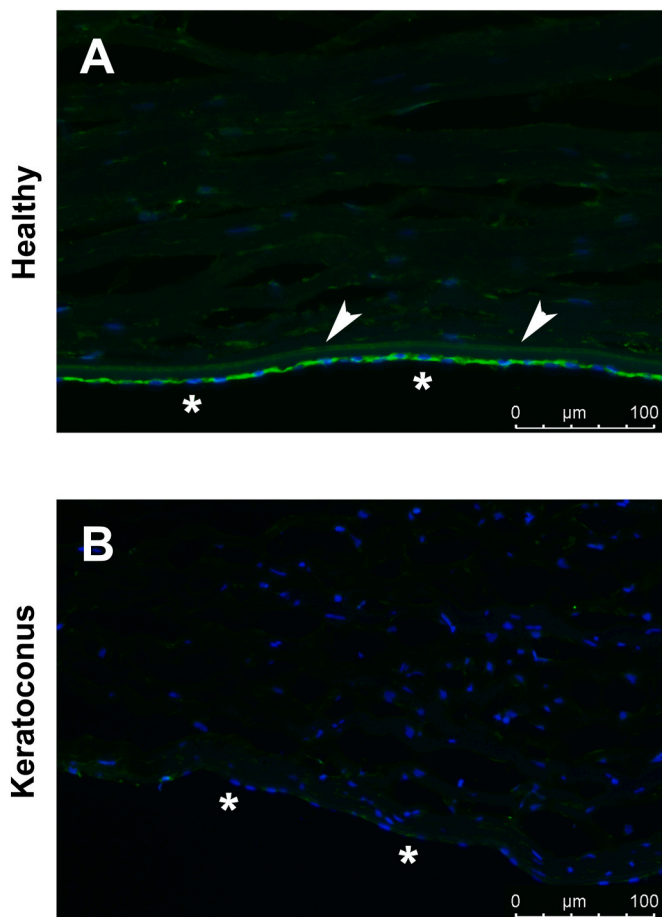

Fig. 16. Loss of elastin in the pre-Descemet's layer/Dua's layer (PDL/DL) in keratoconus. (A) Immunohistology showing the elastin in the PDL/DL and the anterior part of the Descemet's membrane (DM) of a healthy donor cornea (arrowheads). Endothelial cells (EC) with some associated elastin are seen lining the DM (*). (B) Immunohistology of a cornea from keratoconus showing loss of elastin stain in the PDL/DL, DM and elastin associated with the EC (*).

elastin content of the PDL/DL is shown to be affected and degraded in keratoconus (Lewis et al., 2016; Mohammed et al., 2018).

White et al. (2017) demonstrated a lack of elastic fibres in the PDL/DL of keratoconic eyes in comparison to that in the PDL/DL of normal corneas. Our recent studies on immunostaining for elastin in normal and keratoconic corneas has shown near total loss of elastin in the PDL/DL in advanced cases where a corneal transplant was indicated and the tissue obtained for examination (Fig. 16 A, B). This is an important association suggesting that degradation of elastin in the PDL/DL can potentially play a role in the pathogenesis of keratoconus and should be considered amongst the numerous other genetic, biochemical, structural and inflammatory and other factors that have been described (Dua et al., 2022).

6.4. Infectious keratitis

Infectious keratitis (IK) is the most common cause of corneal blindness in the world (Ting et al., 2021b, 2021c; Ung et al., 2019). It can be caused by a wide variety of organisms, including bacteria, fungi, viruses, and parasites, with a range of clinical presentations affecting all layers of the cornea. Certain organisms such as fungi have a propensity for affecting the deeper cornea with resultant deep stromal infiltrate and endothelial plaque (Jin et al., 2021; Ting et al., 2021a).

The increased recognition of PDL/DL may provide explanations to some of the corneal findings observed in IK. Osborne et al. (2005) previously reported a case of double hypopyon secondary to *Pseudomonas aeruginosa* keratitis, for which the hypopyon was present in both the mid-to-deep stromal area and the anterior chamber. Agrawal et al. (2012) similarly reported a case of pseudo-hypopyon (or intracorneal hypopyon) in a case of suture-related IK. On a closer look at the published figures in these studies, it is interesting to note that the lower edge of the hypopyon always assumed a smooth and circular appearance and locates within the area of a type 1 BB (within 8–9 mm of the central cornea), raising the possibility that these intracorneal hypopyons may be present in the cleavage plane between the posterior stroma and the PDL/DL. Histopathological studies have shown that a split between posterior stroma and PDL/DL and/or DM may occur in severe fungal keratitis (Liu et al., 2015). We have three cases of fungal keratitis where the AS-OCT clearly shows a detachment of the PDL/DL (type 1 DMD) with accumulation of debris in the interface (Fig. 17 A, B). Another clinical incident, observed with chronic corneal abscesses is an internal rupture of the abscess with rapid appearance of a hypopyon. This could also be preceded by the accumulation of 'pus' in the plane between PDL/DL and stroma. It is only a matter of time when similar findings will be noted and reported by others. Recognition of such manifestations help improve our understanding of the clinical characteristics and behaviour of IK and could inform future treatment strategies. Like intracorneal hypopyon, intracorneal hemorrhage too can accumulate in the plane of cleavage anterior to the PDL/DL (Fig. 18 A, B).

6.5. Retro corneal membrane

Retro corneal membrane was first described in 1901 by Ernst Fuchs (Fuchs, 1901; Brown and Kitano, 1966), who observed the presence of this feature in 50% of the failed PK specimen. As the first successful PK
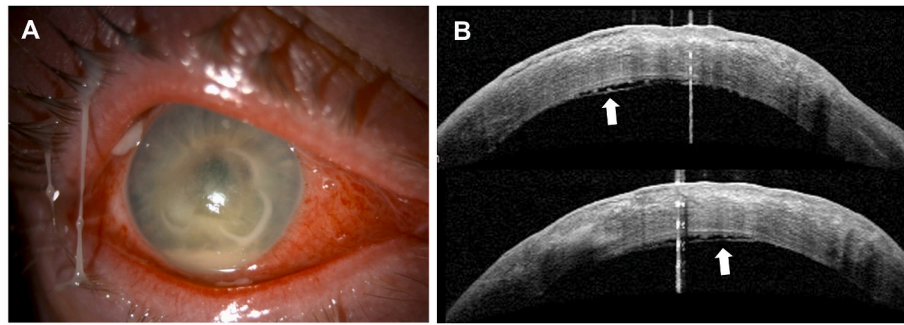
Fig. 17. Separation of the pre-Descemet's layer/Dua's layer (PDL/DL) in infectious keratitis. (A) Diffuse slit lamp image of an eye with chronic fungal keratitis. (B) Optical coherence tomogram (OCT) showing a type 1 Descemet's membrane detachment with accumulation of inflammatory debris in the cleavage plane (arrow).
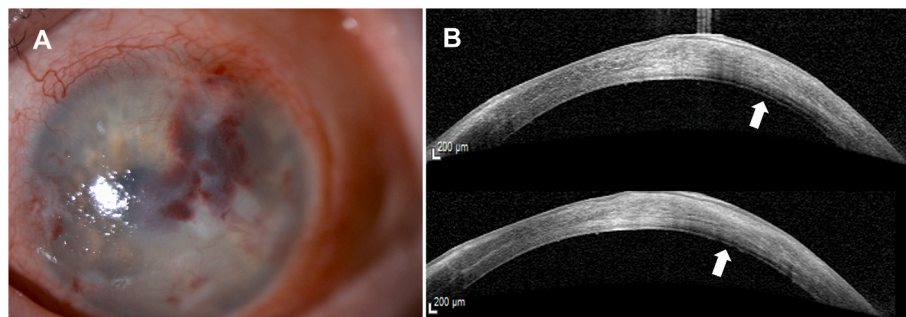

Fig. 18. Separation of the pre-Descemet's layer/Dua's layer (PDL/DL) with intracorneal hemorrhage. (A) Intracorneal hemorrhage in a vascularized cornea treated with fine needle diathermy occlusion of the vessels. (B) Optical coherence tomograms showing separation of the PDL/DL with a hypo reflective space (blood) anterior to it (arrows).

was performed in 1905, the images of failed PK illustrated in Fuchs' account in 1901 must have been from previous failed attempts. The high rate of retrocorneal membrane in failed graft was further observed in a subsequent study of 170 failed corneal grafts (Kremer et al., 1993). Among the 170 failed grafts, chronic or severe anterior chamber inflammation, peripheral anterior synechiae, vitreous attachment to corneal wound, and tube-shunt surgery appeared to be significant risk factors for retro corneal membrane (Brown and Kitano, 1966). While not fully understood, three patho-physiological mechanisms of retro corneal membrane have been postulated, including: (1) epithelial ingrowth/-downgrowth; (2) keratocytic/fibrous downgrowth; and (3) fibrous metaplasia of the corneal endothelium.

In a clinicopathological study of 28 eyes with retro corneal membrane, Jakobiec & Bhat (Jakobiec and Bhat, 2010) observed several histopathological phenotypes, ranging from thin, delicate, fibrillar retro-Descemet membrane to thick keratocytic retro-Descemet membrane that resulted in contraction of the DM, giving rise to an undulating appearance. In some of the cases reported there was evident positive staining of keratocytic cells for alpha-smooth muscle actin (aSMA) in the pre-Descemet deep stroma indicating the involvement of the PDL/DL. The commonly described "endothelial plaque" or "retro corneal plaque/membrane" often adopts a homogeneous and smooth anterior and posterior edge at the deep stroma, for which the location is often restricted to the type 1 BB zone, based on our personal observation (Fig. 19 A, B) and other studies (Takezawa et al., 2017). It is possible that a type 1 DMD in the context of chronic corneal inflammation and oedema can provide the scaffold for a retro corneal membrane to develop and that the type 1 DMD with keratocytic and inflammatory cell infiltration can itself be the 'the retro corneal membrane'. Thus far, the evidence for this is indirect but sufficient to propose this as a viable hypothesis for some cases of retrocorneal membranes warranting further studies.
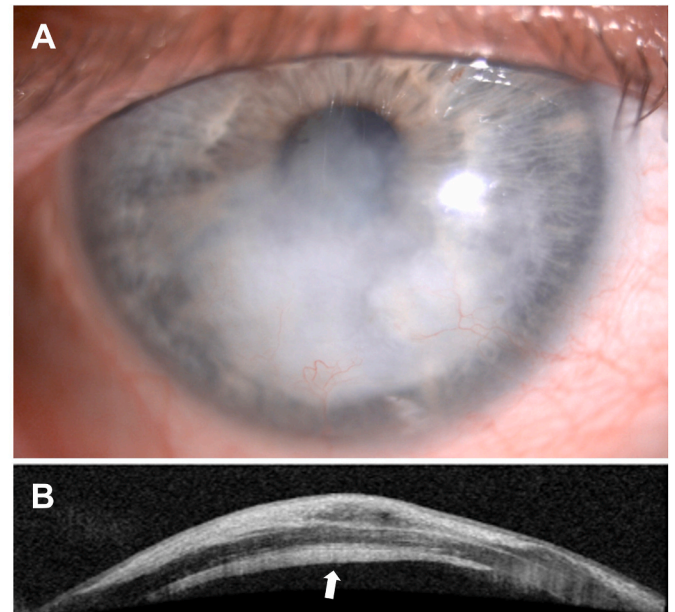
Fig. 19. The pre-Descemet's layer/Dua's layer (PDL/DL) in retrocorneal membranes. (A) Slit lamp diffuse view of a scarred and vascularized cornea post chronic infection. (B) Optical coherence tomography revealed a dense hyper reflective retro corneal plaque that assumes the shape of the cleavage plane between the PDL/DL and the posterior stroma (arrow).

6.6. Descemetocele/pre-descemetocele

It has always been taught that a descemetocele is a rare clinical sign that is characterised by an anterior herniation of the DM via an area of

severe stromal thinning, due to the unopposed outward exertion of intraocular pressure (Agarwal et al., 2021). Not uncommonly, Descemetoceles may progress to corneal perforation if not managed immediately (Ozdemir et al., 2018). The discovery of the PDL/DL had immediately raised the possibility that a Descemetocele may not always be an exposure of the DM only. Initial observation with this in mind had indeed proved this to be the case, hence in our first report on the PDL/DL (Dua et al., 2013) we had indicated that the PDL/DL could be forming a covering layer in some cases of Descemetoceles. A case where video graphic evidence of this was available, was mentioned in a letter (Dua et al., 2014d). It was common teaching that "Descemetoceles resist perforation" implying an inherent strength in the DM. However, it is now clear that in many cases the covering of the PDL/DL confers strength to the DM, which on its own would not withstand the internal (intraocular) and external (friction of lid blinks) forces that a descemetocele would be subjected to (AlTaan et al., 2018). So far three types of Descemetoceles have been identified, 1. Herniation of the Descemet's membrane with the overlying PDL/DL (Type 1); 2. A true herniation of the Descemet's membrane (Type 2); and 3. Herniation of the Descemet's membrane with the overlying PDL/DL and a variable amount of corneal stroma (Type 3). Clinically all three types appear similar on the slit lamp but are readily distinguishable on OCT (Table 3).

Not surprisingly a 'pre-descemetocele', indicating a herniation of the DM covered by the PDL/DL, was reported as a distinct clinical entity with AS-OCT evidence (Narang et al., 2017). Appreciation of the difference between pre-descemetocele and descemetocele may provide useful guidance to the clinicians in stratifying the need and urgency for intervention with use of a bandage contact lens, glue application or surgical tectonic grafting, which are the common approaches to management. In addition to its strength, the PDL/DL may also be more resistant to enzymatic degradation. Collagen cross linking has been shown to increase the resistance of corneal collagen to enzymatic degradation (Spoerl et al., 2004). Given its proximity to the DM and potential exposure to aqueous humour constituents it is likely that the PDL/DL is in a greater crosslinked state than the rest of the cornea. A 'pre-Descemetocele' could represent a stage in the evolution of a Descemetocele.

6.7. Corneal dystrophies and deposits

Corneal dystrophies refer to a group of non-inflammatory, inherited corneal disorders that are characterised by bilateral, progressive corneal changes and/or deposits (Soh et al., 2020). Based on the anatomical level of involvement, they can be broadly classified into several categories, namely: (1) epithelial and subepithelial dystrophies; (2) epithelial-stromal TGFB1 dystrophies; (3) stromal dystrophies; and (4) endothelial dystrophies (Soh et al., 2020).

By and large, when DALK was carried out with the formation of a type 1 BB for granular or lattice dystrophies, the host PDL/DL has been reported to be clear. However, studies where the same procedure was performed for advanced dystrophies, the involvement of the PDL/DL was reported in TGFB1 stromal dystrophies (Oke et al., 2020; Pantanelli et al., 2014). Oke et al. (2020) reported the recurrence of granular corneal dystrophy (GCD) at the posterior graft-host interface after a type 1 BB, suggesting the involvement of PDL/DL. We similarly observed a

case of recurrent GCD involving the PDL/DL, after a manual DALK. In the repeat DALK surgery, we were not able to achieve either a type 1 or a type 2 BB despite multiple attempts. Subsequently, a manual dissection of the deep stroma was performed to reach the cleavage plane and separation along the plane was achieved leaving behind the host PDL/DL. Scattered GCD was identified on the surface of the PDL/DL (Fig. 20A–D).

A significant proportion of recurrent GCD deposits following keratoplasty, occur in the superficial anterior cornea where they are deposited by the regenerated host epithelial cells that carry the same gene defect. The epithelium is packed with granular deposits which migrate into the anterior stroma. These situations can be managed by alcohol delamination of the deposit laden epithelium. The new regenerated epithelium remains clear for months or years and the procedure can be repeated multiple times (Avadhanam et al., 2016). A fair amount of granular deposits are also derived from the host keratocytes in the unoperated corneas, as well as in the transplanted corneas (DALK or PK), which are re-populated by host keratocytes over time. The PDL/DL being either acellular or pauci-cellular, usually does not contain granular deposits. In advanced cases or in recurrent cases, the deposits accumulate in the interface between donor stroma and the host PDL/DL and eventually in the PDL/DL suggesting a migration of the deposits and/or keratocytes into the layer.

Macular corneal dystrophy (MCD) is autosomal recessive, non-TFGB1 corneal stromal dystrophy with abnormality in proteoglycan synthesis due to mutation in the carbohydrate sulfotransferase 6 gene (Aggarwal et al., 2018). The corneas are affected by stromal opacities involving the stroma and extending to the DM and endothelium (Lin et al., 2016; Zhang et al., 2019). However, isolated involvement of the DM and/or PDL/DL in MCD has been described, suggesting that MCD may be a stromal-endothelial dystrophy (Shi et al., 2017). Surgeons have demonstrated considerable skill in excising the PDL/DL affected by macular dystrophy, from the DM after obtaining a type 1 BB during DALK and effectively converting it to a type 2 outcome (Tolees, 2017).

Pre-Descemet's corneal dystrophy (PDCD) is another rare type of corneal dystrophy, characterised by the presence of multiple, tiny, polychromatic deposits at the posterior cornea, immediately at the pre-DM level (Soh et al., 2020). PDCD can occur either in isolation or in association with X-linked ichthyosis [due to mutation in steroid sulfatase (STS) gene] (Boere et al., 2020; Choo et al., 2021; Shi et al., 2017). The deposits are usually visualized as a homogeneous band of hyper-reflectivity affecting the posterior 50–70 μm of the cornea, in which the PDL/DL layer is likely to be involved (Boere et al., 2020; Malhotra et al., 2015). Interestingly, the peripheral cornea (within the 2–3 mm perilimbal zone) is usually clear (Shi et al., 2017), which approximately corresponds to the type 1 BB zone. In addition, co-occurrence of polychromatic lenticular deposits at the anterior sub-capsular region was reported in PDCD with PRDX3 mutation (Choo et al., 2021). Both PDL/DL and lens capsule are rich in elastin (Mohammed et al., 2018), and the co-involvement of both structures in this case suggests a potential affinity of these deposits to elastin. Similarly, cornea farinata – a relatively common age-related corneal condition – is characterised by diffuse, multiple, fine grey-white deposits localised at the deep stromal area just anterior to the DM, indicating the involvement of PDL/DL layer (Kobayashi et al., 2003). Mutation in STS gene has been implicated in the manifestation of cornea farinata (Klintworth, 2009), suggesting that both PDCD and cornea farinata may belong to the same spectrum of disease but with varying phenotypic severity.

On the other hand, Lisch and Vossmerbaeumer recently described a case of biclonal Lewis syndrome (Lewis et al., 1975; Lisch and Vossmerbaeumer, 2020), which consists of a classic triad of biclonal gammopathy of undetermined significant hypercupremia (high serum level of copper), and paraproteinemic keratopathy with brownish-golden discoid opacification at the level of PDL/DL and DM (likely caused by copper infiltration). In addition, anterior and posterior

Table 3
Clinical types of Descemetoceles on optical coherence tomography examination.

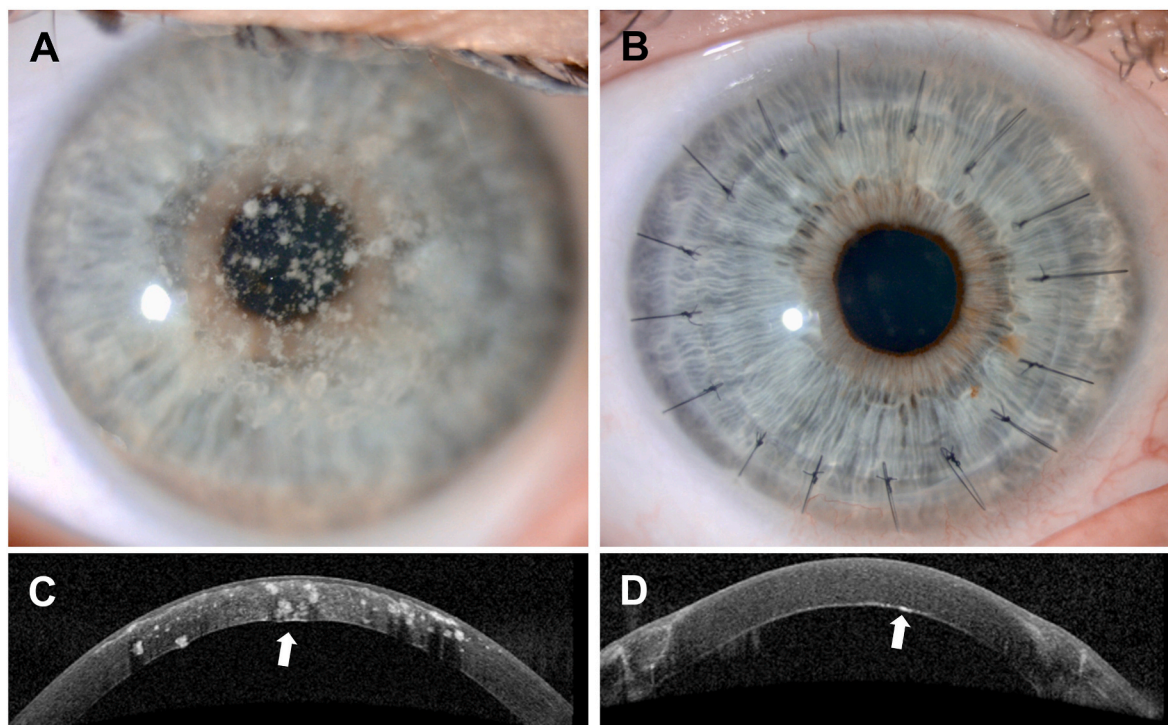| Descemetocele Type	Description
Type 1	Herniation of the Descemet's membrane with the overlying pre-Descemet's layer/Dua's layer
Type 2	Herniation of Descemet's membrane only
Type 3	Herniation of the Descemet's membrane with the overlying pre-Descemet's layer/Dua's layer and a variable amount of corneal stroma.

Fig. 20. Involvement of the pre-Descemet's layer/Dua's layer (PDL/DL) in advanced granular corneal dystrophy. (A) Diffuse slit lamp view of the cornea showing advanced granular dystrophy. (B) The same eye post deep anterior lamellar keratoplasty (DALK). (C) Optical coherence tomogram (OCT) showing involvement of the entire corneal stroma right down to the PDL/DL (arrow). (D) OCT of the eye post DALK showing residual granular material in the PDL/DL (arrow).

lens capsules are shown to be similarly affected by extensive copper infiltration in Lewis syndrome. The predilection of copper to the PDL/DL/DM and lens capsules might be linked to the role of copper in the synthesis of elastin (Harris et al., 1980).

7. Surgical applications of the PDL/DL

7.1. Deep anterior lamellar keratoplasty (DALK)

For several decades, DALK has been the preferred mode of corneal transplantation in diseases involving the stroma with healthy endothelium such as stromal dystrophies corneal scars and ectatic diseases. DALK offers a stronger graft-host junction specially if it is achieved with a type 1 BB in which the resilient PDL/DL is retained. It also offers a marked reduction in rejection related failure due to absence of endothelial rejection, although, epithelial and stromal rejection can still occur (Borderie et al., 2011; Watson et al., 2006). DALK is associated with a potentially quicker visual rehabilitation, early suture removal and a shorter duration of steroid use. Furthermore, the reduced risk of rejection has encouraged surgeons to manage induced astigmatism with phakic implants and refractive lens exchange using toric implants (Malheiro et al., 2019; Schiano Lomoriello et al., 2018).

The principle of DALK techniques is to replace all the host (diseased) stroma with healthy stroma from a donor cornea. Anwar's big bubble technique, where air is injected deep into the host stroma to separate it from PDL/DL - DM remains the most popular technique. However, the dynamics of the separation of the stroma from the deeper tissue was unclear and the procedure ill understood or misunderstood. The discovery of the PDL/DL and the natural cleavage plane between it and deep stroma provided a clear understanding of what was exactly happening during surgery and as a consequence allowed surgeons to maximise the attainment of the desired outcome and minimise risks and complications. This knowledge was fundamental to the technique and helped make DALK safer.

It dispelled the myth that DALK by the BB technique was a DM baring

technique (Anwar and Teichmann, 2002a; Dua et al., 2013). It explained the appearance of three different types of BB, types 1, 2 and mixed. It corrected the assumption that the 'thin walled BB' and the 'double bubble' were due to a split between the banded and non-banded zones of the DM. It provided an explanation of the relative toughness of the eye after DALK compared to PK and demonstrated that this was the case with a type 1 BB because of the toughness of the PDL/DL and probably not with a type 2 BB, where only the host DM is retained.

It illustrated that approximately 80–85% of BB were of the type 1 and the remaining were type 2 or mixed in eye bank eyes and up to 60% type 1 in reported case series (Dua et al., 2015a; Goweida et al., 2020). It taught us how the different types of BB behave during DALK. Though it is not possible to predict which type of BB one would get in a given patient, it is easy to tell which type of BB one has obtained, on the basis of the characteristics of the PDL/DL (Dua et al., 2015b). The surface of a type 1 BB is 'rough looking' as a result of the broken strands of collagen fibres, whereas the surface of a type 2 BB is very smooth and featureless (Fig. 21 A, B). The white margin of a type 1 BB (Fig. 22A) is produced by the stretching of the fibres along the circumference caused by the intra bubble air pressure. This is an optical phenomenon as the white margin disappears or becomes markedly less obvious as soon as the pressure in the bubble is reduced by removing the needle.

Type 2 BB and type 2 components of mixed BB are very fragile and susceptible to rupture and tears (Fig. 22B–D). With a type 2 BB, when the DM is nicked or punctured intraoperatively, the tear extends rapidly and often circumferentially all along the circumference of the trephination for 360° (Fig. 23 A, B). This can be avoided by repeated release of aqueous through the paracentesis and keeping the eye soft and the DM flaccid. A tense DM, of 15–20 μm, holding the eye pressure, which is normally supported by the approximately 550 μm thickness of the cornea, is like a tense inflated balloon that can burst when punctured.

A type 1 BB on the other hand is more robust and resilient. A tear in the PDL/DL + DM does not usually extend and DALK can be successfully completed, despite a 'large' peripherally located tear. The edges of the tear in a type 1 BB do not scroll like the edges of the DM tear in a type 2
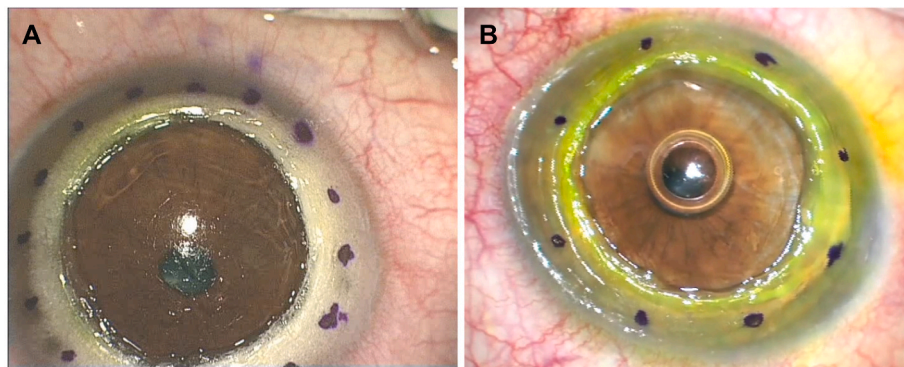
Fig. 21. Difference between type 1 and type 2 big bubbles (BB) in deep anterior lamellar keratoplasty (DALK). (A) A type 1 BB with the rough looking anterior surface of the PDL/DL produced by separated strands of collagen tissue. (B) A type 2 BB with a comparatively very smooth and featureless anterior surface of the DM. PDL/DL = pre- Descemet's layer/Dua's layer. DM = Descemet's membrane.
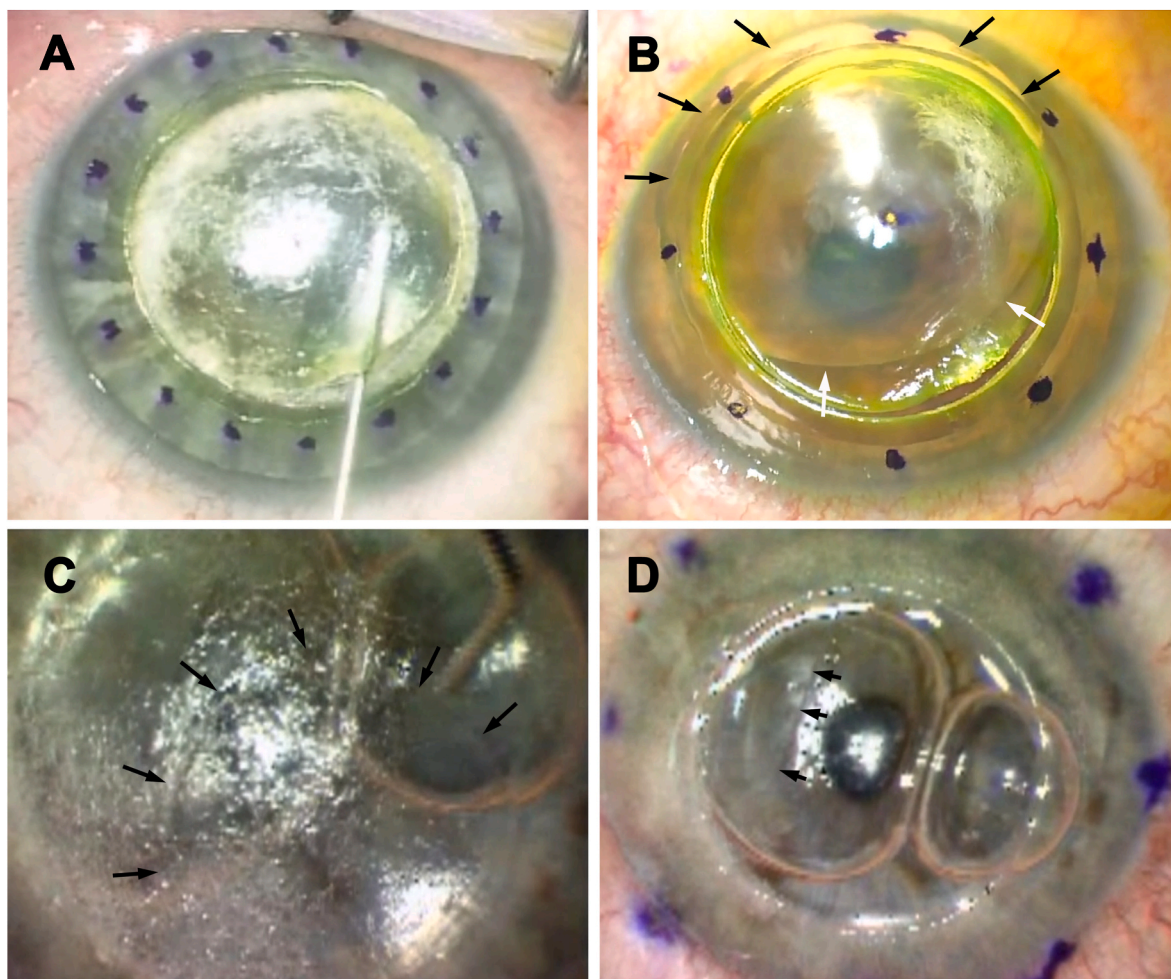

Fig. 22. Types of big bubbles (BB) seen during deep anterior lamellar keratoplasty (DALK). (A) Type 1 BB with the classic white ring. (B) Type 2 BB with a clear margin. The margin of the BB under the host rim is marked with black arrows and the margin inside the trephination ring is marked with white arrows. (C) A mixed BB. A small type 1 component remaining after leakage of air is marked with arrows. (D) After exposing the pre-Descemet's layer/Dua's layer, a partial type 2 component is seen (arrows). The big air bubbles are in the anterior chamber.

BB because in the former the DM is attached to and tamponaded by the PDL/DL (Fig. 24 A, B). At the end of the operation, often air has to be injected in the anterior chamber to keep the PDL/DL apposed to the donor cornea and reduce risk of a double anterior chamber (Sati et al., 2020). This however, can cause the Urrets Zavalia syndrome if pupil block glaucoma is induced by the air (Bozkurt et al., 2013; Niknam and Rajabi, 2009).

Knowledge of the maximum diameter of the type 1 BB has informed us that with large trephination diameters in the host cornea (more than approximately 8.5–9 mm) or with eccentric trephinations, the BB will not reach the edge of the trephination and a manual dissection may have to be carried out at the attached periphery. It has also illustrated clearly,
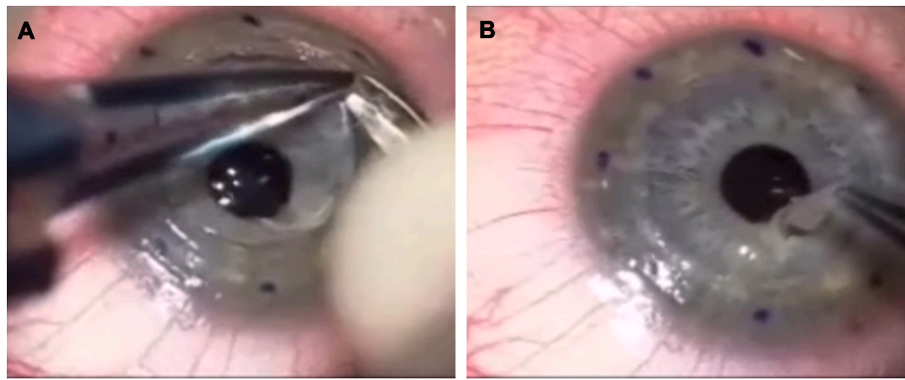
Fig. 23. Fragility of a type 2 big bubble (BB). (A) Trimming the edge of the host rim with a type 2 BB. (B) A slight nick to the Descemet's membrane (DM) resulted in rupture of the BB and a complete circumferential tear along the rim. The DM was lifted out with a forceps.
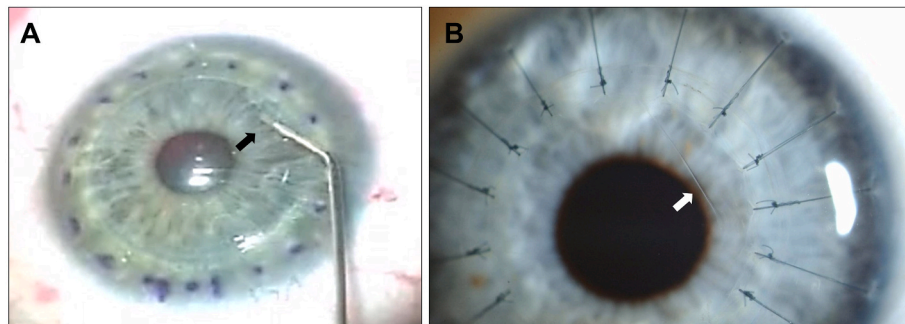

Fig. 24. Type 1 BB with tear in the pre-Descemet's layer/Dua's layer (PDL/DL). (A) A small tear (with a canula tip passed through it) is seen in a type 1 BB (arrow). (B) The operation was completed successfully. Post operatively a wrinkle in the PDL/DL adjacent to the tear, was the only visible clinical sign (arrow).

that with a type 2 BB, which usually extends to inner limbus, there is a high risk of the paracentesis needle puncturing and rupturing the DM. This can be avoided by releasing the air from the top before performing paracentesis or by performing a vertical paracentesis at the limbus. The same applies to the type 2 component of a mixed BB. In case of a partial

mixed BB, the paracentesis can be performed at the site away from the type 2 component. Knowledge of the anatomy of a mixed BB and the behaviour of the PDL/DL has informed us that the type 2 component can be deflated, if needed, by making a small puncture with a 26 or 27-gauge needle, in the PDL/DL at the periphery of the air bubble trapped
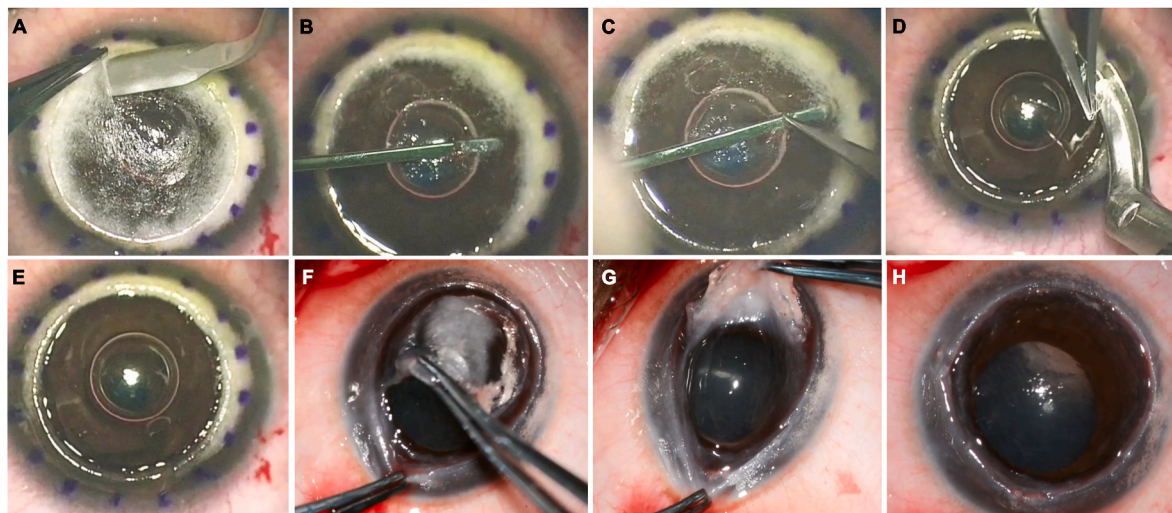
Fig. 25. The cleavage plane between the posterior stroma and the pre-Descemet's layer/Dua's layer (PDL/DL). (A) When a BB does not form the cleavage, plane can be accessed by spiral lamellar dissection of the deep stroma. (B) A small nick can be made in small bubble in the deep stroma and a spatula introduced to explore the plane. (C) When the cleavage plane is reached the spatula is used to separate the PDL/DL over 360°. (D) The PDL/DL is then cut into four quadrants and excised. (E) The anterior surface of the layer is exposed in the area of trephination. It is exactly the same as that achieved with a type 1 BB. (F) After deep trephination the deep stroma can be physically peeled off with force. (G) Considerable force may be required as seen by the ovalisation of the circular trephine cut. (H) The PDL/DL is completely exposed with the trephined cornea. (F, G, H are courtesy of Dr Rishi Swarup, Swarup Eye Centre, Hyderabad, India. Deep anterior lamellar keratoplasty by visco dissection was attempted in keratoconus prior to mechanical peeling).

between DM posteriorly and PDL/DL anteriorly.

Awareness of the cleavage plane between the PDL/DL and the posterior stroma has enabled successful separation of the PDL/DL to the desired diameter, even when a very small type 1 BB has been attained or no bubble obtained at all. In the former situation, the small type 1 BB can be punctured and the space expanded with the injection of viscoelastic and further extended by passing a blunt spatula along the cleavage plane, to the trephination edge. In the latter situation, the stromal layers can be painstakingly dissected off manually until access to the cleavage plane is achieved at one point, from which the entire plane can be dissected by a combination of viscoelastic injection and blunt-spatula dissection. It is also possible to forcibly peel off the stroma from the PDL/DL as illustrated (Fig. 25A–H). With the advent of intraoperative OCT, it is now possible to directly observe many of the above
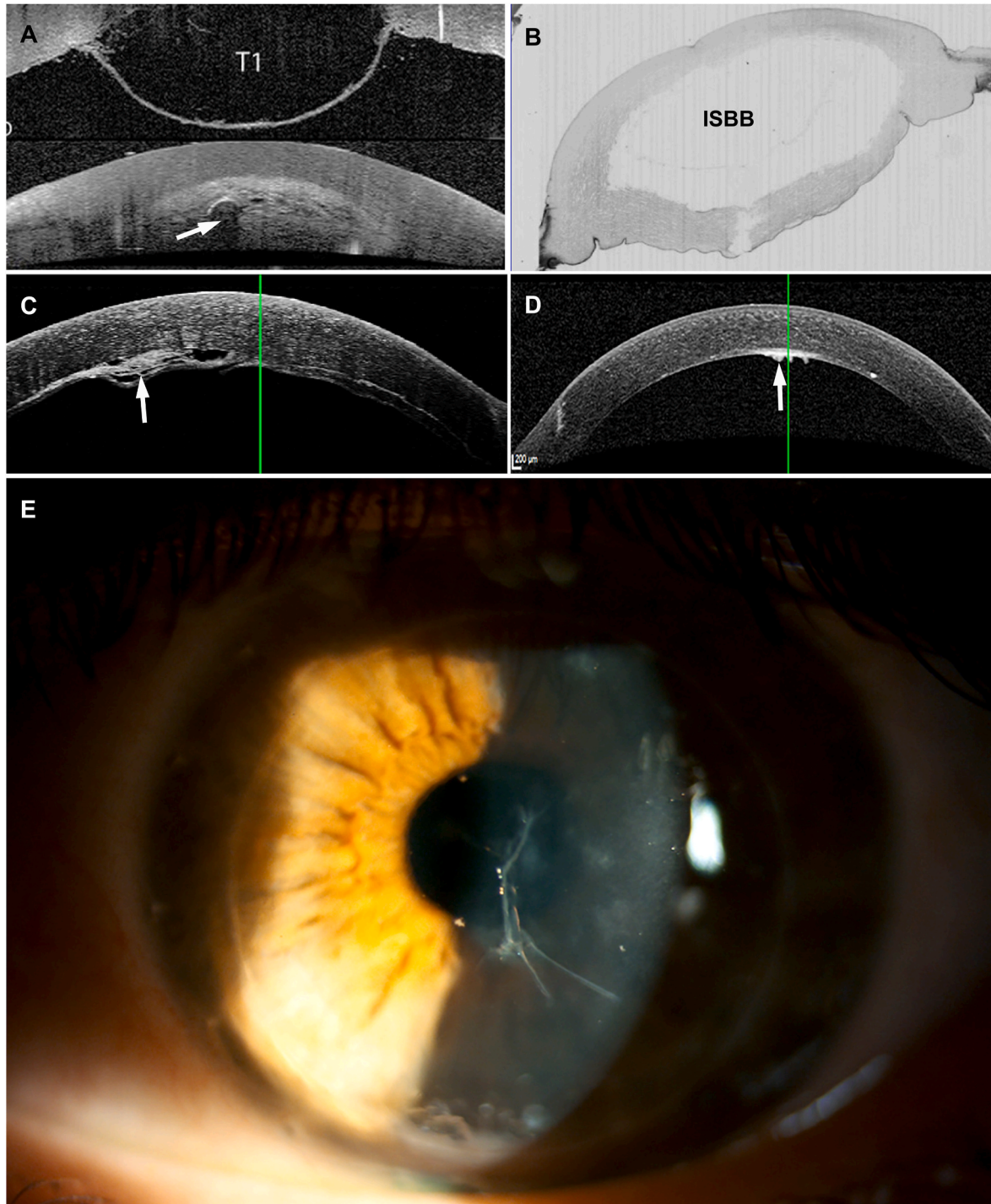
Fig. 26. Big bubbles with viscoelastic injection. (A) Optical coherence tomography (OCT) of a type 1 big bubble (BB) (T1) created by injecting cohesive viscoelastic deep in the corneal stroma. The arrow points to the depth of the needle (hypo reflective circle) in the stroma, which is hyper reflective on account of the spreading viscoelastic. (B) Mid stromal injection of viscoelastic results in the creation of an intra stromal big bubble (ISBB), which has a variable amount of stroma posteriorly, and can be misleading as intraoperatively resembles a type 1 BB. (C) Retained viscoelastic is hyper reflective on OCT taken one week post operatively. (D) OCT of the same eye four months post operatively still shows retained viscoelastic between the grafted cornea and the PDL/DL. (E) Diffuse slit lamp view of the cornea at 4 months showing scarring and a stromal haze.

characteristics and undertake the manoeuvres described during surgery (Lang et al., 2020; Steven et al., 2014).

The demonstration of fenestrations at the periphery of the PDL/DL along the circumference and their variation in relation to the peripheral termination of the DM, has helped to understand how air travels from the stroma to the plane between the DM and PDL/DL despite the fact that the PDL/DL is impervious to air, and also why air bubbles often appear in the anterior chamber during BB DALK (Dua et al., 2018). In some cases when the PDL/DL is thin, as in advanced keratoconus, a type 1 BB can cause it to dehisce and air escapes through the PDL/DL to create the type 2 component of a mixed BB.

7.2. DALK with visco-dissection

As an alternative to air, injection of a cohesive viscoelastic agent has been used in DALK and reported to be comparatively safer (Melles et al., 2000). However, this was prior to the discovery of the PDL/DL and the DM baring BB that was reportedly achieved, was probably a type 1 BB and hence safer. The dynamics of the movement of viscoelastic in the stroma is different to that of air. Air is believed to move through spaces around keratocytes but the increased viscosity of the viscoelastic agent makes this passage slow and often incomplete. To reach the plane of cleavage anterior to the PDL/DL, the viscoelastic has to be injected very deep in the stroma. When a BB is not achieved, manual dissection often leaves behind a layer of deep stroma of variable thickness (Fig. 26 A, B). This can contain residual viscoelastic substance impregnated in it, which can take a long time, up to months to clear and cause scarring (Scorcia et al., 2018) (Fig. 26C–E). With this technique there is some inconsistency in the understanding of the type of BB achieved. It is difficult to envisage how an injection of viscoelastic can create a type 2 BB by the same mechanism as with air. The size of the fenestrations and the viscosity of the agent would both severely restrict the access of the viscoelastic to the plane between the PDL/DL and DM. Ross et al. (2018) through *ex vivo* experiments in human eyes using viscoelastic injections, made some important observations: A type 2 BB did not form. The behaviour of the viscoelastic varied according to the depth of injection. Injection in the anterior stroma resulted in the dispersion of viscoelastic in the adjacent compact stroma with lateral spread but minimal posterior spread. Injection in the mid-stroma resulted in the formation of 'intra-stromal BB' (ISBB), which equated to the accumulation of a large blob of viscoelastic in a cavity created by the tearing apart of the stromal lamellae. Clinically, intraoperatively the ISBB gave the semblance of a type 1 BB. It is likely that surgeons treated this as a type 1 BB and performed DALK, leaving behind a variable amount of stroma of irregular thickness in some instances. Injection of viscoelastic in the deep stroma was the only occasion when a type 1 BB was formed.

7.3. DALK triple procedure

Patients with combined cataract and corneal stromal opacity have always presented challenges, especially the poor visibility through the scarred cornea. Often surgeons have opted for a 2-stage procedure wherein the DALK procedure is performed first followed a year later by the cataract procedure after removal of all sutures. This involves two intraocular procedures with the associated risks, additional cost and delayed visual recovery on account of the cataract, which could get worse over the time to full suture removal. Others have opted for a combined penetrating graft with cataract surgery. This has a greater risk of complications (Greene and Mian, 2013) including endothelial rejection, which is not an issue with the DALK procedure.

The discovery of the PDL/DL and the knowledge that it is tough enough to withstand a pressure of 700 mm Hg, has allowed surgeons to perform a combination of a type 1 BB DALK with phacoemulsification in the same setting (Dua et al., 2013; Zaki et al., 2014). This was termed the DALK-triple procedure. In such patients when a type 1 BB is achieved, the scarred stroma can be removed and the phacoemulsification with

implant (and anterior vitrectomy if needed) can be carried out under the PDL/DL, which is usually transparent and often not affected by the scarring that involves the rest of the cornea. However, if a type 2 BB is formed, performing the phacoemulsification procedure under the DM is very risky with potential of rupture of DM with the least manipulation and with serious consequences (AlTaan et al., 2018; Zaki et al., 2014) (Oie and Nishida, 2017) (Fig. 27 A, B).

7.4. Mini DALK (Fogla)

Dr. Rajesh Fogla has shared a case (personal communication) of a pre-Descemetocoele that developed following collagen cross-linking for keratoconus. The corneal stroma surrounding the pre-Descemetocele was undermined by blunt dissection in a centrifugal direction. A 4 mm trephination, centered on the pre-Descemetocele was made in the stroma, to the depth of the PDL/DL and excised. The exposed area thus resembled a mini type 1 BB. A corneal disc of corresponding diameter was punched from a donor cornea, stripped off its DM and sutured in place. This is a novel approach to the management of a pre-Descemetocele, which restores normal anatomy, unlike cyanoacrylate gluing, which is the usual approach in the management of such a lesion (Fig. 28A–D).

7.5. Pre-Descemet's endothelial keratoplasty

To treat patients with corneal endothelial pathology such as Fuchs dystrophy or corneal decompensation post-surgery, the two main surgical procedures performed are Descemet's stripping endothelial keratoplasty (DSEK) which can be performed manually with or with microkeratome, "Descemet's stripping automated endothelial keratoplasty" (DSAEK) and DMEK. Though PK offers better visual results then DSAEK, the advantages of quicker visual rehabilitation and reduced rejection rate have led to change of practice towards endothelial transplantation. Ultrathin DSAEK has shown promising results with better visual outcome and faster recovery then DSAEK (Dickman et al., 2016) and nearly comparable visual results to DMEK (Busin et al., 2013). Although technically challenging with a steep learning curve due to the difficulties with working with thinner tissue including challenges associated with preparation, insertion and unfolding in the recipient eye (Dapena et al., 2011), DMEK offers the best visual outcome, fastest recovery and least rejection (Hos et al., 2019; Marques et al., 2019; Romano et al., 2020).

With the discovery of the PDL/DL, Dua et al. proposed the use of a tissue composite of the PDL/DL + DM + EC for endothelial keratoplasty (Dua et al., 2013). This can be obtained by creating a type 1 BB in the donor cornea and excising the posterior wall with a trephine of appropriate size or cutting along the perimeter of the BB at its attachment to the cornea. Later Agarwal and Dua reported outcomes of the procedure in human eyes and called it Pre-Descemet's endothelial keratoplasty (PDEK) (Agarwal et al., 2014). The PDL/DL splits the DM reducing it tendency to scroll compared to DMEK tissue, and as a consequence it is easier to unscroll in the eye thus reducing the endothelial cell loss associated with manipulations required to unscroll the tissue (Agarwal et al., 2014; Bennett et al., 2015; Dua et al., 2016; Guerra et al., 2011). The splinting effect of the PDL affords some rigidity to the tissue making it easier to handle, position and centre in the eye (Fig. 9).

Younger donors with their greater endothelial cell density are desirable for EK. One limitation of DMEK is the difficulty encountered in stripping the DM from young donors. Even when successfully obtained, the DM is thinner and forms tight scrolls compared to older donors. PDEK tissue can be obtained from very young donor eyes thus providing a clear advantage (Agarwal et al., 2015, 2017). A type 1 bubble has been demonstrated in eyes as young as 3 weeks, 1 year, and 2 years (Fig. 29 A, B) This procedure has demonstrated that the cleavage plane between PDL/DL and the posterior stroma is a feature present since birth.

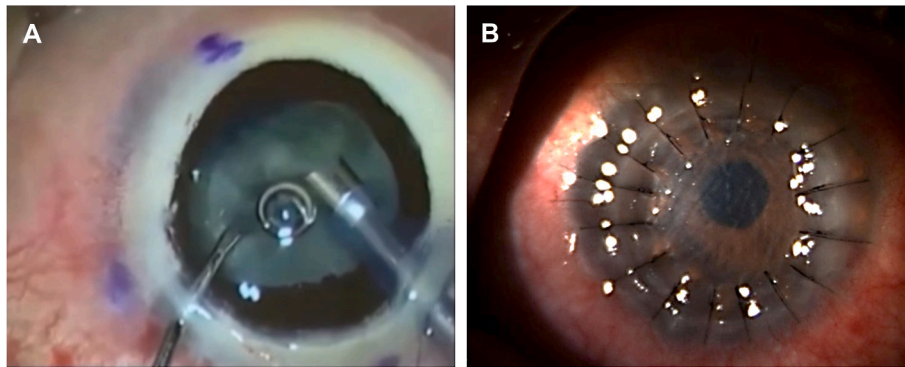Interestingly, pneumo-dissection was a method used to obtain EK

Fig. 27. Deep anterior lamellar keratoplasty (DALK) – Triple procedure. (A) Phacoemulsification being performed under the pre-Descemet's layer/Dua's layer (PCL/DL) during the DALK operation. The PDL/DL can withstand the pressure of phacoemulsification. (B) Post-operative image after a successful DALK-Triple procedure.
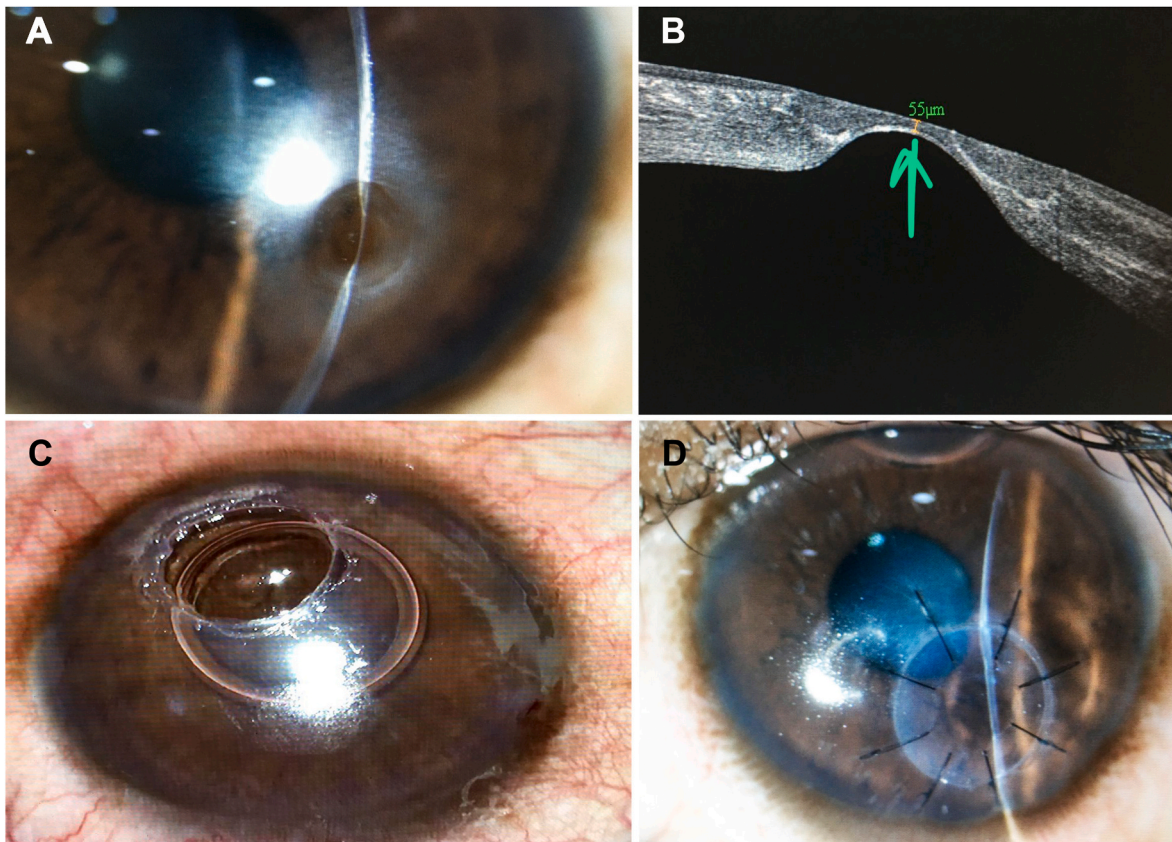

Fig. 28. Mini Deep anterior lamellar keratoplasty (DALK) (Fogla). A slit view of a pre-Descemetocele showing marked thinning over the lesion. (B) Optical coherence tomogram demonstrating the presence of the pre-Descemet's layer/Dua's layer (PDL/DL) covering the ectatic Descemet's membrane (total thickness 55 μm, arrow). (C) Intra operative view showing a 4 mm trephination to the depth of the PDL/DL including the surrounding stroma. (D) Post-operative view of the mini DALK procedure with the graft in situ. (courtesy Dr Rajesh Fogla, Hyderabad, India).

tissue before the discovery of the PDL/DL. It is not surprising that both types of BB were obtained but were all described as providing DMEK tissue and transplanted as such. Clearly some had obtained PDEK tissue and performed PDEK. Other modifications like the inclusion of the scleral rim in a small sector were introduced (Busin et al., 2010a, 2010b, 2011; Studeny et al., 2010). For PDEK, the formation of a type 2 BB would be an undesirable outcome as this would yield DMEK tissue and the formation of a mixed BB would make the donor tissue less than ideal. Knowledge of the fenestrations at the periphery of the PDL/DL and the understanding of the dynamics of the formation of a type 2 BB, provided the basis for the development of a fool proof way of consistently preventing the formation of a type 2 BB. We created a clamp, the PDEK

clamp (Dua and Said, 2017), in which the donor tissue is clamped before injecting air. The clamp shuts all the fenestrations and prevents escape of air. All injected air accumulates in the donor corneal stroma and, in a controlled manner, a type 1 BB can be created. Insertion of the needle tip in the cavity of the BB and further injection of air allows expansion of the BB to its maximum diameter. A critical intra tissue pressure of air is needed to create a type 1 BB. Without the clamp, air escapes from the periphery and the consequent loss of pressure has to be compensated for by further forceful injection of air to reach the critical pressure. This is difficult to control and at times, when the injection velocity is excessive, a type 1 BB forms and bursts very quickly. This is avoided with the use of the clamp. As the fenestrations are clamped shut, no air escapes under
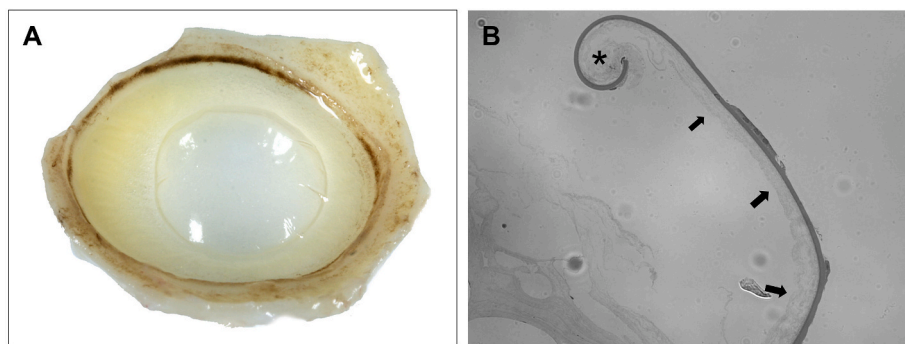
Fig. 29. Pre-Descemet's layer/Dua's layer (PDL/DL) in infants. (A) A type 1 big bubble (BB) in a 3 weeks old donor eye. (B) Light photomicrograph demonstrating the acellular PDL/DL (arrows) in a 2 years old donor eye in which a type 1 BB was created. The Descemet's membrane and PDL/DL scroll with the endothelium outside (*). (tissue material for 'B' courtesy of Dr Amar Agarwal, Hyderabad, India).

the DM either, preventing formation of a type 2 BB (Ross et al., 2020).

Pereira et al. described a simpler technique for preventing the formation of a type 2 BB. By scoring the DM 360° along the periphery before injecting air, it creates an escape route for air emerging from the fenestrations. The air does not access the plane anterior to the DM, preventing the formation of a type 2 BB. They achieved a type 1 BB in 100% of eyes in which the DM was scored while only in 53% of eyes in which the DM was not scored. The remaining 47% of eyes which were not scored developed a type 2 or mixed bubbles (Pereira et al., 2021). This technique however, does not allow for a controlled build of intra tissue pressure, which is an advantage of the PDEK clamp as described above.

Both PDEK and DMEK tissue produced by pneumo-dissection have been shown to have a similar endothelial cell count (AlTaan et al., 2015) and can be stored in organ culture for a week without any effect on endothelial count (Gamaleldin et al., 2016). PDEK has been performed in complex cases of pseudophakic bullous keratopathy or in combination with other complex surgical procedures, including pupilloplasty and glued-on intraocular lens implants (Bedard and Hou, 2021; Huang et al., 2018; Narang et al., 2015; Narang and Agarwal, 2019; Sharma et al., 2021; Tsatsos et al., 2019).

In the context of endothelial keratoplasty it has been noted by surgeons that during DSEK, in some cases, the host DM removed is quite thick. This has been shown to be a combination of DM and PDL/DL as in a type 1 DMD. This aspect of endothelial keratoplasty has now become evident and highlights the role of the PDL/DL in improving our understanding of intraoperative findings (Sharma et al., 2020).

7.6. Management of acute corneal hydrops

ACH is usually self-limiting with a mean healing time of two to four months (Gaskin et al., 2014). Pneumo-descematopexy with intracameral injections of either air or gas (e.g. SF6, C2F6 or C3F8) is a popular intervention (Miyata et al., 2002; Panda et al., 2007; Ting and Srinivasan, 2014), wherein the air/gas serves as an internal tamponade and impedes further influx of aqueous into the corneal stroma as well as to re-attach the PDL/DL and DM. However, resolution of acute hydrops after air/gas injection ranged from 3 to 4 weeks post injection in most reports (Miyata et al., 2002; Panda et al., 2007). Rajaraman et al. reported resolution of the corneal oedema in ACH with the use of full thickness compression sutures + intracameral C3F8 injection in an average of 8.87 ± 4.98 days (Rajaraman et al., 2009). Endothelial cell injury from full thickness corneal sutures is a concern.

In 2015, Yahia Cherif (Yahia Chérif et al., 2015) reported a successful novel technique of managing ACH by applying 10/0 nylon compression sutures to include the torn edges of the PDL but not the DM (they termed it pre-DM sutures) combined with intracameral air injection, and reported resolution of the corneal oedema starting from the first day of

surgery with complete resolution of the oedema within 15 days in 6 out of 7 eyes. They have reinforced their results with OCT images before and on the day of the surgery. They inferred from their success, evidence of the existence of the PDL/DL and its role in the pathophysiology and management of ACH. Zhao et al. compared the resolution of acute hydrops with thermokeratoplasty versus pre-DM compression sutures with intracameral air injection and although resolution of ACH was reported in 2 weeks with both procedures, they concluded that pre-DM sutures with air injection can better maintain the corneal morphology and results in significant reduction of K-max and K-mean at 6 months follow-up. Thus, highlighting the importance of the PDL/DL in restoring a more anatomical resolution of ACH and creating a less visually significant scar. This technique allows further visual rehabilitation with RGP lenses reducing the need for corneal grafts (Mandathara et al., 2017; Zhao et al., 2020).

Regardless of how the ACH resolves, the corresponding healed area of the PDL/DL, though functionally restores endothelial cell function and corneal clearing, is physically never back to its normal self. This is demonstrated by the fact that an attempt to undertake DALK by air injection invariably results in a tear at the healed site and escape of air into the anterior chamber without formation of a type 1 BB. The same is noted in donor eyes with previous cataract surgery where an attempt to produce a type 1 BB *ex vivo* results in rapid air escape through the incision site (main wound or side-ports (Fig. 15).

8. The controversy

In science, anything new has a mixed reception of broadly four types. 1. It cannot be true, hence summarily dismissed; 2. It has been described before, referring to work that was close but usually not close enough; 3. "I have described it before"; and the silent majority who adopt the wait-and-see approach and some start generating evidence themselves. It is not surprising that the first publication of the paper on the PDL/DL had all four responses. The reactions ranged from curiosity, cautious acceptance and enthusiasm to skepticism and vigorous dissent. Criticism, fair criticism that is structured and constructive, is essential for progress but unsubstantiated, opinionated and baseless criticism (see epilogue below) is unhelpful and delays and detracts from progress.

Some questions asked were valid and constructive, like "the average age of patients was over 70 years. If the layer is a true layer it should be present in children as well?" "That this is a random separation of the deep stroma and the layer is nothing more than "residual stroma" seen in the DALK procedure. Another issue raised was related to the presence or absence of keratocytes in the layer as it was originally described as an "acellular layer". A major issue that attracted comments, was the attribution of the name "the pre-Descemet's layer (Dua's layer)" both within the scientific community and the lay public.

Some of these issues have been unequivocally resolved but are of

historic interest. For others the evidence is building. An account of the controversy as the story unfolded, and the evidence generated, is narrated below.

8.1. Is it present in the paediatric age group?

The first evidence came from a 9-year-old child who underwent an 'uneventful' DALK procedure in one eye. Postoperatively there was persistent corneal oedema that failed to clear. The surgeon, Dr Vinay Pillai from Thiruvananthapuram, India, presented the AS-OCT to Prof Dua for comments. It revealed the appearance of a mixed BB with an intact and continuous PDL/DL but a detached and torn DM, the edges of which were curled in the classic manner (Fig. 30 A, B). It was immediately apparent that the PDL/DL had separated as it would do in a type 1 BB but the DM too had detached and torn, making it a mixed BB. As the PDL/DL was intact, there was no aqueous leakage or collapse of the anterior chamber intraoperatively. The full thickness graft was performed and the presence of the PDL/DL was demonstrated by histological examination (Fig. 30 C, D). We have demonstrated the PDL/DL in a 3-week-year-old eye and in paediatric eyes aged 1, 2 and 4 years old (Fig. 29) Others have used young donor eyes for performing the PDEK procedure (Agarwal et al., 2015, 2017).

8.2. Is it "residual stroma"?

We first presented evidence of the presence of the layer, then termed the pre-Descemet's stromal layer, from eye bank eyes injected with air at two international scientific meetings (Dua HS. The Royal College of Ophthalmologists, Annual Congress Symposium - Evolving Techniques in Corneal Surgery – Layer by Layer, ICC Birmingham, 22-24th May 2007; and Dua HS. Societa Italiana Cellule Staminali e Superficie Oculare, VI CONGRESSO S.I.C.S.S.O. Lecce. June 14–16, 2007).

In 2010–11, reports appeared in the literature of the presence of "residual stroma" in DALK surgery (Jafarinasab et al., 2010; McKee et al., 2011). Following publication of our paper, a letter was published claiming that the authors had first described the layer, which was merely "residual stroma". They also contended that "if the pre-Descemet stroma is so "distinct" and "well defined" that it constitutes an entirely new corneal layer, then differences should be clearly seen without pneumodissection" (McKee et al., 2014). In our reply we had pointed out that the layer was not a random separation of "residual stroma" but a unique layer. We had evidence that when the layer ("residual stroma") was excised after creating a type 1 BB, another BB could not be created and air escaped from multiple spaced in the posterior corneal stroma (Dua et al., 2014d).

In a conclusive study, we demonstrated that ablation of the PDL/DL by phototherapeutic keratectomy in human corneal discs, without pneumodissection, followed by air injection, a BB could not be produced in any case. A key point which was not addressed by any of the critics, which we had established as a characteristic of the PDL/DL, was the fact that it was impervious to air. Unlike in the corneal stroma where air percolates through the tissue, in all probability around keratocytes, it does not pass through the PDL/DL. Photoablation of the layer destroys the ability to create a BB, demonstrating that the PDL/DL is unique (Dua
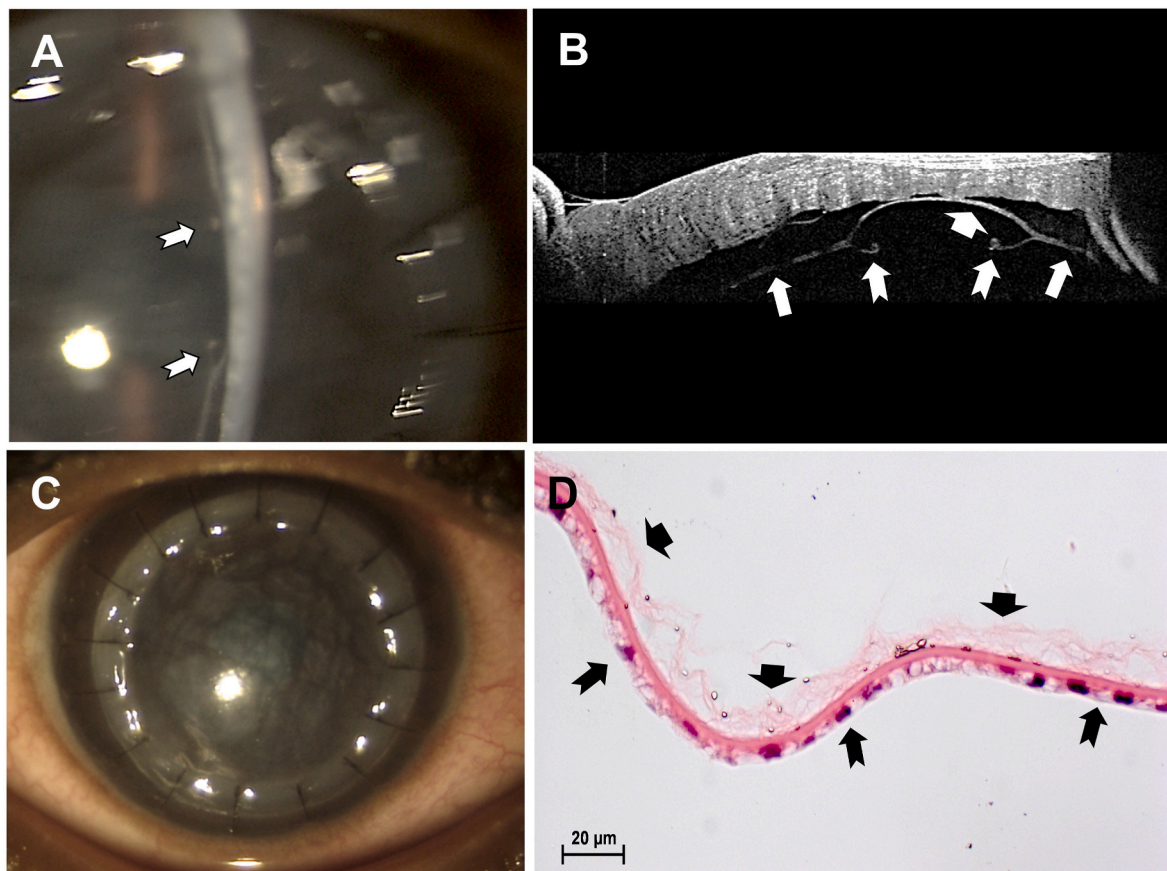
Fig. 30. The pre-Descemet's layer/Dua's layer (PDL/DL) in a 9 years old child. (A) Slit view of the donor cornea after deep anterior lamellar keratoplasty (DALK). The cornea is oedematous and a detachment of the DM with a tear with scrolled edges is seen (arrowheads). (B) Optical coherence tomogram showing a mixed rhegmatogenous Descemet's membrane detachment (DMD). The PDL/DL is intact (broad arrow). The DM has a tear with scrolled edges (arrowheads). The type 1 component of the DMD is seen on either side of the mixed component (arrows). (C) The DALK was replaced by penetrating keratoplasty and the PDL/DL and DM excised. (D) Histology of the excised tissue shows the PDL/DL (broad arrows) lining the anterior surface of the DM (arrowheads) (Hematoxylin and Eosin stain). DM = Descemet's membrane. (Images courtesy of Dr. Vinay Pillai, Thiruvananthapuram, India.)

et al., 2015c) (Fig. 31A and B).

8.3. Is it acellular?

The absence/paucity of keratocytes between the collagen lamellae of the PDL/DL has been a subject of debate. Our original publication demonstrated the presence of keratocytes on the anterior surface of the posterior wall of type 1 BB, the PDL/DL, as well as on the strands of collagen bridging the posterior and anterior walls, but no such cells could be detected within the collagen lamellae of the layer by light and electron microscopy, 4',6-diamidino-2-phenylindole (DAPI) staining, and CD34 staining (Dua et al., 2013, 2014a) (Fig. 7). Marshall and Grindle published an account of the fine structure of the cornea during development. They have included several images in their paper, which show a clear acellular zone anterior to the DM in the different age groups studied. The authors however, did not comment on this zone, which we now know corresponds to the PDL/DL (Marshall and Grindle, 1978) (Fig. 1).

The scarcity/absence of keratocytes on the innermost corneal layer is supported by the observations of Bizheva el al (Bizheva et al., 2016). Using ultrahigh-resolution OCT analysis of *in vivo* healthy corneas and taking the most posterior keratocyte and DM locations as the anterior and posterior references for the measurement of PDL/DL thickness, they reported an average thickness of 6.5 ± 1.4 μm (range 4.7–9.67). It is important to note that these measurements were in living eyes with OCT and the ones previously reported in fixed tissue sections. In contrast to these observations, Schlotzer-Schrehardt et al. have shown the presence of keratocytes as close as 2 μm from the DM using TEM (Schlötzer-Schrehardt et al., 2015), as have Lombardo et al. using two-photon optical microscopy (Lombardo et al., 2016). Jester et al. (2013) reconstructed the posterior cornea based on second harmonic generated signals and demonstrated keratocytes within 5 μm of the DM. These conflicting descriptions suggest that there could be a lower density of keratocytes within the PDL, rather than complete acellularity. However, in some instances one may be visualising keratocyte processes rather than keratocytes. The cell nuclei could be further away from the DM with the process(es) extending closer. We undertook whole mount staining of the PDL/DL and could not find any nuclei over large areas (Fig. 7.).

Dua et al. (2013) had reported that the separation of the PDL/DL from the deep stroma occurs along the last row of keratocytes, hence the cells were seen mostly on the anterior surface of PDL/DL and in the strands. Keratocytes are not arranged in straight lines in the corneal stroma, like soldiers in a formation. The last row would represent a line connecting all the posterior-most keratocytes, which in three dimensions would translate to a plane along which the PDL/DL separates from the deep stroma. The nomenclature is further confounded by the

argument whether the PDL/DL is a "zone", "layer" or "sheet" without specifying what each of these terms mean. The Bowman's is also referred to, interchangeably, as the "zone", "layer" or "membrane". This does not change what it is.

8.4. Was it described before?

Apart from the description of "residual stroma" mentioned above, there was one account by J Murphy posted online in the Review of Optometry on the 12th of July 2013 entitled "More details on Dua's layer of the Cornea" with the subtext "Perhaps discovered two decades ago …" (Murphy, 2013). In what is a balanced report it is stated that "Incidentally, Dr. Dua may not have been the first to report this layer. A paper published in 1991 by Perry Binder, MD, describes a network of fibers located at the interface of the posterior stroma and DM, although it was not identified as a distinct corneal layer". The author misrepresented Binder's work that had reported hitherto unknown attachments between the Descemet's membrane and the posterior stroma (Binder et al., 1991). These attachments would indeed be to the PDL/DL rather than the posterior stroma.

8.5. What's in a name?

A major part of the controversy revolved around the name, "Dua's Layer". One objected strongly (Schwab, 2013) against the name and the finding, though strangely without referring to either. Some suggested someone else's name and claimed priority (McKee et al., 2014) and others objected more courteously (Jester et al., 2013).

The fact is simpler than the perception. Over the years when we were working on the layer and its implication with regard to lamellar surgery was becoming evident, team members would refer to the layer as "Prof's layer or Prof Dua's layer". When the first few drafts of the paper were written, the title was "Human corneal anatomy re-defined: a novel pre-Descemet's layer". In the presentations on the work in 2007 it was termed "The pre-Descemet's stromal layer" and "the pre-Descemet's layer". When the final draft was ready for submission and approval of authors was being sought, a co-author, inserted the name in parenthesis "(Dua's layer)" at the end of the title. The argument put forward with some vigour was that Descemet's name was being invoked for a layer that had nothing to do with the Descemet's membrane. This view prevailed and the paper was published. The paper and the revision had gone through the rigorous peer review process of the journal before being finally accepted. At least four individuals, the reviews, the section editor and the editor did not see the name in the same light as the aforementioned authors. It is very likely, in fact almost certain, and I say this without hindsight, that if the deletion of the name was put forth as a comment for consideration, the revised version would not have had it

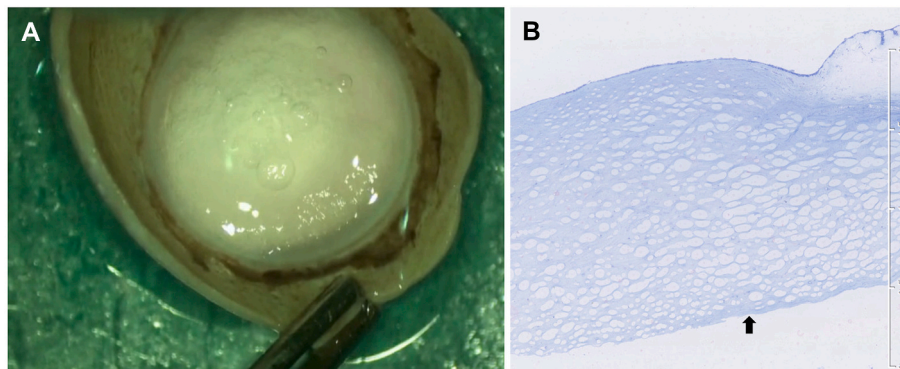**Fig. 31.** The pre-Descemet's layer/Dua's layer (PDL/DL) is not 'residual stroma'. (A) Air bubbles escape from multiple sites after laser ablation (15–20 μm) (phototherapeutic keratectomy) of the posterior surface of a sclero corneal disc. A type 1 big bubble could not be created. (B) Histology of the tissue shows the intact PDL/DL (arrow) and the ablated area on the left of the arrow (toluidine blue stain).

(and the course of history would have changed). In the correspondence that ensued post publication, on two separate occasions we stated "As acknowledged at the outset, the name was a misfortune, compounded by the extraordinary media interest. Let this not detract from the fact that this finding has considerably improved our understanding of DALK and will in all probability do so to our understanding of posterior corneal pathology." (Dua et al., 2014c) and "The circumstances leading to the addition of "(Dua's layer)" at the end of the title and the use of the name to describe the layer seemed right at the time, but with hindsight are regretted. It is very likely that, like the rest of us, the proclaimed guardians of professional righteousness use instruments and devices every week, named after somebody by the same body but felt this unworthy of comment. The extraordinary media coverage of our paper did not help".

Nevertheless, we proposed the term "pre-Descemet's layer" thereby invoking an existing eponym or "the pre-posterior limiting lamina layer" (Dua et al., 2014d). In an attempt to separate the controversy over the name from the relevance of the findings, we had concluded our letter with the following comment "More is yet to come and, through this process of questions, challenge, and debate (with decorum), may the truth emerge." In concordance with the report published in the Lancet in 1975, it was proposed that the layer be termed the "pre-posterior limiting lamina layer". ("Classification and nomenclature of morphological defects", 1975) In relation to disease with eponyms it was contended in the Lancet that "The possessive use of an eponym should be discontinued, since the author neither had nor owned the disorder".

In September and November 2017 the American Association of Ophthalmic Oncologists and Pathologists (AAOOP) contacted me (HSD) to propose the inclusion of the new finding of the PDL/DL in the 8th edition of the textbook on Ocular Pathology. As they had noted the histologic description of the region by Dr. Ben Fine on page 176 and a Figs. 9–26 illustrating it on page 177 in the Second Edition of the textbook, Ocular Histology: A Text and Atlas, by Drs, Fine and Yanoff, they had considered naming it the 'Fine-Dua' corneal layer in the revised textbook. Subsequently the term 'Dua-Fine layer' was approved at the business meeting of the AAOOP.

The support and recognition from a very distinguished and prestigious organisation was exceptionally good news. It signalled a shift in the discussion from whether there was a layer or not, to what it should be called? We have used the term Dua-Fine layer, alongside the PDL/DL in our presentations and publications since, as in this paper, and will continue to do so. A review of the chapter published in 1979 does show the layer clearly, however, there was no mention of the specialized layer in the text, nor was attention drawn to it in the image. Moreover, the image was adapted/modified from McTigue JW, Trans. Am Ophthalmol Soc. 65:591. 1965. A similar clear illustration of the layer is visible in the images published by Marshall and Grindle (1978), one year earlier. They too did not make any mention or comment on this area of the posterior cornea. As this is a discovery, it should be seen in any and all published images of the posterior cornea.

The controversy over the name had other anecdotal consequences. At the meeting of COECSA (College of Ophthalmologists of Eastern, Central and Southern Africa, (21–23 August 2013) in Rwanda, I (HSD) had presented the concept of using the PDL/DL together with the DM and endothelium as tissue for endothelial keratoplasty "as it scrolls less and would be easier to handle and unscroll in the eye, conserving endothelial cells" with *ex vivo* video demonstration of the technique using human corneal discs. During the lunch break, I shared my ex-vivo video with Dr. Amar Agarwal (Chennai, India) and we discussed the concept in detail. A few weeks later, in a long-distance call from India, he informed me that he had performed the operation in a couple of patients with very good results. He was going to call the procedure DLEK, (Dua's layer endothelial keratoplasty). I cautioned him about the controversy over the name and advised that we should avoid it. Moreover, the acronym DLEK (Deep lamellar endothelial keratoplasty) was already taken. We decided to call it PDEK (pre-Descemet's endothelial keratoplasty) and the first

publication describing this technique appeared in the BJO with Agarwal and Dua as joint first authors (Agarwal et al., 2014). Dr Agarwal was the first surgeon to perform PDEK in a human though others had performed it before, thinking that they were performing DMEK (See Surgical applications). This story elicited an interesting comment from a team member, "when the dust settles, PDEK will be known as Prof Dua's endothelial keratoplasty and the PDL will be Prof Dua's layer"!!

The term 'pre-Descemet' or 'pre-Descemetic' has been used as a descriptor long before the discovery of the PDL/DL, for example in 'pre-Descemet corneal dystrophy'. Sarnicola V et al. (Sarnicola et al., 2010) used the term Descemetic DALK (dDALK) to describe all cases where a BB was created with injection of air, viscoelastic or by hydro-delamination. They assumed that in all these cases the DM was bared. When a BB was not attained, deep dissection was carried out to reach as close to the DM as possible, invariably leaving behind some stroma. This was termed pre-Descemetic DALK (pdDALK). We now know that in a majority of their dDALK cases, the DM was not bared. When the difference between type 1 and type 2 BB was published in 2013, the terminology dDALK and pdDALK was accepted as not a true representation of what was intended and that the type 1 BB was a pdDALK. The terms subtotal anterior lamellar keratoplasty (STALK) (for type 1 BB and manual DALK) and total anterior lamellar keratoplasty (TALK) (for a type 2 DALK) were proposed (Sarnicola et al., 2019).

8.6. Acceptance of the 'layer' and its name

Comments were not exclusively controversial. Natalia Skuza, Poland, presented a patient who had chemical injury with a silver nitrate solution. Histology of the injured cornea showed that most of the cornea was stained brown from the injury, except a narrow strip, corresponding to the PDL/DL which remained unstained (Fig. 32). It was suggested that this illustrated a unique difference of the PDL/DL compared to the stroma.

Distinguished researchers in the field, who might have had initial reservations have adopted the term 'pre-Descemet's layer' in their reports when referring to this part of the cornea (Lisch and Vossmerbaeumer, 2020; Parker et al., 2019a; White et al., 2017). The terms "Dua's layer" and "Pre-Descemet's layer" have been included in the title, key words and abstracts of over fifty publications (Bizheva et al., 2016; Costet and Touboul, 2016; Daas et al., 2021; Feizi et al., 2014; Gamaleldin et al., 2016; Huang et al., 2018; Koçluk et al., 2016; Narang et al.,
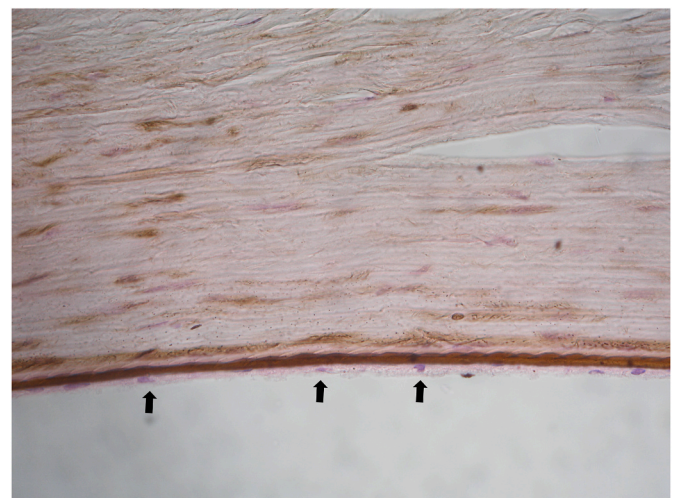
Fig. 32. The pre-Descemet's layer/Dua's layer (PDL/DL) in silver nitrate injury. Histology of the cornea injured with silver nitrate shows a generalised brown discolouration. A clear unstained band, corresponding to the PDL/DL is seen anterior to the Descemet's membrane (arrows). (Image courtesy Natalia Skuza, Poland).

2017; Oie and Nishida, 2017; Rickmann et al., 2021; Sarnicola et al., 2019; Selvan et al., 2018; Sharma et al., 2020; Yahia Chérif et al., 2015) and several ophthalmic text books (Bowling, 2015; Denniston and Murray, 2014; Forrester et al., 2020; Jacob, 2016; Olver et al., 2014; Tandon and Sihota, 2014; Tubbs et al., 2016; Yanoff and Sassani, 2018).

9. Future directions

From the first observations in 2005, through the first presentations in 2007, to the first publication in 2013 the progress can be regarded as slow but not atypical of any novel discovery. Then followed a period of rapid revelation of evidence supporting the existence, characteristics and relevance of the PDL/DL to corneal anatomy, physiology, surgery and pathology. Several authors and groups contributed data, adding to the body of knowledge, providing insights and opening new avenues for exploration. The old adage that the eye cannot see what the mind does not know, no longer applied and numerous publications have appeared in the literature. The evidence thus far indicates that the PDL/DL is an important part of the surgical anatomy of the cornea and plays a distinct role in posterior corneal pathology. However, whether it is a distinctive layer of the cornea, like the other known layers is debated and is a subject of further interrogation and research.

The immediate future will see reports on outcomes of research on the role of the PDL/DL in corneal biomechanics and consequently its impact on conditions such as glaucoma and corneal ectatic disorders. The continuity of the PDL/DL with the TM will be a major incentive to study this feature with regard to IOP and glaucoma. The involvement of the layer in ACH and DMD has already changed age old paradigms. More is yet to come with regard to the pathogenesis and the basis of certain clinical features of keratoconus, corneal dystrophies, corneal infections, corneal melts, Descemetoceles and perforation.

The demonstration of the layer in different species of animals and its comparative anatomy will be a major area of research. The use of air to inflate the stroma and separate the lamellae will be an interesting method to study the intricate structure of the stroma in the anterior, posterior, central and peripheral cornea of animals and humans.

9.1. The PDL/DL in corneal biomechanics and corneal ectasia

The PDL/DL is likely to influence corneal biomechanics of the healthy and diseased cornea. It has recently been demonstrated that the PDL/DL of healthy corneas contains a network of elastic fibers (Lewis et al., 2016), as well as a high content of elastin (Mohammed et al., 2018). Elastic fibers allow for deformability and recoil of biological tissues (Kielty et al., 2002). It is well recognised that posterior corneal bulging is an early manifestation in the development of ectasia/keratoconus (Sedaghat et al., 2021; Tomidokoro et al., 2000). It has also been demonstrated that the PDL/DL elastic network may be deficient in keratoconic subjects (White et al., 2017). We too have shown by immunostaining (section 6.3) that keratoconus corneas (obtained by PK) have a marked reduction in the elastin content of the PDL/DL. This would suggest that the PDL is implicated in the pathophysiology of keratoconus. Both elastin/tropoelastin and fibrillin microfibrils are biologically subjected to an analogous crosslinking process to that of collagen (Lockhart-Cairns et al., 2020). Theoretically, the identification of corneas with subnormal elastin content/function through surrogate biomarkers could allow for detection of susceptibility to ectasia. The same rationale could also be applied to donor corneal buttons for assessment of biomechanical stability prior to PK. Of note is the fact that desmosine and isodesmosine, crosslink peptides that result from elastin breakdown, are currently used as biomarkers of elastin degradation in chronic obstructive pulmonary disease and other respiratory pathologies (Luisetti et al., 2008).

Corneal crosslinking has shown efficacy in halting progression of ectasia (Hashemi et al., 2013). Further work addressing the unique extracellular matrix environment associated with elastin/elastic fibers

(Jensen and Handford, 2016) could potentially give rise to specific crosslinking protocols and extracellular matrix (ECM) modifiers targeting the elastic fiber system. In line with the inherent function of elastic fibers on other biological tissues that are required to sustain repetitive strain (Kielty et al., 2002), the PDL/DL may be involved in the maintenance/recovery of corneal shape under physiological conditions (blinking, heartbeat and IOP) and under higher loads as in eye rubbing. Eye rubbing (or any kind of repetitive trauma to the cornea) contributes to corneal biomechanical decompensation through mechanisms that are still not fully understood (McMonnies, 2009). The concept of "elastin fatigue" was developed to explain the changes in compliance of arterial blood vessels with ageing and in diseased states, where the cumulative effect of forces applied by blood pressure and cardiac pulsation over years, surpasses the capacity of elastic fibers to extend/recoil and fractures them, generating a load transfer to the more rigid collagenous components of the wall (O'Rourke, 2007). This same concept could, in theory, be applied to the PDL/DL, where the repetitive trauma applied by eye rubbing would cause a loss of function of the corneal elastic fiber system, which is particularly prevalent in the PDL/DL, therefore resulting in permanent biomechanical imbalance. While currently available depth-dependent biomechanical assessment technologies characterise the anterior stroma as being 'stiffer' than the posterior stroma in normal corneas (De Stefano et al., 2018; Seiler et al., 2019), one could speculate that the weakening of the PDL/DL elastic system could function as a 'tipping point' for further biomechanical decompensation.

Keratoconus has classically been described as a non-inflammatory disease, but mounting evidence suggests inflammation is involved in the pathogenesis of corneal ectasia (Galvis et al., 2015). Elastic fibers can be degraded by inflammatory agents in other biological tissues, therefore the PDL/DL could equally be subjected to this type of injury, thus impairing corneal biomechanical stability. Interestingly, elastin fragments released after elastic fiber degradation have shown to have pro-inflammatory activity (Duca et al., 2004) which could, as with atherosclerosis and emphysema, contribute to perpetuate the biomechanical insult to corneal stroma secondary to a 'lesion' of the elastic system in the PDL/DL (Gayral et al., 2014; Mehraban et al., 2020). The study of structural, biochemical and biomechanical changes in the PDL/DL in keratoconic and post laser vision correction ectasia, will contribute to further understand the biomechanical implications of the layer and its role in the pathogenesis of these conditions.

9.2. The PDL/DL in relation to intraocular pressure and glaucoma

The relationship of the cornea to IOP and glaucoma has been elaborated in section 4.3. It has been demonstrated that ocular biomechanics play a role in the development of optic nerve damage, as corneal biomechanical characteristics are thought to be a surrogate of lamina cribrosa compliance (Medeiros et al., 2013). The description of the PDL/DL and its embedded elastic system has provided clues towards a distinct, perhaps complementary approach to glaucoma pathophysiology. The trabecular meshwork is connected to the ciliary muscle through a system of tendons supported by an intricate elastic network, which is responsible for the increase in TM permeability upon contraction of the ciliary muscle (Park et al., 2016; Rohen et al., 1981). The continuity of the elastic fiber system throughout the PDL/DL and into the TM seems to add to the functional unit comprised of the TM and ciliary muscle, potentially contributing to TM 'tonus' maintenance in physiological conditions and modulation of aqueous outflow facility in response to IOP fluctuations. This presents a possible rationale for the increased incidence of raised IOP after PK when compared to DALK that cannot be accounted for by the use of steroids alone (Zhang et al., 2013), as the former eliminates the tensile strength provided by the elastic system by transecting the PDL/DL, and could contribute to physical alterations of the TM and irido-trabecular angle distortion. In this regard, one could speculate that therapeutic approaches aiming to 'strengthen'

the PDL/DL could induce an increment in the tensile strength applied to the TM, therefore increasing aqueous drainage and lowering IOP. However, whether alterations in the PDL/DL would increase or decrease IOP remains to be seen and studied. The converse is also part of the jigsaw; keratoconus patients usually record low IOP, which is not just a function of the thin cornea. Thus, a weakening of the PDL/DL could be an option to be tested.

Recent studies have confirmed that mechanical stretching of the TM induces differential expression of ECM genes and changes in protein expression patterns in a segmental fashion, which have the capacity to modulate aqueous outflow (see review by Acott et al., 2021). Similarly to elastin/elastic fibers, we have shown that type VI collagen is also abundant within the PDL/DL and extends into the TM beams (Dua et al., 2014a). Several specific interactions with ECM proteins have been attributed to collagen VI, and in particular with elastic fibers (Everts et al., 1998; Finnis and Gibson, 1997). The role of the collagenous and elastic systems embedded within the PDL/DL requires further clarification, as they could hypothetically function as transducers for IOP changes, thereby inducing ECM remodeling. Further work is currently underway to evaluate the effect of selective PDL/DL sectioning on the expression of different extracellular matrix proteins in the TM and possible effect of ECM protein pattern modification on aqueous outflow capacity and IOP.

Moreker (Moreker et al., 2014) reported results of their video audit on deep sclerectomy in glaucoma patients, which showed that "cases in which the layer was not removed, failed and needed a gonio puncture and cases in which the Dua's layer was removed were successful and did not need a gonio puncture, suggesting that removal of Dua's Layer is essential for success of Deep Sclerectomy, modified or otherwise." Further supporting data was presented in posters by Ghorpade H and Morekar SR in the annual meetings of ASCRS and the All India Ophthalmological Society, in 2020. The association of the PDL/DL with this procedure will no doubt be further explored along the lines described.

In our studies, some scanning electron micrographs of the TM, intact sheets of the PDL/DL were seen to extend into the TM for some distance before ramifying into trabecular beams (Fig. 33A). Such sheets could affect aqueous drainage especially if they covered large areas of the TM. Such sheets could be related to 'membranes' treated by goniotomy in childhood glaucoma can be an area for further research.

9.3. The PDL/DL in corneal surgery and corneal pathology

With the improved understanding of the principles of lamellar corneal surgery, both anterior and posterior, the techniques, outcomes and uptake of these procedures will continue to improve.

With increased understanding of the surgical anatomy and the science behind DALK surgery, the uptake of this procedure in increasing. In the UK, the average uptake of DALK v PK for keratoconus is 50:50 with some centres reporting 70:30. In the USA (EBAA report 2021) 586, 372 and 425 DALKs were performed in 2019, 2020 and 2021 respectively.

These figures are to be viewed in the light of the pandemic where EK procedures too showed a dip. DALK has a steep learning curve, hence some centres are slower to adopt this technique but the numbers are increasing. Both techniques, using air bubble and visco bubble are being used with increasing confidence.

Of the three techniques of endothelial keratoplasty, DMEK, DSAEK/ Ultrathin DSAEK and PDEK, the former two are the most commonly performed procedures. In DMEK there is no stromal tissue at all, in DSAEK a variable amount of stromal tissue including the PDL/DL is retained (usually around 150 μm) whereas in ultrathin DSAEK the residual stroma including the PDL/DL is less than 100 μm, usually around 80 μm. Visual outcomes of ultrathin DSAEK are reported to be similar to DMEK (Busin et al., 2013; Dickman et al., 2016) despite the presence of the residual stroma and PDL/DL. Visual results after PDEK, where the PDL/DL adds no more than 20 μm to the thickness of DMEK tissue, too are reported to be similar to DMEK though the numbers are much smaller compared to UT-DSAEK and DMEK. A study on corneal densitometry after PDEK showed no difference in PDEK grafts with 20/20 vision and control unoperated eyes (Kumar et al., 2020). Similarly, no change in posterior corneal curvature, total corneal power and best fit sphere were noted after PDEK (Nidhi et al., 2022), which are noted with DSAEK but not with DMEK, showing a similar outcome of PDEK as with DMEK in relation to these parameters. Pre-prepared and loaded PDEK tissue is being provided by eye banks such as Lions Eye Institute for Transplant & Research and Vision Share (Ophthalmology Management, 2017; Nariani et al., 2016; CRSToday, 2017). According to the Eye Bank Association of America statistical report 2021, a total of 57 PDEKs were performed with EBAA provided tissue between 2017 and 2021. It is estimated that around 10% of donated eyes are from children. Such eyes are not suited for DMEK because of the thinner and more adherent DM. These eyes can be specifically targeted to provide PDEK tissue, with the potential to provide better very long term graft survival compared to current DMEK tissue. Studies on long-term follow up of PDEK graft from young donors will undoubtedly be in the offing.

As the understanding of corneal pathology in the context of the PDL and the cleavage plane improves, more reports are likely to appear in the literature demonstrating the role of the PDL in different conditions as described in the paper and possibly others. Histological demonstration of the three types of Descemetoceles/pre-Descemetoceles to corroborate the OCT findings is required. Anecdotally, we have observed intracorneal hypopyon to be the accumulation of inflammatory exudate in the plane anterior to the PDL/DL and a distinct separation of the PDL/DL from the posterior stroma in three cases of fungal keratitis. If this observation is substantiated by more cases, it can become one of the OCT features that supports the clinical diagnosis of fungal keratitis.

Studies on the management of the different types of DMD are underway and initial observations are that type 1 DMD, especially if long standing, is very difficult to re-attach compared to type 2 DMD (Table 2). Strategies like controlled incisions at the periphery of type1 DMD will be tested clinically and evolve to replace the current approach of intracameral air or gas injection in all cases. The role of the PDL/DL in acute
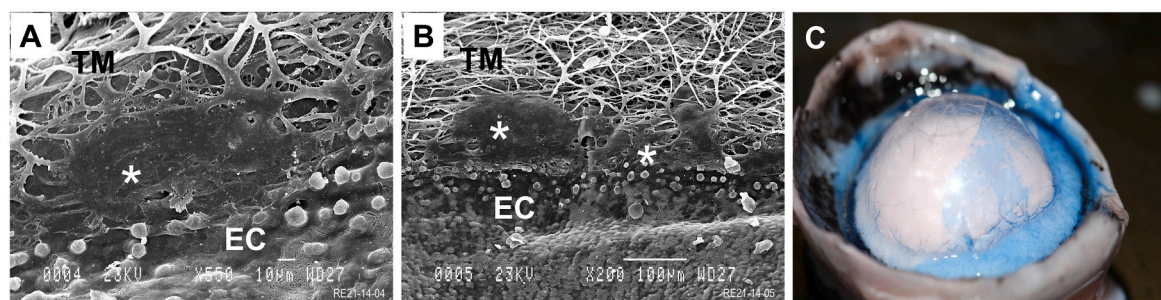

Fig. 33. The pre-Descemet's layer/Dua's layer (PDL/DL) - Future directions. (A) and (B). Intact sheets of the PDL/DL (*) extending into the trabecular zone are seen in the scanning electron micrographs. TM = trabecular meshwork. EC = endothelial cells. (C) A type 1 big bubble created in dog's eye.

hydrops in keratoconus will be elaborated further as more data from high resolution OCT imaging of the condition accumulates.

9.4. The PDL/DL in animal eyes

Our early studies on bovine, porcine, equine and dog eyes have shown the presence of the PDL/DL and in particular the formation of type 1 BB in dog eyes (Fig. 33B) (Kafarnik et al., 2022). Equine eyes are similar but not identical. In dog eyes, only type 1 BB was seen in ex-vivo studies. This will form a sound basis for veterinary surgeons to attempt DALK in canine eyes. More data and information on the layer and relevance to veterinary ophthalmology will emerge with exploration of the PDL/DL across species.

9.4.1. Epilogue

History has an uncanny knack of revealing the truth. Those who can 'predict history' before it is created have foresight. Those who deny history when it is created lack insight. Denial creates controversy and can distort and delay acceptance of facts but it cannot deny the emergence of the truth. In some respects this is a human trait that works to ensure that facts are accepted as facts only after rigorous scrutiny and reproducibility. It also has an adverse effect on discoverers, inventors and innovators as some may not survive the delay thus introduced or the vitriolic criticism thrown at them. History is littered with individuals whose names have been immortalised not for what they have achieved but for criticism of their contemporaries who were well before their time, who had 'foresight'. Three examples, from many, stand out:

It is common knowledge that in the early part of the 17th century the Church held the view, which was embodied in the scriptures, that the Sun moved around the Earth. In 1633, Galileo was accused of heresy and prosecuted for his belief that the Earth moved around the Sun and was not the centre of the universe. "*On June 22, 1633, the Church handed down the following order: "We pronounce, judge, and declare, that you, the said Galileo … have rendered yourself vehemently suspected by this Holy Office of heresy, that is, of having believed and held the doctrine (which is false and contrary to the Holy and Divine Scriptures) that the sun is the center of the world, and that it does not move from east to west, and that the earth does move, and is not the center of the world.*" He "spent the rest of his life under house arrest. It took more than 300 years for the Church to admit that Galileo was right and to clear his name of heresy" (History.com, 2020, 2018).

Harold Ridley's invention of the intraocular lens is a poignant example. The following account is derived from David J Apple's book entitled "Sir Harold Ridley and his fight for sight: He changed the world so that we may better see it" He referred to the period covering the trials and tribulations of the innovation of the lens implant as a cure for cataract, as "The golden age of ophthalmology and the visual sciences." Despite, as Howard Fine put it in his foreword to the book, the "Forces that interact to either retard or suppress or to facilitate and enhance any truly new and innovative development". It took 40 years for the established academic community to accept the concept and the practice. This took its toll on the small community of pioneers who braved the vitriol and "The skepticism, which had been drilled into us by our professors and international doyens of ophthalmology for many years." (in the preface by Apple DJ) and ploughed ahead. Critical comments enumerated in the book include "Rayner should be prosecuted for supplying implants", "Dr. Ridley, why don't you GO HOME", "The IOL and the phacoemulsification procedure that goes with it represent a time bomb." They sure did, when it exploded, it smeared egg on many faces.

It includes two telling quotes by Harold Ridley: "I had 25 years in the wilderness, and a whole generation of cataract patients who might have enjoyed full visual rehabilitation, instead suffered the abnormalities of aphakia" and "As a result of the failure of British Ophthalmology to join and support the pioneers, Britain lost its rightful place in a new and developing field. Through this failure, a whole generation of British men and women who underwent cataract surgery between 1951 and 1975

were denied the full treatment which was then becoming available" (Apple, 2006).

Russell Foster's story of the photosensitive retinal ganglion cells is more recent but equally relevant. It demonstrates that history repeats itself and that lessons from history are seldom learnt. When he discovered the Light-sensitive ganglion cells in the retina (photosensitive retinal ganglion cells) and presented it to the scientific community, it must have felt like walking from "light" into a dark abyss. Dr Russell Foster, CBE, recalls in his book (Foster, 2022): "To my genuine surprise, this suggestion was first met with undiluted contempt by many in the vision community. On one occasion I gave a scientific talk, and a member of the audience shouted "bullshit" before walking out, and another time a very angry individual shouted "Are you seriously trying to tell us that after 150 years of research on the eye we have all missed an entire class of photoreceptor"? My early grant proposals were rejected because our results were just not believed. One particularly painful reason for rejection was: "Why is Foster looking for novel photoreceptors within the eye, when we know the light sensors are located behind the knee"? This reviewer was referring, with heavy sarcasm, to the discredited study where the researchers had claimed the existence of light sensitive cells behind the skin of the knee. With the courage of his conviction he and his team, and others who took note, established the facts by accumulating evidence. The rest is History but no one should bet on History not repeating itself.

In 2016, writing about photosensitive retinal ganglion cells, Figueiro M quotes W.I.B. Beveridge thus: "The reception of an original contribution to knowledge can be divided into three phases. First, it is ridiculed as untrue, impossible, or useless. Second, people then acknowledge that there may be something to the idea, but declare it would never be of any practical use. Third and finally, when the discovery has received general recognition, people say that the idea is not original and had been anticipated by others." Figueiro added "The reception of the newly discovered intrinsically photosensitive retinal ganglion cell (ipRGC) in the mammalian retina was no different. What is now considered general knowledge was ridiculed in the early 1990s" (Figueiro, 2016).

To make a point, if we go with the story that Newton conceived of Gravity when he saw an apple falling from a tree, it would be disingenuous for all those who saw an apple fall before Newton, to say that "we saw it fall first" and claim credit.

What might be the motive, if any behind such blind-folded criticism and non-acceptance or even complete unwillingness to rationally consider the evidence presented? The obvious and understandable reason is that the evidence presented is novel, contrary to convention but not (yet) comprehensive; but total denial in the presence of reasonable evidence is difficult to explain. One could naively expect that envy, ego, professional rivalry, prejudices, beliefs, opinions, and other similar emotions do not influence such decisions in scientific minds, but the fact is that they do. This is a human trait. Mark Twain said "There are basically two kinds of people in this world. Those who accomplish something and those that claim to. The first group is less crowded." However, human kind is not binary. In amongst the manifold other kinds of people are a third kind, who are first on the scene with their criticism and comments, unsubstantiated by existing evidence or personal contribution. Genuine scientific disagreement and opposing views are part of the scientific rigour. But if it is genuine, these individuals should be the first to raise their hand to acknowledge the new facts, rather than respond with a deafening silence.

Science progresses in small increments, constantly refining, remodeling and reshaping small Truths in its quest for The Truth. Along the way, some discarded truths are resurrected and established 'truths' are rejected. "Today is the Yesterday of Tomorrow" (hsd).

Author statement

All authors contributed to the content of the manuscript as detailed below:

Harminder S Dua: Contributed to the concept, outline, writing, reviewing and editing, critical discussion related to sections of the paper and images and part referencing.

Rui Freitas: Contributed to the writing of the paper, complete referencing, reviewing and editing and writing of some sections. Part of the work will go towards his post graduate thesis.

Imran Mohammed: Contributed to the selection, montaging, labelling of images and legends.

Darren SJ Ting: Contributed to the writing of the section on clinic-pathological applications and to the related images.

Dalia G Said: Contributed to the writing of the paper, reviewing the paper and montaging of images and writing the section on surgical applications.

All authors proof read the entire manuscript suggested changes, corrections and amendments. All authors are part of the team that undertook the experiments and research that provided the evidence contained in the manuscript.

Declaration of competing interest

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References

Abahussin, M., Hayes, S., Cartwright, N.E.K., Kamma-Lorger, C.S., Khan, Y., Marshall, J., Meek, K.M., 2009. 3D collagen orientation study of the human cornea using X-ray diffraction and femtosecond laser technology. Invest. Ophthalmol. Vis. Sci. 50, 5159–5164.

Acott, T.S., Vranka, J.A., Keller, K.E., Raghunathan, V.K., Kelley, M.J., 2021. Normal and glaucomatous outflow regulation. Prog. Retin. Eye Res. 82, 100897.

Agarwal, Amar, Agarwal, Ashvin, Narang, P., Kumar, D.A., Jacob, S., 2015. Pre-Descemet endothelial keratoplasty with infant donor corneas: a prospective analysis. Cornea 34, 859–865.

Agarwal, Amar, Dua, H.S., Narang, P., Kumar, D.A., Agarwal, Ashvin, Jacob, S., Agarwal, Athiya, Gupta, A., 2014. Pre-Descemet's endothelial keratoplasty (PDEK). Br. J. Ophthalmol. 98, 1181–1185.

Agarwal, Amar, Narang, P., Kumar, D.A., Agarwal, Ashvin, 2017. Young donor-graft assisted endothelial keratoplasty (PDEK/DMEK) with epithelial debridement for chronic pseudophakic bullous keratopathy. Can. J. Ophthalmol. 52, 519–526.

Agarwal, R., Nagpal, R., Todi, V., Sharma, N., 2021. Descemetocele. Surv. Ophthalmol. 66, 2–19.

Aggarwal, S., Peck, T., Golen, J., Karcioglu, Z.A., 2018. Macular corneal dystrophy: a review. Surv. Ophthalmol. 63, 609–617.

Aghamohammadzadeh, H., Newton, R.H., Meek, K.M., 2004. X-ray scattering used to map the preferred collagen orientation in the human cornea and limbus. Structure 12, 249–256.

Agrawal, A., Pratap, V.B., Suman, S., Pal, V.K., 2012. Corneal stromal pseudohypopyon in a pseudophakic patient. Nepal. J. Ophthalmol. 4, 174–175.

Al-Torbak, A.A., Al-Motowa, S., Al-Assiri, A., Al-Kharashi, S., Al-Shahwan, S., Al-Mezaine, H., Teichmann, K., 2006. Deep anterior lamellar keratoplasty for keratoconus. Cornea 25, 408–412.

Alio, J.L., Shah, S., Barraquer, C., Bilgihan, K., Anwar, M., Melles, G.R.J., 2002. New techniques in lamellar keratoplasty. Curr. Opin. Ophthalmol. 13, 224–229.

AlTaan, S.L., Gupta, A., Sidney, L.E., Elalfy, M.S., Agarwal, A., Dua, H.S., 2015. Endothelial cell loss following tissue harvesting by pneumodissection for endothelial keratoplasty: an ex vivo study. Br. J. Ophthalmol. 99, 710–713.

AlTaan, S.L., Mohammed, I., Said, D.G., Dua, H.S., 2018. Air pressure changes in the creation and bursting of the type-1 big bubble in deep anterior lamellar keratoplasty: an ex vivo study. Eye 32, 146–151.

Amayem, A.F., Anwar, M., 2000. Fluid lamellar keratoplasty in keratoconus. Ophthalmology 107, 76–79.

Andreanos, K.D., Hashemi, K., Petrelli, M., Droutsas, K., Georgalas, I., Kymionis, G.D., 2017. Keratoconus treatment algorithm. Ophthalmol. Ther. 6, 245–262.

Anwar, M., 2007. Big-Bubble technique. In: Fontana, L., Tassinari, G. (Eds.), Atlas of Lamellar Keratoplasty. Fabiano, San Giovani, pp. 125–136.

Anwar, M., 1974. Technique in lamellar keratoplasty. Trans. Ophthalmol. Soc. U. K. 94, 163–171.

Anwar, M., 1972. Dissection technique in lamellar keratoplasty. Br. J. Ophthalmol. 56, 711–713.

Anwar, M., Teichmann, K.D., 2002a. Big-bubble technique to bare Descemet's membrane in anterior lamellar keratoplasty. J. Cataract Refract. Surg. 28, 398–403.

Anwar, M., Teichmann, K.D., 2002b. Deep lamellar keratoplasty: surgical techniques for anterior lamellar keratoplasty with and without baring of Descemet's membrane. Cornea 21, 374–383.

Apple, D.J., 2006. Sir Harold Ridley and His Fight for Sight: He Changed the World So that We May Better See it. Slack.

Archila, E.A., 1984. Deep lamellar keratoplasty dissection of host tissue with intrastromal air injection. Cornea 3, 217–218.

Asejczyk-Widlicka, M., Śródka, D.W., Kasprzak, H., Pierscionek, B.K., 2007. Modelling the elastic properties of the anterior eye and their contribution to maintenance of image quality: the role of the limbus. Eye 21, 1087–1094.

Avadhanam, V., Messina, M., Said, D.G., Dua, H.S., 2016. Alcohol delamination of corneal epithelium in recurrent granular dystrophy. Ophthalmology 123, 2050–2052.

Barraquer, J.I., 1972. Lamellar keratoplasty. (Special techniques). Ann. Ophthalmol. 4, 437–469.

Bedard, P., Hou, J.H., 2021. Characterization of endothelial cell loss in pre-descemet endothelial keratoplasty graft preparation. Cornea 40, 364–369.

Bennett, A., Mahmoud, S., Drury, D., Cavanagh, H.D., Mcculley, J.P., Petroll, W.M., Mootha, V.V., 2015. Impact of donor age on corneal endothelium-descemet membrane layer scroll formation. Eye Contact Lens 41, 236–239.

Bhandari, V., Ganesh, S., 2015. Corneal hydrops in pellucid marginal degeneration: a case series. Case Rep. Ophthalmol. 6, 191–199.

Binder, P.S., Rock, M.E., Schmidt, K.C., Anderson, J.A., 1991. High-voltage electron microscopy of normal human cornea. Investig. Ophthalmol. Vis. Sci. 32, 2234–2243.

Bizheva, K., Haines, L., Mason, E., MacLellan, B., Tan, B., Hileeto, D., Sorbara, L., 2016. In vivo imaging and morphometry of the human pre-Descemet's layer and endothelium with ultrahigh-resolution optical coherence tomography. Investig. Ophthalmol. Vis. Sci. 57, 2782–2787.

Boere, P.M., Bonnet, C., Frausto, R.F., Fung, S.S.M., Aldave, A.J., 2020. Multimodal imaging of pre-descemet corneal dystrophy associated with X-linked ichthyosis and deletion of the STS gene. Cornea 39, 1442–1445.

Borderie, V.M., Guilbert, E., Touzeau, O., Laroche, L., 2011. Graft rejection and graft failure after anterior lamellar versus penetrating keratoplasty. Am. J. Ophthalmol. 151.

Bowling, B., 2015. Kanski's Clinical Ophthalmology: A Systematic Approach, Expert Consult. Elsevier.

Bozkurt, K.T., Acar, B.T., Acar, S., 2013. Fixed dilated pupilla as a common complication of deep anterior lamellar keratoplasty complicated with Descemet membrane perforation. Eur. J. Ophthalmol. 23, 164–170.

Bron, A., Tripathi, R., Tripathi, B., 1998. Wolff's Anatomy of the Eye and Orbit, 8Ed. Taylor & Francis.

Brown, S.I., Kitano, S., 1966. Pathogenesis of the retrocorneal membrane. Arch. Ophthalmol. 75, 518–525 (Chicago, Ill. 1960).

Busin, M., Madi, S., Santorum, P., Scorcia, V., Beltz, J., 2013. Ultrathin descemet's stripping automated endothelial keratoplasty with the microkeratome double-pass technique: two-year outcomes. Ophthalmology 120, 1186–1194.

Busin, M., Patel, A.K., Scorcia, V., Galan, A., Ponzin, D., 2010a. Stromal support for Descemet's membrane endothelial keratoplasty. Ophthalmology 117, 2273–2277.

Busin, M., Scorcia, V., Patel, A.K., Salvalaio, G., Ponzin, D., 2011. Donor tissue preparation for Descemet membrane endothelial keratoplasty. Br. J. Ophthalmol. 95, 1172–1173.

Busin, M., Scorcia, V., Patel, A.K., Salvalaio, G., Ponzin, D., 2010b. Pneumatic dissection and storage of donor endothelial tissue for Descemet's membrane endothelial keratoplasty: a novel technique. Ophthalmology 117, 1517–1520.

Butler, T.K.H., Dua, H.S., Edwards, R., Lowe, J.S., 2001. In vitro model of infectious crystalline keratopathy: tissue architecture determines pattern of microbial spread. Investig. Ophthalmol. Vis. Sci. 42, 1243–1246.

Cai, C.X., Fitch, J.M., Svoboda, K.K.H., Birk, D.E., Linsenmayer, T.F., 1994. Cellular invasion and collagen type IX in the primary corneal stroma in vitro. Dev. Dynam. 201, 206–215.

Chen, Si, Liu, X., Wang, N., Wang, X., Xiong, Q., Bo, E., Yu, X., Chen, Shufen, Liu, L., 2017. Visualizing micro-anatomical structures of the posterior cornea with micro-optical coherence tomography. Sci. Rep. 7, 1–10.

Choo, C.H., Boto de Los Bueis, A., Chung, D.D., Aldave, A.J., 2021. Confirmation of PRDX3 c.568G>C as the genetic basis of punctiform and polychromatic pre-descemet corneal dystrophy. Cornea 39, 779–781.

Classification and nomenclature of morphological defects, 1975. Lancet 1 (7905), 513.

Costet, C., Touboul, D., 2016. [Viscodissection of dua's layer after partial big bubble]. J. Fr. Ophtalmol. 39, 404.

Crawford, A., Patel, D., McGhee, C., 2013. A brief history of corneal transplantation: from ancient to modern. Oman J. Ophthalmol. 6.

CRSToday, 2017. PDEK with a graft prepared by an Eye Bank. https://crstoday.com/articles/2017-apr/pdek-with-a-graft-prepared-by-an-eye-bank. (Accessed 9 December 2022).

Daas, L., Hamon, L., Ardjomand, N., Safi, T., Seitz, B., 2021. [Excimer laser-assisted DALK: a case report from the homburg keratoconus center (HKC)]. Ophthalmologe 118, 1245–1248.

Dai, Y., Liu, Z., Wang, W., Han, X., Jin, L., Chen, X., Jin, G., Wang, L., Zhang, E., Qu, B., Liu, J., Congdon, N., He, M., Luo, L., Liu, Y., 2021. Incidence of incision-related descemet membrane detachment using phacoemulsification with trapezoid vs conventional 2.2-mm clear corneal incision: a randomized clinical trial. JAMA Ophthalmol 139, 1228–1234.

Dapena, I., Ham, L., Droutsas, K., Van Dijk, K., Moutsouris, K., Melles, G.R.J., 2011. Learning curve in descemet's membrane endothelial keratoplasty: first series of 135 consecutive cases. Ophthalmology 118, 2147–2154.

De Stefano, V.S., Ford, M.R., Seven, I., Dupps, W.J., 2018. Live human assessment of depth-dependent corneal displacements with swept-source optical coherence elastography. PLoS One 13, e0209480.

Denniston, A., Murray, P., 2014. Oxford Handbook of Ophthalmology, Oxford Handbook of Ophthalmology. Oxford University Press, Oxford, UK.

Deol, M., Taylor, D.A., Radcliffe, N.M., 2015. Corneal hysteresis and its relevance to glaucoma. Curr. Opin. Ophthalmol. 26, 96–102.

Dickman, M.M., Kruit, P.J., Remeijer, L., van Rooij, J., Van der Lelij, A., Wijdh, R.H.J., van den Biggelaar, F.J.H.M., Berendschot, T.T.J.M., Nuijts, R.M.M.A., 2016. A randomized multicenter clinical trial of ultrathin descemet stripping automated endothelial keratoplasty (DSAEK) versus DSAEK. Ophthalmology 123, 2276–2284.

Dodson, J.W., Hay, E.D., 1971. Secretion of collagenous stroma by isolated epithelium grown in vitro. Exp. Cell Res. 65, 215–220.

Dua, H.S., Faraj, L.A., Branch, M.J., Yeung, A.M., Elalfy, M.S., Said, D.G., Gray, T., Lowe, J., 2014a. The collagen matrix of the human trabecular meshwork is an extension of the novel pre-Descemet's layer (Dua's layer). Br. J. Ophthalmol. 98, 691–697.

Dua, H.S., Faraj, L.A., Kenawy, M.B., AlTaan, S., Elalfy, M.S., Katamish, T., Said, D.G., 2018. Dynamics of big bubble formation in deep anterior lamellar keratoplasty by the big bubble technique: in vitro studies. Acta Ophthalmol. 96, 69–76.

Dua, H.S., Faraj, L.A., Said, D.G., 2015a. Duas layer: discovery, characteristics, clinical applications, controversy and potential relevance to glaucoma. Expet Rev. Ophthalmol. 10, 531–547.

Dua, H.S., Faraj, L.A., Said, D.G., 2014b. Dua's layer: its discovery, characteristics and applications. In: Á., M., Martínez, y arquitectura corneal, C.P.B.T.-B. (Eds.), del Buey Sayas, Biomecánica Y Arquitectura Corneal. Elsevier, Madrid, pp. 35–47.

Dua, H.S., Faraj, L.A., Said, D.G., Gray, T., Lowe, J., 2014c. Re: jester et al.: lessons in Corneal Structure and Mechanics to Guide the Corneal Surgeon (Ophthalmology 2013;120:1715-1717). Ophthalmology 121, e18.

Dua, H.S., Faraj, L.A., Said, D.G., Gray, T., Lowe, J., 2014d. Author reply. Ophthalmology 121, e25–e26.

Dua, H.S., Faraj, L.A., Said, D.G., Gray, T., Lowe, J., 2013. Human corneal anatomy redefined: a novel pre-descemet's layer (Dua's Layer). Ophthalmology 120, 1778–1785.

Dua, H.S., Katamish, T., Said, D.G., Faraj, L.A., 2015b. Differentiating type 1 from type 2 big bubbles in deep anterior lamellar keratoplasty. Clin. Ophthalmol. 9, 1155–1157.

Dua, H.S., Mastropasqua, L., Faraj, L., Nubile, M., Elalfy, M.S., Lanzini, M., Calienno, R., Said, D.G., 2015c. Big bubble deep anterior lamellar keratoplasty: the collagen layer in the wall of the big bubble is unique. Acta Ophthalmol. 93, 427–430.

Dua, H.S., Said, D.G., 2021. Deep anterior lamellar keratoplasty (DALK): science and surgery. In: Albert, D., Miller, J., Azar, D., Young, L.H. (Eds.), Albert and Jakobiec's Principles and Practice of Ophthalmology. Springer International Publishing, Cham, pp. 1–22.

Dua, H.S., Said, D.G., 2017. Pre-Descemets endothelial keratoplasty: the PDEK clamp for successful PDEK. Eye 31, 1106–1110, 2017 317.

Dua, H.S., Said, D.G., 2016. Clinical evidence of the pre-Descemets layer (Dua's layer) in corneal pathology. Eye 30, 1144–1145.

Dua, H.S., Sinha, R., D'Souza, S., Potgieter, F., Ross, A., Kenawy, M., Scott, I., Said, D.G., 2020. Descemet membrane detachment": a novel concept in diagnosis and classification. Am. J. Ophthalmol. 218, 84–98.

Dua, H.S., Termote, K., Kenawy, M.B., Said, D.G., Jayaswal, R., Nubile, M., Mastropasqua, L., Holland, S., 2016. Scrolling characteristics of pre-descemet endothelial keratoplasty tissue: an ex vivo study. Am. J. Ophthalmol. 166, 84–90.

Dua, H., Ting, D., Al-Aqaba, M., Said, D., 2022. Pathophysiology of keratoconus. In: Izquierdo, L., Henriquez, M., Mannis, M.J. (Eds.), Keratoconus Diagnosis and Management. Elsevier - Health Sciences Division, pp. 51–63.

Duca, L., Floquet, N., Alix, A.J.P., Haye, B., Debelle, L., 2004. Elastin as a matrikine. Crit. Rev. Oncol. Hematol. 49, 235–244.

Ehrlich, M.I., Phinney, R.B., Mondino, B.J., Pettit, T.H., 1988. Techniques of lamellar keratoplasty. Int. Ophthalmol. Clin. 28, 24–29.

Esporcatte, L.P.G., Salomão, M.Q., Lopes, B.T., Vinciguerra, P., Vinciguerra, R., Roberts, C., Elsheikh, A., Dawson, D.G., Ambrósio, R., 2020. Biomechanical Diagnostics of the Cornea, vol. 7. Eye Vis., London, England.

Everts, V., Niehof, A., Jansen, D., Beertsen, W., 1998. Type VI collagen is associated with microfibrils and oxytalan fibers in the extracellular matrix of periodontium, mesenterium and periosteum. J. Periodontal. Res. 33, 118–125.

Faraj, L.A., Hashmani, K., Khatib, T., Al-Aqaba, M., Dua, H.S., 2012. The changing face of corneal graft rejection. Br. J. Ophthalmol. 96, 1049–1050.

Farlex, 2012. Layers. Farlex partner medical dictionary. https://medical-dictionary.thefreedictionary.com/layers. (Accessed 22 December 2022).

Feizi, S., Faramarzi, A., Javadi, M.A., Jafarinasab, M.R., 2014. Modified big-bubble deep anterior lamellar keratoplasty using peripheral air injection. Br. J. Ophthalmol. 98, 1597–1600.

Feneck, E.M., Lewis, P.N., Meek, K.M., 2020. Identification of a primary stroma and novel endothelial cell projections in the developing human cornea. Invest. Ophthalmol. Vis. Sci. 61, 5.

Figueiro, M., 2016. Discovery of the photosensitive retinal ganglion cell. Redefining humans' biological response to light. Architect. https://www.architectmagazine.com/technology/lighting/discovery-of-the-photosensitive-retinal-ganglion-cell_o. (Accessed 1 January 2022).

Finnis, M.L., Gibson, M.A., 1997. Microfibril-associated glycoprotein-1 (MAGP-1) binds to the pepsin-resistant domain of the alpha3(VI) chain of type VI collagen. J. Biol. Chem. 272, 22817–22823.

Fontana, L., Tassinari, G., 2007. Descemet's membrane baring techniques. In: Fontana, L., Tassinari, G. (Eds.), Atlas of Lamellar Keratoplasty. Fabiano, San Giovani, pp. 125–174.

Forrester, J.V., Dick, A.D., McMenamin, P.G., Roberts, F., Pearlman, E., 2020. The Eye E-Book: Basic Sciences in Practice, fifth ed. Elsevier Health Sciences.

Foster, R., 2022. Life Time: the New Science of the Body Clock and How it Can Revolutionise Your Sleep and Health. Penguin Random House, UK (in press).

Fuchs, E., 1901. I. Zur keratoplastik. Ophthalmologica 5, 1–5.

Gain, P., Jullienne, R., He, Z., Aldossary, M., Acquart, S., Cognasse, F., Thuret, G., 2016. Global survey of corneal transplantation and eye banking. JAMA Ophthalmol 134, 167–173.

Galst, J., Maryshev, Y., 2009. Ophthalmology numismatics. Vladimir petrovich Filatov. Arch. Ophthalmol. 127 (9), 1160.

Galvis, V., Sherwin, T., Tello, A., Merayo, J., Barrera, R., Acera, A., 2015. Keratoconus: an inflammatory disorder? Eye 29, 843–859.

Gamaleldin, S.A., Salama, M.M., Elshazly, M.I., 2016. Seven-day storage of pneumatically dissected Descemet's endothelial grafts with and without Dua's layer. Acta Ophthalmol. 94, e130–e134.

Gaskin, J., Patel, D., McGhee, C., 2014. Acute corneal hydrops in keratoconus - new perspectives. Am. J. Ophthalmol. 157, 921–928.

Gatzioufas, Z., Labiris, G., Stachs, O., Hovakimyan, M., Schnaidt, A., Viestenz, A., Käsmann-Kellner, B., Seitz, B., 2013. Biomechanical profile of the cornea in primary congenital glaucoma. Acta Ophthalmol. 91, e29–e34.

Gayral, S., Garnotel, R., Castaing-Berthou, A., Blaise, S., Fougerat, A., Berge, E., Montheil, A., Malet, N., Wymann, M.P., Maurice, P., Debelle, L., Martiny, L., Martinez, L.O., Pshezhetsky, A.V., Duca, L., Laffargue, M., 2014. Elastin-derived peptides potentiate atherosclerosis through the immune Neu1-PI3Kγ pathway. Cardiovasc. Res. 102, 118–127.

Ghanem, A., Mokbel, T., 2010. Correlation of central corneal thickness and optic nerve head topography in patients with primary open-angle glaucoma. Oman J. Ophthalmol. 3, 75–80.

Gharebaghi, D., Fallahi, B., Javadzadeh, A., Amiraslanzadeh, G., 2009. Spontaneous corneal hydrops and perforation in pellucid marginal degeneration; a case report. J. Ophthalmic Vis. Res. 4, 174–176.

Gorski, M., Shih, C., Savoie, B., Udell, I., 2016. Spontaneous descemet membrane detachment 20 Years after penetrating keratoplasty for keratoconus. Cornea 35, 1023–1025.

Goweida, M.B., Abuelkheir, A., Abdel, W., El-Menawy, R., Mahmoud, S., 2020. Dynamics of big bubble formation during deep anterior lamellar keratoplasty in eyes with advanced keratoconus. Clin. Ophthalmol. 14, 4305–4310.

Greene, J.B., Mian, S.I., 2013. Cataract surgery in patients with corneal disease. Curr. Opin. Ophthalmol. 24, 9–14.

Güell, J.L., Aristizábal-Montes, D., 2014. Visco-bubble technique for deep anterior lamellar keratoplasty. J Emmetropia 5, 65–68.

Guerra, F.P., Anshu, A., Price, M.O., Giebel, A.W., Price, F.W., 2011. Descemet's membrane endothelial keratoplasty: prospective study of 1-year visual outcomes, graft survival, and endothelial cell loss. Ophthalmology 118, 2368–2373.

Gunasekaran, S., Sharma, N., Titiyal, J.S., 2014. Management of traumatic wound dehiscence of a functional graft 34 years after penetrating keratoplasty. BMJ Case Rep. https://doi.org/10.1136/bcr-2014-205903, 2014 Dec 2. bcr2014205903.

Harris, E.D., Rayton, J.K., Balthrop, J.E., DiSilvestro, R.A., Garcia-de-Quevedo, M., 1980. Copper and the synthesis of elastin and collagen. Ciba Found. Symp. 79, 163–182.

Hashemi, H., Seyedian, M.A., Miraftab, M., Fotouhi, A., Asgari, S., 2013. Corneal collagen cross-linking with riboflavin and ultraviolet a irradiation for keratoconus: long-term results. Ophthalmology 120, 1515–1520.

Hay, E.D., Revel, J.P., 1969. Fine structure of the developing avian cornea. Monogr. Dev. Biol. 1, 1–144.

Hayashi, M., Ninomiya, Y., Hayashi, K., Linsenmayer, T.F., Olsen, B.R., Trelstad, R.L., 1988. Secretion of collagen types I and II by epithelial and endothelial cells in the developing chick cornea demonstrated by in situ hybridization and immunohistochemistry. Development 103, 27–36.

Herndon, L.W., Weizer, J.S., Stinnett, S.S., 2004. Central corneal thickness as a risk factor for advanced glaucoma damage. Arch. Ophthalmol. 122, 17–21.

Hirano, K., Sugita, J., Kobayashi, M., 2002. Separation of corneal stroma and Descemet's membrane during deep lamellar keratoplasty. Cornea 21, 196–199.

History.com, 2020. Galileo is accused of heresy. History. https://www.history.com/this-day-in-history/galileo-is-accused-of-heresy. (Accessed 1 January 2022).

History.com, 2018. 8 Things You May Not Know about Galileo. History. https://www.history.com/news/8-things-you-may-not-know-about-galileo. (Accessed 1 January 2022). accessed.

Hos, D., Matthaei, M., Bock, F., Maruyama, K., Notara, M., Clahsen, T., Hou, Y., Le, V.N. H., Salabarria, A.C., Horstmann, J., Bachmann, B.O., Cursiefen, C., 2019. Immune reactions after modern lamellar (DALK, DSAEK, DMEK) versus conventional penetrating corneal transplantation. Prog. Retin. Eye Res. 73, 100768.

Huang, T., Jiang, L., Zhan, J., Ouyang, C., 2018. [Pre-descement membrane endothelial keratoplasty for treatment of patients with corneal endothelial decompensation]. Zhonghua. Yan Ke Za Zhi. 54, 105–110.

Jacob, S., 2016. Mastering Endothelial Keratoplasty: DSAEK, DMEK, E-DMEK, PDEK, Air Pump-Assisted PDEK and Others. Springer India.

Jacob, S., Agarwal, A., Chaudhry, P., Narasimhan, S., Chaudhry, V.N., 2015. A new clinico-tomographic classification and management algorithm for Descemet's membrane detachment. Cont. Lens Anterior Eye 38, 327–333.

Jafarinasab, M.R., Rahmati-Kamel, M., Kanavi, M.R., Feizi, S., 2010. Dissection plane in deep anterior lamellar keratoplasty using the big-bubble technique. Cornea 29, 388–391.

Jakobiec, F.A., Bhat, P., 2010. Retrocorneal membranes: a comparative immunohistochemical analysis of keratocytic, endothelial, and epithelial origins. Am. J. Ophthalmol. 150, 230–242.e2.

Jensen, S.A., Handford, P.A., 2016. New insights into the structure, assembly and biological roles of 10-12 nm connective tissue microfibrils from fibrillin-1 studies. Biochem. J. 473, 827–838.

Jester, J.V., Murphy, C.J., Winkler, M., Bergmanson, J.P.G., Brown, D., Steinert, R.F., Mannis, M.J., 2013. Lessons in corneal structure and mechanics to guide the corneal surgeon. Ophthalmology 120, 1715–1717.

Jin, X., Jin, H., Shi, Y., Zhang, N., Zhang, H., 2021. Clinical observation of corneal endothelial plaques with fungal and bacterial keratitis by anterior segment optical coherence tomography and in vivo confocal microscopy. Cornea 41, 1426–1432.

Jonas, J.B., Stroux, A., Velten, I., Juenemann, A., Martus, P., Budde, W.M., 2005. Central corneal thickness correlated with glaucoma damage and rate of progression. Invest. Ophthalmol. Vis. Sci. 46, 1269–1274.

Joseph, A., Hossain, P., Jham, S., Jones, R.E., Tighe, P., McIntosh, R.S., Dua, H.S., 2003. Expression of CD34 and L-selectin on human corneal keratocytes. Invest. Ophthalmol. Vis. Sci. 44, 4689–4692.

Kafarnik, C., Lana, A., Faraj, L.A., Ting, D.S.J., Goh, J.N., Said, D.G., Dua, H.S., 2022. Ex-vivo demonstration of canine corneal pre-Descemet's anatomy using pneumodissection as for the big bubble technique for deep anterior lamellar keratoplasty. Sci. Rep. (in press).

Kamiya, K., Ayatsuka, Y., Kato, Y., Shoji, N., Miyai, T., Ishii, H., Mori, Y., Miyata, K., 2021. Prediction of keratoconus progression using deep learning of anterior segment optical coherence tomography maps. Ann. Transl. Med. 9, 1287–1287.

Keane, M., Coster, D., Ziaei, M., Williams, K., 2014. Deep anterior lamellar keratoplasty versus penetrating keratoplasty for treating keratoconus. Cochrane Database Syst. Rev. (7) 2014 Jul 22.

Keeler, R., Singh, A.D., Dua, A., Dua, H.S., 2013. Leeching blood. Br. J. Ophthalmol. 97, 679.

Kielty, C.M., Sherratt, M.J., Shuttleworth, C.A., 2002. Elastic fibres. J. Cell Sci. 115, 2817–2828.

Klintworth, G.K., 2009. Corneal dystrophies. Orphanet J. Rare Dis. 4, 7.

Kobayashi, A., Ohkubo, S., Tagawa, S., Uchiyama, K., Sugiyama, K., 2003. In vivo confocal microscopy in the patients with cornea farinata. Cornea 22, 578–581.

Koçluk, Y., Burcu, A., Sukgen, E.A., 2016. Demonstration of cornea Dua's layer at a deep anterior lamellar keratoplasty surgery. Oman J. Ophthalmol. 9, 179–181.

Kotecha, A., 2007. What biomechanical properties of the cornea are relevant for the clinician? Surv. Ophthalmol. 52 (Suppl. 2), 109–114.

Kremer, I., Rapuano, C.J., Cohen, E.J., Laibson, P.R., Eagle, R.C., 1993. Retrocorneal fibrous membranes in failed corneal grafts. Am. J. Ophthalmol. 115, 478–483.

Kumar, D.A., Agarwal, A., Jaganathasamy, N., 2020. Densitometry analysis of corneal backscatter after pre-descemet endothelial keratoplasty for pseudophakic bullous keratopathy. Cornea 39, 30–38.

Lang, S.J., Heinzelmann, S., Böhringer, D., Reinhard, T., Maier, P., 2020. Indications for intraoperative anterior segment optical coherence tomography in corneal surgery. Int. Ophthalmol. 40, 2617–2625.

Lee, W.B., Jacobs, D.S., Musch, D.C., Kaufman, S.C., Reinhart, W.J., Shtein, R.M., 2009. Descemet's stripping endothelial keratoplasty: safety and outcomes: a report by the American Academy of Ophthalmology. Ophthalmology 116, 1818–1830.

Lewis, P.N., White, T.L., Young, R.D., Bell, J.S., Winlove, C.P., Meek, K.M., 2016. Three-dimensional arrangement of elastic fibers in the human corneal stroma. Exp. Eye Res. 146, 43–53.

Lewis, R.A., Falls, H.F., Troyer, D.O., 1975. Ocular manifestations of hypercupremia associated with multiple myeloma. Arch. Ophthalmol. 93, 1050–1053 (Chicago, Ill. 1960).

Lin, Z.N., Chen, J., Cui, H.P., 2016. Characteristics of corneal dystrophies: a review from clinical, histological and genetic perspectives. Int. J. Ophthalmol. 9, 904–913.

Linsenmayer, T.F., Gibney, E., Gordon, M.K., Marchant, J.K., Hayashi, M., Fitch, J.M., 1990. Extracellular matrices of the developing chick retina and cornea. Localization of mRNAs for collagen types II and IX by in situ hybridization. Invest. Ophthalmol. Vis. Sci. 31, 1271–1276.

Lisch, W., Vossmerbaeumer, U., 2020. Corneal opacity and copper levels of the Lewis syndrome after systemic chemotherapy. Am. J. Ophthalmol. Case Reports 20, 100926.

Liu, Z., Zhang, P., Liu, C., Zhang, W., Hong, J., Wang, W., 2015. Split of Descemet's membrane and pre-Descemet's layer in fungal keratitis: new definition of corneal anatomy incorporating new knowledge of fungal infection. Histopathology 66, 1046–1049.

Lockhart-Cairns, M.P., Newandee, H., Thomson, J., Weiss, A.S., Baldock, C., Tarakanova, A., 2020. Transglutaminase-mediated cross-linking of tropoelastin to

fibrillin stabilises the elastin precursor prior to elastic fibre assembly. J. Mol. Biol. 432, 5736–5751.

Lombardo, M., Parekh, M., Serrao, S., Ruzza, A., Ferrari, S., Lombardo, G., 2016. Two-photon optical microscopy imaging of endothelial keratoplasty grafts. Graefe's Arch. Clin. Exp. Ophthalmol. 255, 575–582.

Luisetti, M., Ma, S., Iadarola, P., Stone, P.J., Viglio, S., Casado, B., Lin, Y.Y., Snider, G.L., Turino, G.M., 2008. Desmosine as a biomarker of elastin degradation in COPD: current status and future directions. Eur. Respir. J. 32, 1146–1157.

M'ilroy, J.H., 1906. On the presence of elastic fibres in the cornea. J. Anat. Physiol. 40, 282–291.

Mackool, R.J., Holtz, S.J., 1977. Descemet membrane detachment. Arch. Ophthalmol. 95, 459–463 (Chicago, Ill. 1960).

Malheiro, L., Coelho, J., Neves, M.M., Gomes, M., Oliveira, L., 2019. Phakic intraocular lens implantation after deep anterior lamellar keratoplasty: retrospective case series analysis with long-term follow-up. Clin. Ophthalmol. 13, 2043–2052.

Malhotra, C., Jain, A.K., Dwivedi, S., Chakma, P., Rohilla, V., Sachdeva, K., 2015. Characteristics of pre-descemet membrane corneal dystrophy by three different imaging modalities-in vivo confocal microscopy, anterior segment optical coherence tomography, and scheimpflug corneal densitometry analysis. Cornea 34, 829–832.

Manche, E.E., Holland, G.N., Maloney, R.K., 1999. Deep lamellar keratoplasty using viscoelastic dissection. Arch. Ophthalmol. 117, 1561–1565 (Chicago, Ill. 1960).

Mandathara, P.S., Stapleton, F.J., Willcox, M.D.P., 2017. Outcome of keratoconus management: review of the past 20 Years' contemporary treatment modalities. Eye Contact Lens 43, 141–154.

Mangouritsas, G., Morphis, G., Mourtzoukos, S., Feretis, E., 2009. Association between corneal hysteresis and central corneal thickness in glaucomatous and non-glaucomatous eyes. Acta Ophthalmol. 87, 901–905.

Marques, R.E., Guerra, P.S., Sousa, D.C., Gonçalves, A.I., Quintas, A.M., Rodrigues, W., 2019. DMEK versus DSAEK for Fuchs' endothelial dystrophy: a meta-analysis. Eur. J. Ophthalmol. 29, 15–22.

Marshall, J., Grindle, C.F.J., 1978. Fine structure of the cornea and its development. Trans. Ophthalmol. Soc. U. K. 98, 320–328.

Marty, A.S., Burillon, C., Desanlis, A., Damour, O., Kocaba, V., Auxenfans, C., 2016. Validation of an endothelial roll preparation for Descemet Membrane Endothelial Keratoplasty by a cornea bank using "no touch" dissection technique. Cell Tissue Bank. 17, 225–232.

McKee, H.D., Barua, A., Brahma, A.K., Jhanji, V., Carley, F.M., 2012a. Rupture pressure of the posterior lamella obtained during big-bubble deep anterior lamellar keratoplasty. Cornea 31, 1285–1287.

McKee, H.D., Irion, L.C.D., Carley, F.M., Brahma, A.K., Jafarinasab, M.R., Rahmati-Kamel, M., Kanavi, M.R., Feizi, S., 2014. Re: dua et al.: human corneal anatomy redefined: a novel pre-Descemet layer (Dua's layer), 2013 Ophthalmology 120, 1778–1785. Ophthalmology 121.

McKee, H.D., Irion, L.C.D., Carley, F.M., Jhanji, V., Brahma, A.K., 2013. Dissection plane of the clear margin big-bubble in deep anterior lamellar keratoplasty. Cornea 32, e51–e52.

McKee, H.D., Irion, L.C.D., Carley, F.M., Jhanji, V., Brahma, A.K., 2012b. Donor preparation using pneumatic dissection in endothelial keratoplasty: DMEK or DSEK? Cornea 31, 798–800.

McKee, H.D., Irion, L.C.D., Carley, F.M., Jhanji, V., Brahma, A.K., 2011. Residual corneal stroma in big-bubble deep anterior lamellar keratoplasty: a histological study in eye-bank corneas. Br. J. Ophthalmol. 95, 1463–1465.

McMonnies, C.W., 2009. Mechanisms of rubbing-related corneal trauma in keratoconus. Cornea 28, 607–615.

Medeiros, F.A., Alencar, L.M., Zangwill, L.M., Bowd, C., Sample, P.A., Weinreb, R.N., 2009. Prediction of functional loss in glaucoma from progressive optic disc damage. Arch. Ophthalmol. 127, 1250–1256 (Chicago, Ill. 1960).

Medeiros, F.A., Meira-Freitas, D., Lisboa, R., Kuang, T.M., Zangwill, L.M., Weinreb, R.N., 2013. Corneal hysteresis as a risk factor for glaucoma progression: a prospective longitudinal study. Ophthalmology 120, 1533–1540.

Mehraban, S., Gu, G., Ma, S., Liu, X., Turino, G., Cantor, J., 2020. The proinflammatory activity of structurally altered elastic fibers. Am. J. Respir. Cell Mol. Biol. 63, 699–706.

Melles, G.R.J., Lander, F., Rietveld, F.J.R., Remeijer, L., Beekhuis, W.H., Binder, P.S., 1999. A new surgical technique for deep stromal, anterior lamellar keratoplasty. Br. J. Ophthalmol. 83, 327–333.

Melles, G.R.J., Remeijer, L., Geerards, A.J.M., Beekhuis, W.H., 2000. A quick surgical technique for deep, anterior lamellar keratoplasty using visco-dissection. Cornea 19, 427–432.

Miyata, K., Tsuji, H., Tanabe, T., Mimura, Y., Amano, S., Oshika, T., 2002. Intracameral air injection for acute hydrops in keratoconus. Am. J. Ophthalmol. 133, 750–752.

Mohammed, I., Ross, A.R., Britton, J.O., Said, D.G., Dua, H.S., 2018. Elastin content and distribution in endothelial keratoplasty tissue determines direction of scrolling. Am. J. Ophthalmol. 194, 16–25.

Moreker, S.R., Ghorpade, H., Moreker, M., 2014. Dua's layer and success of non penetrating glaucoma filtering surgery. Br. J. Ophthalmol. https://bjo.bmj.com/con tent/98/5/691.responses#duas-layer-and-success-of-non-penetrating-glaucoma-filte ring-surgery. (Accessed 1 January 2022).

Morrison, J.C., Swan, K.C., 1982. Full-thickness lamellar keratoplasty. A histologic study in human eyes. Ophthalmology 89, 715–719.

Moshirfar, M., Jarstad, A., Khalifa, Y.M., 2013. Descemet membrane endothelial keratoplasty: why does the donor tissue roll? Cornea 32, e52–e53.

Murphy, J., 2013. More Details on Dua's Layer of the Cornea. Rev. Optom. http://www. reviewofoptometry.com/content/d/web_exclusives/c/41849. (Accessed 28 November 2021). accessed.

Najmi, H., Mobarki, Y., Mania, K., Altowairqi, B., Basehi, M., Mahfouz, M.S., Elmahdy, M., 2019. The correlation between keratoconus and eye rubbing: a review. Int. J. Ophthalmol. 12, 1775–1781.

Narang, P., Agarwal, A., 2019. Triple procedure for pseudophakic bullous keratopathy in complicated cataract surgery: glued IOL with single-pass four-throw pupilloplasty with pre-Descemet's endothelial keratoplasty. J. Cataract Refract. Surg. 45, 398–403.

Narang, P., Agarwal, A., Kumar, D., 2017. Predescemetocele: a distinct clinical entity. Indian J. Ophthalmol. 65, 1224–1226.

Narang, P., Agarwal, Amar, Dua, H.S., Kumar, D.A., Jacob, S., Agarwal, Ashvin, 2015. Glued intrascleral fixation of intraocular lens with pupilloplasty and pre-descemet endothelial keratoplasty: a triple procedure. Cornea 34, 1627–1631.

Nariani, A., Kumar, D.A., Agarwal, A., Perry, I., Tramber, M., Kuo, A.N., Daluvoy, M.B., Carlson, A.N., Kim, T., 2016. Eye Bank graft preparation for pre-descemet's endothelial keratoplasty. Invest. Ophthalmol. Vis. Sci. 57, 1222.

Newton, R.H., Meek, K.M., 1998. Circumcorneal annulus of collagen fibrils in the human limbus. Investig. Ophthalmol. Vis. Sci. 39, 1125–1134.

Nidhi, K., Ashok Kumar, D., Sinha, N., Agarwal, A., 2022. Posterior corneal curvature changes after pre-descemet's endothelial keratoplasty: a prospective analysis. Cornea 41, 1525–1529.

Niknam, S., Rajabi, M.T., 2009. Fixed dilated pupil (urrets-zavalia syndrome) after deep anterior lamellar keratoplasty. Cornea 28, 1187–1190.

O'Rahilly, R., 1983. The timing and sequence of events in the development of the human eye and ear during the embryonic period proper. Anat. Embryol. 168, 87–99.

O'Rourke, M.F., 2007. Arterial aging: pathophysiological principles. Vasc. Med. 12, 329–341.

Oie, Y., Nishida, K., 2017. Triple procedure: cataract extraction, intraocular lens implantation, and corneal graft. Curr. Opin. Ophthalmol. 28, 63–66.

Oke, I., Haddad, N., Lee, H.J., 2020. Granular corneal dystrophy recurrence at the posterior graft-host interface after type 1 big bubble deep anterior lamellar keratoplasty. Am. J. Ophthalmol. Case Reports 20, 100960.

Olver, J., Cassidy, L., Jutley, G., Crawley, L., 2014. Ophthalmology at a Glance. Wiley. Ophthalmology Management, 2017. Corneal graft tissue now comes prepared, preloaded. https://www.ophthalmologymanagement. com/issues/2017/february-2017/corneal-graft-tissue-now-comes-prepared,-p reloaded/. (Accessed 9 December 2022).

Osborne, S.A., Al Ahmar, B., Gerges, S., 2005. A case of "double hypopyon" secondary to Pseudomonas aeruginosa keratitis in a patient with longstanding rubeotic glaucoma. Acta Ophthalmol. Scand. 83, 510–511.

Ozdemir, E.S., Burcu, A., Akkaya, Z.Y., Ornek, F., 2018. Surgical outcomes of perforated and unperforated corneal descemetocele. Int. Ophthalmol. 38, 327–335.

Panda, A., Aggarwal, A., Madhavi, P., Wagh, V.B., Dada, T., Kumar, A., Mohan, S., 2007. Management of acute corneal hydrops secondary to keratoconus with intracameral injection of sulfur hexafluoride (SF6). Cornea 26, 1067–1069.

Pantanelli, S.M., Herzlich, A., Yeaney, G., Ching, S.T., 2014. Recurrence of granular corneal dystrophy type I deposits within host stroma after non-descemet baring anterior lamellar keratoplasty. Cornea 33, 1348–1351.

Park, C.Y., Lee, J.K., Kahook, M.Y., Schultz, J.S., Zhang, C., Chuck, R.S., 2016. Revisiting ciliary muscle tendons and their connections with the trabecular meshwork by two photon excitation microscopic imaging. Invest. Ophthalmol. Vis. Sci. 57, 1096–1105.

Parker, J., Birbal, R., van Dijk, K., Oellerich, S., Dapena, I., Melles, G.R.J., 2019a. Are descemet membrane ruptures the root cause of corneal hydrops in keratoconic eyes? Am. J. Ophthalmol. 205, 147–152.

Parker, J., Birbal, R.S., van Dijk, K., Oellerich, S., Dapena, I., Melles, G.R.J., 2019b. Are descemet membrane ruptures the root cause of corneal hydrops in keratoconic eyes? Am. J. Ophthalmol. 205, 204–205.

Pereira, N.C., Forseto, A., dos, S., Maluf, R.C.P., Dua, H.S., 2021. Pre-Descemet's endothelial keratoplasty: a simple, Descemet's membrane scoring technique for successful graft preparation. Br. J. Ophthalmol. 106, 786–789.

Perrella, G., Brusini, P., Spelat, R., Hossain, P., Hopkinson, A., Dua, H.S., 2007. Expression of haematopoietic stem cell markers, CD133 and CD34 on human corneal keratocytes. Br. J. Ophthalmol. 91, 94–99.

Polack, F.M., 1971. Lamellar keratoplasty. Malbran's "peeling off" technique. Arch. Ophthalmol. 86, 293–295 (Chicago, Ill. 1960).

Price, M.O., Gupta, P., Lass, J., Price, F.W., 2017. EK (DLEK, DSEK, DMEK): new frontier in cornea surgery. Annu. Rev. Vis. Sci. 3, 69–90.

Quantock, A.J., Young, R.D., 2008. Development of the corneal stroma, and the collagen-proteoglycan associations that help define its structure and function. Dev. Dynam. 237, 2607–2621.

Rajaraman, R., Singh, S., Raghavan, A., Karkhanis, A., 2009. Efficacy and safety of intracameral perfluoropropane (C3F8) tamponade and compression sutures for the management of acute corneal hydrops. Cornea 28, 317–320.

Reinhart, W.J., Musch, D.C., Jacobs, D.S., Lee, W.B., Kaufman, S.C., Shtein, R.M., 2011. Deep anterior lamellar keratoplasty as an alternative to penetrating keratoplasty a report by the american academy of ophthalmology. Ophthalmology 118, 209–218.

Rich, L.F., MacRae, S.M., Fraunfelder, F.T., 1988. An improved method for lamellar keratoplasty. CLAO J. Off. Publ. Contact Lens Assoc. Ophthalmol. Inc 14, 42–46.

Rickmann, A., Wahl, S., Katsen-Globa, A., Schulz, A., Pütz, N., Szurman, P., 2021. Cleavage plane after liquid-bubble preparation of Descemet's membrane. Acta Ophthalmol. 99, e937–e942.

Rohen, J.W., Futa, R., Liitjen-Drecoll, E., 1981. The fine structure of the cribriform meshwork in normal and glaucomatous eyes as seen in tangential sections. Invest. Ophthalmol. Vis. Sci. 21, 574–585.

Romano, V., Pagano, L., Gadhvi, K.A., Coco, G., Titley, M., Fenech, M.T., Ferrari, S., Levis, H.J., Parekh, M., Kaye, S., 2020. Clinical outcomes of pre-loaded ultra-thin DSAEK and pre-loaded DMEK. BMJ open Ophthalmol 5, e000546.

Romero-Jiménez, M., Santodomingo-Rubido, J., Wolffsohn, J.S., 2010. Keratoconus: a review. Cont. Lens Anterior Eye 33, 157–166.

Ross, A.R., Said, D.G., Colabelli Gisoldi, R.A.M., Nubile, M., El-Amin, A., Gabr, A.F., Abd Ed-Moniem, M., Mencucci, R., Pocobelli, A., Mastropasqua, L., Dua, H.S., 2020. Optimizing pre-Descemet endothelial keratoplasty technique. J. Cataract Refract. Surg. 46, 667–674.

Ross, A.R., Said, D.G., El-Amin, A., Altaan, S., Cabrerizo, J., Nubile, M., Hogan, E., Mastropasqua, L., Dua, H.S., 2018. Deep anterior lamellar keratoplasty: dissection plane with viscoelastic and air can be different. Br. J. Ophthalmol. 102, 1646–1652.

Said, D.G., Faraj, L., Elalfy, M.S., Miri, A., Maharajan, S.V., Dua, H.S., 2013. Atypical hydrops in keratoconus. Int. Ophthalmol. 34, 951–955.

Samuels, B., 1928. Detachment of descemet's membrane. Trans. Am. Ophthalmol. Soc. 26, 427–437.

Sarnicola, E., Sarnicola, C., Cheung, A.Y., Holland, E.J., Sarnicola, V., 2019. Surgical corneal anatomy in deep anterior lamellar keratoplasty: suggestion of new acronyms. Cornea 38, 515–522.

Sarnicola, V., Toro, P., Gentile, D., Hannush, S.B., 2010. Descemetic dalk and predescemetic dalk: outcomes in 236 cases of keratoconus. Cornea 29, 53–59.

Sati, A., Moulick, P.S., Shankar, S., 2020. Triple anterior chamber following deep anterior lamellar keratoplasty: an unknown complication. Med. J. Armed Forces India 76, 217.

Schiano Lomoriello, D., Savini, G., Naeser, K., Colabelli-Gisoldi, R.M., Bono, V., Pocobelli, A., 2018. Customized toric intraocular lens implantation in eyes with cataract and corneal astigmatism after deep anterior lamellar keratoplasty: a prospective study. J. Ophthalmol. 1649576.

Schlötzer-Schrehardt, U., Bachmann, B.O., Tourtas, T., Torricelli, A.A.M., Singh, A., González, S., Mei, H., Deng, S.X., Wilson, S.E., Kruse, F.E., 2015. Ultrastructure of the posterior corneal stroma. Ophthalmology 122, 693–699.

Schwab, I.R., 2013. Who's on first? Ophthalmology 120, 1718–1719.

Scorcia, V., Luca, V. De, Lucisano, A., Carnevali, A., Scalzo, G.C., Bovone, C., Busin, M., 2018. Results of viscobubble deep anterior lamellar keratoplasty after failure of pneumatic dissection. Br. J. Ophthalmol. 102, 1288–1292.

Sedaghat, M.R., Momeni-Moghaddam, H., Roberts, C.J., Maddah, N., Ambrósio, R., Hosseini, S.R., 2021. Corneal biomechanical parameters in keratoconus eyes with abnormal elevation on the back corneal surface only versus both back and front surfaces. Sci. Rep. 11, 11971.

Seiler, T.G., Shao, P., Eltony, A., Seiler, T., Yun, S.H., 2019. Brillouin spectroscopy of normal and keratoconus corneas. Am. J. Ophthalmol. 202, 118–125.

Selvan, H., Patil, M., Yadav, S., Tandon, R., 2018. Triple chamber: a clinical rarity after deep anterior lamellar keratoplasty and role of optical coherence tomography in management. Int. Ophthalmol. 38, 2683–2687.

Sharma, N., Devi, C., Agarwal, R., Bafna, R.K., Agarwal, A., 2021. i-PDEK: microscope-integrated OCT-assisted pre-Descemet endothelial keratoplasty. J. Cataract Refract. Surg. 47, e44–e48.

Sharma, N., Swarup, R., Bali, S.J., Maharana, P., Titiyal, J.S., Vajpayee, R.B., 2013. Management of intra-descemet membrane air bubble in big-bubble deep anterior lamellar keratoplasty. Cornea 32, 1193–1195.

Sharma, V.K., Sinha, R., Sati, A., Agarwal, M., 2020. Was it thickened Dua's layer? Clinical, tomographical, and histopathological correlation: a case report. Eur. J. Ophthalmol. 32, 87–90.

Shi, H., Qi, X. feng, Liu, T. tao, Hao, Q., Li, X. hong, Liang, L. ling, Wang, Y. miao, Cui, Z. hua, 2017. In vivo confocal microscopy of pre-Descemet corneal dystrophy associated with X-linked ichthyosis: a case report. BMC Ophthalmol. 17, 29.

Shimmura, S., Shimazaki, J., Omoto, M., Teruya, A., Ishioka, M., Tsubota, K., 2005. Deep lamellar keratoplasty (DLKP) in keratoconus patients using viscoadaptive viscoelastics. Cornea 24, 178–181.

Singh, R., Gupta, N., Vanathi, M., Tandon, R., 2019. Corneal transplantation in the modern era. Indian J. Med. Res. 150, 7–22.

Singhal, D., Sahay, P., Goel, S., Asif, M.I., Maharana, P.K., Sharma, N., 2020. Descemet membrane detachment. Surv. Ophthalmol. 65, 279–293.

Smolek, M.K., McCarey, B.E., 1990. Interlamellar adhesive strength in human eyebank corneas. Investig. Ophthalmol. Vis. Sci. 31, 1087–1095.

Soh, Y.Q., Kocaba, V., Weiss, J.S., Jurkunas, U.V., Kinoshita, S., Aldave, A.J., Mehta, J.S., 2020. Corneal dystrophies. Nat. Rev. Dis. Prim. 6.

Spoerl, E., Wollensak, G., Seiler, T., 2004. Increased resistance of crosslinked cornea against enzymatic digestion. Curr. Eye Res. 29, 35–40.

Stechschulte, S.U., Azar, D.T., 2000. Complications after penetrating keratoplasty. Int. Ophthalmol. Clin. 40, 27–43.

Steven, P., Le Blanc, C., Lankenau, E., Krug, M., Oelckers, S., Heindl, L.M., Gehlsen, U., Huettmann, G., Cursiefen, C., 2014. Optimising deep anterior lamellar keratoplasty (DALK) using intraoperative online optical coherence tomography (iOCT). Br. J. Ophthalmol. 98, 900–904.

Studeny, P., Farkas, A., Vokrojova, M., Liskova, P., Jirsova, K., 2010. Descemet membrane endothelial keratoplasty with a stromal rim (DMEK-S). Br. J. Ophthalmol. 94, 909–914.

Takezawa, Y., Suzuki, T., Shiraishi, A., 2017. Observation of retrocorneal plaques in patients with infectious keratitis using anterior segment optical coherence tomography. Cornea 36, 1237–1242.

Tandon, R., Sihota, R., 2014. Parson's Diseases of the Eye - E-Book, twenty-second ed. Elsevier Health Sciences.

Ting, D.S.J., Galal, M., Kulkarni, B., Elalfy, M.S., Lake, D., Hamada, S., Said, D.G., Dua, H. S., 2021a. Clinical characteristics and outcomes of fungal keratitis in the United Kingdom 2011-2020: a 10-year study. J. fungi (Basel, Switzerland) 7, 966.

Ting, D.S.J., Ho, C.S., Cairns, J., Elsahn, A., Al-Aqaba, M., Boswell, T., Said, D.G., Dua, H. S., 2021b. 12-year analysis of incidence, microbiological profiles and in vitro

antimicrobial susceptibility of infectious keratitis: the Nottingham Infectious Keratitis Study. Br. J. Ophthalmol. 105, 328–333.

Ting, D.S.J., Ho, C.S., Deshmukh, R., Said, D.G., Dua, H.S., 2021c. Infectious keratitis: an update on epidemiology, causative microorganisms, risk factors, and antimicrobial resistance. Eye 35, 1084–1101.

Ting, D.S.J., Bandyopadhyay, J., Patel, T., 2019a. Microbial keratitis complicated by acute hydrops following corneal collagen cross-linking for keratoconus. Clin. Exp. Optom. 102, 434–436.

Ting, D.S.J., Said, D.G., Dua, H.S., 2019. Are descemet membrane ruptures the root cause of corneal hydrops in keratoconic eyes? Am. J. Ophthalmol. 205, 204.

Ting, D.S.J., Srinivasan, S., 2014. Pneumodescemetopexy with perfluoroethane (C2F6) for the treatment of acute hydrops secondary to keratoconus. Eye 28, 847–851.

Tolees, S., 2017. DALK for macular dystrophy BB type 2. YouTube. https://www.youtube.com/watch?v=wK7S_NNg-5A. (Accessed 17 December 2021).

Tomidokoro, A., Oshika, T., Amano, S., Higaki, S., Maeda, N., Miyata, K., 2000. Changes in anterior and posterior corneal curvatures in keratoconus. Ophthalmology 107, 1328–1332.

Toole, B.P., Trelstad, R.L., 1971. Hyaluronate production and removal during corneal development in the chick. Dev. Biol. 26, 28–35.

Tsatsos, M., Mironidou, M., Jacob, S., Ziakas, N., 2019. Factors influencing corneal predescemetic endothelial keratoplasty (PDEK) graft creation: it's all in a bubble. Hellenic J. Nucl. Med. 22 (Suppl. 2), 42–46.

Tubbs, R., Shoja, M., Loukas, M., 2016. Bergman's Comprehensive Encyclopedia of Human Anatomic Variation. Wiley.

Tuft, S.J., Gregory, W.M., Buckley, R.J., 1994. Acute corneal hydrops in keratoconus. Ophthalmology 101, 1738–1744.

Ung, L., Bispo, P.J.M., Shanbhag, S.S., Gilmore, M.S., Chodosh, J., 2019. The persistent dilemma of microbial keratitis: global burden, diagnosis, and antimicrobial resistance. Surv. Ophthalmol. 64, 255–271.

Watson, S.L., Tuft, S.J., Dart, J.K.G., 2006. Patterns of rejection after deep lamellar keratoplasty. Ophthalmology 113, 556–560.

White, T.L., Lewis, P.N., Young, R.D., Kitazawa, K., Inatomi, T., Kinoshita, S., Meek, K. M., 2017. Elastic microfibril distribution in the cornea: differences between normal and keratoconic stroma. Exp. Eye Res. 159, 40–48.

Yahia Chérif, H., Gueudry, J., Afriat, M., Delcampe, A., Attal, P., Gross, H., Muraine, M., 2015. Efficacy and safety of pre-Descemet's membrane sutures for the management of acute corneal hydrops in keratoconus. Br. J. Ophthalmol. 99, 773–777.

Yanoff, M., Sassani, J.W., 2018. Ocular Pathology. Elsevier Health Sciences.

Yoeruek, E., Bayyoud, T., Hofmann, J., Szurman, P., Bartz-Schmidt, K.U., 2012. Comparison of pneumatic dissection and forceps dissection in descemet membrane endothelial keratoplasty: histological and ultrastructural findings. Cornea 31, 920–925.

Zaki, A.A., Elalfy, M.S., Said, D.G., Dua, H.S., 2014. Deep anterior lamellar keratoplasty—triple procedure: a useful clinical application of the pre-Descemet's layer (Dua's layer). Eye 29, 323–326.

Zarei-Ghanavati, S., Zarei-Ghanavati, M., Sheibani, S., 2010. Traumatic wound dehiscence after deep anterior lamellar keratoplasty: protective role of intact descemet membrane after big-bubble technique. Cornea 29, 220–221.

Zhang, W., Kassels, A.C., Barrington, A., Khan, S., Tomatsu, S., Alkadi, T., Aldave, A., 2019. Macular corneal dystrophy with isolated peripheral Descemet membrane deposits. Am. J. Ophthalmol. Case Reports 16, 100571.

Zhang, Y.M., Wu, S.Q., Yao, Y.F., 2013. Long-term comparison of full-bed deep anterior lamellar keratoplasty and penetrating keratoplasty in treating keratoconus. J. Zhejiang Univ. - Sci. B 14, 438–450.

Zhao, Z., Wu, S., Ren, W., Zheng, Q., Ye, C., Kim, A.D., Jhanji, V., Wang, M.T.M., Chen, W., 2020. Compression sutures combined with intracameral air injection versus thermokeratoplasty for acute corneal hydrops: a prospective-randomised trial. Br. J. Ophthalmol. 105, 1645–1650.