Dua’s Layer: its discovery, characteristics and applications

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ABSTRACT: Definitive treatment for loss of corneal transparency or for corneal distortion is a corneal transplant. When the sight affecting pathology lies in the corneal stroma, deep anterior lamellar keratoplasty (DALK) allows retention of the healthy recipient endothelium and Descemet’s layer. The “Big Bubble” (BB) technique described by Mohamed Anwar is the most popular Descemet’s baring technique worldwide. It involves the injection of air into the corneal stroma, thus stripping the DM from the deep stroma and allowing excision of the affected tissue. Over nearly a decade of performing DALK by the BB technique, a number of clues pointed to a novelty in the surgical anatomy of the posterior cornea which, after characterisation, was termed the Dua’s layer. This report summarizes the events that suggested the existence, provided evidence, and finally led to the characterisation of the Dua’s layer, along to its clinical relevance and the applications of this discovery.

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Loss of corneal transparency or corneal distortion leading to visual impairment and blindness can occur for two main reasons – stromal disease with or without scarring and endothelial disease with consequent corneal hydration. The definitive treatment for these conditions is a corneal transplant. The first human corneal transplant was performed more than a hundred years ago1. The initial emphasis was on lamellar grafts wherein the anterior scarred layer of the cornea was replaced by a similar layer of clear tissue taken from a donor cornea. Full thickness corneal grafts or penetrating keratoplasty, to manage both stromal and endothelial disease, then became the norm and remained as the standard of care for most of the one hundred years, despite its many well recognised problems. Major problems include a prolonged visual recovery time, weaker structural integrity of the eye rendering it susceptible to rupture following trivial trauma, high astigmatism and graft rejection and failure.

Inventions and innovations in eye banking, technology and instrumentation, particularly in the last decade have enabled refinements in corneal transplantation that address many of the problems mentioned above. When the endothelium is affected, specific replacement of the endothelial layer can be undertaken in the procedures termed Descemet’s stripping endothelial keratoplasty (DSEK) and Descemet’s membrane endothelial keratoplasty (DMEK)2,3. Endothelial transplantation allows for very rapid visual recovery, is astigmatically neutral, does not weaken the eye and also appears to have a reduced incidence of rejection.

When the sight affecting pathology lies in the corneal stroma, it is no longer necessary to replace the entire cornea. Deep anterior lamellar keratoplasty (DALK) allows retention of the healthy recipient endothelium and Descemet’s layer while replacing the entire stroma and epithelium2-5. This virtually eliminates the risk of rejection related graft failure.
and also leaves the eye “more secure” than after a penetrating keratoplasty. Most of the techniques deployed to perform DALK attempt to reach the Descemet’s membrane (DM), i.e. remove the entire stroma. One approach, known as the “Big Bubble” (BB) technique, involves the injection of air into the corneal stroma, which when successful, strips the DM from the deep stroma allowing excision of the affected stroma whilst retaining the recipient DM and endothelium. This can also be achieved with the injection of viscoelastic substances or balanced salt solution. Collectively these techniques are referred to as “Descemet’s baring techniques” where it is claimed that the cleavage occurs as a plane that enables the DM to be laid bare.

**THE “BIG BUBBLE” TECHNIQUE FOR DALK**

Understanding this technique is important for the understanding of the clues that led to the discovery of the Duas layer and its clinical relevance. The “Big Bubble” (BB) technique described by Mohamed Anwar (Figure 1) is the most popular technique worldwide that is used for separating the DM from the corneal stroma. A central trephination (between 7 mm and 8.5 mm) to deep lamellar depth is carried out with a manual or a vacuum trephine (Figure 1A). A 27 or 30 gauge, angled sharp pointed needle, bevel down (or a blunt ended cannula with a hole on the lower surface, near the tip) mounted on a 5 or 10 ml Luer lock syringe filled with air, is passed through the deep stroma starting in the groove made by the trephination, towards the central part of the cornea (Figure 1B). Air is forced into the stroma until a large bubble is seen to form and allowed to expand till it extends just beyond the trephine mark (Figure 1C). Approximately half to two thirds thickness of the anterior stroma outlined by the trephine mark is then dissected off (Figure 1D). Some aqueous is released through a paracentesis to reduce the eye pressure (Figure 1E) and the roof of the bubble, made of deep stroma is punctured with a sharp knife (Figure 1F). A spatula is inserted in the plane between the deep stroma and the DM and the stroma incised in a cruciate manner to divide the deep stroma into four quadrants (Figure 1G). Each quadrant is then excised with a pair of curved corneal scissors (Figure 1H), laying bare the DM (Figure 1I). The donor button, approximately 0.25 mm larger than the host trephine diameter, is stripped off its DM (Figure 1J), placed on the exposed recipient’s DM (Figure 1K) and sutured to the recipient rim (Figure 1L). There are several variations to the above technique but the principles remain as described above. For example,
instead of inserting a spatula in the plane between deep stroma and DM, the space is filled with a cohesive viscoelastic agent and divided into quadrants with a pair of scissors; some surgeons may dissect off the top half or two thirds of the trephined cornea and then inject air in the deep stroma to obtain the BB. Others insert the needle or cannula of the air filled syringe tangentially along the periphery of the cornea rather than radially towards the pupil margin.

c) However, on occasions when the BB did not reach the edge of the trephine mark, the deep stroma (roof of the bubble) was seen to be attached to the host’s ‘DM’ by fine strands of tissue which had to be mechanically broken or cut to extend the cleavage plane to the trephine mark before excising the respective quadrant of deep stroma (Figure 2C). This begged the question that if the plane of cleavage with the BB technique is the same as when DM is peeled off the donor button; why do strands appear when separating stroma from the DM and not when separating DM from stroma?

d) When there was an inadvertent micro or macro perforation of the deep stroma during the operation, the torn ‘DM’ did not scroll like the DM is known to do (Figure 2D and 2E).

e) During suturing of full thickness corneal donor buttons in PK, a distinct edge of the ‘DM’ is very often seen as the needle emerges from deep stroma, anterior to DM. This edge is attributed to the DM. However, in DALK, when the DM was peeled off the donor button, the edge was still present suggesting that it is caused by something else (Figure 2F).

f) We and several surgeons have noted and commented on the better structural integrity of the globe after a DALK compared to a PK. DALK eyes could withstand a degree of blunt trauma without rupture unlike eyes that have undergone PK. It was counter intuitive to attribute this additional strength entirely to the retained host DM.

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**CLINICAL CLUES THAT SUGGESTED THE EXISTENCE OF DUA’S LAYER**

Over nearly a decade of performing DALK by the BB technique, one of us (HSD) noted a number of clues that pointed to a novelty in the surgical anatomy of the posterior cornea (Figure 2). Surgeons who perform DALK by the BB technique can easily relate to these clues.

a) Intra-operatively, the exposed ‘DM’ felt more resilient than DM, and could withstand pressure or force applied by a blunt instrument or swab (Figure 2A).

b) The DM could in the vast majority of cases, be peeled off the donor corneal button very easily and smoothly (Figure 2B). This suggested that the plane of cleavage between DM and corneal stroma was smooth and uniform offering little resistance during separation.

c) However, on occasions when the BB did not reach the edge of the trephine mark, the deep stroma (roof of the bubble) was seen to be attached to the host’s ‘DM’ by fine strands of tissue which had to be mechanically broken or cut to extend the cleavage plane to the trephine mark before excising the respective quadrant of deep stroma (Figure 2C). This begged the question that if the plane of cleavage with the BB technique is the same as when DM is peeled off the donor button; why do strands appear when separating stroma from the DM and not when separating DM from stroma?

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f) We and several surgeons have noted and commented on the better structural integrity of the globe after a DALK compared to a PK. DALK eyes could withstand a degree of blunt trauma without rupture unlike eyes that have undergone PK. It was counter intuitive to attribute this additional strength entirely to the retained host DM.

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**Figure 2.** Clinical clues suggesting the presence of Dua’s layer (DL). A. The exposed “Descemets membrane” (DM) is tougher and more resilient than true DM. B. DM strips off easily from the donor lenticule without any strands extending between DM and overlying stroma. C. When the big bubble does not extend to the trephine mark the stroma has to be mechanically separated from the underlying “DM”. Strands of collagen are seen to extend between stroma and “DM”, which have to be mechanically severed or cut. D. A cannula is inserted through a tear in the “DM” during DALK following an inadvertent tear intra-operatively. The “DM” has not scrolled. E. Post operatively, the same eye as in E, shows a linear fold unlike the double contour line of a torn DM. F. When a suture is passed through the donor lenticule from which the DM has been stripped, a distinct edge, normally attributed to the DM is still visible. All these clues suggested the presence of another layer besides the DM.
All the above were considered as clinical clues and not evidence. Some had substance but others were mere clinical impressions and anecdotes. Collectively however, they raised sufficient doubt on the concept of “Descemet’s baring” in DALK leading to the hypothesis that there exists in the posterior corneal stroma adjacent to the DM, a distinct layer of tissue, which provides a plane of cleavage between its anterior surface and deep stroma in DALK. This hypothesis was proposed and early clinical and histological evidence of its existence was provided at the annual congress of The Royal College of Ophthalmologists UK, in May 2007 and further evidence presented at the Societa Italiana Cellule Staminali e Superficie Oculare, VI Congreso S.I.C.S.S.O. Lecce, June 14-16, 2007.

THE EVIDENCE

Ex-vivo experiments were performed on human sclero-corneal discs not suitable for transplantation; that were maintained in organ culture medium and those dissected from “fresh” (within 24 hours of enucleation) donor whole globes. Air was injected in the deep stroma to simulate the surgical DALK procedure. Air was noted to spread from the point of injection anteriorly, circumferentially and posteriorly to fill the corneal stroma and eventually result in the formation of a BB. Three types of bubbles could be produced. A Type 1 bubble, which starts in the centre of the cornea by the coalescence of multiple smaller bubbles (Figure 3A) and expands centrifugally and posteriorly to assume a well circumscribed dome shaped appearance. It reaches a maximum diameter of ≤ 9 mm and maximum height (measured from epithelium) of 5.5 mm. A Type 2 bubble, which is a thin walled bubble that starts at the periphery of the sclero-corneal disc and enlarges on continuing injection of air to spread across almost the entire surface of the sclero-corneal disc (Figure 3B) reaching a diameter of 10-11 mm and a maximum thickness of 6.5 mm. Occasionally this Type 2 BB starts as two or more small bubbles at the periphery which enlarge centrally to meet and coalesce. At times both Type 1 and Type 2 BB can appear in the same sclero-corneal disc. These are termed “Mixed bubbles” (Figure 3C). The two bubbles in mixed-BB can be in various combinations, a complete Type 1 BB and a partial Type 2 BB (most common), a complete Type 1 and a complete Type 2 BB and rarely a complete Type 2 with a partial Type 1 BB. Type 1 BB was the most common (approximately 80%) and mixed BB the least.

The DM could be completely peeled off a Type 1 BB without deflating the BB (Figure 4A) suggesting that the posterior wall of the Type 1 B, in addition to the DM is made of another distinct layer of tissue. This layer was termed the pre-Descemet’s layer (Dua’s layer) (DL). Equally it was possible to first peel off the DM from the sclero-corneal disc and then inject air to create a complete Type 1 BB indicating that the DM is not essential for the creation of a Type 1BB. When an attempt was made to peel the DM off a Type 2 BB it deflated immediately indicating that a Type 2 BB is a pre-Descemetic collection of air. Similarly, when the DM was peeled off a mixed BB the Type 2 component deflated but the Type 1 component remained intact. With continued injection of air, a Type 1 BB (with DM peeled off) became tense but did not extend beyond a maximum of 9 mm diameter. At a pressure of around 700 mm Hg (with the tip of the injecting needle inserted through the posterior stroma to appear in the centre of the BB) the Type 1 BB burst with a popping sound. The bursting pressure of a Type 2 BB was variable and much less because at times the DM burst and on other occasions it disinserted along a segment of its peripheral attachment. This indicates that DL like the DM is impervious to air. This is an important characteristic of the DL as compared to the rest of the stroma where air moves in all directions. The tissue of DL presented as a glistening, pliable, resilient and tough layer (Figure 4B). Tugging or pulling on the DL with a forceps after removing the DM resulted in the formation of striae that could be seen extending from

Figure 3. Types of ‘Big bubbles’ (BB). A. A type-1 BB, which is centrally located and does not extend for more than 9mm in diameter. This is a pre-Dua’s layer BB (reproduced from the authors’ own publication in the Journal Ophthalmology 2013 [Reference 9]). B. A type-2 BB which is thin walled and covers the entire posterior surface of the cornea. This is a pre-Descemetic BB. C. Mixed BB – a central type-1 BB is associated with a peripheral partial type-2 BB.
the tip of the forceps radially across the boundary of the BB to the limbus. This indicated that the DL extended to the limbus but the zone between the edge of the BB and the limbus was firmly attached to the underlying stroma. This attachment could not be broken either by injection of air or by attempts to mechanically and physically peel of the DL from the edge of the BB outwards. When DL from a Type 1 BB was excised along its circumference, further injection of air did not result in creation of another BB indicating that the DL is not a random separation of some posterior stromal lamellae of the corneal stroma.

Characteristics of Dua’s layer (Description derived from the authors’ own recent publications references)7-9

The clinical characteristics of DL as described above and the histological features are largely derived from examination of the part of DL that makes up the posterior wall of the Type 1 BB with and without the DM attached to it. Though the vast majority of eye donors in which it was demonstrated were in the age range of 50 to 94 years, it has also been demonstrated in infants and children.

Histological examination by light and electron microscopy confirmed that the posterior wall of a Type 1 BB is made of a collagenous layer of tissue (DL) and the DM and endothelium. Strands of collagen extend from the adjacent stroma into the DL (Figure 5A). The mean ± SD thickness of DL is 10.15 ± 3.6 microns (range 6.3 to 15.83) compared to DM which showed a mean thickness of 10.97 ± 2.36 microns (range 7.8 to 13.98). The DL is made of collagen bundles organised as tightly packed thin lamellae numbering 5-8 (Figure 5B). DM peeled from DL and from mixed BB does not show any split between banded and non-banded zones (Figure 5C). The collagen fibres are largely oriented longitudinally and transversely with some running in an oblique direction (Figure 5D). Long spacing collagen is prominent in DL towards its posterior aspect closer to the apposition with DM (Figures 5D, 5E). Occasionally broken strands of collagen can be seen on the anterior surface of DL and appear as a recoiled clump of collagen (Figure 5F). In comparison, the corresponding width of the corneal stroma anterior to DL in un-inflated control eyes shows only 3-5 lamellae. The fibril diameter in DL measures around 21.70 ± 2.43 nm and in the corneal stroma immediately overlying the DL it is 24.20 ± 2.68 nm. This difference is statistically significant with the DL fibrils being narrower. The inter-fibrillar distance is the same in the DL (9.64 ± 7.74 nm) as in the posterior stroma (10.09 ± 7.91 nm). However as the inter-fibrillar distance is measured between centres of adjacent fibrils the space between the narrower fibrils is greater in DL than in the posterior corneal stroma. This can allow for greater ‘filling-in’ with proteoaminoglycans and give it a gel like structure which in turn could explain why it is impervious to air. On scanning electron microscopy the anterior surface of DL shows parallel bundles of collagen regularly arranged while the posterior surface shows a smooth pleated pattern made of coarse bundles of collagen (Figure 6A) and differs from the appearances of the deep stroma (Figure 6B) and DM (Figure 6C). Strands of collagen bundles bridge the space between the DL and the stromal bed as demonstrated by both scanning and transmission electron microscopy (Figure 5A, 6D). This explains the strands seen intra-operatively as described above (see also clinical implications). Unlike the corneal stroma, DL that forms the posterior wall of a Type 1 BB did not demonstrate any keratocytes. Histology of mixed bubbles shows that
Figure 5. A. Light photomicrograph of a Type-1 BB from which the Descemets membrane (DM) has been peeled off centrally to reveal the Dua’s layer (DL). Strands of collagen bundles (S) are seen extending from the stroma to DL. The separation occurs along the last row of keratocytes (arrow). B-F. Transmission electron micrographs: B. DL is made of multiple thin lamellae closely applied to Descemets membrane (DM). An endothelial cell (EC) is seen on the posterior surface of DM. Bar = 10 microns. C. The posterior wall of a Type-2 BB showing histological features of DM; the banded (BZ) and non-banded (NBZ) zones and endothelium. The same features were seen in DM peeled off a Type-1 BB. There was no split in BZ and NBZ. Bar = 5 microns. D. DL from a Type-1 BB made of multiple compact lamellae with collagen bundles running in longitudinal, transverse and oblique directions. Bar = 5 microns. E. DL from a Type-1 BB with long spacing collagen (arrow) adjacent to the DM. Bar = 1 micron. This is also evident as dark material in (D). F. A recoiled broken strand is seen on DL as a clump of collagen (between the two arrows). Some keratocyte cellular debris is also present. Reproduced from the authors’ own publication in the Journal Ophthalmology 2013.
Figure 6. Scanning electron micrographs (SEM). A. The anterior surface (star) and posterior surface (triangle) of Dua’s layer (DL) showing parallel and regularly arranged collagen bundles and a smooth pleated pattern respectively. The edge of the Descemets membrane is also seen. B. The stromal bed of a type-1 big bubble showing a criss-cross pattern with gaps related to passage of air (arrows). This contrasts with the appearance of the anterior surface of DL seen in ‘a’. C. The anterior surface of the Descemets membrane (DM) presents as a very smooth surface. D. Strands of collagen are seen extending from the stromal bed to the anterior surface of DL. Ends of broken strands are visible as small round dots (arrow). E. The end of the cleavage created by the air bubble between DL and posterior stroma is visible. DL is seen as a distinct and compact layer. Reproduced from the authors’ own publication in the Journal Ophthalmology 2013.

Immunohistology demonstrates that DL, like corneal stroma, is primarily composed of collagen I. Collagens, IV, V and VI were present in DL of which IV and VI were more in DL compared to adjacent deep corneal stroma. Collagen V was weakly positive in both DL and stroma. There is no difference in the intensity of staining for proteoglycans lumican, mimecan and decorin in DL and corneal stroma. Absence of keratocytes was also noted by the lack of CD34 positivity in DL.

In summary, evidence from the ex-vivo experiments on human donor eyes therefore suggest that following intra-stromal injection of air “DL” separates no greater than 9 mm forming the type 1 bubble, which commences centrally by the accumulation and coalescence of
several small bubbles and expands centrifugally; that DL extends to the periphery but is firmly adherent to the peripheral stroma, that it is impervious to air, that the DM can be peeled off the type 1 bubble without deflating it, that the presence of DM is not essential for the formation of a type 1 bubble, that mixed bubbles can occur and are not due to a split between banded and non-banded zones of the DM, that the DL that forms the wall of the type 1 bubble is devoid of keratocytes, that it contains a fair amount of type VI collagen and long spacing collagen, that it is not a random separation of the posterior stroma leaving behind some “residual stroma” but a distinct layer that cannot be re-created by blowing out further “residual stroma” after excising the first one (the DL), that the diameter of the fibrils in the DL is significantly smaller than that of the posterior cornea, and that the pre-Descemetic (Type 2) bubbles start predominantly at the periphery and spread across the entire posterior surface of the cornea".

Dua’s Layer and Trabecular Meshwork

Further interesting information has emerged upon examination of the termination of DL at the periphery along the circumference of the cornea at it junction with the limbus. By light microscopy of cross sections of the cornea it can be seen that DL, which is visible as a band of tissue anterior to DM extends peripherally beyond the termination of DM, initially as a compact band, which then spreads out in a triangular manner with the fibres diverging further as they approach the sclera, ciliary body and root of the iris (Figure 7). Scanning electron microscopy shows that the trabecular beams emerge radially from the anterior surface of the peripheral edge of Schwalbe’s line or zone and are wrapped around by the peripheral part of the DM (Figure 8). However, when the DM is stripped off the posterior surface of the cornea, DL can be seen as a smooth sheet, which begins to split into radially oriented broad beams which in turn divide and subdivide to form narrower beams which interconnect and intertwine with similar adjacent beams as they extend towards the iris and ciliary body, constituting the trabecular meshwork (TM). This observation is substantiated by transmission electron microscopy (TEM) (Figure 9).

TEM of the peripheral part of the cornea confirms that DL continues beyond the termination of DM (Figure 9A). The compact lamellar arrangement of DL begins to open approximately 350 microns central to the termination of the DM. Trabecular cells are seen in the peripheral cornea in DL extending to a mean of 322 µm (range 260 to 390 µm) central to the termination of DM (Figure 9B-G). Here they are associated with deposition of basement membrane which separate lamellae of DL. Attachments of the trabecular cells to the basement membrane and to each other are visible within the periphery of DL. (Figure 9E-G). The morphology of trabecular cells seen in DL is distinct from keratocyte cell bodies that are seen further anterior to DM in the posterior corneal stroma. (Figure 9E). Whilst nuclei of trabecular cells and keratocytes can be demonstrated by DAPI staining (nuclear stain) the trabecular cells are negative for CD34 (keratocyte marker) which stains all the keratocytes in the corneal stroma. The presence of trabecular cells in DL corresponds to the point at which the collagen lamellae of DL began to split and separate. The 5 to 8 lamellae of DL separate in the antero-posterior direction and also split into narrower bands which intertwine and cross each other as they extend towards the sclera, ciliary body and iris root. The majority of the trabecular beams that form the TM posterior to the canal of Schlemm are made of collagen fibres arising from the posterior aspect of DL. The anterior collagen fibres, together with some fibres of the deep limbus/sclera form the anterior wall of the canal of Schlemm. Long spacing collagen that has been described in DL is also abundant in the TM. Elastic fibres are present in the TM but not in DL.

The separation of DL collagen lamellae into broad and narrow beams along the circumference of the termination of DL and their continuity with the TM beams seen on TEM establish that the TM is a continuation of DL. SEM of DL after stripping off the DM illustrates that DL continues imperceptibly with the broader beams of the apical part of the TM.

Figure 7. Light photomicrograph illustrating Dua’s layer extending beyond the termination of the DM (black arrow) and fanning out as the trabecular meshwork. From the point indicated by the white arrow the stromal fibres can be seen to diverge and spread out as the beams of the trabecular meshwork before attaching to the ciliary body and the iris. CB, ciliary body; S, canal of Schlemm. Reproduced from the authors’ own publication in the British Journal of Ophthalmology, 2014."
The demonstration of the presence of trabecular cells within the peripheral cornea in DL is another important observation. These cells are surrounded by basement membrane with which they establish attachments, within DL. TM beams are known to be enveloped with a basal lamina to which trabecular/endothelial cells attach. The presence of these cells with basement membrane in the periphery of DL strongly suggests that the formation of the TM beams commences in DL, approximately 500 microns central to the termination of the DM. Staining for laminin, which is a basement membrane component, is strongly positive in the TM and the peripheral DL corroborating the above observation. The central collagen core of the TM beams is acellular. This is consistent with the finding that no keratocytes are noted in DL either. This suggests that the formation of the TM commences in the peripheral cornea, central (anterior) to the termination of the DM and not at the termination of the DM. As DL becomes thinner towards the periphery the distance between the last row of keratocytes and the DM reduces compared to the central part of DL.

Thus, while the bulk of the corneal stroma merges at the periphery with the scleral stroma the collagen of DL continues as the TM. The significance of the observations and the importance of the TM in relation to glaucoma, a blinding disease, should influence the direction of some relevant research in determining interactions between the cornea and the TM.

**CLINICAL IMPLICATIONS AND APPLICATIONS**

Identification of the DL and its relationship to the different types of big bubbles formed on injection of air is clinically very relevant to the DALK procedure. Surgeons have long recognised the formation of a central BB with a white ring as the commonest type of BB. This is clearly a Type 1 BB. The interesting clinical understanding here is that most surgeons make a trephine

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**Figure 8.** Scanning electron micrographs (SEM). **A.** The peripheral cornea with Descemet’s membrane (DM) in situ. The endothelial cells stop (thick arrow) just before the zone with curly lines (star). The trabecular beams are seen to emerge from the peripheral termination of the DM (thin arrow) and can be seen to be mainly radially oriented. The beams are generally thicker near their origin and become narrower distal to the termination of the DM. The beams can be seen to divide, subdivide and interconnect. Bar = 100 µm. **B.** SEM of periphery of Dua’s layer (DL) from which the DM has been removed. DL is visible as a homogenous sheet. There is a tear in DL (arrow) through which the distinctly different architecture of the underlying stroma is visible. At the periphery, DL tissue spreads out as the trabecular beams as described in (A). Bar = 100 µm. **C.** The transition of DL into the trabecular beams is clearly visible. Bar = 10 µm. **D.** The splitting of DL into broad sheets which narrow, subdivide and make connections with adjacent beams to form the trabecular meshwork is clearly seen. Near the edge of DL they appear as broad(er) sheets which narrow distally to become more like beams. Bar = 10 µm. *Reproduced from the authors’ own publication in the British Journal of Ophthalmology, 2014.*
incision of between 7.5 to 8.5 mm diameter and ‘stop’ the BB when it reaches the trephine mark. The fact is that the BB stops itself. It does not extend beyond 9 mm hence it is important that DALK greater than 9 mm should not be attempted as air separation of DL from the deep stroma will not occur to the trephine incision and mechanical separation with a spatula will have to be attempted. This carries a risk of perforation of DL. An ideal size for DALK, based on this observation would be no greater than 8 mm. Even so, in some instances the BB may not be centred on the trephine incision and for varying clock hours along the circumference mechanical separation will be required. Strands of collagen tissue are seen extending between the anterior surface of the DL and the deep stroma, which have to be physically broken or cut. This also indicates that the plane of separation of a Type 1 BB is not between deep stroma and DM but rather between deep stroma and DL. The demonstration of DL also explains the reason for the classical white ring seen surrounding most big bubbles. In Type 1 BB the air separating DL from deep stroma stretches the attachments between DL and the stroma. As these break the BB enlarges and so does the surrounding white ring. When the separation reaches its maximum, the stretching of tissue between the central DL and the stroma at the edge of the BB produces the white ring. As soon as the stretching force is released or eased by withdrawal of the needle or escape of air, the white ring markedly fades in whiteness or disappears, supporting the explanation offered.

Figure 9. Transmission electron micrographs (TEM) A. Montage of TEM sections from peripheral part of cornea to trabecular meshwork. Duâ’s layer (DL) fibres anterior to Descemets membrane (DM) are seen (double arrows) to split and separate central to the termination of DM (single arrow) and continue to diverge to form the narrow beams of the trabecular meshwork. This illustrates that the starting point of the trabecular meshwork is anterior to the DM, in DL of the peripheral cornea. The dark black bands correspond to the grid of the TEM. Bar (seen on the black bands) = 5 µm. B. A large trabecular cell (Star) is seen anterior to DM within the collagen of DL. The cell is surrounded with basement membrane (BM) (black arrows). No keratocytes are visible in the adjacent stroma on either side. Bar = 10 µm. C. A large trabecular cell (star) with a prominent nucleus is seen anterior to DM in the collagen of DL. There is BM material between it and the collagen tissue to which it is closely applied. No keratocytes are visible in the adjacent stroma on either side. Bar = 2 µm. D. A large trabecular cell is seen anterior to DM in the collagen of DL. The cell body of a keratocyte (arrow) is seen approximately 15 microns from the DM in the posterior corneal stroma but no keratocyte is visible in DL. Bar = 5 µm. E. Distinct layers of BM are seen separating the collagen of DL anterior to DM. The cell body of a transversely sectioned trabecular cell in DL with attachments to BM (arrows) is also seen. Bar = 2 µm. F. Cell-cell adhesions are distinctly visible (arrows) between cells in DL. Bar = 1 µm. G. Cell BM attachments are distinctly seen (arrows) in DL. DM = Descemet’s membrane. Cell-cell and cell basement membrane attachments of the kind seen in (F) and (G) are not usually associated with keratocytes; Bar = 1 µm. Reproduced from the authors’ own publication in the British Journal of Ophthalmology, 2014.
Surgeons performing DALK by the big bubble technique have also reported the occasional sudden appearance of a big bubble that extends to the periphery in one or more quadrants without the classical white ring. Terms such as “explosive bubble” or “glassy bubble” have been used to explain this type of bubble. This is clearly the Type 2 or pre-Descemet BB that extends to the periphery and as there is an easily separable plane of cleavage between DM and DL, a white ring does not appear.

For almost as long as the big bubble DALK procedure has been in vogue, surgeons have commented on the appearance of distinctly visible ‘double bubbles’ thought admittedly rare. The explanation offered and accepted for this has been a split between banded and non-banded zones of the DM, usually partial with air accumulating between deep stroma and DM and in a pocket between the split banded and non-banded zones of DM. The knowledge of DL has not made it absolutely clear that such a split does not occur but rather the double bubbles
are the Mixed BB as described above, one between deep stroma and DL and the other between DL and DM. The latter can be partial or complete.

Clinical experience has also taught us that during BB DALK, if the “DM” is punctured, at times it scrolls in the classical manner described for tears in DM and at times it does not. This difference can now be attributed to punctures/tears in DM vs punctures/tears in DL. In the latter situation, the opening tends not to spontaneously extend and DALK can be completed despite the tear. The risk of double chamber too is less compared to a DM tear. Furthermore, descriptions of “bursting of the DM” following intraoperative puncture have been reported. Once again, it is now apparent that this is more likely to be a phenomenon associated with a Type 1 BB than with a Type 2 BB.

Intraoperative signs such as the appearance of a BB from the periphery spreading centrally, absence of a white ring, rapid expansion of the BB which extends well beyond the trephine mark, a very smooth surface of the anterior wall of the BB after removal of the anterior stroma (as against the rough appearance related to broken strands of stroma of DL) are indicative of the presence of a Type 2 BB. A spatula may be inserted between the anterior wall of the BB and the deep stroma along the circumference of the trephine mark. If it passes readily in the plane the BB wall is likely to be DM and not DL. Knowledge of the presence of a Type 1 or Type 2 BB helps the surgeon to take additional precautions, such as repeated release of aqueous through the paracentesis to lower eye pressure and taking care not to touch the larger Type 2 BB with the tip of the instrument used to make the paracentesis opening, thus making the operation safer. There are no definitive tips that can predict the appearance of the Type 1 bubble but insertion of the needle or cannula as deep as possible helps. For a long time surgeons have known that a DALK eye is less susceptible to rupture following trauma compared to a PK eye. This was attributed to the retention of the recipient’s DM. However, we now know that it is the eyes where DALK has been completed with a Type 1 BB, i.e where DL is retained, that are stronger due to the strength afforded by the DL. When air was injected in the stroma of corneal buttons obtained from eyes that had undergone phacoemulsification surgery it was noted that the site of cataract incision and side port(s) remained weak and that air leaked from these sites restricting the size of the bubble formed due to loss of air pressure in the stroma. This would apply to any full thickness penetrating injury to the cornea thus telling us that DALK should be performed with caution in eyes with previous penetrating injury (surgical or traumatic) as internal leakage of air and failure to obtain a BB or one of adequate size is a definitive risk.

It is postulated that in patients with keratoconus, DL stretches along with the DM and that acute hydrops is not just due to a tear in the DM but also due to a tear or dehiscence in DL. Discontinuity of both DM and DL, in the background of abnormal collagen related to keratoconus is more likely result in sudden hydration of the stroma rather than a tear in DM alone. It is also postulated that Descemetocoeles, which are believed to offer some resistance before perforation, retain this strength due to a covering of DL. When DL is damaged or destroyed by proteolytic enzymes, the DM rapidly perforates. Anecdotal evidence to demonstrate presence of a DL covering over DM in Descemetocoeles is available. Macular dystrophy, which presents as opacities across the entire stroma, has been shown to affect DL as well. When DALK by BB technique is carried out for macular dystrophy, residual opacities are present in the retained DL with a Type 1 BB. Some surgeons have left these and complete the operation as normal and other have painstakingly dissected off DL (converting the Type 1 BB to a Type 2 BB) to remove all opacities. Thus for macular dystrophy, a Type 2 bubble may be more appropriate though the advantage of removing DL in these patients has yet to be demonstrated. In our experience and that of a high volume DALK surgeon (Dr. Tarek Katamish, Cairo) in most patients with post infectious corneal scarring: removal of the affected stroma by the big bubble technique reveals a clear (unscarred) DL. This is an interesting observation which could be related to the paucity of keratocytes in this layer, which would otherwise undergo transformation to myofibroblasts and fibroblasts following infection and inflammation and lead to scarring. Thus the knowledge of the existence of DL is informing our understanding of posterior corneal pathology.

The knowledge of this layer and understanding of its biomechanical properties in terms of its strength, flexibility and resilience has encouraged surgeons to perform innovative surgical procedures. After removal of the corneal stroma by the big bubble technique in cases with corneal scarring one surgeon (Dr. Ahmad Atef, Cairo) has successfully performed a phacoemulsification procedure with lens implant in two patients). This procedure termed the DALK-Triple procedure\textsuperscript{13} is a direct clinical application of the understanding of the biomechanical properties and strength of the DL. Drs. Agarwal A and Dua HS have developed an innovative procedure for endothelial transplant termed pre-Descemet’s endothelial keratoplasty (PDEK) wherein a donor endothelial graft is prepared by harvesting a lenticule composed of DL + DM + endothelium\textsuperscript{14}. This is relatively easier to handle, tends to scroll less and can be harvested from eyes of any age (as young as 1 year).
How this layer affects and contributes to the biomechanics of the posterior cornea remains to be determined. Experiments towards ascertaining this are underway. Unlike DL, the TM also has a network of elastic (like) fibres that are linked to the tendon of the ciliary muscle and find attachment in the corneal stroma. It is believed that the ciliary muscle tone can directly affect the TM beams and influence the flow of aqueous. Biomechanical properties of the eye are formed, both Type 1 and Type 2 is likely to reveal the extension of DL in to the core of the TM beams. Eyes with glaucoma and thin corneas are known to have greater visual field loss at presentation and greater shallowing of the cup after treatment indicating greater displacement (compliance) of the lamina cribrosa. This is attributed to the biomechanics of a thin cornea. A recent study comparing DALK with penetrating keratoplasty (PK) for keratoconus reported that incidence of high intraocular pressure and secondary glaucoma was significantly higher in patients undergoing PK. In PK DL is completely transected along the graft host junction while in DALK DL is preserved. The effect of DL on posterior corneal biomechanics and on the TM and consequently on aqueous drainage will be an area of investigation. It is our experience and observation and that of several other surgeons that during the DALK procedure escape of small air bubbles into the anterior chamber of the eye through the trabecular meshwork is a common occurrence. As DL is impervious to air and the corneo-scleral trabecular meshwork has pores, air injected into the stroma can only escape into the anterior chamber through the pores at the extreme periphery. This also supports the association between the DL and the trabecular meshwork beams.

Ongoing work on the dynamics of the big bubble formation, both Type 1 and Type 2 is likely to reveal further interesting aspects related to the microanatomy of the respective parts of the cornea and is also likely to provide information on the structure and arrangement of collagen fibres in the substantia propria (stroma) of the cornea.

REFERENCES