

Six Plus One: Pre-Descemet's Layer of the Cornea

Jamil Hasanov V*

Senior consultant in Cornea, Glaucoma and Trauma department, Zarifa Aliyeva National Eye Centre, Baku, Azerbaijan

***Corresponding Author:** Jamil Hasanov V, Senior consultant in Cornea, Glaucoma and Trauma department, Zarifa Aliyeva National Eye Centre, Baku, Yasamal, Azerbaijan.

Received: October 04, 2016; **Published:** October 26, 2016

Abstract

Purpose: To define layers of the cornea and the air bubbles that form between them in the “big-bubble” deep anterior lamellar keratoplasty.

Methods: Ninety-one donor corneascleral buttons were mounted on an artificial anterior chamber and air was forcefully injected into the stroma to achieve formation of a “big bubble” as in the deep anterior lamellar keratoplasty procedure. A pair of corneal scissors was used to cut and excise the stroma, exposing pre-Descemet's layers or Descemet membranes.

Results: We obtained pattern one (n = 58), which was the classic, circular, silver sheen of the central Descemet's membrane detached from the stroma (“big bubble”), and pattern two (n = 33), which was a combination of the big bubble and unusual bubble between Descemet's membrane and the transparent layer of corneal collagen. The air cleavage occurred between the posterior stroma and the pre-Descemet's layer in the big bubble and between the pre-Descemet's layer and the Descemet membrane in the formation of the unusual bubble that was confirmed by histology. Mechanisms used to form the air bubbles were different and independent. Pre-Descemet's layer ($9.7 \pm 4.8 \mu\text{m}$) was impervious to the air wall in the big bubble and in the unusual bubble, and this layer separated the two bubbles in pattern two.

Conclusion: Pre-Descemet's layer is the main layer in the formation of big and unusual bubbles. The stages and the characteristics of DALK with the AAC were similar to those of the human eye, and we believe that the rates of formation of big and unusual bubbles would be similar *in vivo*.

Keywords: Deep Anterior Lamellar Keratoplasty; Big Bubble; Unusual Bubble; Pre-Descemet's Layer; Artificial Anterior Chamber

Abbreviations

DALK: Deep anterior lamellar keratoplasty; DM: Descemet's Membrane, AAC: Artificial Anterior Chamber; MVR: Microvitoretinal Knife, DLEK: Deep Lamellar Endothelial Keratoplasty

Introduction

DALK can be an effective treatment for any pathology of the pre-Descemet's cornea as long as the patient has an intact and functioning endothelium. Anwar and Teichmann described a “big bubble” technique in which air is injected to detach the central DM from the stroma [1]. The procedure produces good visual results with low rates of complications and minimal risk of graft rejection [2]. During cornea fellowship in LV Prasad Eye Institute we used a training model to acquire skills and confidence for performing DALK [3]. Using this model, we observed the same features and complications of this technique and recorded simultaneous formations of the big bubble and the unusual bubble. This research was designed to define the layers and the air bubbles that form between them.

Materials and Methods

Study performed from February to April 2008 at the LVPEI. For procedure, used the AAC, 8 - mm and 9 - mm corneal trephine blades, 350- μm guarded knife, curved corneal dissector, corneal scissors, MVR knife, 27-gauge cannula, and 91 donor corneascleral buttons

(age 5 - 68 years, stored for 3 - 10 days at 4°C in McCarey Kaufman medium) not suitable for keratoplasty, that were collected from the Ramayama International Eye Bank.

Procedure

All procedures performed by one doctor (H.J.V.). The donor corneascleral button clamped onto the AAC with endothelial surface facing downwards. The chamber inflated by infusing balanced salt solution until normal pressure obtained at digital evaluation (Figure 1A and 1B). A circular template made with the 8.0 - mm or 9 - mm corneal trephine blade placed on the corneal epithelial surface (Figure 1C and 1D). The partial-thickness 4 - mm groove made by a 350- μ m guarded knife. Further stromal dissection performed with a DLEK curved corneal dissector and the anterior corneal lamella excised with corneal scissors (Figure 1E, 1F, 1G).

A nonpenetrating stromal corneal "nick" created with the MVR knife placed slightly inside the limiting ring. A blunt 27 - gauge cannula attached to a 5 - ml syringe filled with air inserted through the nick and into the residual stromal thickness. The tip of the cannula gripped by a needle holder (Figure 1H and 1I).



Figure 1: **A-B:** The donor corneascleral button clamped and fixated onto the. **C-D:** A circular template created with 8.0-mm. **E:** A partial-thickness 4-mm groove made by the 350- μ m guarded knife. **F:** Stromal dissection performed with DLEK curved corneal dissector. **G:** Anterior corneal lamella excised with corneal scissors. **H:** A 27-gauge cannula attached to a 5-ml syringe filled with air inserted into the residual stromal thickness. **I:** Tip of the cannula gripped by a needle holder.

The air forcefully injected once or a few times into the stroma until the big bubble formation achieved (Figure 2A, 2B, 2C). When the big bubble achieved, intrachamber pressure decreased and the nick created in the residual stromal bed with the MVR knife, allowing air to escape and allowing the big bubble to collapse. A pair of blunt-tip corneal scissors used to cut and excise the residual stroma (Figure 2D, 2E, 2F).

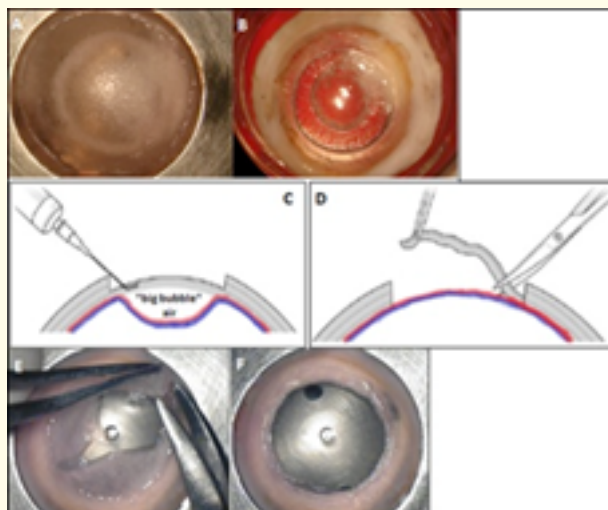


Figure 2: Formation of a big bubble. The sclerocorneal disc is shown from the epithelial (**A**) and from the endothelial side (**B**). **C:** The big bubble formed by separating a thin layer of corneal collagen (pre-Descemet's layer, red line) from the inner stroma (DM, blue line). **D-E:** The residual stroma excised by corneal scissors to expose the pre-Descemet's layer. **F:** The sclerocorneal disc from the epithelial side with a bared pre-Descemet's layer.

Surprisingly, in some cases after excising residual stroma, we found another air bubble between the DM and the transparent layer, which we called the unusual bubble (Figure 3A, 3B, 3C, 3D, 3E).

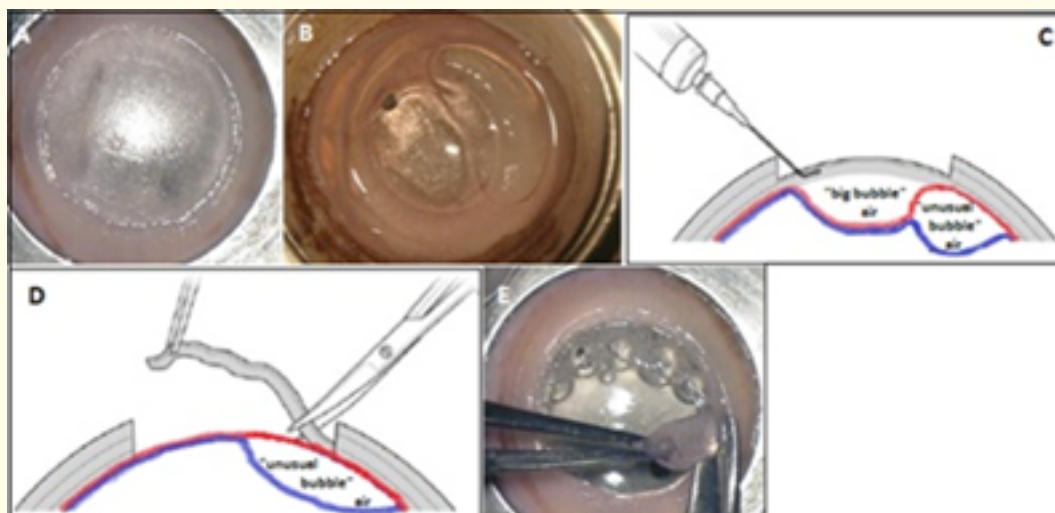


Figure 3: Formation of the big bubble and the unusual bubble. The sclerocorneal disc is shown from the epithelial (**A**) and from the endothelial side (**B**). The big bubble occupies the center of the sclerocorneal disc and is well-circumscribed and circular. The unusual bubble (crescent shape) occupies the peripheral quadrant of the sclerocorneal disc and lies over the big bubble. **C:** The pre-Descemet's layer (red line) is impervious to air and separates the big bubble and the unusual bubble. **D:** The residual stroma excised by corneal scissors to expose the pre-Descemet's layer. **E:** Transparent, thin-walled unusual bubble.

Again, a nick created in the thin layer of corneal collagen (pre-Descemet's layer) with the MVR knife, allowing air to escape and allowing the unusual bubble to collapse. Furthermore, a pair of blunt-tip corneal scissors used to cut and excise the pre-Descemet's layer to expose the DM (Figure 4A, 4B, 4C, 4D, 4E). After the procedure, corneal samples underwent histological examination. All stages of the procedure were recorded and analyzed.

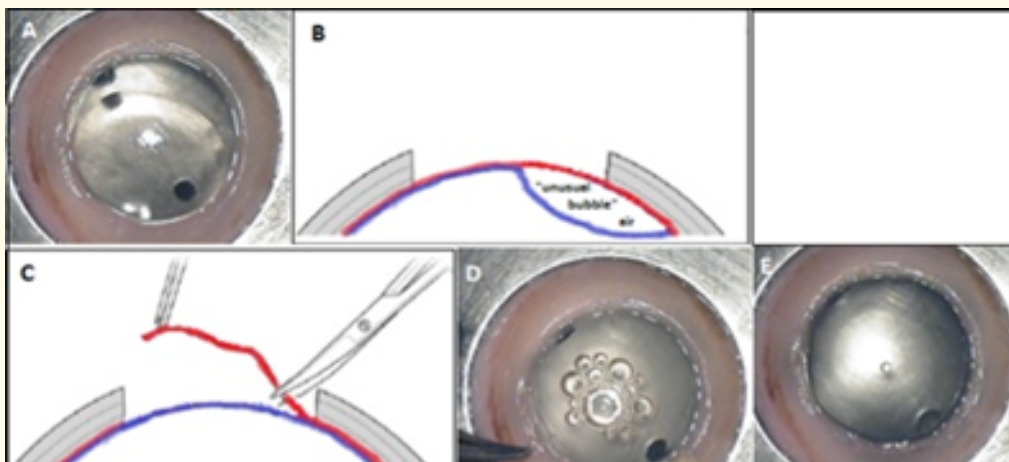


Figure 4: A-B: Transparent, thin-walled unusual bubble. C: Pre-Descemet's layer (red line) excised with corneal scissors to expose the DM (blue line). D: Pre-Descemet's layer is peeled with forceps. E: The donor corneascleral disc from the epithelial side with the bared DM.

Results and Discussion

We obtained two patterns. Pattern one (n = 58) was the classic, circular, silver sheen of the big bubble that develops in the center of the cornea and extends to the margins created by trephination by approximately 1 mm (Video 1). Pattern two (n = 33) was a combination of the big bubble and the unusual bubble. The unusual bubble was manifested in the form of a gentle wave, rapidly flowing from the limb toward the center of the cornea (Video 2). Histological examination confirmed that combination cleavage occurred between the posterior stroma and pre-Descemet's layer and also between pre-Descemet's layer and the DM in pattern two (Figure 5A, 5B, 5C). Pre-Descemet's layer measured $9.7 \pm 4.8 \mu\text{m}$.

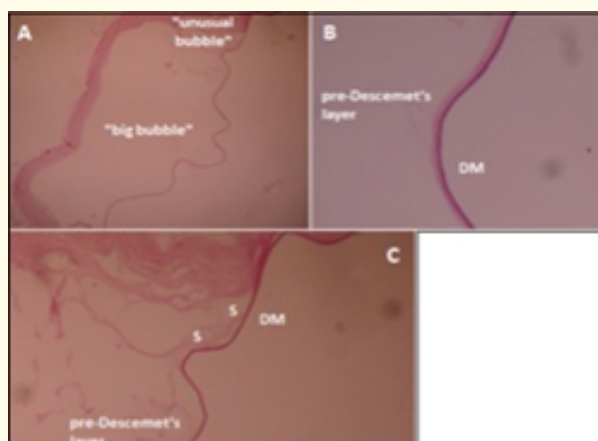


Figure 5: A: Light photomicrograph of resin section stained with hematoxylin and eosin showing a big bubble and an unusual bubble (pattern two). B: DM can be seen on the posterior surface of the pre-Descemet's layer. C: Strands of collagen bundles (S) are seen bridging the stromal bed and the posterior surface of the pre-Descemet's layer.

Collagen fibrils in the human stroma had a uniform diameter of 25 to 35 nm and ran parallel to each other with somewhat regular spacing to form flat lamellar bundles [4]. The posterior lamellae bundles of the stroma are wider and thicker (100–200 μ m wide and 1.0–2.5 μ m thick) than the anterior (0.5–30 μ m wide and 0.2–1.2 μ m thick). They arranged parallel to the plane of the corneal curvature and course directly across the full width of the cornea without a break, having their origins in fibers that wind around the limb at the corneoscleral junction [4-7]. Collagen fibrils in the innermost layer of the stroma were not aligned in bundles. Individual fibrils run in various directions and are interwoven to form a thin sheet of collagen networks [4]. The attachment of the DM to the posterior stroma appeared to be accomplished, in part, by fibers 22.3 nm in diameter that ran perpendicular to the DM [8]. The depths of penetration of the fibers into the DM range from 0.16 to 0.21 mm [8]. They are frequently associated with a dense, amorphous mass (approximately 0.5 μ m) at the interface between the DM and the posterior stroma [4]. Although these fibers were found across the entire cornea, they were observed more frequently in the peripheral third [8]. Similar to other techniques of dissection DM from the stroma, DALK with the big bubble technique is likely to leave a thin layer of the corneal stroma in place [9-13]. We obtained 58 in pattern one and 33 in pattern two (big bubble and unusual bubble combination) big bubble formations where after excision stroma observed thin layer of the corneal stroma over DM (Figure 2). In pattern two only after excision corneal stroma and a thin layer corneal collagen we observed clear DM (Figure 4).

On 91 corneascleral buttons clearly demonstrated that a big bubble is formed by cleavage occurring in a plane along the pre-Descemet's layer and the stroma as compared with the unusual bubble ($n = 33$), which is formed by the separation of the DM only. Histological examination is showing a big bubble and an unusual bubble (pattern two), where DM located on the posterior surface of the pre-Descemet's layer (Figure 5).

During the DALK big bubble technique, the cornea cleaves not between the DM and the stroma but rather between the most posterior layer of the corneal stroma and the overlying majority of the stroma, thus helping to prove our results [14].

We detected different and independent mechanisms for formation of the air bubbles. During formation of the big bubble, accumulation of air in the plane between pre-Descemet's layer and the stroma increases the air pressure, which helps the cleavage. The cleavage circularly and equally begins to grow from the center of the cornea to the limb. Under a surgical microscope, the process looks like a semi-opaque ring that expands from the center of the cornea toward the periphery and has a white outline (**Video 3**). The same thing is happening *in vivo*. The diameter of the big bubble or cleavage depends on the air pressure (increasing air pressure increases the air bubble size), the diameter of the lamellar keratectomy created by partial-thickness trephination, which is performed before injecting air (the larger the diameter of the lamellar keratectomy, the less supporting tissue), and the diameter of the recipient cornea (the larger the diameter of the recipient cornea, the greater the distance to the limbus). We can assume since adhesion of the innermost lamellae of the stroma in the periphery much tighter than in the center, the diameter of the big bubble ($n = 91$) in the study was ≤ 9.5 mm [8].

During the formation of the unusual bubble, the air that reaches the corneoscleral zone (for DALK surgeons, any sector typically in the range from 4 to 8 o'clock) with high speed spreads along the limbal zone and accumulates there with increasing air pressure, creating cleavage between the DM and pre-Descemet's layer (for DALK surgeons, any sector usually in the range from 10 to 2 o'clock). Cleavage begins to grow from the limb to the center of the cornea in the form of an increasing wavelength, the size of which depends on air pressure (**Video 4**). The formation of an unusual bubble is much faster than that of a big bubble. *In vivo*, identification and evaluation of the splitting without using special equipment (ultrasound biomicroscopy or anterior segment optical coherence tomography) are difficult.

In pattern two pre-Descemet's layer was common and impermeable to the air wall that separates the big and unusual bubbles, strongly indicating that it is a sovereign layer. We achieved 91 big bubbles, because of the impermeability of air through the pre-Descemet's layer, and only 33 unusual bubbles because ingress of air at the periphery which led separation DM from pre-Descemet's layer. This phenomenon could suggest the air permeability through the pre-Descemet's layer at the limbus than in the center of the cornea.

The cornea comprises six layers: the epithelium; the basement membrane of the epithelium; Bowman's layer, as a modification of the anterior stroma; the stroma proper; DM, the large basement membrane of the endothelium; and the endothelium [15]. The corneal structure including the innermost part of the stroma, the pre-Descemet's layer, has been described previously [4,8,14]. To our knowledge, this is the first study to use 91 donor corneascleral buttons and AACs where in 33 have been separated the pre-Descemet's layer from the stroma and the DM to show its role in the formation of the big bubble and of the unusual bubble. We can assume that the stroma is covered with cell-free layers in a sandwich pattern outside by Bowman's layer and inside the pre-Descemet's layer. Despite its small thickness, such a structure provides the cornea with resistance to various influences. A modern concept of the structure of the cornea suggests the following seven layers: the epithelium; the basement membrane of the epithelium; Bowman's layer; the stroma proper; pre-Descemet's layer; DM; and the endothelium.

Conclusion

Pre-Descemet's layer is the main layer in the formation of big and unusual bubbles. The stages and the characteristics of DALK with the AAC were similar to those of the human eye, and we believe that the rates of formation of big and unusual bubbles would be similar *in vivo*.

Conflict of interest

I don't have to disclose.

Bibliography

1. Anwar M and Teichmann KD. "Big-bubble technique to bare Descemet's membrane in anterior lamellar keratoplasty". *Journal of Cataract & Refractive Surgery* 28.3 (2002): 398-403.
2. Luengo-Gimeno F, *et al.* "Evolution of deep anterior lamellar keratoplasty (DALK)". *The Ocular Surface* 9.2 (2011): 98-110.
3. Fontana L, *et al.* "Experimental model for learning and practicing big-bubble deep anterior lamellar keratoplasty". *Journal of Cataract & Refractive Surgery* 34.4 (2008): 710-711.
4. Komai Y, *et al.* "Three-dimensional organization of collagen fibrils in the human cornea". *Investigative Ophthalmology & Visual Science* 32 (1991): 2244-2258.
5. Newton RH and Meek KM. "Circumcorneal annulus of collagen fibrils in the human limbus". *Investigative Ophthalmology & Visual Science* 39.7 (1998): 1125-1134.
6. Daxer A and Fratzl P. "Collagen fibril orientation in the human corneal stroma and its implications in keratoconus". *Investigative Ophthalmology & Visual Science* 38.1 (1997): 121-129.
7. Kokott W. "Über mechanisch-funktionelle Strukturen des Auges". *Albrecht Von Graefe's Archive for Ophthalmology* 138.4 (1938): 424-485.
8. Binder PS, *et al.* "High-voltage electron microscopy of normal human cornea". *Investigative Ophthalmology and Visual Science* 32.8 (1991): 2234-2243.
9. Manche EE, *et al.* "Deep lamellar keratoplasty using viscoelastic dissection". *Archives of ophthalmology* 117.11 (1999): 1561-1565.
10. Melles GR, *et al.* "A quick surgical technique for deep, anterior lamellar keratoplasty using visco-dissection". *Cornea* 19.4 (2000): 427-432.

11. Sugita J and Kondo J. "Deep lamellar keratoplasty with complete removal of pathological stroma for vision improvement". *British Journal of Ophthalmology* 81.3 (1997): 184-188.
12. Jafarinasab MR, *et al.* "Dissection plane in deep anterior lamellar keratoplasty using the big-bubble technique". *Cornea* 29.4 (2010): 388-391.
13. McKee HD, *et al.* "Residual corneal stroma in big-bubble deep anterior lamellar keratoplasty: a histological study in eye-bank corneas". *British Journal of Ophthalmology* 95.10 (2011): 1463-1465.
14. Dua HS, *et al.* "Human corneal anatomy redefined: a novel pre-Descemet's layer (Dua's layer)". *Ophthalmology* 120.9 (2013): 1778-1785.
15. McTigue JW. "The human cornea: a light and electron microscopic study of the normal cornea and its alterations in various dystrophies". *Transactions of the American Ophthalmological Society* 65 (1967): 591-660.

Volume 4 Issue 1 October 2016

© All rights reserved by Jamil Hasanov V.