Eye (2018) 32, 146–151

www.nature.com/eye

LABORATORY STUDY

Air pressure changes in the creation and bursting of the type-1 big bubble in deep anterior lamellar keratoplasty: an *ex vivo* study

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Abstract

Purpose **To measure the pressure and volume of air required to create a big bubble (BB) in simulated deep anterior lamellar keratoplasty (DALK) in donor eyes and ascertain the bursting pressure of the BB.**

Patients and methods **Twenty-two human sclera-corneal discs were used. Air was injected into the corneal stroma to create a BB and the pressure measured by means of a pressure converter attached to the system via a side port. A special clamp was designed to prevent air leak from the periphery of the discs. The pressure at which air emerged in the corneal tissue; the bursting pressure measured after advancing the needle into the bubble cavity and injecting more air; the volume of air required to create a BB and the volume of the BB were ascertained.**

Results **Type-1 BB were achieved in 19 and type-2 BB in 3 eyes. The maximum pressure reached to create a BB was 96.25+/− 21.61 kpa; the mean type-1 intrabubble pressure was 10.16 +/− 3.65 kpa. The mean bursting pressure of a type-1 BB was 66.65 +/− 18.65 kpa, while that of a type-2 BB was 14.77 +/− 2.44 kpa. The volume of air required to create a type-1 BB was 0.54 ml and the volume of a type-1 BB was consistently 0.1 ml.**

Conclusions **Dua's layer baring DALK can withstand high intraoperative pressures compared to Descemet's membrane baring DALK. The study suggests that it could be safe to undertake procedures such as DALK-triple with a type-1 BB but not with a type-2 BB.**

Eye (2018) **32,** 146–151; doi:10.1038/eye.2017.121; published online 30 June 2017

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Received: 15 January 2017
Accepted in revised form: 4 May 2017
Published online: 30 June 2017

Introduction

Deep anterior lamellar keratoplasty (DALK) has replaced penetrating keratoplasty as the procedure of choice in surgical management of eyes with diseases affecting the corneal stroma and affecting sight such as scars, dystrophy or ectasia. The big bubble (BB) technique[1] is the most popular technique wherein air is injected in the corneal stroma to separate either the Descemet's membrane (DM) or the DM together with a layer of deep corneal stroma termed the pre-Descemet's layer (Dua's layer–DL). This allows excision of the affected stroma and recipient epithelium and replacement with healthy stroma and epithelium from a cadaver donor.

When air is injected in the corneal stroma, it either cleaves the DL from the deep stroma to create a big bubble termed type-1 or it accesses the plane between DM and DL to create a thin walled bubble termed type-2. The wall of a type-1 BB is made of DL and DM, while that of a type-2 BB is made of DM alone and is more vulnerable to major tears or bursting during surgery. Often during injection of air, tiny bubbles escape from the peripheral cornea, in the vicinity of the trabecular meshwork, into the anterior chamber and can cause post-operative raised intraocular pressure.[2–4]

Dua *et al*[5] have reported that DL is a strong and resilient layer with bursting pressure 1.45 bars. On the basis of the above information, Zaki AA *et al*[6] described a combination of DALK with phacoemulsification and lens implant, termed the DALK-Triple procedure. When confronted with patients requiring DALK, who also had dense cataracts, they were able to perform cataract surgery under the exposed DL of a type-1 BB. They reported that DL could withstand all pressure fluctuations associated with the phacoemulsification procedure, and that, despite stromal scarring requiring keratoplasty, the DL was remarkably clear in most cases.[6] In one instance, they attempted DALK-Triple under the DM (type-2 BB), which burst promptly during injection of viscoelastic in the anterior chamber.

In this study, we report the pressure and volume of air required to create the BB, the volume and pressure of air in the type-1 BB and the bursting pressure of the type-1 BB.

Materials and methods

Tissue samples

Twenty-two human sclera-corneal discs from eye bank donor eyes that were not suitable for transplantation were used. The sclera-corneal discs were maintained in organ culture in Eagle's minimum essential medium with 2% foetal bovine serum for 4–8 weeks post-mortem. Donor details are given in Table 1.

Experiment to measure pressure

Air injection The sclera-corneal disc was placed endothelial side up in a petri dish and kept moist with balanced salt solution. In fifteen samples, under an operating microscope, a 30 gauge needle, bent to an angle of 135°, bevel up, attached to a 20 ml syringe was passed from the scleral rim into the corneal stroma and advanced to the centre of the disc. The needle was passed close to the endothelial surface without perforating it. Air was injected with force to overcome the tissue resistance, until a big bubble was formed. The type of the bubble was determined, type-1 or type-2. The position of the needle tip was kept constant in the centre of the sclera-corneal disc in mid stroma.

Pressure measurement An electronic pressure gauge/converter device was used (Keller, K-114, Winterthur, Switzerland). The tube from the device was linked to the side arm of a three-way cannula attached between the syringe and needle. The injecting needle was attached to the front end of the cannula and a 20 ml syringe to the other end. The device was also connected to a personal computer (PC) via a USB port. The USB link also powered the device. Pressure readings were recorded in real time and transmitted as serial RS485 half-duplex signals to the PC, where the pressure was displayed as a continuous trace on the screen by the software associated with the K-114 device. (Figure 1) The pressure recorded was that in the syringe during injection of air. In validation experiments, when the needle was not inserted in tissue and the piston was advanced rapidly, the pressure recorded was between 0 and 1, indicating that the resistance offered by the needle was not relevant to the pressures measured (data not shown).

The maximum pressure required to create the bubble was recorded. The plunger was then released and allowed to attain a stable position. The needle tip was advanced to lie inside the BB and the bubble inflated till its wall was

Table 1 Donor details for the sclera-corneal discs used in the experiments

Sample no.	*Eye bank no.*	*Type of big bubble (BB)*	*Sex*	*Age*	*Date of death*	*Cause of death*
E1955	M20599B	T1BB	F	67	08/05/2014	Stroke
E2168	M21433B	T1BB	F	60	29/12/2014	Other (unknown)
E2182	M21468A	T1BB	F	58	07/01/2015	Cancer
E2183	M21468B	T1BB	F	58	07/01/2015	Cancer
E2246	M21715A	T1BB	F	69	15/03/2015	Chronic obstructive pulmonary disease
E2187	M21447B	T1BB	F	65	02/01/2015	Pending
E2385	M22280B	T1BB	M	73	29/06/2015	Respiratory failure
E2347	M22237A	T1BB	F	52	17/06/2015	Encephalopathy
E2278	M22072B	T1BB	F	80	07/05/2015	Sepsis
E2276	M22016B	T1BB	M	74	01/05/2015	Brain damage hypoxia
E2275	M22016A	T1BB	M	74	01/05/2015	Brain damage hypoxia
E2309	M21828B	T1BB	M	72	02/04/2015	Cronic obstructive pulmonary disease
E2326	M22034A	T2BB	F	75	04/05/2015	Myocardial infarction
E2348	M22237B	T2BB	F	52	17/06/2015	Encephalopathy
E2384	M22333A	T2BB	F	68	14/07/2015	Myocardial infarction
E2677	M22933B	T1BB	F	81	29/12/2015	Myocardial infarction
E2675	M22956B	T1BB	M	53	02/01/2016	Unknown
E2674	M22956A	T1BB	M	53	02/01/2016	Unknown
E2678	M22913A	T1BB	F	44	14/12/2015	Intracranial heamorrhage
E2679	M22913B	T1BB	F	44	14/12/2015	Intracranial heamorrhage
E2829	M23226B	T1BB	M	80	14/03/2016	Cancer
E2836	M23301B	T1BB	F	88	03/04/2016	Old age

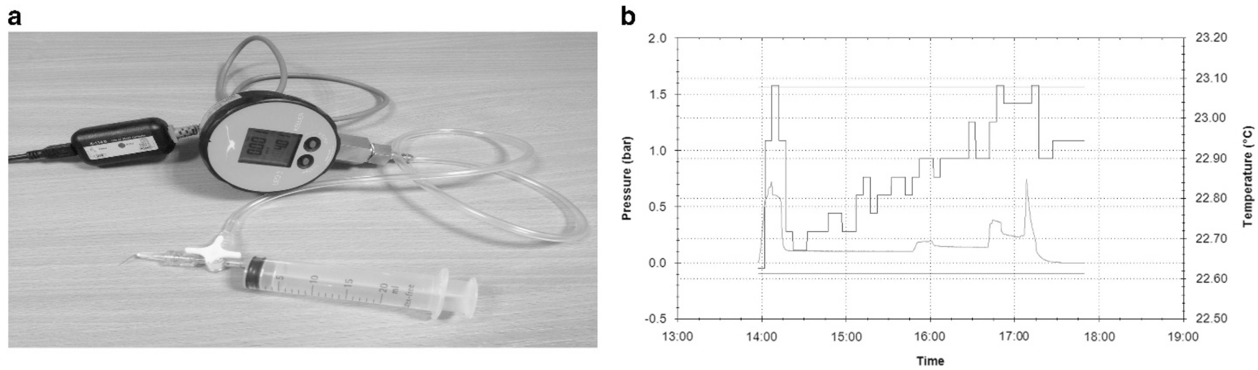

Figure 1 (a) Pressure converter system K-144, (b) Real pressure record over time (red graph), the temperature of the pressure sensor (blue graph), the maximum pressure (pink graph), the minus pressure (green line). A full colour version of this figure is available at the *Eye* journal online.

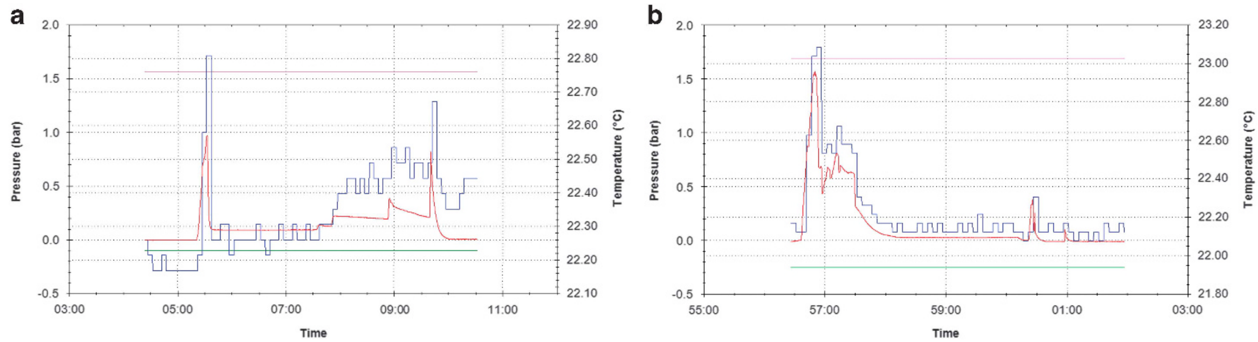

Figure 2 (a) Pressure change over time (red line) in T1BB. (b) Pressure change over time (red line) in T2BB.

taut. The pressure recorded at this point was taken as the base line intrabubble pressure. With the needle tip in the BB, the piston was pushed further with force until the BB burst. This recorded the bursting pressure of the bubble (Figures 2a and b).

Experiment to measure volume

As air leaked through multiple points along the circumference of the corneal periphery, a clamp was designed to block the holes and stop air leak. In seven samples, the sclera-corneal discs were clamped in a circular clamp of 10 mm diameter that prevented air escape from the periphery. A 30 gauge needle attached to a 1 ml syringe (internal diameter 5 mm) filled with air was passed into the corneal stroma from the scleral rim as described above. During injection, the maximum compression of air (position of piston) at the time air just started to appear in the corneal stroma was recorded. The piston was held in place until a type-1 BB was formed. The pressure on the piston was released and piston was allowed to reverse to a stable position. The volume of air lost in the cornea was ascertained from the final position of the piston. The BB diameter was measured with a pair of surgical callipers. The needle was then advanced into the BB and all the air aspirated until the BB had completely collapsed. This provided a measure of the volume of air in the big bubble. The pressure (above atmosphere) in the syringe at the point where air started to emerge in the tissue from the needle tip was deduced by the formula $P1V1 = P2V2$, where P1 is the initial pressure (atmospheric) and V1 the initial volume (1 ml), and P2 is the final pressure (unknown) and V2 the final volume (mean 0.54 ml, see results).

Results

The average age of donors was 66 years (range; 52–80 years). There were 15 females and 7 males.

Pressure measurements

Twelve type-1 and 3 type-2 BB were obtained (Table 2). The mean pressure attained to create a BB was 96.25+/− 21.61 kpa (range 90–130 kpa). For type-1 BB, the mean intrabubble pressure was 10.16 +/− 3.65 kpa (range 5.2–18 kpa) and the bursting pressure was 66.65 +/− 18.65kpa (range 40–110kpa). The median bursting pressure was 68.5 kpa (Table 2). Accurate measurements of type-2 BB could not be obtained as when advancing the

Table 2 Measurements of the big bubble

Sample no.	Diameter (mm)	Intrabubble pressure (Kpa)	Bursting pressure (kpa)
T1BB			
E1955	nm	nm	45
E2168	7	12	60
E2182	9	13	80
E2183	8.5	14	73
E2246	8.5	11.6	66
E2187	8.5	18	40
E2309	8.5	7.5	110
E2275	8.5	7.5	78
E2276	8.5	7.5	55
E2278	nm	5.2	71
E2347	8.5	6.8	76.8
E2385	8.5	8.7	45
T2BB			
E2326	10	nm	17
E2348	10.5	nm	12
E2384	10.5	nm	12.7

Abbreviations: Kpa, kilopascal; nm, not measured.

needle into the bubble cavity, while the needle was still in the stroma, the type-2 BB burst in one case and the DM disinserted (separated along its peripheral attachment to the stroma) in one sector before the bubble could be inflated enough to make the DM taut. The mean pressure at the time of the type-2 BB burst/disinserted was 14.77 +/− 2.44 kpa (range 12.0–17.0 kpa) (Table 2).

Volume measurements

In the bubble volume experiment, the maximum compression of air required to create type-1 BB was 0.54 +/− 0.07 ml (range 0.5–0.7 ml), the volume of air lost in the cornea was 0.38 +/− 0.06 ml (range 0.3–0.5 ml) and the average volume of the BB was 0.1 ml. The mean pressure in the syringe at which air started to emerge in the tissue, as calculated from the volume compression, was 131.82+/− 50.58 kpa (range 101.28–236.3 kpa above atmosphere). The difference in pressures measured directly with the gauge and by this method were not statistically significant ($P=0.25$) (Figure 3).

Statistical methods

The data was normally distributed as confirmed by Levene's test. Statistical analysis between two groups was performed by the unpaired student *t*-test using Graphpad prism version 5.0. (Graphpad software, La Jolla, CA, USA). $P<0.05$ was considered statistically significant.

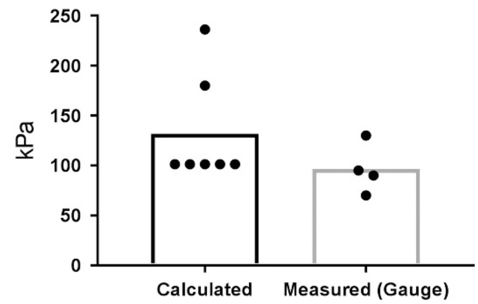

Figure 3 Compares the pressure calculated from the volume compression of the syringe and that measured directly with gauge ($P=0.25$).

Discussion

In DALK by the BB technique, when air is injected in the corneal stroma, a type-1 BB forms by air cleaving in the plane of deep stroma and DL, with a posterior displacement of DL and DM. The cleavage and displacement are related to the pressure of air in the corneal stroma and in the BB. As the BB expands posteriorly, the intrabubble pressure is countered by the intraocular pressure, which can rise up to 70 mm of mercury (authors' unpublished observations). This counter pressure and the closed space within which the BB expands limits the posterior expansion of the BB in the eye thus rupture of a type-1 BB during inflation is unlikely and has not been reported. However, when the type-1 BB is deflated and the corneal stroma anterior to it is removed, the DL+DM bulge anteriorly to assume a convex dome shape. Any pressure applied to the DL+DM from within the eye, as during the DALK-triple procedure, would cause the layers to expand outward, into the atmosphere and theoretically reach a bursting point. In this study, we set out to ascertain the minimum and mean popping (bursting) pressure of the layers to establish whether it would always be safe to perform cataract surgery under DL+DM after creating a type-1 BB.

The pressure converter K-114 allowed us to measure in real time the pressure at the tip of the needle during the creation of a BB. On initiation of injection, air is compressed in the syringe on account of the tissue resistance offered by the corneal stroma at the site of the tip of the needle. Once this is overcome, air starts to enter the stroma separating the lamellae, and the intrastromal pressure builds up as the cornea gets completely aerated. At a critical tissue pressure, the air forces its way to the plane anterior to DL and cleaves this away from deep stroma as a type-1 BB. The volume of air required to achieve the critical tissue pressure depends on the escape of air through the trabecular meshwork or through distinct peripheral holes in the stroma, during injection.[7–9] This confounder was eliminated by the use of the clamp, which prevented any escape of air, thus giving us an accurate measure of the mean tissue pressure required to create a BB overcoming tissue resistance, which was 96.25 +/− 21.61 kpa. It has been recently demonstrated that air injected in the corneal stroma follows a consistent path regardless of the location, direction of bevel, and depth of the needle tip in the stroma.[9]

Once a type-1 BB was created, the intrabubble pressure was ascertained by advancing the needle into the cavity of the BB. This measured 10.16 +/− 3.65 kpa. In the *ex vivo* situation of this study, it was possible to expand the type-1 BB to its bursting point by continued forceful injection of air with the needle positioned in the cavity of the bubble. This situation would simulate increased intraocular pressure exerted on the layers during phacoemulsification carried out under the layers (DALK-triple). The lowest pressure at which a type-1 BB burst was 40 kpa and the highest was 110 kpa. The mean bursting pressure was 66.65 +/− 18.65 kpa. Although we reported the bursting pressure in our original paper,[5] we refined the measurement by placing the needle tip in the type-1 BB while increasing the pressure to the bursting point. This approach eliminated any variations induced by the resistance of the stroma to the passage of air. Any effect of variable leakage of air from the periphery of the sclera-corneal disc was prevented by the use of the clamp. In addition, the accuracy of the measurements was enhanced by using the continuous digital pressure recording device.

A number of studies have reported the variations in intraocular pressure during phacoemulsification. By direct measurements during surgery, Zhao Y *et al*[10] found that the IOP fluctuated from 13–96 mm Hg (1.8–13.5 kpa). Khng C *et al* stated that IOP exceeded 60 mm Hg (8.4 kpa) and the highest IOP occurred during hydro-dissection, viscoelastic injection and intraocular lens insertion.[11] Vasavada V *et al* compared the impact of different fluidic parameters on intraoperative IOP and found that the minimum IOP in the low and high parameters groups was 35 mm Hg (4.9 kpa) and 34.5 mm Hg (4.8 kpa), respectively, and the maximum IOP in the low and high parameters groups was 69 (9.7 kpa) and 85 (11.9 kpa) mm Hg respectively.[12] In another study, Kamae KK *et al*[13] monitored IOP during IOL implantation and found that the mean and peak IOPs exceeded 60 mm Hg (8.4 kpa) during IOL implantation. In comparison, the data on bursting pressure of the DL+DM generated in this study show that the pressures attained during cataract surgery are several times less than what is required to burst the layers under which phacoemulsification can be carried out in the DALK-triple procedure. Even the lowest bursting pressure had a safety margin of over 25 kpa (177.5 mm Hg) compared to the highest pressure reached during phacoemulsification. This would indicate that DALK-triple is a viable option with regard to the risk of inadvertent rupture of the DL+DM layers intraoperatively.

When cataract and DALK surgery are required simultaneously, if the cornea is clear, one could consider performing phacoemulsificaton as the first step and DALK as the second step of the same procedure. However, when the cornea is scarred to an extent that visualisation is poor, a triple-DALK would be the preferred option. With triple-DALK, when air injection fails to produce a type-1 BB, manual dissection allows access to the plane between the deep stroma and DL. Once the opaque cornea, related to the aeration of the stroma anterior to the DL, is removed, the transparent DL allows phacoemusification to be carried out.

We were able to create both type-1 and type-2 BB, as reported by Dua *et al*; however, the type-1 BB was more consistent, occurring in 86.4% of the 22 sclera-corneal discs. The data provided in this study can help us develop an automated system whereby we can produce big bubbles *in vivo* with improved consistency.

Summary

What was known before

- Dua's layer is a tough, resilient and elastic layer, which can be bared during DALK by the pneumo-dissection big bubble technique.
- Reports showed that DALK-triple procedure can be performed safely with phacoemulsification by baring Dua's layer due to its resiliency and strength.

What this study adds

- The maximum pressure required to burst Dua's layer is less than that reached during phacoemulsification surgery, which makes it possible to perform DALK-triple safely.
- Descemet's membrane bursts easily at a very low pressure. This makes it risky to attempt the DALK-triple with type-2 BB because the intraocular pressure during phacoemulsification is higher than what can be withsood by the Descemet's membrane.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was funded by The Royal College of Surgeons, Edinburgh, Scotland, Royal Blind Scotland and The Elizabeth C King Trust, Pittsburgh, USA.

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