Comparison of corneal densitometry between big-bubble and visco-bubble deep anterior lamellar keratoplasty

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ABSTRACT

Aims To evaluate deep corneal densitometry and visual outcomes after big-bubble (BB-DALK) and visco-bubble (VB-DALK) deep anterior lamellar keratoplasty performed in patients with keratoconus.

Methods Prospective comparative study of 50 advanced keratoconic patients who underwent DALK surgery; 25 eyes (group I) were completed with BB-DALK and 25 eyes (group II) with VB-DALK after the failure of pneumatic dissection. Best spectacle-corrected visual acuity (BSCVA), corneal tomographic parameters and endothelial cell count were recorded 1, 3, 6, 12 and 24 months after surgery. Densitometric analysis of the deep corneal interface was obtained using Scheimpflug tomography at each visit; values recorded were compared between the two groups and statistically analysed.

Results BSCVA was significantly better in the BB-DALK group than the VB-DALK group (0.39±0.29 vs 0.65±0.23 logarithm of the minimum angle of resolution, respectively) for the first 3 months; and in the same time period, densitometry was significantly higher in the VB-DALK group than those recorded in the BB-DALK group (23.97±5.34 vs 17.13±4.44 grayscale units). However, densitometric values and visual acuity did not differ significantly in the two groups at 1 year. No statistically significant difference for the other variables analysed at any time frame was found.

Conclusion The use of viscoelastic substance in the VB-DALK technique may induce modification of interface stromal reflectivity resulting in reduced visual acuity up to 3 months postoperatively. However, this initial negative effect on the interface quality does not affect the long-term visual outcome, with densitometric values and visual outcomes similar in the two groups from 6 months postoperatively.

INTRODUCTION

Deep anterior lamellar keratoplasty (DALK) is increasingly favoured over penetrating keratoplasty (PK) in the surgical treatment of anterior corneal disease because it offers similar visual outcomes while preserving healthy endothelium. The main advantages of DALK over PK include elimination of endothelial immunological rejection and complications related to 'open sky' surgery.[1] However, perhaps the largest barrier to adopting DALK over PK has been the technical challenge encountered in achieving successful dissection of the anterior stroma from the predescemetic layer (PDL).

Various surgical approaches have been described to successfully achieve lamellar separation of the anterior stroma, including manual dissection,[1] pneumatic dissection,[2 3] saline injection[4] or the use of viscoelastic substance.[5–7]

Big-bubble (BB-DALK) is the most widely adopted technique, which allows the exposure of PDL or Descemet's membrane (DM) by means of air injection between anterior stroma and PDL or DM.[8 9] In cases whereby pneumatic dissection fails, a visco-bubble (VB-DALK) technique has been proposed as a valid alternative to manual dissection, achieving the exposure of PDL in a very high rate of cases.[6 10–12]

Although both techniques achieve separation of the same deep plane, in a previous study, we reported a statistically significant difference in terms of visual recovery between two groups of eyes dissected with ophthalmic viscoelastic substance (OVD) and those dissected with air.[12] This finding was limited to the first few months postoperatively, and we hypothesised a potential transient modification of the interface between donor stroma and host PDL, induced by minimal persistence of OVD.

In order to verify this hypothesis, we undertook a densitometric analysis of the host-donor interface by means of Scheimpflug tomography (Pentacam HR; Oculus, Wetzlar, Germany). This non-contact method provides measurements of corneal topography, pachymetry and densitometry, and has been used in the past to detect a difference in backscatter light and monitoring corneal transparency.[13] In this study, we compared the results between two groups of eyes which underwent a BB-DALK or VB-DALK during planned DALK keratoconus.

MATERIAL AND METHODS

In this prospective comparative study, we included 50 consecutive keratoconic patients intolerant to spectacles and/or contact lens who underwent a BB-DALK (group I=25 consecutive eyes) or VB-DALK after the failure of pneumatic dissection (group II=25 consecutive eyes). All surgery was performed between March 2014 and September 2016 at the University of Magna Graecia, Catanzaro, Italy. Patients with full-thickness corneal opacities, previous ocular surgery, glaucoma, cataract, amblyopia, retinal diseases or any prior ocular trauma were excluded.

All patients underwent complete ophthalmic examination, including slit-lamp examination, best

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spectacle-corrected visual acuity (BSCVA), refraction, tonometry, funduscopy and endothelial specular microscopy (EM-3000; Tomey, Erlangen, Germany). Visual acuity values were converted from Snellen to logarithm of the minimum angle of resolution (LogMAR) units for statistical analysis. Corneal tomography and pachymetry were obtained using a high-resolution rotating Scheimpflug camera (Pentacam HR, Oculus). An add-on tool of the standard software of the Pentacam system was used to generate corneal densitometry maps. The programme captured 25 images (1003×520 pixels) over different meridians; first identifying the corneal apex, and then analysing an area from the centre up to a maximum of 12 mm total in diameter. Densitometric measurements were expressed in standardised grayscale units, ranging from a minimum light scatter of 0 (maximum transparency) to a maximum light scatter of 100 (minimum transparency), according to a previously described scale.[14 15]

Layer densitometry may be calculated for four concentric radial zones: 0–2 mm; 2–6 mm; 6–10 mm and 10–12 mm. Furthermore, based on corneal depth, further subanalyses can be conducted on the anterior (superficial 120 µm), central (between anterior and posterior cut-off depths) and posterior stroma (deep 60 µm); these are the only three depths of analysis available in the default software settings and are not modifiable by the operator. In this study, we measured data of the posterior scans for only the first two zones, excluding those of the relative peripheral area (6 to –10 and 10 to –12 mm). The peripheral values were excluded because they are outside the optical zone and potentially include areas affected by potential artefact such as corneal scarring. The anterior and central depth values were excluded because we were interested in the interface between donor and host, which is within the deep 60 µm. The corneal densitometric values were compared between the two groups.

All surgeries were performed by a single surgeon (VS) according to standardised technique widely described in the past.[16]

All surgical procedures were performed under local anaesthesia obtained with a peribulbar injection of a mixture of lidocaine hydrochloride 2% and bupivacaine hydrochloride 0.5%. Shortly before surgery, a single drop of tropicamide 1% (Visumidriatic 1%; Visufarma Spa, Italy) was instilled into the lower fornix to obtain mydriasis. After marking the geometric centre of the cornea, a disposable Hessburg-Barron suction trephine (Katena Products, Denville, New Jersey, USA) was used to create a circular incision in the recipient cornea, usually 8.75–9.00 mm in diameter and approximately 80% of the corneal thickness in depth; the peripheral pachymetric map obtained before surgery by corneal tomographer served as a reference. A Melles blunt dissection spatula (D.O.R.C., Zuidland, the Netherlands) was used to enter the stroma at the base of the corneal incision and advanced centripetally; the triangled spatula was then exchanged with a blunt spatula (Model AE-2900; Asico, Illinois, USA) that was further advanced 1 mm along the same plane. Then, a blunt 27-gauge Fontana cannula (Janach srl, Italy), connected with a 5 mL Luer-lock syringe filled with air, was inserted into the path previously created and air was gently injected until a big bubble was formed and reached the edge of trephination. A temporal paracentesis was created to reduce the intraocular pressure, and the pupil was constricted with intracameral injection of acetylcholine chloride (Miovisin 20 mg; Farmigea Spa, Italy). In cases of successful bubble formation, the superficial stroma was manually dissected with a crescent knife and removed. The residual bed was punctured with a 30° blade to collapse the bubble, and blunt-tipped corneal scissors were used to divide the residual stroma into four quadrants, which were then excised baring the posterior lamella completely. The donor cornea was punched from the endothelial side with a Barron donor punch (Katena Products) to the same diameter as the trephination. The endothelium and DM were gently stripped off with a dry Weck-Cel sponge after staining with 0.06% trypan blue dye (VisionBlue; D.O.R.C.). Four interrupted 10–0 nylon sutures secured the graft on the recipient bed; finally, surgery was completed with 2 running 10–0 nylon sutures. When pneumatic dissection failed, the procedure was continued attempting at obtaining a bubble formation by means injection of OVD (I-SPACE Laboratoires VIVACY sodium hyaluronate 1,55%, La Ravoire, France) according to the following surgical steps: removal of about 80% of the anterior emphysematous tissue by means of hand dissection; insertion of a 27-gauge anterior chamber cannula, connected to a syringe containing OVD into the same track previously used for the air injection; gentle injection of the OVD into the deep stroma until a bubble was noted and its extension up to the trephination edge. Once the bubble had formed, all the remaining surgical steps and postoperative management did not differ from those of a BB-DALK; suture removal was completed in all patients between 10 and 12 months after the surgery.

The patients were divided into two groups depending on the surgical technique employed to expose the PDL or DM plane; group I (n=25) included eyes completed with BB-DALK; group II (n=25) comprised eyes completed with OVD injection (VB-DALK) after the initial failure of pneumatic dissection. All operations were recorded and the videos reviewed postoperatively to confirm classification of the type of bubble obtained using Dua's classification.[17] Preoperative data and that obtained at 1, 3, 6, 12 and 24 months after surgery were compared between the two groups.

Statistical analysis was performed using SPSS Statistics V.20 platform (IBM). For the analysis of quantitative variables, we used average mean values±SD, while categorical variables were analysed by counts and percentages. For the analysis of quantitative measures, we used the Student's t-test for normally distributed variables. P values <0.05 were considered to be statistically significant.

RESULTS

The present study included 50 patients: 28 males (56%) and 22 females (44%). The average age was 43.66 (±15.67 SD) years in BB-DALK group and 38.56 (±11.51 SD) years in VB-DALK group. All the bubbles obtained with both procedures were classified as type I, that is, the plane was obtained between stroma and PDL.[17]

Peripheral microperforations of DM occurred in two cases (one for each group) during completion of the stromal excision; all of them were managed conservatively.

There were no statistically significant differences preoperatively between BB-DALK and the VB-DALK group with regard to mean keratometric values (64.19±7.43 vs 65.20±6.87 Diopters (D)), mean thinnest point (364.47±59.98 vs 362.38±25.48 µm) or mean endothelial cell density (2538.24±588.31 vs 2559.23±486.92 cells/mm^2). These data are displayed in table 1.

The BSCVA was significantly better in the BB-DALK group than VB-DALK group at 1 month (0.40±0.19 vs 0.67±0.29 LogMAR, p=0.0003) and 3 months (0.39±0.29 vs 0.65±0.23 LogMAR, p=0.001) postoperatively. No statistically significant difference in BSCVA was found at later follow-up intervals at 6, 12 and 24 months (p=0.84, p=0.83 and p=0.80 respectively).

Postoperative mean keratometric astigmatism, pachymetry and endothelial cell density did not significantly differ

Table 1 Preoperative and postoperative main data of big-bubble and visco-bubble DALK

Measures	Big-bubble DALK	Visco-bubble DALK	P value (Student's t-test)
Preoperative mean corneal curvature (D)	64.19±7.43	65.20±6.87	0.62
Postoperative (1 year) mean corneal curvature (D)	43.86±2.54	45.17±1,79	0.11
Preoperative thinnest point (µm)	364.47±59.98	362.38±25.48	0.95
Postoperative thinnest point (µm)	521.35±52.75	509.65±28.82	0.26
Preoperative ECD (cells/mm^2)	2538.24±588.31	2559.23±486.92	0.8756
Postoperative ECD (cells/mm^2)	2286.41±475.98	2299.31±674.76	0.9088
BSCVA 1 month (LogMAR)	0.40±0.19	0.67±0.29	0.0003*
BSCVA 3 months (LogMAR)	0.39±0.29	0.65±0.23	0.001*
BSCVA 6 months (LogMAR)	0.19±0.23	0.21±0.45	0.84
BSCVA 12 months (LogMAR)	0.11±0.94	0.15±0.27	0.83
BSCVA 24 months (LogMAR)	0.10±0.34	0.13±0.51	0.80

Data are expressed as mean±SD.
*Statistically significant (<0.05).
BSCVA, best spectacle-corrected visual acuity; D, diopters; DALK, deep anterior lamellar keratoplasty; ECD, endothelial cell density; LogMAR, logarithmic of minimum angle of resolution.

between the two groups during the follow-ups. One year after surgery, following complete suture removal, the corneal curvature was 43.86±2.54 D in BB-DALK group and 45.17±1.79 D in VB-DALK (p=0.11). Central pachymetric values were 521.35±52.75 µm in BB-DALK group and 509.65±28.82 µm in VB-DALK group (p=0.26), while endothelial cell density was 2286.41±475.98 cells/mm^2 in the BB-DALK versus 2299.31±674.76 cells/mm^2 in the VB-DALK (p=0.9088).

Corneal densitometric analysis of the posterior corneal layer performed one and 3 months postoperatively recorded values significantly higher in VB-DALK group than in BB-DALK group (p<0.05) in both of the two zones analysed (0 to –2 mm and 2 to –6 mm). However, densitometric values became comparable between the two groups in the latest follow-up examinations, as summarised in table 2; other densitometric values were also reported in tables 3 and 4 but no significant differences were noted.

Table 2 Densitometric analysis of posterior corneal layer after big-bubble and visco-bubble DALK

Densitometry (GSUs)		Big-bubble DALK	Visco-bubble DALK	P value (Student's t-test)
1 month	0–2 mm	19.42±5.79	28.12±6.74	*0.0006**
	2–6 mm	17.20±3.54	23.32±6.23	*0.0099**
3 months	0–2 mm	17.13±4.44	23.97±5.34	*0.0048**
	2–6 mm	15.35±2.40	21.87±4.58	*0.0069**
6 months	0–2 mm	16.43±4.03	18.56±3.57	*0.3517*
	2–6 mm	14.59±2.21	15.74±2.59	*0.6142*
12 months	0–2 mm	14.30±2.84	14.23±3.47	*0.9749*
	2–6 mm	12.67±2.42	13.03±2.73	*0.8735*
24 months	0–2 mm	13.54±3.18	13.41±3.62	*0.9532*
	2–6 mm	11.99±3.44	12.79±3.57	*0.7267*

Data are expressed as mean±SD.
*Statistically significant (<0).
DALK, deep anterior lamellar keratoplasty;GSUs, grayscale units.

Table 3 Densitometric analysis of anterior layer (120 µm) after big-bubble and visco-bubble DALK

Densitometry (GSUs)		Big-bubble DALK	Visco-bubble DALK	P value (Student's t-test)
1 month	0–2 mm	36.72±11.34	40.37±14.62	*0.1138*
	2–6 mm	33.90±10.91	37.80±13.65	*0.0961*
3 months	0–2 mm	32.51±11.39	36.74±13.63	*0.0683*
	2–6 mm	31.74±11.50	34.79±10.68	*0.1842*
6 months	0–2 mm	31.61±10.07	34.30±10.17	*0.2407*
	2–6 mm	31.60±9.57	31.61±10.66	*0.9950*
12 months	0–2 mm	28.61±7.75	30.07±9.40	*0.5239*
	2–6 mm	27.67±7.20	29.09±7.53	*0.5335*
24 months	0–2 mm	24.38±5.85	26.84±7.58	*0.2842*
	2–6 mm	23.85±7.13	25.77±6.17	*0.4030*

Data are expressed as mean±SD.
* Statistically significant (P <0.05)
DALK, deep anterior lamellar keratoplasty; GSUs, grayscale units.

DISCUSSION

In the last decade, several techniques of DALK have been proposed, with varying success in the treatment of keratoconus and other corneal stromal pathology.[1] The original technique of BB-DALK introduced by Anwar described the injection of air to facilitate dissection of the stromal tissue from the underlying DM. In cases of failure of pneumatic dissection, the use of visco-elastic substance has been reported as a safe technique to achieve plane separation, with a reduced risk of conversion in PK.[11 12] When compared with air, the high viscosity of OVD prevents its permeation through the residual stroma; allowing bubble formation in most of those cases where the pneumatic attempt has previously failed.

Although the dissection plane established by both techniques is the same, in a recent study, we reported an initial visual impairment in those eyes where the OVD is used. This finding suggests a potentially negative optical impact on the interface created, which can sometimes appear slightly opaque during slit lamp examination (figure 1).[12]

In order to identify and quantify the modifications induced by the surgical intervention, we performed a densitometric analysis of the stromal interface using a Scheimpflug-based imaging system (figure 2). This method has been validated as a well-standardised

Table 4 Densitometric analysis of md-stromal layer after big-bubble and visco-bubble DALK

Densitometry (GSUs)		Big-bubble DALK	Visco-bubble DALK	P value (Student's t-test)
1 month	0–2 mm	23.57±4.34	26,86±10.25	*0.1530*
	2–6 mm	21.30±6.93	25.49±9.40	*0.0713*
3 months	0–2 mm	22.65±4.54	25.61±11.61	*0.1973*
	2–6 mm	20.62±3.62	23.12±9.01	*0.2759*
6 months	0–2 mm	21.50±4.90	25.22±11.30	*0.1071*
	2–6 mm	19.82±3.83	22.53±8.02	*0.2374*
12 months	0–2 mm	19.96±4.31	23.41±11.13	*0.1338*
	2–6 mm	18.40±3.55	21.50±7.37	*0.1776*
24 months	0–2 mm	18.40±3.67	21.15±8.33	*0.2294*
	2–6 mm	16.71±2.60	19.38±6.36	*0.2456*

Data are expressed as mean±SD.
*Statistically significant (P <0.05)
DALK, deep anterior lamellar keratoplasty; GSUs, grayscale units.

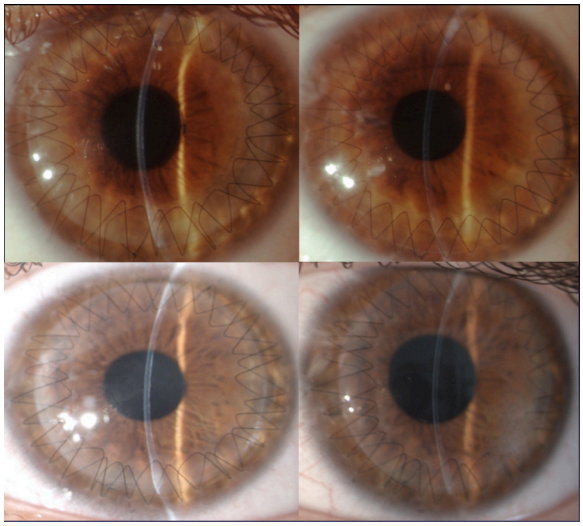

Figure 1 Postoperative slit-lamp appearance of two eyes that underwent deep anterior lamellar keratoplasty (DALK). One month after visco-bubble DALK (upper left), a faint haze of the predescemetic layer is noted; instead, at same follow-up interval, a perfectly clear interface is evidenced after big-bubble DALK (upper right). A different case of clinically more significant opacity of the deep interface in an eye that received visco-bubble DALK (bottom left) and its complete resolution six months after the surgery (bottom right).

tool for assessing corneal backscattering and long-term follow-up of several surgical procedures (ie, corneal collagen crosslinking, refractive surgery and endothelial keratoplasty).[15 18–20] In this study, the densitometric analysis of the posterior corneal layer revealed hyperreflectivity of the stromal interface in the group of VB-DALK which was not repeatable in the group of BB-DALK. A possible explanation for this different result is minimal infiltration of the OVD in the thin, acellular layer of collagen, which characterises the PDL layer of the type I bubble.[7]

With regard to being certain of the dissection plane achieved in these series, high-resolution tomography scans were performed at the level of the recipient bed which confirmed the presence of a very thin, linear and regular residual posterior layer compatible with a PDL dissection as intraoperatively deduced. Furthermore, the absence of any intrastromal bubbles, irregular stromal tissue or folds ruled out potential inadvertent predescemetic viscodissection.

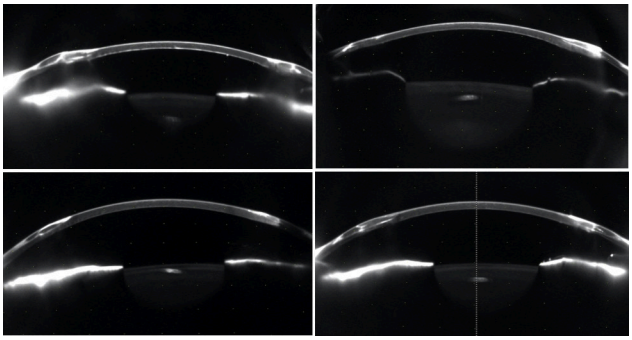

Figure 2 Densitometric scans of deep stromal interface obtained with Scheimpflug tomography. Hyperreflectivity of the predescemetic layer is detected 1 month after visco-bubble DALK (top left) that completely disappeared at 6-month postoperative follow-up (bottom left). No evidence of reflectivity modification is noted in case of big-bubble DALK at same examination times (up and bottom right). DALK, deep anterior lamellar keratoplasty.

In addition, the presence of a healthy endothelial layer, as detected by endothelial specular microscopy, established that alteration of densitometric values was not related to endothelial disease, as reported in another study.[21]

Although this densitometric discrepancy between the two groups was transient and limited to the first six postoperative months, this was in keeping with the poorer visual acuity achieved by the VB-DALK group during the same time frame. This outcome is similar to that reported by Melles with the OVD-assisted lamellar dissection technique, although the recovery of good visual acuity in our series was much quicker. The amount of residual stromal tissue, that is, only Dua's layer rather than the variable and thicker manually dissected stroma, may account for the faster resolution of acuity in our series.

In the VB-DALK group, no type 2 or mixed bubble type occurred, thus precluding necessary consideration on the impact of the OVD interaction with the DM and its effect on the new interface produced.

In almost half of the cases of our series, the interface modification could not be detected accurately by slit-lamp examination but only identified by densitometric analysis. This suggests its potential value in all patients with poor visual outcomes after lamellar corneal surgery despite relatively good transparency on clinical examination alone. Furthermore, if we extrapolate this finding to posterior lamellar surgery it may add weight to the argument of using air over OVD to maintain the anterior chamber during descemetorhexis.

Further studies examining different stromal pathologies are required to improve our understanding regarding the interaction of viscoelastic with the deep corneal stroma. In addition, other OVD with different rheologic properties could be tested to evaluate any difference in densitometric and functional interface alterations.

In conclusion, corneal densitometry allows a quantitative evaluation of the corneal stromal interface created with DALK. The higher densitometry observed with VB-DALK when compared with BB-DALK correlates with an initially poorer postoperative visual acuity which normalises by 6 months postoperatively.

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