Stromal peeling for deep anterior lamellar keratoplasty in post-penetrating keratoplasty eyes

Cristina Bovone,[1,2,3] Yoav Nahum,[4,5] Vincenzo Scorcia,[6] Giuseppe Giannaccare,[6] Rossella Spena,[1,2,3] James Myerscough,[2,3,7] Angeli Christy Yu,[1,2,3] Massimo Busin[1,2,3]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/bjophthalmol-2020-316653).

[1]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy
[2]Department of Ophthalmology, Ospedali Privati Forlì "Villa Igea", Forlì, Italy
[3]Istituto Internazionale per la Ricerca e Formazione in Oftalmologia (IRFO), Forlì, Italy
[4]Department of Ophthalmology, Rabin Medical Center, Petah Tikva, Israel
[5]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[6]Department of Ophthalmology, Magna Graecia University of Catanzaro, Catanzaro, Italy
[7]Department of Ophthalmology, Southend University Hospital, Southend, United Kingdom

Correspondence to
Dr Massimo Busin, Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara 47122, Italy; mbusin@yahoo.com

Received 23 April 2020
Revised 20 October 2020
Accepted 7 November 2020
Published Online First 26 November 2020

To cite: Bovone C, Nahum Y, Scorcia V, *et al*. *Br J Ophthalmol* 2022;**106**:336–340.

ABSTRACT
Background/aims To evaluate the clinical outcomes of deep anterior lamellar keratoplasty performed by stromal peeling in eyes that have previously undergone penetrating keratoplasty (PK) for keratoconus.
Methods Standardised stromal exchange included (1) 9 mm trephination of the recipient bed outside the old PK wound, (2) creation of a partial anterior corneal flap through lamellar dissection across the PK wound, (3) opening the stromal component of the old PK wound using blunt-tipped Vannas scissors until a plane of separation is reached, (4) severing the attachment of the PK surgical scar from the recipient host, (5) peeling the stroma of the PK graft from the underlying tissue and (6) suturing the donor anterior corneal lamella prepared by microkeratome dissection (450 µm depth, 9 mm diameter). Main outcome measures were success rate, best spectacle-corrected visual acuity (BSCVA) and endothelial cell loss (ECL).
Results Of 21 post-PK eyes, stromal exchange succeeded in all but three cases, which were converted to a two-piece mushroom PK. After complete suture removal, mean BSCVA significantly improved from 0.95±0.39 logMAR preoperatively to 0.23±0.17 logMAR ($p<0.001$). Mean ECL was 5.4±23.2%. Double anterior chamber formation occurred in eight cases (44%), which all resolved after a single re-bubbling.
Conclusion In post-PK eyes, stromal exchange can be performed by means of simple peeling without deep anterior lamellar dissection of the previous PK graft. Large-diameter (9 mm) repeat keratoplasty through stromal peeling yields excellent visual outcomes and minimal ECL. Double anterior chamber formation may complicate the postoperative course, but prompt intervention allows successful management.

INTRODUCTION
Over the last decades, improvements in the surgical and postoperative management of corneal grafts have resulted in increased long-term survival after penetrating keratoplasty (PK).[1] However, several years after PK, recurrent ectasia can adversely affect vision even in the presence of functional corneal endothelium.[1] Since the survival of a full-thickness graft successively decreases after every subsequent repeat PK,[2] it would be desirable to selectively remove diseased stroma during re-grafting. Specifically, deep anterior lamellar keratoplasty (DALK) would obviate the introduction of new endothelium, which is the prime alloimmune target for immunologic rejection leading to graft failure.[3]

Modifications in DALK have been previously described for eyes with failed PK.[4 5] However, these techniques are more technically challenging than conventional DALK due to alterations in the normal corneal anatomy after PK.

We present herein the clinical outcomes of a 9 mm DALK through exchange of diseased stroma by means of simple stromal peeling of the PK graft along a natural plane of separation without performing additional pneumatic-assisted, hydro-assisted, viscoelastic-assisted or manual deep lamellar dissection of the PK graft.

MATERIALS AND METHODS
This interventional case series evaluated the outcomes of DALK through stromal peeling for eyes that have previously undergone PK for keratoconus. The interventions were performed by three surgeons (CB (n=5) and MB (n=10) at Ospedali Privati Forlì "Villa Igea" (Forlì, Italy), and VS (n=6) at University Eye Hospital of "Magna Graecia" University (Catanzaro, Italy)) between June 2015 and May 2017. The study was compliant to the tenets of the 2013 Declaration of Helsinki and was approved by the local ethics committee (Comitato Etico, Ospedali Privati Forlì). Detailed informed consent was obtained from all participants.

All eyes required repeat keratoplasty for unsatisfactory best spectacle-corrected visual acuity (BSCVA) (Snellen ≤20/100) and poor contact lens tolerance. In all cases, the endothelium was clinically functional with a preoperative endothelial cell density of at least 600 cells/mm^2, the threshold we arbitrarily considered sufficient to maintain corneal deturgescence.

Main outcome measures were success rate of stromal peeling, BSCVA and endothelial cell density (ECD). Preoperatively, each patient underwent complete ophthalmological evaluation including slit-lamp examination, BSCVA testing, manifest refraction, funduscopy, non-contact specular microscopy and anterior segment optical coherence tomography (AS-OCT) (Casia; Tomey, Tokyo, Japan). Intraoperative and postoperative complications were recorded. BSCVA was assessed using the Snellen visual acuity chart and converted to logarithm of the minimum angle of resolution (logMAR) units. ECD was evaluated via non-contact specular microscopy (EM-3000; Tomey, Erlangen, Germany) using automatic focusing and digital capture of 15 images of the central cornea. All operated eyes were evaluated annually after surgery.

Surgical technique

The surgical steps are demonstrated in online supplemental video 1 and figure 1. In all patients, anaesthesia and akinesia were obtained using peribulbar injection of 10 mL of 0.75% ropivacaine solution.

A guarded suction trephine was calibrated within 150 µm from the thinnest point pachymetry value measured by AS-OCT at 9 mm diameter. Using a pre-calibrated trephine, initial partial-thickness trephination of the recipient cornea was performed outside the PK graft at the 9 mm zone (figure 1A,B). From the base of the trephination, lamellar dissection using a crescent blade was performed tangentially across the old PK wound to circumferentially create a partial anterior corneal flap (figure 1C,D). While lifting the anterior corneal flap, blunt-tipped Vannas scissors were used to enter and open the old PK wound until a natural plane of separation was identified (figure 1E,F). This was characterised by a reduction in resistance. The plane could be recognised by its smooth, glossy and translucent appearance.

As one of the blades of the Vannas scissors could be inserted into the plane of separation without any resistance, the old PK scar was cut to sever the attachment of the old donor button to the recipient bed. The blade was then advanced along the plane of separation and the procedure was repeated as many times as necessary to completely sever the old PK surgical scar (figure 1G,H). The stroma of the PK button was then simply peeled off from its underlying tissue bed (figure 1I,J).

Finally, a 9 mm donor corneal button was prepared by microkeratome dissection (450 µm depth) and secured to the recipient bed using 16-bite double-running continuous sutures (figure 1K,L).

Postoperative care

At the end of the procedure, triamcinolone acetonide and gentamicin sulphate 0.3% were injected subconjunctivally. A fixed combination of dexamethasone phosphate 0.1% and netilmicin sulphate 0.3% ophthalmic solution (Netildex; Sifi, Catania, Italy) was administered every 2 hours and tapered to four times daily over the first postoperative month. Subsequently, antibiotic treatment was discontinued and dexamethasone phosphate 0.1% eyedrops (Etacortilen; Sifi) was slowly tapered to once daily over 4 to 5 postoperative months and discontinued after 13 months. In all patients, the first running suture was removed 3 to 4 months postoperatively, while the second was removed within 12 months from surgery.

If a micro-perforation occurred, the procedure was completed after air fill of the anterior chamber, which was partially released 2 hours postoperatively. In cases with significant macro-perforation, the procedure was converted to a two-piece mushroom PK, according to the technique described previously.[6] The mushroom graft consisted of a smaller posterior lamella of the same diameter of the old PK graft (6.5 to 8 mm) and a larger anterior lamella of 9 mm diameter.

Statistical methods

All data collected in the study were entered into an electronic database via Microsoft Excel 2013 (Microsoft, Redmond, WA) and analysed with Minitab Software (V.17; Minitab, State College, PA). Values were expressed as mean±SD for continuous variables and individual counts and percentages for categorical variables. Continuous data were analysed using Student's t-test while χ^2 test was used for the analysis of categorical variables. P value of less than 0.05 was considered statistically significant.

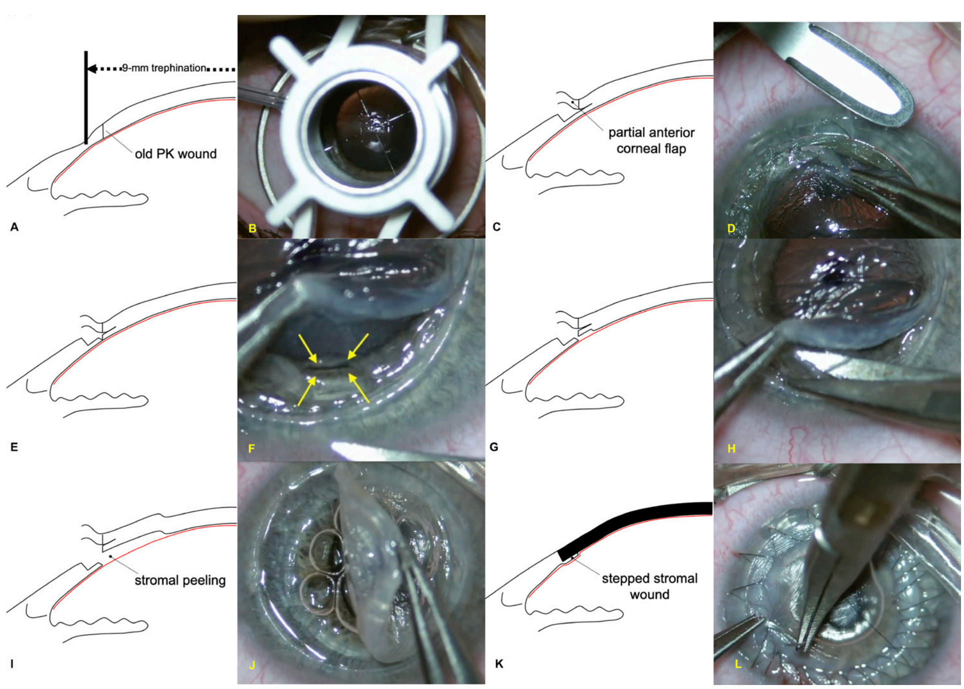

Figure 1 Schematic diagram and intraoperative images of the surgical steps of stromal peeling for deep anterior lamellar following penetrating keratoplasty (PK): (A, B) 9 mm trephination outside the old PK wound. (C, D) Creation of a partial anterior corneal flap through lamellar dissection across the PK wound. (E, F) Opening the stromal component of the old PK wound using blunt Vannas scissors to reach the natural plane of separation. (G, H) Cutting the circumference of the old PK scar to sever the attachment of the stroma of the previous graft from the recipient host. (I, J) Stromal peeling of the PK graft. (K, L) Suturing of the donor graft. Schematic diagram illustrated by ACY.

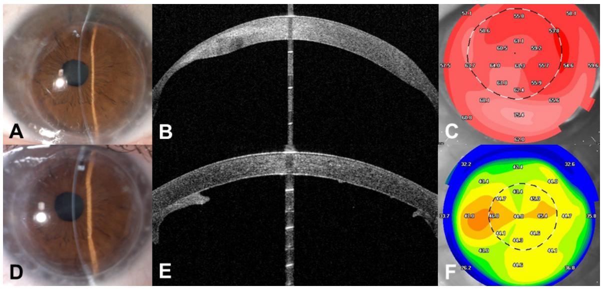

Figure 2 Clinical picture (A), anterior segment optical coherence tomography (B) and corneal topography (C) before and 3 years after (D, E and F) stromal peeling performed in a 62-year-old patient with recurrent ectasia after penetrating keratoplasty for keratoconus. Normalisation of peripheral corneal thickness and marked flattening of the cornea were achieved.

RESULTS

This series included 21 eyes of 21 patients with a mean age at surgery of 59±9 years. Thirteen (62%) were males and eight (38%) were females. Figure 2 shows the preoperative and postoperative clinical pictures (figure 2A and D), corneal topography (figure 2B and E) and AS-OCT images (figure 2C and F) of a typical patient who underwent stromal peeling for post-PK recurrence of ectasia in the residual recipient bed.

Stromal peeling was successfully completed in 18 of 21 cases (85.7%), while the procedure was converted to a two-piece mushroom PK due to significant macro-perforation in 3 of 21 eyes (14.3%). The success rate of stromal peeling did not differ significantly among the three surgeons ($p=0.569$). A peripheral micro-perforation occurred in 2 of 21 eyes (9.5%), but did not prevent successful completion of stromal peeling in these cases.

Mean follow-up was 36±5 months. Within 1 month from complete suture removal, mean BSCVA significantly improved from 0.95±0.39 logMAR preoperatively to 0.23±0.17 logMAR ($p<0.001$). Three years after surgery, 7 of 18 eyes (39%) reached Snellen BSCVA ≥20/25, 17 (89%) reached ≥20/40 and all eyes reached ≥20/50. Mean refractive astigmatism (RA) decreased significantly from 7.4±3.0 dioptres (D) preoperatively to 3.7±1.6 D after complete suture removal ($p<0.001$). One patient required corneal-relaxing incisions to manage postoperative astigmatism. RA ≥4.5 D was observed in only two eyes (11%) at final follow-up. Keratometric astigmatism (KA) was not significantly different from RA, averaging 3.8±1.5 D. ECD averaged 1095±384 cells/mm^2 1 year postoperatively. Mean endothelial cell loss at 1 year was 5.4±23.2% compared with baseline values. In four cases, ECD increased by 6% to 17%.

Double anterior chamber formation occurred in 8 (44%) eyes and was observed as early as 1 to 42 days postoperatively. Each case was managed successfully by a single air injection into the anterior chamber and the grafts were clear at all succeeding follow-ups. Comparing eyes that underwent descemetopexy for double anterior chamber formation and those that did not require any additional intervention, descemetopexy was not found to significantly influence BSCVA ($p=0.82$), RA ($p=0.62$), KA ($p=0.78$) or ECD ($p=0.17$).

Cataract formation was identified in 4 eyes (21%) preoperatively and in an additional 3 (16%) cases within 3 years postoperatively. Sequential cataract surgery via phacoemulsification with posterior chamber intraocular lens implantation was performed uneventfully in six (32%) eyes. Of the six eyes undergoing cataract surgery, the final ECD after sequential stromal peeling and cataract surgery averaged 1230±281 cells/mm^2 with a mean cell loss of 4.1%±3.8% from the preoperative value. No episode of immunological rejection or recurrence of stromal disease was observed postoperatively among any patients in this series. Graft survival at 3 years was 100%.

DISCUSSION

Eyes that have previously undergone PK for keratoconus may develop unsatisfactory vision due to re-emergence of pathological ectasia after a period of latency.[7 8] Although repeat PK can be performed for visual rehabilitation of these eyes, DALK can be alternatively performed to selectively replace the diseased stroma while leaving the functional endothelium of the old PK graft intact. Unlike PK, DALK does not pose an additional risk for endothelial immunologic rejection that can cause graft failure. Since both cumulative graft survival and median survival time decrease with every repeat PK procedure,[2 9] avoiding unnecessary replacement of host endothelium is particularly important considering that most patients with stromal disease as primary indications are younger and may possibly require multiple grafts throughout their lifetime.

The current techniques for conventional DALK are technically challenging to perform in post-PK grafts. Manual stromal dissection with large diameter DALK for post-PK eyes has been described by Lake *et al*,[5] but was associated with relatively suboptimal visual outcomes, likely due to an irregular stromal interface or an incomplete stromal dissection. Although we have previously demonstrated that pneumatic dissection can be achieved even in post-PK grafts,[4] it is not always possible to prevent the bubble from expanding into the PK wound, therefore requiring conversion to PK. In contrast, the technique used in this study allows removal of the diseased stroma through controlled manual peeling along a natural plane of separation with a resulting graft–host interface that is compatible with excellent visual outcomes. As opposed to conventional DALK, additional instruments such as a spatula, cannula or blade are not required during peeling of the deep anterior stroma of the old PK graft.

Following the removal of the anterior stroma, the resulting tissue bed appears as a strong, homogenous and impervious layer, which is akin to the pre-Descemet's layer–Descemet's membrane (DM)–endothelium complex observed after type 1 big bubble formation. Even without performing pneumatic-assisted, hydro-assisted or viscoelastic- assisted dissection, stromal peeling can be easily performed due to the absence of adherence between the stroma of the PK graft and the underlying layer, likely caused by postoperative alterations in the stromal microarchitecture. Moreover, there is some indirect clinical evidence supporting our observation of weakened interlaminar attachment in post-PK corneas. First, spontaneous DM detachment is a rare but well-known complication that may occur years after PK.[10] Second, selective stromal peeling has been obtained unintentionally during repeat PK and, when gone unnoticed, has led to inadvertent retention of host DM with subsequent postoperative formation of a double anterior chamber.[11] Further histological studies would be useful to clarify the ultrastructural composition of the remaining layer and to provide validation for the existence of a true plane of separation.

For eyes with recurrent ectatic corneal disease extending beyond the PK wound (figure 2A-C), simply repeating PK of the same diameter would pose significant challenges during fixation of the new graft onto a severely thinned recipient bed. Furthermore, simple exchange of an old PK graft would not address the underlying corneal ectasia of the recipient bed and therefore has a limited effect on astigmatic correction. Though a large-diameter PK may be performed to address these issues,[12] it carries a greater risk for immunological endothelial rejection and subsequent graft failure related to increased antigenic load. Instead, a large-diameter lamellar graft can be performed to maximise the removal of the pathological ectasia, while reducing the additional risk for both immune rejection and repeat recurrence of ectasia.

The presence of a stepped stromal wound confers several advantages (figures 1K and 2E). First, the peripheral stromal crown protects the recipient bed from DM perforation during suturing and reduces the tendency to make superficial loose suture passes that can cause corneal neovascularisation. Second, it provides additional tectonic support in cases of peripheral thinning, which may prevent the repeat recurrence of ectasia. Third, similar to stepped PK wounds, the lap-joint configuration results in increased surface area of donor–host stromal contact that could confer greater mechanical stability and prevent would dehiscence.[13] Finally, in the event of a significant intraoperative complication, the crown allows conversion to a two-piece mushroom PK rather than to a 9 mm conventional PK.[6]

With regards to ECD, cell loss caused by this procedure was limited, and we have not recorded a single case of endothelial failure, even after subsequent cataract surgery. In the subgroup of patients who had undergone cataract surgery, endothelial cell loss was less than 5%, suggesting that planned sequential lens surgery is possible after stromal peeling in PK. Although the disparity in preoperative and postoperative ECD may be due to underestimation of ECD secondary to poor stromal quality and OCT image acquisition, the paradoxical increase of ECD in some cases could also be related to the replacement of the protruding cone with a graft of normal curvature, thus reducing the posterior corneal surface area spanned by endothelial cells.

In terms of complications, double anterior chamber formation was observed more frequently in this series than after conventional DALK. Even in the absence of micro-perforations, alterations in the corneal anatomy after PK result in a weaker cleavage plane that may be more prone to microleaks leading to double anterior chamber formation. Although this series also included cases in the early phase of the surgeons' learning curves for this specific technique, further mechanistic studies may help explain why this complication occurs more commonly in post-PK eyes than treatment-naïve eyes. Nevertheless, close monitoring during the early postoperative period is essential for prompt anterior chamber re-bubbling, which resulted in successful re-attachment in all eyes with this complication.

Moreover, although the series included the first consecutive cases of stromal peeling in post-penetrating keratoplasty eyes with recurrence of ectasia, all surgeries were performed by surgeons experienced with lamellar corneal procedures, each of whom have completed at least 100 DALK procedures. Since the success rates did not differ significantly among surgeons, we believe that this technique is fairly reproducible among surgeons who have achieved sufficient proficiency in conventional DALK surgery.

There are valid concerns for possible conversion to full-thickness keratoplasty due to a significant macro-perforation. In our practice, should unsalvageable complications occur during large-diameter DALK, the procedure is converted to two-piece mushroom PK instead of a full-thickness PK. Although the proximity of the larger 9 mm anterior lamella (mushroom 'hat') to the limbal vascular arcade may theoretically increase the risk of immunological rejection, we have not observed an increased incidence of immunologic rejection in the primary series of eyes that we have operated on using this technique,[14 15] and even among those with vascularised corneas and therefore at high risk for immunologic rejection.[16] Reduced endothelial transplantation in mushroom PK may confer a lower risk of immunologic rejection related to less antigenic load.[14–16]

The limitations of the study include a small sample size and limited follow-up. Larger studies with longer follow-up are required to validate our initial findings, especially with regards to the risk of recurrent ectasia and graft failure. As our understanding of the corneal biomechanics in lamellar surgery continues to evolve, these preliminary results provide valuable additional information.

In conclusion, diseased corneal stroma can be removed by means of simple peeling along a natural plane of separation without deep anterior lamellar dissection of the PK graft. Large diameter (9 mm) grafting after stromal peeling in eyes that have previously undergone PK for keratoconus yields excellent visual outcomes with minimal endothelial cell loss.

Contributors Concept and design of the study: CB, VS and MB. Data acquisition: YN, GG, RS and JM. Data analysis/interpretation: JM, ACY and MB. Drafting the manuscript: CB, YN, VS, GG, RS and JM. Critical revision of manuscript: ACY and MB. Statistical analysis: YN and ACY. Final approval: CB, YN, VS, GG, RS, JM, ACY and MB.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Approval was obtained from the Comitato Etico of Ospedali Privati Forlì, Forlì, Italy (CEOPF2013-003).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information.

ORCID iDs
Yoav Nahum http://orcid.org/0000-0002-0246-4787
Vincenzo Scorcia http://orcid.org/0000-0001-6826-7957
Giuseppe Giannaccare http://orcid.org/0000-0003-2617-0289
Angeli Christy Yu http://orcid.org/0000-0001-5654-3942
Massimo Busin http://orcid.org/0000-0002-1921-715X

REFERENCES

1 Pramanik S, Musch DC, Sutphin JE, *et al*. Extended long-term outcomes of penetrating keratoplasty for keratoconus. *Ophthalmology* 2006;113:1633–8.
2 Kelly T-L, Williams KA, Coster DJ, *et al*. Corneal transplantation for keratoconus: a registry study. *Arch Ophthalmol* 2011;129:691–7.
3 Pleyer U, Schlickeiser S. The taming of the shrew? The immunology of corneal transplantation. *Acta Ophthalmol* 2009;87:488–97.
4 Scorcia V, Beltz J, Busin M. Small-bubble deep anterior lamellar keratoplasty technique. *JAMA Ophthalmol* 2014;132:1369–71.
5 Lake D, Hamada S, Khan S, *et al*. Deep anterior lamellar keratoplasty over penetrating keratoplasty for host rim thinning and ectasia. *Cornea* 2009;28:489–92.
6 Busin M, Madi S, Scorcia V, *et al*. A two-piece microkeratome-assisted mushroom keratoplasty improves the outcomes and survival of grafts performed in eyes with diseased stroma and healthy endothelium (an American Ophthalmological Society thesis). *Trans Am Ophthalmol Soc* 2015;113:T1.
7 Reinhart WJ, Musch DC, Jacobs DS, *et al*. Deep anterior lamellar keratoplasty as an alternative to penetrating keratoplasty a report by the American Academy of Ophthalmology. *Ophthalmology* 2011;118:209–18.
8 Bergmanson JPG, Goosey JD, Patel CK, *et al*. Recurrence or re-emergence of keratoconus—what is the evidence telling us? Literature review and two case reports. *Ocul Surf* 2014;12:267–72.
9 Bersudsky V, Blum-Hareuveni T, Rehany U, *et al*. The profile of repeated corneal transplantation. *Ophthalmology* 2001;108:461–9.

10 Gorski M, Shih C, Savoie B, *et al*. Spontaneous Descemet membrane detachment 20 years after penetrating keratoplasty for keratoconus. *Cornea* 2016;35:1023–5.
11 Lifshitz T, Oshry T, Rosenthal G. Retrocorneal membrane after penetrating keratoplasty. *Ophthalmic Surg Lasers* 2001;32:159–61.
12 Skeens HM, Holland EJ. Large-diameter penetrating keratoplasty: indications and outcomes. *Cornea* 2010;29:296–301.
13 Busin M, Leon P, Nahum Y, *et al*. Large (9 mm) deep anterior lamellar keratoplasty with clearance of a 6-mm optical zone optimizes outcomes of keratoconus surgery. *Ophthalmology* 2017;124:1072–80.
14 Scorcia V, Busin M. Survival of mushroom keratoplasty performed in corneas with postinfectious vascularized scars. *Am J Ophthalmol* 2012;153:44–50.
15 Myerscough J, Roberts H, Yu AC, *et al*. Five-year outcomes of converted mushroom keratoplasty from intended deep anterior lamellar keratoplasty (DALK) mandate 9-mm diameter DALK as the optimal approach to keratoconus. *Am J Ophthalmol* 2020;220:9–18.
16 Yu AC, Friehmann A, Myerscough J, *et al*. Initial high-dose prophylaxis and extended taper for mushroom keratoplasty in vascularized herpetic scars. *Am J Ophthalmol* 2020;217:212–23.