5-year Outcomes of Converted Mushroom Keratoplasty from Intended DALK Mandate 9mm Diameter DALK as the Optimal Approach to Keratoconus

James Myerscough, FRCOphth, Harry Roberts, MSc MD FRCOphth, Angeli Christy Yu, MD, Mohamed Elkadim, MSc, Cristina Bovone, MD, Massimo Busin, MD

AMERICAN JOURNAL OF OPHTHALMOLOGY

PII: S0002-9394(20)30360-3

DOI: https://doi.org/10.1016/j.ajo.2020.07.009

Reference: AJOPHT 11449

To appear in: American Journal of Ophthalmology

Received Date: 22 April 2020 Revised Date: 2 July 2020 Accepted Date: 3 July 2020

Please cite this article as: Myerscough J, Roberts H, Yu AC, Elkadim M, Bovone C, Busin M, 5-year Outcomes of Converted Mushroom Keratoplasty from Intended DALK Mandate 9mm Diameter DALK as the Optimal Approach to Keratoconus, *American Journal of Ophthalmology* (2020), doi: https://doi.org/10.1016/j.ajo.2020.07.009.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Elsevier Inc. All rights reserved.

**Purpose:** To compare the 5-year outcomes of 9mm deep anterior lamellar keratoplasty (DALK) for keratoconus against those converted to mushroom keratoplasty (MK).

**Design:** Retrospective cohort study

Methods:

<u>Setting:</u> Ospedali Privati "Villa Igea", Department of Ophthalmology, Forlì, Italy <u>Patient or Study Population:</u> The medical records of all attempted DALKs (416 eyes) for the indication of keratoconus performed between January 2012 and January 2018 were evaluated, 68 (16.4%) of which were converted to MK and analysed as a separate cohort. The mean follow-up time was 33.8±15.1 months.

<u>Intervention or Observation Procedure(s):</u> 9 mm DALK and MK (9 mm anterior lamella with 6 mm posterior lamella)

Main Outcome Measures: Best corrected visual acuity (BCVA), refractive astigmatism, endothelial cell loss (ECL) at 5 years.

**Results:** Average BCVA at 5 years was  $0.06\pm0.07$  in DALK group and  $0.09\pm0.15$  in the MK group (p=0.88). Refractive astigmatism following suture removal (all visits later than 12 months) was slightly less in the DALK cohort (5-year DALK=2.16D $\pm$ 1.40, MK 3.02D $\pm$ 0.89, p=0.04, mean difference 0.86D (95%CI 0.71-1.01)). ECL was significantly higher in the MK group than in the DALK group at all follow up intervals (5-year DALK = 19.36% $\pm$ 21.47, MK 56.61% $\pm$  15.82, p<0.001). Total all-cause graft failure rate at 5 years was 0.58% for DALK (2/348 cases) and 5.88% for (4/68 cases).

**Conclusions:** Excellent 5-year visual and clinical outcomes associated with a two-piece mushroom keratoplasty in cases converted from intended DALK mandate large diameter DALK (9mm) as the optimal surgical approach to keratoconus.

Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

5-year Outcomes of Converted Mushroom Keratoplasty from Intended DALK Mandate 9mm Diameter DALK as the Optimal Approach to Keratoconus American Journal of Ophthalmology

James Myerscough FRCOphth<sup>1-4</sup>, Harry Roberts MSc MD FRCOphth<sup>1</sup>, Angeli Christy Yu MD<sup>2,4,5</sup>, Mohamed Elkadim MSc<sup>2,4,6</sup>, Cristina Bovone MD<sup>2,4,5</sup>, Massimo Busin MD<sup>2,4,5</sup>.

#### From:

<sup>1</sup>Southend University Hospital, Southend, United Kingdom;

Correspondence and reprint requests to: Massimo Busin, MD, Ospedali Privati Forlì "Villa Igea", Dept. of Ophthalmology, 47122 Forlì, ITALY. Tel.:01139-347-2449343; FAX: 01139 0543 419566; E-mail: mbusin@yahoo.com

Short title: Mushroom Keratoplasty Versus DALK for Keratoconus

<sup>&</sup>lt;sup>2</sup>Ospedali Privati Forlì "Villa Igea", Department of Ophthalmology, Forlì, Italy;

<sup>&</sup>lt;sup>3</sup> Vision and Eye Research Institute, School of Medicine, Anglia Ruskin University, Cambridge, United Kingdom

<sup>&</sup>lt;sup>4</sup>Istituto Internazionale per la Ricerca e Formazione in Oftalmologia (IRFO), Forlì, Italy; <sup>5</sup>University of Ferrara, Department of Morphology, Surgery and Experimental Medicine, Ferrara. Italy

<sup>&</sup>lt;sup>6</sup>Department of Ophthalmology, Faculty of Medicine, Tanta University, Egypt.

Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

#### INTRODUCTION

In the management of keratoconus, deep anterior lamellar keratoplasty (DALK) allows the selective removal of diseased stroma, whilst preserving healthy host endothelium eliminating the risk of endothelial rejection. Since the largest contributing component to the overall corneal optical power is the anterior corneal curvature it is desirable from a refractive point of view to maximize the diameter of the graft utilized during DALK. However, since the risk of intraoperative conversion from intended DALK to penetrating keratoplasty (PK) is reported to occur in up to a quarter of cases, and the risk of endothelial rejection increases for full-thickness grafts larger than 8 mm, wide diameter DALK is not widely performed as the technique of choice.

In order to be able to confidently attempt wide diameter DALK, it is therefore essential in cases where conversion to PK is necessary to have a technique that still maximizes the refractive benefit of the initially performed wide diameter trephination, whilst minimizing the risk of endothelial failure or rejection conventionally associated with wide diameter grafts. As previously described, a two-piece microkeratome-assisted mushroom keratoplasty (MK) with an anterior lamella of 9 mm and a posterior lamella of 6 mm combines the increased survival rate of minimal endothelial replacement with the visual outcomes of a large diameter anterior lamella graft. Accordingly, it is our technique of choice in cases in which intended DALK requires intraoperative conversion to PK.

The outcomes of conventional PK when compared with DALK as primary procedures have been well reported.<sup>8,9</sup> However, there is generally a lack of evidence as to whether the outcomes in eyes that were intended to undergo DALK yet required conversion to PK are worse than those that had successfully completed DALK.<sup>6,9</sup> In particular, studies have not compared long term clinical and refractive outcomes for wide diameter grafts. In order to routinely attempt wide diameter DALK, confidence that the respective outcomes of such patients are comparable to planned DALK is imperative.

The purpose of this study was therefore to evaluate and compare the 5-year survival rate and visual outcomes between keratoconic eyes that had successfully completed DALK as intended, and those in which conversion from DALK to MK was mandated.

#### **METHODS**

# Study Design

This was an institutional retrospective cohort study performed at Ospedali Privati Forlì "Villa Igea" (Forlì, Italy). The medical records of all attempted DALKs for the indication of keratoconus, regardless of severity of the cone and presence of the scar, performed between January 2012 and January 2018 were evaluated. Keratoconic eyes with clinically significant endothelial disease such as concomitant Fuchs endothelial dystrophy were offered MK to simultaneously address both pathologies and were thus excluded from the analysis. All data had been initially collected prospectively and entered into the institutional database. The study followed the tenets of the 2013 Declaration of Helsinki and was approved by the local ethics committee of Ospedali Privati Forlì (Forlì, Italy).

All operations were performed by either a senior surgeon or by a fellow supervised by a senior surgeon.

# Surgical Technique

DALK was performed as per our previously described technique<sup>10</sup>. Briefly, a deep trephination of 9 mm diameter was carried out by a vacuum trephine (Moria, Antony, France) calibrated within 100 µm from the thinnest pachymetry value at the 9 mm zone measured by anterior segment optical coherence tomography (CASIA, Tomey, Tokyo, Japan). In cases with highly irregular peripheral corneal thickness (>100 µm difference in corneal thickness at 9 mm zone), peripheral intrastromal hydration using 1 mL of normal saline for every clock hour of corneal thinning was performed to increase stromal volume and allow deep trephination. 11 A blunt probe was advanced 1 mm centripetally from the base of the trephination. The probe was replaced by a cannula which was advanced 1 mm further along the same track created by the probe, before performing pneumatic dissection. Then en bloc anterior keratectomy was performed starting from the base of deep trephination,, i.e. within 100 µm from the thinnest paghymetric value at 9.0mm, thus resulting in a residual bed 9 mm in diameter and about 100-150 µm in thickness. At this point the central 6 mm of the bubble roof was removed with baring of the optical zone at the level of pre-Descemet's layer or Descemet's Membrane (DM), depending on the plane of dissection achieved. A 9 mm anterior lamellar graft was prepared by means of a 400 µm microkeratome head and sutured into place.

When a perforation occurred during trephination, the site was initially sutured before attempting pneumatic dissection. The sutured wound allowed completion of the procedure since bubble formation typically does not extend as far as the 9 mm site of trephination. When microperforation occurred during lamellar dissection, the procedure was completed after air fill of the anterior chamber, and DALK was completed as per our standard technique.

Intraoperative conversion to mushroom shaped penetrating keratoplasty
In cases in which there was either unsatisfactory clearance of the optical zone of a full
thickness opacity or a macroperforation of the DM, the procedure was converted to a
two-piece microkeratome-assisted mushroom shaped penetrating keratoplasty, as per

Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

our previously described technique<sup>8</sup>. Having performed a 9 mm diameter anterior keratectomy, the central 6 mm optical zone was excised, leaving a 1.5 mm posterior corneal crown of approximately 100-150 µm thickness. Using the automated lamellar keratoplasty system (ALTK; Moria SA, Antony, France), the donor cornea was split into anterior and posterior lamellae using a 350 µm microkeratome head. Both anterior and posterior lamellae were punched to the corresponding diameters of the recipient beds of 9 and 6 mm respectively. The donor stem of the mushroom (endothelium and deep stroma) was placed into the central hole of the recipient bed without sutures, and the anterior lamella was placed on top, before being sutured into position with double running sutures. To complete the intervention, the anterior chamber was filled with balanced saline solution. All cases of postoperative double anterior chamber formation (in DALK) or posterior lamella detachment (in MK) underwent rebubbling of the anterior chamber with complete air fill, which was partially released after 2 hours. <sup>12</sup>

# Statistical Analysis

All data collected in the study was entered into an electronic database via Microsoft Excel 2007 (Microsoft Corp., Redmond, WA), and statistical analyses were performed using SPSS Statistics Version 16 (IBM, Armonk, New York, USA). Chi-square testing was used to determine the significance of difference between nominal variables. Continuous variables were reported as mean ± standard deviation and Wilcoxon signed-rank test was used for comparison. Correlations were examined with Spearman rank-correlation test. P values less than 0.05 were considered statistically significant.

Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

#### **RESULTS**

During the study period there were 416 9 mm DALKs attempted in total, 68 of which (16.4%) were converted to MK. The indications for conversion to MK included failure to clear the optical zone of full thickness opacity and/or macroperforation. The mean follow-up time was 33.8±15.1 months. The mean age was 38±15years with no significant difference between DALK and MK groups (p=0.74).

The visual outcomes of the two groups are illustrated in table 1. Eyes that developed graft failure (7 eyes total across both groups) were excluded from analysis of visual outcome but are included for the rest of the analysis. Briefly, there was no significant difference in best-corrected visual acuity (BCVA) between DALK and MK at any timepoint recorded. Average BCVA at 5 years was  $0.06\pm0.07$  in DALK group and  $0.09\pm0.15$  in the MK group (p=0.88) (Table1). At 18 months (all with sutures out), 93.82% of DALK patients saw 20/40 or better compared with 95.85% of MK (Figure 1) no statistically significant difference was found between DALK and MK groups (p = 0.67; chi-square test). Only 0.77% and 2.07% of cases respectively had BCVA worse than 20/80.

Refractive astigmatism at all time points following suture removal (all visits later than 12 months) was slightly lower in the DALK group when compared with the MK group. Mean difference ranged from 0.46 Diopter (D) to 0.86D across all time points after suture removal, all are statistically significant (Table 1). At 18 months (all with sutures out), 89.94% of DALK patients had 4D or less of refractive astigmatism compared with 92.65% of MK (Figure 2). Only 1.72% and 1.47% of cases respectively had refractive astigmatism greater than 8D. High levels of astigmatism (greater than 4.5D) was observed in 35/313 (11%) eyes in the DALK group and 5/63 (8%) eyes of the MK group; this difference was not statistically significant (p=0.49).

Endothelial cell loss (ECL) was significantly higher in the MK group than in the DALK group at all follow up intervals (Table 2). The mean annual ECL in MK was 11.4 %, and in DALK was 4.7 %. ECD was relatively stable in the DALK group after the first year (Figure 3) with mean ECL at the 5th year of follow up only 2.44% of year one ECD (in comparison to 29.73% in MK group). There were no cases of endothelial failure in the MK group other than 2 resulting from episodes of endothelial rejection.

Post-operative complications are listed in table 3. Eyes that underwent MK were more prone to development of cataract, endothelial rejection and graft failure than DALK eyes, which had a higher incidence of rebubbling for DM detachment. Only 1 case developed posterior lamella detachment after MK. Kaplan-Meier survival curve (Figure 4, Table 4A) shows significantly higher survival of DALKs than MK grafts (p<0.001), however, after 2 years survival probability remains stable in both DALK and MK. Table 4B details cause for graft failure in the 6/416 cases where it occurred.

#### DISCUSSION

This paper describes the 5-year follow up results of 416 keratoplasties for keratoconus, all intended as 9 mm DALK, with 68 (16.4%) requiring conversion to a two-piece mushroom shaped full thickness keratoplasty (previously described in detail) for reasons of DM macroperforation or full thickness opacity within the optical zone.8 Those converted to MK had no difference in the BCVA at any time point, but had significantly greater refractive astigmatism at time points after sutures removed from 18 months to 5 years (5 year DALK=2.16D±1.40, MK 3.02D±0.89, p=0.04, mean difference 0.86D, (95%CI 0.71-1.01)). This difference has limited clinical significance among postkeratoplasty eyes considering that in approximately 90% of cases in this series, refractive astigmatism could be corrected with spectacles, with no significant differences in rates of high astigmatism (>4.5 D) between the two groups. Total rates of failure from all causes by 5 years were 2/348 DALK (0.58%) and 4/68 MK (5.88%). Large diameter DALK (8.50-9.00mm) has been shown to have superior refractive outcomes over smaller diameter grafts, whilst avoiding an increased risk of stromal rejection which, in any case, is manageable medically in the majority of cases. 10,13,14 Such diameters are larger than those conventionally performed for PK due to concerns of the increased risk of endothelial rejection. In the first instance, macroperforation of the DM during DALK can still result in successful DALK. 15,16 Nevertheless, DALK still has a relatively high rate of conversion to full thickness keratoplasty; 16.4% in this series and between 14.9-35.3% in others. <sup>6,9,15</sup> In such cases, conversion to a 9mm PK is significantly larger than the conventionally considered optimal compromise of 8-8.25mm diameter full-thickness grafts, sized to simultaneously minimize postoperative refractive error and the risk of immunologic rejection. A strategy to manage conversion from DALK to PK has therefore evolved using a two-piece mushroom shaped graft. which aims to minimize the endothelium transplanted in these keratoconic patients with otherwise healthy host endothelium to a 6mm posterior lamella,8 whilst still benefitting from the wide anterior lamellar refractive surface of 9mm. This strategy permits 9mm DALK being safely attempted in all keratoconic patients in the knowledge that cases requiring conversion still perform well with excellent outcomes at 5 years. In this series, most cases that required conversion were associated with manual lamellar dissection. Additionally, we have previously described significant independent risk factors associated with conversion from DALK to PK (in reducing order of risk) including occurrence of a type 2 bubble during pneumatic dissection, the need for manual dissection, the presence of corneal scarring and relative surgeon inexperience.<sup>17</sup> Comparing the outcomes of the MK with the DALK cohorts, the MK group had statistically higher refractive astigmatism, however achieved the same BCVA. This finding reinforces our clinical experience that the microkeratome-dissected interface present in MK does not result in inferior visual outcomes when compared with the interface created by pneumatic dissection during DALK. This is unsurprising given the clinical appearance of the interface postoperatively as can be seen in Figure 5. The presence of any stromal interface may cause some degree of light scattering depending on the optical quality of the surfaces in contact. As shown after laser in situ keratomileusis or various other types of lamellar keratoplasty, microkeratome-assisted dissection produces smooth regular interfaces of a quality compatible with excellent vision while light scattering is usually of no clinical significance.<sup>8,10,18,19</sup> However, further

### Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

comparative analysis of induced higher order aberrations and light scatter is necessary to evaluate differences between the two procedures. Notably, the use of viscoelastic during lamellar dissection for DALK often induces transient interface haze, thereby affecting early postoperative visual outcomes.<sup>20</sup>

ECL at 2 years following DALK was 17.70% vs 46.24% following MK (P<0.001) (Table 2), as to be expected from studies comparing DALK with PK.<sup>21</sup> However the ECL reported for the MK cohort is sampled from the 6mm central stalk, which represents only 25% of the total endothelial population of the cornea<sup>8</sup> with the remaining healthy host endothelium being left intact. Since it has been shown that host endothelium may migrate across the posterior graft-host junction; preserving an even greater area of peripheral host will only provide a greater reservoir of native healthy endothelium.<sup>22</sup> This may account for why there was not a single episode of graft failure in the MK group secondary to endothelial decay in the absence of rejection. In comparison to other studies which have reported no worse outcomes for their cohorts converted to PK compared to uncomplicated DALK, 6,9 the greater failure rates of 4/68 (5.88%) in MK cohort is fact more consistent with reported failures rates of DALK in the literature. <sup>6,23</sup> One failure was due to traumatic dehiscence, one due to infection and two were subsequent to episodes of endothelial rejection (Table 4B); all were regrafted with good outcomes. In both cases of graft failure following endothelial rejection, only the 6 mm posterior lamella was exchanged with a similar one prepared by means of microkeratome-assisted dissection, punched to a 6 mm diameter and delivered bimanually through a 3 mm nasal clear-cornea incision, as per our standard DSAEK technique. 19

As expected given the larger diameter, the BCVA and refractive astigmatism outcomes of both 9mm DALK and MK groups compare favorably with a 2017 systematic review of DALK and PK for keratoconus. <sup>24</sup> Furthermore, respective graft survival probabilities of 99.42% and 94.12% at 5 years also compare favorably with the literature, with a meta-analysis reporting average DALK survival rate to be 100%, 92% and 90% (86.0–94.8%) at 1, 3 and 5 years respectively. <sup>16</sup>

Same size donor and recipient trephines and punches were used for the 9 mm anterior and the 6 mm posterior lamellae. In the deep plane, this results in the diameter of the donor button being slightly smaller than that of the recipient bed, thus avoiding formation of clinically evident Descemet folds, which may affect vision.

With the 2-piece MK. although the proximity of the larger 9-mm anterior lamella (mushroom "hat") to the limbal vascular arcade may theoretically increase the risk of immune rejection, we have not observed an increased risk of stromal rejection even among high-risk eyes. <sup>8,13,25</sup> On the other hand, the smaller 6-mm posterior lamella (mushroom "stem") decreases the endothelial antigenic load, therefore reducing the primary alloimmune target for endothelial rejection. In addition, as at least 2/3 of the healthy host endothelium is preserved with this design, even if an immunologic rejection were to occur, endothelial cell migration from the large healthy recipient bed could theoretically replace all the donor rejected endothelium, similarly to what is seen in Descemet stripping without endothelial keratoplasty. <sup>26</sup>

Clearance of central corneal stroma in DALK and excision of central full-thickness cornea in MK were limited to the 6 mm optical zone, resulting in a posterior stromal crown (Figure 5), about 1.5 mm in width. This rim of overlap between the posterior

## Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

surface of the anterior lamellar graft and the peripheral residual host stroma results in a wound configuration with several advantages. First, the manually dissected plane results in a far greater surface area of donor-host stromal contact compared with vertical wound in conventional PK or DALK, theoretically inducing superior wound strength and improved corneal biomechanics, as well as allowing earlier suture removal (all sutures are removed before 12 months). Although the thickness of posterior stromal shoulder cannot be determined exactly, aiming at a precise thickness is of negligible clinical importance, as stromal remodelling of the posterior shoulder occurs modifying over time both the thickness and the shape of this part of the residual bed.<sup>27</sup> Second, in cases of post-keratoplasty astigmatism this plane also facilitates the safe creation of predictable, deep and consistent in-the-wound blunt relaxing incisions without risk of perforation into the anterior chamber.<sup>28</sup> Finally, large diameter grafting according to the techniques employed in this series maximize removal of ectatic tissue while extending "capping" of the residual bed further into the corneal periphery, Theoretically this may also reduce the rate of late ectasia recurrence which is typically characterized by progressive thinning of, the residual recipient bed, thus resulting in elongation and slippage of the original PK wound. <sup>8,29,30</sup> Since some cones can extend beyond an 8mm trephination, it is likely that ectasia recurrence is further reduced by the fact that 9mm grafts are more likely to excise the entire cone in such patients. However, longer followup is required to evaluate recurrence of ectasia in these cases.

The present study has several limitations. First, it has a retrospective design, which may decrease the validity of the study findings. However, all of the previously mentioned studies on the same topic have also had a retrospective design, most with lower sample sizes. Including a large number of patients in our study can compensate, at least to some extent, for the limitations inherent to the retrospective design. Besides, all data was entered prospectively at regular intervals post-operatively, with consistent results up to 5 years. Studies with even longer follow up would be interesting in future to determine whether the rate of ectasia recurrence is indeed lower in eyes with wide diameter grafts or those with increased surface area of the graft-host interface. In conclusion, the excellent 5-year visual and clinical outcomes associated with a two-piece mushroom keratoplasty in cases converted from intended DALK mandate large diameter DALK (9mm) as the optimal surgical approach to keratoconus.

Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

### **ACKOWLEDGEMENTS/DISCLOSURE:**

- a) No support, financial or otherwise was received for this study
- b) Massimo Busin has received (2006-2016) reimbursement of travel expenses and royalties from Moria (Antony, France). Harry Roberts has undertaken paid consultancy work for Alcon Inc (Fort Worth, TX, USA) in the past 12 months and has received honoraria from Thea Pharmaceuticals Ltd (Keele, UK). The other authors have no financial interest to disclose.
- c) No other acknowledgements are necessary

#### REFERENCES

- 1. Anwar M, Teichmann KD. Big-bubble technique to bare Descemet's membrane in anterior lamellar keratoplasty. *J Cataract Refract Surg* 2002;28(3):398-403.
- 2. Melles GR, Lander F, Rietveld FJ, Remeijer L, Beekhuis WH, Binder PS. A new surgical technique for deep stromal, anterior lamellar keratoplasty. *Br J Ophthalmol* 1999;83(3):327-333.
- 3. Gonzalez A, Price MO, Feng MT, Lee C, Arbelaez JG, Price FW. Immunologic rejection episodes after deep anterior lamellar keratoplasty: incidence and risk factors. *Cornea* 2017;36(9):1076-1082.
- 4. Seitz B, Langenbucher A, Küchle M, Naumann GOH. Impact of graft diameter on corneal power and the regularity of postkeratoplasty astigmatism before and after suture removal. *Ophthalmology* 2003;110(11):2162-2167.
- 5. Huang T, Hu Y, Gui M, Zhang H, Wang Y, Hou C. Large-diameter deep anterior lamellar keratoplasty for keratoconus: visual and refractive outcomes. *Br J Ophthalmol.* 2015;99(9):1196-1200.
- 6. Gadhvi KA, Romano V, Fernández-Vega Cueto L, Aiello F, Day AC, Allan BD. Deep anterior lamellar keratoplasty for keratoconus: multisurgeon results. *Am J Ophthalmol.* 2019;201:54-62.
- 7. Williams KA, Lowe M, Bartlett C, et al. Risk factors for human corneal graft failure within the Australian corneal graft registry. *Transplantation* 2008;86(12):1720-1724.
- 8. Busin M, Madi S, Scorcia V, Santorum P, Nahum Y. A two-piece microkeratomeassisted 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.
- 9. Donoso R, Díaz C, Villavicencio P. Comparative study of keratoconus between Anwar's deep anterior lamellar keratoplasty versus converted penetrating keratoplasty. *Arch Soc Esp de Oftalmol* 2015;90(6):257-263.
- 10. Busin M, Leon P, Nahum Y, Scorcia V. Large (9 mm) Deep anterior lamellar keratoplasty with clearance of a 6-mm optical zone optimizes outcomes of keratoconus surgery. *Ophthalmology* 2017;124(7):1072-1080.
- 11. Bovone C, Myerscough J, Friehmann A, Elkadim M, Parmeggiani F, Busin M. Peripheral intrastromal hydration facilitates safe, deep trephination in corneas of irregular thickness. *Cornea* 2020;39(2):207-209.

- 12. Myerscough J, Bovone C, Mimouni M, Elkadim M, Rimondi E, Busin M. Factors predictive of double anterior chamber formation following deep anterior lamellar keratoplasty. *Am J Ophthalmol* 2019;205:11-16.
- 13. Giannaccare G, Weiss JS, Sapigni L, et al. Immunologic stromal rejection after deep anterior lamellar keratoplasty with grafts of a larger size (9 mm) for various stromal diseases. *Cornea* 2018;37(8):967-972.
- 14. Feizi S, Javadi MA, Daryabari SH. Factors influencing big-bubble formation during deep anterior lamellar keratoplasty in keratoconus. *Br J Ophthalmol* 2016;100(5):622-625.
- 15. Huang OS, Htoon HM, Chan AM, Tan D, Mehta JS. Incidence and outcomes of intraoperative Descemet membrane perforations during deep anterior lamellar keratoplasty. *Am J Ophthalmol* 2019;199:9-18.
- 16. Kodavoor SK, Deb B, Ramamurthy D. Outcome of deep anterior lamellar keratoplasty patients with intraoperative Descemet's membrane perforation: A retrospective cross-sectional study. *Indian J Ophthalmol*. 2018;66(11):1574-1579.
- 17. Myerscough J, Friehmann A, Bovone C, Mimouni M, Busin M. Evaluation of the risk factors associated with conversion of intended deep anterior lamellar keratoplasty to penetrating keratoplasty. *Br J Ophthalmol* 2020;104(6):764-767.
- 18. McLaren JW, Bourne WM, Maguire LJ, Patel SV. Changes in keratocyte density and visual function five years after laser in situ keratomileusis: femtosecond laser vs mechanical microkeratome. *Am J Ophthalmol* 2016;160(1):163-170.
- 19. Busin M, Madi S, Santorum P, et al. Ultrathin Descemet's stripping automated endothelial keratoplasty with the microkeratome double-pass technique: two-year outcomes. *Ophthalmology* 2013;120 (6):1186–1194.
- 20. Scorcia V, De Luca V, Lucisano A, et al. Comparison of corneal densitometry between big- bubble and visco-bubble deep anterior lamellar keratoplasty. *Br J Ophthalmol* 2020;104(3):336-340.
- 21. Chen G, Tzekov R, Li W, Jiang F, Mao S, Tong Y. Deep anterior lamellar keratoplasty versus penetrating keratoplasty: A meta-analysis of randomized controlled trials. *Cornea* 2016;35(2):169-174.
- 22. Imaizumi T. Movement of corneal endothelium after penetrating keratoplasty. Observation of sex chromatin as a cell marker. *Nippon Ganka Gakkai Zasshi*. 1990;94(10):928-936.

### Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

- 23. Keane M, Coster D, Ziaei M, Williams K. Deep anterior lamellar keratoplasty versus penetrating keratoplasty for treating keratoconus. *Cochrane Database of Syst Rev.* https://doi.org/10.1002/14651858.CD009700.pub2. 2014.07.22.
- 24. Henein C, Nanavaty MA. Systematic review comparing penetrating keratoplasty and deep anterior lamellar keratoplasty for management of keratoconus. *Contact Lens Anterior Eye* 2017;40(1):3-14.
- 25. 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.* https://doi.org/10.1016/j.ajo.2020.04.016. 2020.04.27.
- 26. Borkar D, Veldman P, Colby KA. Treatment of Fuchs endothelial dystrophy by Descemet stripping without endothelial keratoplasty. *Cornea* 2016;35(10):1267-1273.
- 27. Yu AC, Mattioli L, Busin M. Optimizing outcomes for keratoplasty in ectatic corneal disease. *Curr Opin Ophthalmol*. doi: 10.1097/ICU.000000000000666. 2020.05.13.
- 28. Elkadim M, Myerscough J, Bovone C, Busin M. A novel blunt dissection technique to treat modified deep anterior lamellar keratoplasty (DALK)-associated high astigmatism. *Eye (Lond)*. https://doi.org/10.1038/s41433-019-0686-6. 2019.11.21.
- 29. Raecker ME, Erie JC, Patel SV, McLaren JW, Hodge DO, Bourne WM. Long-term keratometric changes after penetrating keratoplasty for keratoconus and Fuchs endothelial dystrophy. *Am J Ophthalmol*. 2009;147(2):227-233.
- 30. Patel SV, Malta JB, Banitt MR, et al. Recurrent ectasia in corneal grafts and outcomes of repeat keratoplasty for keratoconus. *Br J Ophthalmol*. 2009;93(2):191-197.

Myerscough et al. – Mushroom Keratoplasty Versus DALK for Keratoconus

#### **LEGEND**

- Figure 1: Best-corrected visual acuity after complete suture removal (1.5-year follow-up). BCVA = best corrected visual acuity; DALK = Deep anterior lamellar keratoplasty; MK = Mushroom keratoplasty.
- Figure 2: Refractive astigmatism after complete suture removal (1.5-year follow-up). DALK = Deep anterior lamellar keratoplasty; MK = Mushroom keratoplasty.
- Figure 3: Mean endothelial cell density over 5 years following deep anterior lamellar keratoplasty and mushroom keratoplasty. DALK = deep anterior lamellar keratoplasty, ECD = endothelial cell density, MK = mushroom keratoplasty
- Figure 4: Kaplan-Meier survival curve of deep anterior lamellar keratoplasty and mushroom keratoplasty. DALK = Deep anterior lamellar keratoplasty; MK = Mushroom keratoplasty.
- Figure 5: Photos of deep anterior lamellar keratoplasty (DALK) (A, B, C, D) and mushroom keratoplasty (MK) (E, F, G, H) for keratoconus. A, E) Color photographs 5 years postoperatively. B, F) High zoom optical section 5 years postoperatively. C, G) Anterior segment optical coherence tomography 5 years after DALK. D, H) Schematic diagrams.

Table 1: Best spectacle corrected visual acuity and refractive astigmatism

		Follow-up duration (year)							
		Preoperative	0.5	1	1.5	2	3	4	5
BCVA (logMAR)	DALK	0.66±0.30	0.23±0.15	0.16±0.17	0.12±0.15	0.08±0.13	0.09±0.16	0.09±0.13	0.06±0.07
	MK	0.71±0.28	0.28±0.19	0.17±0.16	0.08±0.09	0.10±0.10	0.10±0.11	0.09±0.11	0.09±0.15
	p- value	0.23	0.26	0.89	0.46	0.28	0.54	0.81	0.88
RA (D)	DALK	4.76±2.13	3.64±1.80	3.60±1.78	3.03±1.27	2.74±1.30	2.53±1.25	2.39±1.57	2.16±1.40
	MK	4.86±2.45	3.81±0.82	3.51±1.58	3.49±1.36	3.27±1.50	3.31±1.61	3.09±1.66	3.02±0.89
	p- value	0.77	0.72	0.58	0.041	0.033	0.028	0.039	0.042

BCVA=best spectacle corrected visual acuity; DALK=deep anterior lamellar keratoplasty; D= diopter; logMAR=logarithm of minimum angle of resolution; MK=mushroom keratoplasty; RA=refractive astigmatism

Table 2: Endothelial cell density and endothelial cell loss

		DALK			MK		p-value
	Number	ECD	ECL	Number	ECD	ECL	
	of eyes	(cells/mm <sup>2</sup> )	(%)	of eyes	(cells/mm <sup>2</sup> )	(%)	
Preoperative	348	2431±334	-	68	2370±238	-	0.63
6 months	348	2113±430	13.07	68	1575±434	31.57	< 0.001
1 year	348	2005±401	14.04	68	1463±294	33.53	< 0.001
18 months	313	2000±395	17.50	59	1347±381	43.15	< 0.001
2 years	278	2001±356	17.70	53	1274±304	46.24	< 0.001
3 years	192	1967±413	17.65	29	1158±279	51.13	< 0.001
4 years	110	1960±375	19.08	16	1072±260	54.74	< 0.001
5 years	53	1956±522	19.36	5	1028±375	56.61	< 0.001

DALK=deep anterior lamellar keratoplasty; ECD=endothelial cell density; ECL=endothelial cell loss; MK=mushroom keratoplasty

**Table 3: Postoperative complications** 

	Deep anterior lamellar keratoplasty	Mushroom keratoplasty	
Detachment	16 (4.60%) 1 failed (residual bed)	1 (1.47%) (posterior lamella)	
Persistent epithelial defect	1 (0.29%)	0 (0%)	
Glaucoma	2 (0.58%)	0 (0%)	
High astigmatism	35 (10.06%)	5 (7.35%)	
Cataract	9 (2.59 %)	5 (7.35%)	
Wound dehiscence	7 (2.01%)	2 (2.94%) 1 failed	
Immune rejection	22 (6.31%)	6 (8.82%)	
Stromal rejection	22 (6.31%)	2 (2.94%)	
Endothelial rejection	0 (0%)	4 (5.88%) 2 failed	
Interface infection	2 (0.58 %) 1 failed	1 (1.47%) 1 failed	

Graft failure	2 (0.58 %)	4(5.88%)
	= (0.00 /0)	(0.0070)

Table 4: (A) Kaplan-Meier analysis of graft survival probability

	1 year	2 years	3 years	4 years	5 years		
DALK (%)	99.42	99.42	99.42	99.42	99.42		
MK (%)	95.59	94.12	94.12	94.12	94.12		
(B) Details of failed cases: (All failed grafts were re-grafted)							
DALK							
1	Persistent Descemet membrane detachment with repeated rebubbling after 1.5 months: endothelial KP (converted to MK) (3-year Snellen BSCVA 0.4)						
2	Interface infection: Therapeutic penetrating keratoplasty: (1-year Snellen BSCVA: 0.4)						
MK							
1	Wound dehiscence secondary to trauma with ruptured globe: Tectonic PK: vascularized corneal opacity						
2	Infection after 3 weeks: Repeat MK (4-year Snellen BSCVA 0.6)						
3	Endothelial rejection at 5 months: Posterior lamella exchange (2-year Snellen BSCVA 0.5)						
4	Endothelial rejection at 2 years: Posterior lamella exchange (1-year Snellen BSCVA 0.9)						

BCVA=best corrected visual acuity; DALK=deep anterior lamellar keratoplasty; MK=mushroom keratoplasty

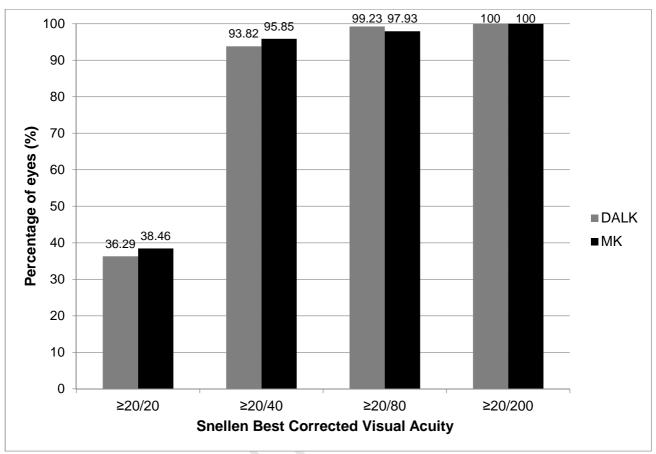


Figure 1: Best-corrected visual acuity after complete suture removal (1.5-year follow-up).

DALK = Deep anterior lamellar keratoplasty; MK = Mushroom keratoplasty.

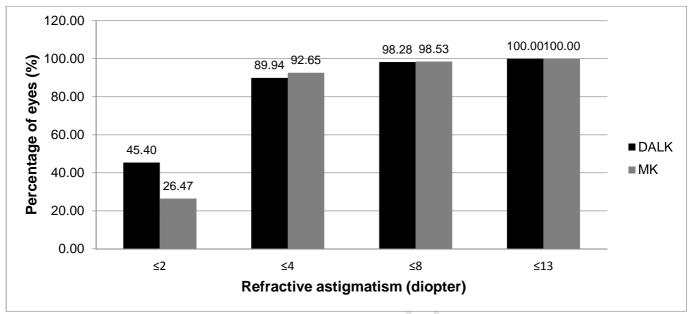


Figure 2: Refractive astigmatism after complete suture removal (1.5-year follow-up). DALK = Deep anterior lamellar keratoplasty; MK = Mushroom keratoplasty.

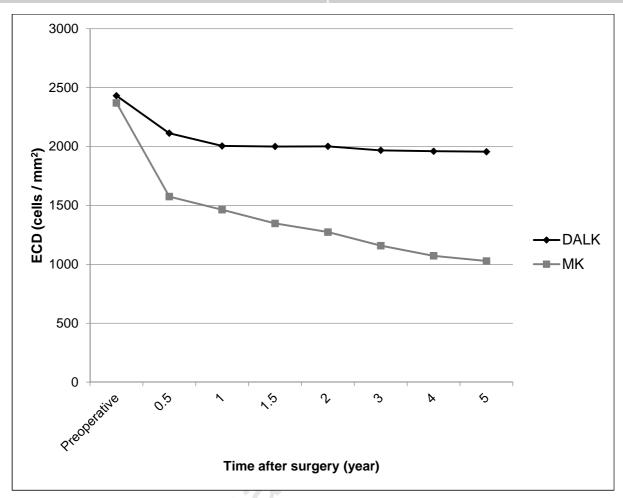


Figure 3: Mean endothelial cell density over 5 years following deep anterior lamellar keratoplasty and mushroom keratoplasty

DALK = deep anterior lamellar keratoplasty, ECD = endothelial cell density, MK =

mushroom keratoplasty

