Title: Femtosecond laser assisted Deep anterior lamellar keratoplasty for keratoconus: multi-surgeon results

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Financial support: This research has received a proportion of its funding from the Department of Health's NIHR Biomedical Research Centre for Ophthalmology at Moorfields Eye Hospital and UCL Institute of Ophthalmology. The views expressed in the publication are those of the authors and not necessarily those of the Department of Health.

Conflict of interest: No conflicting relationship exists for any author

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Abstract

Purpose: To compare the clinical outcomes in femtosecond laser assisted deep anterior lamellar keratoplasty (F-DALK) to manual non-laser deep anterior lamellar keratoplasty (M-DALK) for keratoconus in a multi-surgeon public healthcare setting.

Design: Single-centre, comparative, retrospective cohort analysis.

Methods:

Population: Consecutive cases of keratoconus treated with big-bubble F-DALK from August 1st 2015 to September 1st 2018 and big-bubble M-DALK from September 1st, 2012, to September 31st, 2016. **Setting:** Moorfields Eye Hospital, London. **Observations:** Data on preoperative status, operative details, intraoperative and postoperative complications, secondary interventions, and visual outcomes were archived on a customized spreadsheet for analysis. **Main outcome measures:** Rate of Intra operative perforation and conversion to penetrating keratoplasty (PK) and the percentage of patients, post removal of sutures (ROS), with corrected distance visual acuity (CDVA) ≥20/40.

Results: We analysed 58 eyes of 55 patient who underwent F-DALK and 326 eyes of 309 patients who underwent M-DALK. Intraoperative perforation of Descemet membrane occurred in 24.9% of F-DALK cases compared 45.4% of M-DALK cases (p=0.006). Intraoperative conversion to PK was carried out in 3.4% of F-DALK cases compared to 24.5% of M-DALK cases (p=0.001). Post ROS, 86.5% of F-DALK eyes had a CDVA of ≥20/40 (15±7 months after surgery) compared to 83.7% of M-DALK eyes (p=0.825).

Conclusion: Laser automation of some steps in DALK for keratoconus may reduce the rate of intraoperative Descemet perforation and the conversion to PK in a multi-surgeon setting.

Introduction

Keratoconus is one of the leading indications for corneal transplantation worldwide, accounting for 27% of all corneal transplants in a recent global survey [1]. Penetrating keratoplasty (PK) – full thickness corneal transplantation, and deep anterior lamellar keratoplasty (DALK) – transplantation of a full thickness donor button into a host bed dissected down to the pre-Descemet layer, are the main contemporary corneal transplantation techniques for keratoconus. Both produce good results [2] , but PK is still more widely performed [1] . This is despite advantages for DALK including avoiding an open globe, preserving the host endothelium and preventing endothelial rejection [3] .

Adoption of DALK for keratoconus has been limited by the technical challenges of deep dissection in the host cornea and unfavourable data, reflecting the learning curve in the transition to DALK, in previous transplant registry publications from Australia and the UK [2,4] . Improved techniques for manual pre-Descemet layer dissection [6,5] are now more widely disseminated, and recent results suggest that visual and early graft survival outcomes for DALK and PK are now similar [2,6,7,8,9,10,11]. Manual DALK (M-DALK), using conventional microsurgery, remains technically challenging however, particularly in a multi-surgeon setting. In a review of 357 consecutive cases of DALK for keratoconus performed by 42 surgeons (31 trainees operating under supervision) at Moorfields Eye Hospital using contemporary manual DALK techniques, we found a 45% rate of intraoperative Descemet perforation, with 24% of cases converted to PK [12] .

Automation, or 'robot surgery', is a rapidly developing solution in technically demanding areas of surgery[13,14]. In the context of DALK, optical coherence tomography for accurate pre-operative and intraoperative mapping of corneal dimensions, and femtosecond photodisruptors capable of producing accurately controlled 3-dimensional cut patterns in the cornea, are being combined in a variety of approaches with the aim of enhancing the safety and reproducibility of results in DALK for keratoconus [15 16 17 18 19 20 21 22 23 24 25 26 27 28].

Here we describe a variation of mushroom pattern femtosecond laser assisted DALK (F-DALK) featuring a large diameter (9mm) anterior cap designed to reduce postoperative astigmatism, and a small diameter (6mm) optical zone designed to respect the anatomy of the pre-Descemet layer, which inserts into the anterior corneal stroma at 6-8mm diameter [29]. We hypothesized that confining deep dissection to within the diameter of this natural anatomical plane would help reduce the rate of intraoperative perforation and conversion to PK. Outcomes at one year in consecutive keratoconus cases treated with F-DALK are compared with outcomes for big-bubble M-DALK in similar cases extracted from our previously published series [12] .

Methods

The study was approved as a clinical audit project by the Moorfields Eye Hospital Clinical Audit and Effectiveness Committee. The tenets of the declaration of Helsinki were followed with informed consent for surgery as part of routine clinical care.

The study was a comparative interventional case series, with retrospective review of case notes and electronic operating theatre records and anonymized archiving of study data.

Inclusion criteria

The audit period was August 2015 to September 2018. We identified consecutive cases of keratoconus listed for F-DALK in the audit period from an electronic operating healthcare record system (Open Eyes v1.18, www.openeyes.org.uk). We used an 'intention to treat' protocol in which cases converted to PK were included for study. The indication for surgery was advanced keratoconus (Amsler-Krumeich stage II, III or IV) with poor contact lens tolerance and subjectively inadequate spectacle corrected distance visual acuity (CDVA). As a historical control group, we used data from our previous study [12] of patients undergoing big-bubble M-DALK for Keratoconus during the period September 2012 to September 2016.

Surgical planning

For F-DALK cases we performed optical coherence tomography (OCT) mapping of the host cornea (Casia SS-1000, Tomey, Nagoya, Japan) to identify the thinnest point at a 6mm diameter around the corneal vertex (Fig 1a), and estimated the minimum corneal white to white dimension by superimposing an 8mm slit lamp beam on the host cornea in horizontal and vertical meridians. We programmed a mushroom cut pattern in both donor and host corneas using the Intralase enabled keratoplasty (IEK) tab of treatment planning software on an Intralase iFS femtosecond laser (J&J Vision, Santa Ana, CA) using default energy and spot separation settings throughout.

In the host cornea, we programmed a 6mm diameter posterior side cut, and set the maximum depth at the OCT measured minimum 6mm diameter corneal thickness minus 70µm. We set the depth of the lamellar ring cut at the maximum depth of the posterior side cut minus 50µm (Fig 1b). We then set the diameter of the anterior side cut at 9mm for most cases, reducing to 8.7mm where the minimum white to white measurement was less than 11mm. We set a minimum cut overlap of 20µm in all directions.

In the donor cornea, we programmed a reciprocal mushroom cut pattern with reference to the host cut, setting the anterior side cut diameter at host diameter plus 0.3mm, the posterior side cut diameter at 6mm, and the lamellar ring cut depth at host depth plus 20µm to allow for donor tissue deturgescence post transplantation. We set the posterior depth of the donor tissue posterior side cut at 900µm to ensure clean penetration into the anterior chamber.

We performed the host cut initially under topical anaesthetic (Proxymetacaine hydrochloride 0.5% and Povidone Iodine 5%, Bausch and Lomb UK Ltd, Kingston-upon-Thames, UK) and cut the donor tissue once a satisfactory host cut was confirmed. We marked the anatomical centre of the host cornea with a gentian violet marker and centred the host cut on this mark.

We mounted donor corneal buttons on an artificial anterior chamber (Barron artificial anterior chamber, Katena Products Inc, Parsippany, NJ) using a thin layer of cohesive OVD to cover the anterior surface of the artificial chamber mount and filtered air to bring the chamber to a firm physiological pressure after the locking ring had been engaged symmetrically over the donor corneal limbus. We irrigated the epithelial surface of the donor cornea with balanced salt solution, and dried around the limbus with arrow tip surgical sponges to remove excess fluid, leaving a clear image of a thin meniscus during applanation and host cutting.

After the donor cut was completed, we infused culture medium supplied with the donor tissue gently through the artificial anterior chamber to expel air from beneath the donor corneal endothelium. We then covered the epithelial surface of the cornea with culture medium, and transferred the artificial anterior chamber with the mounted cornea to a sterile anaesthetic tray, covered it with a sterile plastic galley pot, and wrapped the anaesthetic tray with the protected artificial anterior chamber in a sterile theatre trolley cover for transfer from the laser suite to the main operation theatre.

After transferring both the patient and the pre-cut donor corneal button to the operating theatre, we performed surgery under general anaesthetic using a variation of the big-bubble technique described by Anwar and Teichman in which the femtosecond lamellar cut was blunt dissected and marked 360° with gentian violet. We then identified the deep aspect of the posterior side cut with sharp dissection using a bent 27-gauge needle, and passed a blunt trocar to dissect as close as possible to Descemet membrane, before advancing a blunt 27-guage Fontana cannula (Surgistar, Vista, CA) to the centre of the cornea for air dissection aiming to form a big-bubble and dissect down to the pre-Descemet layer in the 6mm central optical zone. Following attempted air dissection, we used the small-bubble technique [30] , in which a small bubble is introduced to the anterior chamber through a paracentesis and the eye is rolled to ensure that the small bubble remains visible in the anterior chamber periphery, to determine whether a big-bubble had been achieved. Where a big-bubble was present, we proceeded as described by Anwar and Teichman to expose the pre-Descemet layer using blunt scissors to clear residual posterior corneal stromal tissue within the 6mm zone. Where no big-bubble was achieved, we proceeded we attempted viscodissection [31] with cohesive OVD. If this too failed, or in cases with air injected directly into the anterior chamber, we proceeded with layer by layer manual dissection to clear the posterior stroma within the optical zone. Following host dissection, we peeled the pre-dissected donor cornea from the mounted corneosceral button, removed the donor Descemet membrane with semi-dry arrow tip sponges, washed the donor cornea in BSS, and secured the donor with 16 interrupted 10-0 nylon sutures or a continuous suture. We injected subconjunctival Cefuroxime (125mg/ml) and Betamethasone (4mg/ml) at termination of surgery.

Details of surgical planning for the Manual DALK cases has been previously described[12]. In this series, we only included cases performed using the big-bubble technique. Surgeons performed a partial-thickness (350-450µm) trephination of variable diameter, between 7.5 and 9.0 mm, using a suction trephine. The size of trephination was determined according to the size of the cone and the horizontal corneal diameter, aiming to include the entire cone within the area of trephination whilst leaving a minimum 1mm boundary of host cornea over 360°. A 27-guage needle or custom air dissection cannula was introduced into the deep stroma starting at the bottom of the trephination groove and advanced toward the center of the cornea. Air was injected progressively into the stroma, with the aim of achieving the formation of a large air bubble between the pre-Descemet layer and the overlying stroma. A peripheral paracentesis was performed to lower the intraocular pressure. Blunt-tipped scissors were used to divide the anterior stroma into four sections, which were then removed, exposing the pre-Descemet layer. Surgeons secured donor buttons with 10-0 nylon sutures in a continuous or interrupted suture pattern. At the end of surgery, surgeons attempted to minimise astigmatism using intraoperative adjustment of continuous sutures adjustment or selective removal and replacement of interrupted sutures. Surgeons injected subconjunctival Cefuroxime (125mg/ml) and Betamethasone (4mg/ml) at termination of surgery.

Postoperative care

In cases of intraoperative perforation, intensive pupil dilation was followed by an anterior chamber air fill, which was reduced at termination of surgery to approximately 60%. Patients were checked 1 hour after surgery prior to discharge to ensure that there was no pupil block. Patients were then asked to posture face up to ceiling, when possible, for the 2 days after surgery, and pupil dilation was maintained for 3 days.

Routine postoperative medication included antibiotic (chloramphenicol 0.5%) eye-drops four times daily for 1 week, and a diminishing regimen of topical steroid medication – typically dexamethasone 0.1% 1-2 hourly for 1 week, reducing over 3-6 months after surgery. All patients were reviewed in the first week after surgery, with a variable follow-up regimen dictated by clinical progress subsequently.

Outcome measures

We archived data retrieved from a retrospective review of case notes and electronic patient records in a customised Excel (Microsoft Corp, Seattle, WA) spreadsheet with forced choice entry criteria. Our primary outcome measures were the rates of intraoperative perforation into the anterior chamber, and intraoperative conversion to penetrating keratoplasty.

We recorded unaided distance visual acuity (UDVA), CDVA and manifest refraction data at the last follow up visit before 12 months post-surgery (early recovery) and at final follow-up, together with the number of glaucoma medications, whether topical steroid medication had been discontinued (yes/no), and whether all sutures had been removed (yes/no). We recorded pre-operative demographic details along with any record in preoperative notes of: atopy, hydrops, previous corneal collagen cross-linking or intracorneal ring segment implantation. We subcategorised atopy into mild atopy (any history of eczema, asthma, hay fever or topical treatment with mast cell degranulation inhibitors) and severe atopy (any record of topical treatment with Cyclosporine A).

Operative details and events we recorded were: the surgeon career grade (consultant/surgeon in training); donor punch diameter (mm); host trephination diameter (mm); intended lamellar dissection technique (big-bubble/Melles/other); big-bubble result (type I/type II/no bubble/bubble rupture/air injected in anterior chamber/trephination into anterior chamber); perforation into the anterior chamber (yes/no); intraoperative conversion to penetrating keratoplasty (yes/no); suture method (continuous/interrupted); and whether or not donor Descemet membrane had been removed.

Early postoperative events we recorded (yes/no) were: a double anterior chamber (fluid in the lamella interface between donor and host cornea); Urrets Zavalia Syndrome (fixed dilated pupil presumed secondary to pupil block glaucoma); and atopic sclerokeratitis (host side inflammation associated with multifocal infiltrates at points of suture entry and suture loosening).

Postoperative interventions we recorded at any time point were: any unscheduled increase in topical steroid medication (transplant rejection); re-suture; air injection into the anterior chamber: the maximum number of glaucoma medications required for intraocular pressure control; glaucoma drainage surgery or cycloablation; cataract surgery; repeat corneal transplantation; and refractive surgery.

In line with Coster et al [4], we defined graft failure as irreversible loss of graft clarity or repeat corneal transplantation.

Data Analysis

Continuous data are shown as the mean±SD. Categorical data are shown as % throughout, where the percentage denominator is the total number of available data points in that category. Accountability data is shown as n (%), where the percentage denominator is the total number of cases studied. Accountability was 100% unless specified. We converted Snellen visual acuities to LogMAR values for statistical comparisons. We checked normality in this data using the Shapiro Wilk test. Two-tailed analyses were used throughout. For continuous data comparisons, we used the t test where there were >30 observations in each group. We used Fisher's exact test for comparisons of categorical data.

We performed statistical tests in Excel (v15.34 for Mac), "www.graphpad.com" or SPSS (IBM, version 26 for Mac)

Results

We identified 58 consecutive cases of F-DALK performed for keratoconus in 55 patients within the audit period: August 2015 to September 2018. In our historical control group, 326 consecutive cases of big-bubble M-DALK were performed for keratoconus in 309 patients within the audit period: September 2012 to September 2016. We have presented summary data in the following tables: preoperative data (table1) intraoperative data (table 2), intraoperative complications (table3), postoperative data (table 4) and post-operative inventions (table 5). The follow-up period for F-DALK cases reported here (15±7 months) was shorter than for M-DALK controls (22±11 months). There were graft failures in our F-DALK series, and the rejection rate in the follow-up period was 15.5%.

Perforation and conversion

In F-DALK cases intraoperative perforations occurred in 15 cases (25.9%). 2 cases (3.4%) were converted to PK. 2 eyes (3.4%) developed a double anterior chamber postoperatively. The double anterior chamber persisted despite treatment with postoperative anterior chamber air injection in 1 case. Descemet's membrane was not removed on the donor cornea intraoperatively in this single case of a persistent double anterior chamber. This patient had a clear donor cornea, but developed an opaque, fibrotic, detached residual host Descemet's membrane which we removed in revision surgery. This eye also developed secondary open angle glaucoma treated with insertion of a glaucoma drainage device.

In M-DALK cases intraoperative perforation occurred in 148 cases (45.4%). Overall, 80 cases (24.5%) were converted to PK intraoperatively. These included 11 cases (3.4%) converted electively when no big-bubble was obtained, and 69 eyes (21.2%) converted to PK after intraoperative perforation. 79 eyes (24.2%) with intraoperative perforation into the anterior chamber were managed without conversion to PK.

In comparison with M-DALK historical control cases, the intraoperative perforation rate was significantly lower in F-DALK (p=0.006). Both the overall rate of intraoperative conversion to PK (p=0.0001) and the rate of intraoperative conversion to PK after perforation into the anterior chamber (p=0.014) were also significantly lower in F-DALK.

Filtering out 4 cases with suspected previous hydrops, the rate of Type 1 big-bubble formation in F-DALK cases was 61.1% (33/54). This was similar (p=0.7652) to the 58.1% rate observed in M-DALK controls (180/310 - excluding 16 cases with suspected hydrops) (Fig 4).

Visual outcomes

Manifest refraction data after removal of corneal sutures was available in 52 eyes treated with F-DALK at final review (15.0±7.3 months). The mean postoperative CDVA was 0.16±0.20. CDVA was ≥20/40 in 86.54% (45/52) of eyes. The mean preoperative CDVA in this group was 0.85±0.34 (Fig 2).

Corneal suture removal was earlier in our F-DALK case series than in our manual DALK series. At 12 months, 62% of F-DALK cases had had sutures removed, whereas only 22% of M-DALK cases had had sutures removed at the same timepoint (p=0.001).

Manifest refraction data after removal of corneal sutures was available in 154 M-DALK eyes at final review (24.9±10.6 months). The mean postoperative CDVA was 0.20±.28. CDVA was

≥20/40 in 83.7% (129/154) of eyes. The mean preoperative CDVA in this group was 0.86±0.38 (Fig 3).

Although there was a trend towards improved final postoperative CDVA after F-DALK, it was not statistically significant (p=0.21). Similarly, there was no significant difference in the number of eyes achieving CDVA ≥20/40 (p=0.825).

Refractive Outcomes

The mean refraction spherical equivalent (MRSE) after suture removal in F-DALK cases was -3.76±3.67D. The mean absolute cylinder was -5.00±3.76D. In M- DALK case series, the mean refraction spherical equivalent after suture removal was -3.42±3.70 D. The mean absolute cylinder was -4.27±2.91D. MRSE results were similar in F-DALK and M-DALK. There was a non-significant trend (p=0.23) towards worse cylinder outcomes in F-DALK despite a larger graft diameter (Table 2).

Discussion

Our data suggest that in a multi-surgeon setting, in which over half the surgery is performed under supervision by corneal fellowship trainees, both the intraoperative perforation rate and the rate of intraoperative conversion to PK are reduced by using a variation of F-DALK in which big-bubble deep dissection is confined within a central 6mm optical zone. Visual and refractive results are similar to those for conventional M-DALK.

A variety of F-DALK techniques has been described over the last 10 years [16]. Early publications from Price et al [24] and Farid and Steinert [19] in 2009 suggested that precise control of dissection depth might increase the rate of big-bubble formation in big-bubble DALK, and that wound strength might be enhanced using a modified side-cut pattern.

Alio et al [17] , using a graded evaluation of scarring at the graft/host junction in slit lamp examination compared eyes treated with mushroom pattern F-DALK (n=25) to M-DALK (n=25). They reported significantly more visible scarring in the F-DALK group, providing indirect evidence of stronger healing.

Alio et al [17] used a mushroom pattern with a 6mm central optical zone in big-bubble DALK similar to ours, but with an 8mm anterior cap and a target posterior side cut depth set at 80% of the thinnest pachymetry. They observed successful big-bubble formation in 80% (20/25) F-DALK and 84% (21/25) M-DALK cases. Operations were all performed by two experienced corneal surgeons. In F-DALK, we used a 9mm anterior cap and a target maximum posterior side cut depth of the thinnest point at the 6mm diameter minus 70µm. This was deeper than thinnest pachymetry in 94% of eyes. Our big-bubble formation rate was lower (56.4%) than that observed by Alio et al in F-DALK despite a deeper target posterior side cut depth. Our big-bubble formation rate was similar (58.4%) in M-DALK controls, and we observed no differences in bubble formation rates between experienced surgeons and surgeons in training operating under supervision (Fig 4) in either case series [12] .

Deeper cannula placement is thought to improve the rate of big-bubble formation in big-bubble DALK [32 33]. These findings suggest that precise definition vertical posterior side cut depth in F-DALK may not translate into precise control of cannula entry depth for air injection. This may be because the depth within the side cut at which the surgeons initiate manual dissection for cannula placement remains poorly controlled. The solution offered by Buzzonetti et al [15] is a femtosecond laser tunnel cut 100µm above the thinnest point to control air injection cannula placement. A big-bubble was achieved in 9/10 keratoconus patients treated using this F-DALK variation.

Although femtosecond photodisruptors are capable of cutting any 3-dimensional pattern in the cornea, current commercially available lasers offer a restricted range of cut shape variations based on commonly used procedures. Buzzonetti et al [15] , using the Intralase iFS, needed a metal mask to shield a ring lamellar cut in order to create a defined depth tunnel. More recently, Liu et al [23], working with the Ziemer LDV Z8 laser (Ziemer Ophthalmic System, Port, Switzerland) used built in intraoperative OCT guidance and dedicated software to create a tunnel cut 3mm in length, 80µm in width, at a 60° downward angle to the applanated horizontal plane. The target depth for the end of this tunnel was 50µm from Descemet's membrane. They achieved a big-bubble in 14 consecutive cases (11 with keratoconus) of F-DALK. Further study is needed to see if these promising results can be replicated.

Although our Type 1 big-bubble formation rate was similar in F-DALK and M-DALK cases, and was not influenced by surgeon experience, the intraoperative perforation rate and the rate of conversion to PK were both significantly reduced in F-DALK cases. Confining dissection to a central 6mm zone, within the diameter of the natural anatomical plane between the pre-Descemet layer and the overlying corneal stroma [29], may make DALK safer. We acknowledge that a learning effect inherent in study designs using historical control cases may introduce bias. But the magnitude of reduction we observed in both the intraoperative perforation rate (almost twice as low) and the rate of conversion to PK (seven times lower) compared with M-DALK suggests a significant clinical gain for F-DALK. Using mushroom pattern F-DALK to combine a small optical zone with a large anterior graft diameter appears may have helped move our multi-surgeon results closer to good results for M-DALK published in single surgeon series [12] .

Recent single surgeon results from Salouti et al are particularly striking. They used Melles technique [12] in both M-DALK historical controls (n=469) and two F-DALK patterns: decagonal (n= 264) and mushroom (n= 153: 9mm anterior diameter; 8mm posterior diameter) created with the Femtec 520F femtosecond laser (Baush and lomb, Munich, Germany). Salouti et al report an intraoperative perforation rate of 1/860. The depth of dissection was not specified, but CDVA at one year (0.17±0.12) for their F-DALK cases was similar to that we report here for a pre-Descemet baring technique (0.16±0.20), implying a low residual stromal thickness [34] .

Salouti et al [27] gathered these data over a 10-year period, and the question of whether such outstanding safety results are repeatable in multi-surgeon series with manual lamellar dissection remains open. Dissection anterior to the pre-Descemet layer may enhance safety and provide greater protection from late traumatic wound dehiscence with little long-term detriment to visual results provided the residual stromal bed thickness is ≤80µm [34]. But

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automating the creation of a uniform, smooth deep stromal interface anterior to the natural anatomical plane of the pre-Descemet layer in keratoconus is technically challenging [35] . Combinations of F-DALK with excimer laser PTK smoothing [25] and excimer laser DALK with no prior femtosecond laser dissection[36] have been described. Whilst the focus of recent F-DALK research has been increasing the rate of big-bubble formation, novel solutions for the automation of other deep dissection techniques merit further investigation.

Astigmatic results were not improved by F-DALK in our series despite a larger (9mm) graft diameter. This may be because the iFS femtosecond laser uses a glass interface with flat applanation. Flat applanation of an irregular ectatic corneal induces distortion, creating a non-circular anterior side cut. There is a trend towards reduced astigmatism in F-DALK studies using lasers with a curved interface [27] [21] [18] [17] [28] (Table 6), for which the applanation effect is similar to that produced in M-DALK by the Hanna trephine (Moria SA, Antony, France) featuring a curved central obturator. Liquid interface femtosecond lasers should eliminate applanation distortion, but are not currently packaged with the software capabilities required to optimise F-DALK.

Informal observations from our F-DALK case series that may be helpful to other surgeons include the following. During donor preparation, filling the artificial anterior chamber with air makes flat applanation at a controlled supraphysiological pressure easier. This is because, gas (air) is compressible, whereas liquids (balanced salt solution, culture medium or OVD) are not. Drying excess fluid with arrow-tip disposable surgical sponges after donor cornea mounting by applying the sponges around the edge of the cornea assists in ensuring that a thin meniscus is clearly visible to demarcate the edge of the applanation zone.

Communication with the eye bank supplying tissue to request that small corneas, or corneas with prominent corneal arcus are avoided helps to reduce problems with completeness of femtosecond dissection. In the postoperative period, suture loosening requiring revision in the operating room occurred in some early cases with a single running continuous 10/0 nylon suture. This may be a vulnerability in larger (9mm) grafts in which suture placement is closer to the limbus for many cases. We switched to the use of interrupted sutures to reduce the frequency of readmission. Although we had no graft failures in the F-DALK series, as with M-DALK, postoperative transplant rejection episodes were common and often associated with poor compliance with medication or early cessation of steroids (Table 4). We and other author[10 12 4 37 38] have observed that rejection episodes in DALK are unusual after the first 2 postoperative years. Based on this, we would recommend continuing low dose topical steroids for 2 years after surgery.

Our results suggest that reducing the diameter of the zone of deep dissection to 6mm using mushroom pattern F-DALK may reduce the risk of intraoperative anterior chamber perforation and conversion to penetrating keratoplasty in a multi-surgeon setting. A larger graft may help to protect from late peripheral ectasia, but did not reduce postoperative astigmatism where flat applanation was used in F-DALK.

M-DALK is a difficult operation to perform with consistent good results. Continued development of systems to automate controlled access to the pre-Descemet layer and enhance cut precision in DALK for keratoconus will help to make optimised outcomes less dependent on individual surgical ability.

Figure 1a.

Schematic showing an 8-point sample of the corneal thickness measured normal to the surface at the 6mm diameter using optical coherence tomography (Casia SS-1000, Tomey, Nagoya, Japan) with the thinnest point highlighted for cut pattern planning in the health record.

Figure 1b.

Schematic of femtosecond laser cut pattern we used in host and donor corneas based on preoperative optical coherence tomography measurements of the host cornea. All cuts were programmed to intersect by a minimum of 20µm.

Figure 2.

CDVA at baseline, within one year (6-12 months after surgery), and last follow-up after removal of sutures (ROS) for Femto-DALK.

Figure 3.

CDVA at baseline, within one year (6-12 months after surgery), and last follow-up after removal of sutures (ROS) for Manual-DALK.

Figure 4.

Intraoperative outcomes in 54 consecutive cases (excluding hydrops cases) of Femto-DALK surgery using the big-bubble technique by surgeon grade (A/C = anterior chamber; Type I = air cleavage plane anterior to the pre-Descemet layer; Type II = air cleavage plane posterior to the pre-Descemet layer.

References

1 . Gain P, Jullienne R, He Z, et al. Global Survey of Corneal Transplantation and Eye Banking. *JAMA Ophthalmol*. 2016;134(2):167–173. doi:10.1001/jamaophthalmol.2015.4776.

2. Reinhart WJ, Musch DC, Jacobs DS, Lee WB, Kaufman SC, Shtein RM. Deep anterior lamellar keratoplasty as an alternative to penetrating keratoplasty a report by the american academy of ophthalmology. *Ophthalmology*. 2011;118(1):209–218. doi:10.1016/j.ophtha.2010.11.002.

3. Borderie VM, Sandali O, Bullet J, Gaujoux T, Touzeau O, Laroche L. Long-term results of deep anterior lamellar versus penetrating keratoplasty. *Ophthalmology*. 2012;119(2):249–255. doi:10.1016/j.ophtha.2011.07.057.

4. Coster DJ, Lowe MT, Keane MC, Williams KA. A comparison of lamellar and penetrating keratoplasty outcomes: a registry study. *Ophthalmology*. 2014;121(5):979–987. doi:10.1016/j.ophtha.2013.12.017.

5. 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. doi:10.1136/bjo.83.3.327.

6. Sarnicola V, Toro P, Sarnicola C, Sarnicola E, Ruggiero A. Long-term graft survival in deep anterior lamellar keratoplasty. *Cornea*. 2012;31(6):621–626. doi:10.1097/ICO.0b013e31823d0412.

7. Feizi S, Javadi MA, Jamali H, Mirbabaee F. Deep anterior lamellar keratoplasty in patients with keratoconus: big-bubble technique. *Cornea*. 2010;29(2):177–182. doi:10.1097/ICO.0b013e3181af25b7.

8. Kubaloglu A, Sari ES, Unal M, et al. Long-term results of deep anterior lamellar keratoplasty for the treatment of keratoconus. *Am J Ophthalmol*. 2011;151(5):760–767.e1. doi:10.1016/j.ajo.2010.11.020.

9. Khattak A, Nakhli FR, Al-Arfaj KM, Cheema AA. Comparison of outcomes and complications of deep anterior lamellar keratoplasty and penetrating

keratoplasty performed in a large group of patients with keratoconus. *Int Ophthalmol*. 2018;38(3):985–992. doi:10.1007/s10792-017-0548-9.

10. Romano V, Iovieno A, Parente G, Soldani AM, Fontana L. Long-term clinical outcomes of deep anterior lamellar keratoplasty in patients with keratoconus. *Am J Ophthalmol*. 2015;159(3):505–511. doi:10.1016/j.ajo.2014.11.033.

11. MacIntyre R, Chow S-P, Chan E, Poon A. Long-term outcomes of deep anterior lamellar keratoplasty versus penetrating keratoplasty in Australian keratoconus patients. *Cornea*. 2014;33(1):6–9. doi:10.1097/ICO.0b013e3182a9fbfd.

12. Gadhvi KA, Romano V, Fernandez-Vega Cueto L, Aiello F, Day AC, Allan BD. Deep Anterior Lamellar Keratoplasty for Keratoconus: Multisurgeon Results. *Am J Ophthalmol*. 2019;201:54–62. doi:10.1016/j.ajo.2019.01.022.

13. Leal Ghezzi T, Campos Corleta O. 30 Years of Robotic Surgery. *World J Surg*. 2016;40(10):2550–2557. doi:10.1007/s00268-016-3543-9.

14. Sheetz KH, Claflin J, Dimick JB. Trends in the Adoption of Robotic Surgery for Common Surgical Procedures. *JAMA Netw Open*. 2020;3(1):e1918911. doi:10.1001/jamanetworkopen.2019.18911.

15. Buzzonetti L, Petrocelli G, Valente P, et al. The Big-Bubble Full Femtosecond Laser-Assisted Technique in Deep Anterior Lamellar Keratoplasty. *J Refract Surg*. 2015;31(12):830–834. doi:10.3928/1081597X-20151111-07.

16. Chamberlain WD. Femtosecond laser-assisted deep anterior lamellar keratoplasty. *Curr Opin Ophthalmol*. 2019;30(4):256–263. doi:10.1097/ICU.0000000000000574.

17. Alio JL, Abdelghany AA, Barraquer R, Hammouda LM, Sabry AM. Femtosecond Laser Assisted Deep Anterior Lamellar Keratoplasty Outcomes and Healing Patterns Compared to Manual Technique. *Biomed Res Int*. 2015;2015:397891. doi:10.1155/2015/397891.

18. Espandar L, Mandell JB, Niknam S. Femtosecond laser-assisted decagonal deep anterior lamellar keratoplasty. *Can J Ophthalmol*. 2016;51(2):67–70. doi:10.1016/j.jcjo.2015.12.001.

19. Farid M, Steinert RF. Deep anterior lamellar keratoplasty performed with the femtosecond laser zigzag incision for the treatment of stromal corneal pathology and ectatic disease. *J Cataract Refract Surg*. 2009;35(5):809–813. doi:10.1016/j.jcrs.2009.01.012.

20. Guindolet D, Nguyen DT, Bergin C, Doan S, Cochereau I, Gabison EE. Double-Docking Technique for Femtosecond Laser-Assisted Deep Anterior Lamellar Keratoplasty. *Cornea*. 2018;37(1):123–126. doi:10.1097/ICO.0000000000001442.

21. Li S, Wang T, Bian J, Wang F, Han S, Shi W. Precisely Controlled Side Cut in Femtosecond Laser-Assisted Deep Lamellar Keratoplasty for Advanced Keratoconus. *Cornea*. 2016;35(10):1289–1294. doi:10.1097/ICO.0000000000000962.

22. Fung SSM, Aiello F, Maurino V. Outcomes of femtosecond laser-assisted mushroom-configuration keratoplasty in advanced keratoconus. *Eye (Lond)*. 2016;30(4):553–561. doi:10.1038/eye.2015.273.

23. Liu Y-C, Wittwer VV, Yusoff NZM, et al. Intraoperative Optical Coherence Tomography-Guided Femtosecond Laser-Assisted Deep Anterior Lamellar Keratoplasty. *Cornea*. 2019;38(5):648–653. doi:10.1097/ICO.0000000000001851.

24. Price FWJ, Price MO, Grandin JC, Kwon R. Deep anterior lamellar keratoplasty with femtosecond-laser zigzag incisions. *J Cataract Refract Surg*. 2009;35(5):804–808. doi:10.1016/j.jcrs.2009.01.011.

25. de Macedo JP, de Oliveira LA, Hirai F, de Sousa LB. Femtosecond laser-assisted deep anterior lamellar keratoplasty in phototherapeutic keratectomy versus the big-bubble technique in keratoconus. *Int J Ophthalmol*. 2018;11(5):807–812.

26. Wade M, Muniz Castro H, Garg S, et al. Long-Term Results of Femtosecond Laser-Enabled Keratoplasty With Zig-Zag Trephination. *Cornea*. 2019;38(1):42–49. doi:10.1097/ICO.0000000000001783.

27. Salouti R, Zamani M, Ghoreyshi M, Dapena I, Melles GRJ, Nowroozzadeh MH. Comparison between manual trephination versus femtosecond laser-assisted deep anterior lamellar keratoplasty for keratoconus. *Br J Ophthalmol*. 2019;103(12):1716–1723. doi:10.1136/bjophthalmol-2018-313365.

28. Shehadeh-Mashor R, Chan CC, Bahar I, Lichtinger A, Yeung SN, Rootman DS. Comparison between femtosecond laser mushroom configuration and manual trephine straight-edge configuration deep anterior lamellar keratoplasty. *Br J Ophthalmol*. 2014;98(1):35–39. doi:10.1136/bjophthalmol-2013-303737.

29. Dua HS, Faraj LA, Said DG, Gray T, Lowe J. Human corneal anatomy redefined: a novel pre-Descemet"s layer (Dua"s layer). *Ophthalmology*. 2013;120(9):1778–1785. doi:10.1016/j.ophtha.2013.01.018.

30. Parthasarathy A, Por YM, Tan DTH. Use of a "small-bubble technique" to increase the success of Anwar"s 'big-bubble technique' for deep lamellar keratoplasty with complete baring of Descemet"s membrane. *Br J Ophthalmol*. 2007;91(10):1369–1373. doi:10.1136/bjo.2006.113357.

31. Shimmura S, Shimazaki J, Omoto M, Teruya A, Ishioka M, Tsubota K. Deep lamellar keratoplasty (DLKP) in keratoconus patients using viscoadaptive viscoelastics. *Cornea*. 2005;24(2):178–181. doi:10.1097/01.ico.0000138843.83044.7d.

32. Yoo Y-S, Whang W-J, Kang M-J, et al. Effect of Air Injection Depth on Big-bubble Formation in Lamellar Keratoplasty: an Ex Vivo Study. *Sci Rep*. 2019;9(1):3785. doi:10.1038/s41598-018-36522-w.

33. Pasricha ND, Shieh C, Carrasco-Zevallos OM, et al. Needle Depth and Big-Bubble Success in Deep Anterior Lamellar Keratoplasty: An Ex Vivo Microscope-Integrated OCT Study. *Cornea*. 2016;35(11):1471–1477. doi:10.1097/ICO.0000000000000948.

34. Ardjomand N, Hau S, McAlister JC, et al. Quality of vision and graft thickness in deep anterior lamellar and penetrating corneal allografts. *Am J Ophthalmol*. 2007;143(2):228–235. doi:10.1016/j.ajo.2006.10.043.

35. Vetter JM, Butsch C, Faust M, et al. Irregularity of the posterior corneal surface after curved interface femtosecond laser-assisted versus microkeratome-assisted descemet stripping automated endothelial keratoplasty. *Cornea*. 2013;32(2):118–124. doi:10.1097/ICO.0b013e31826ae2d8.

36. Alessio G, L'abbate M, Boscia F, Sborgia C, La Tegola MG. Excimer laser-assisted lamellar keratoplasty and the corneal endothelium. *Am J Ophthalmol*. 2010;150(1):88–96.e1. doi:10.1016/j.ajo.2010.01.042.

37. Jones MNA, Armitage WJ, Ayliffe W, Larkin DF, Kaye SB. Penetrating and deep anterior lamellar keratoplasty for keratoconus: a comparison of graft outcomes in the United kingdom. *Invest Ophthalmol Vis Sci*. 2009;50(12):5625–5629. doi:10.1167/iovs.09-3994.

38. Watson SL, Tuft SJ, Dart JKG. Patterns of rejection after deep lamellar keratoplasty. *Ophthalmology*. 2006;113(4):556–560. doi:10.1016/j.ophtha.2006.01.006.

Figure 1a.

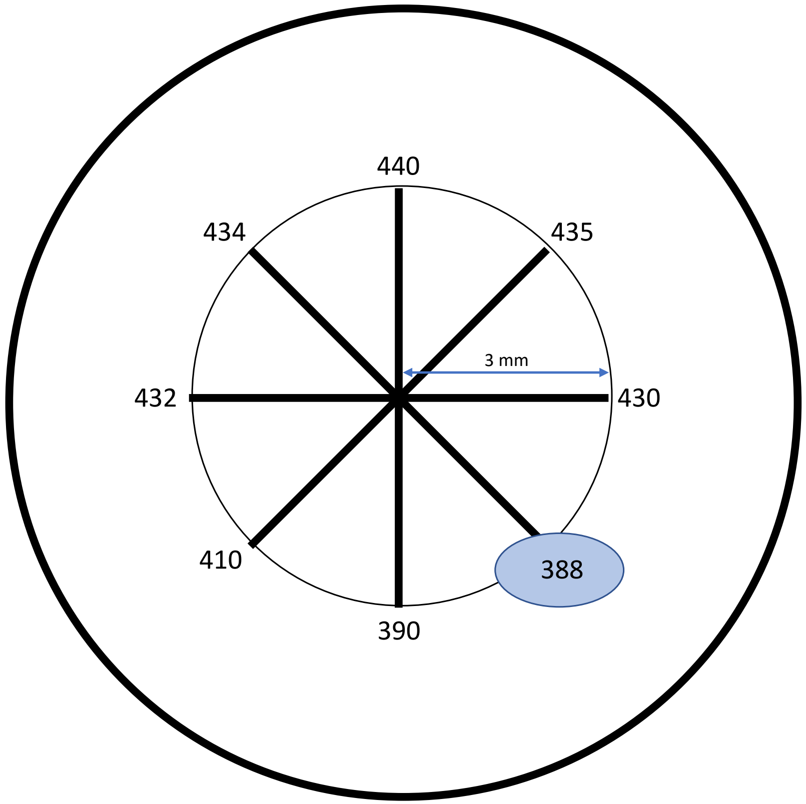
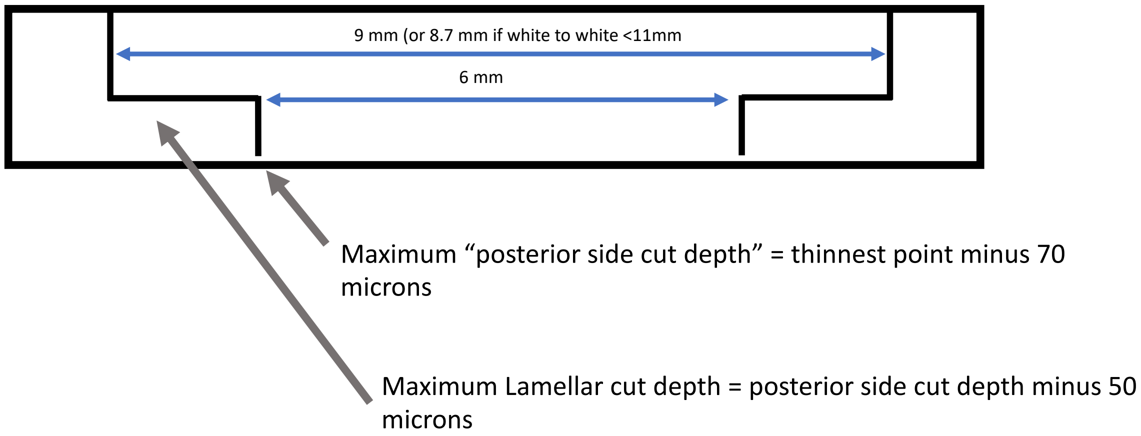


Figure 1b.



9 mm (or 8.7 mm if white to white <11mm
6 mm
Maximum “posterior side cut depth” = thinnest point minus 70 microns
Maximum Lamellar cut depth = posterior side cut depth minus 50 microns

Figure 2.

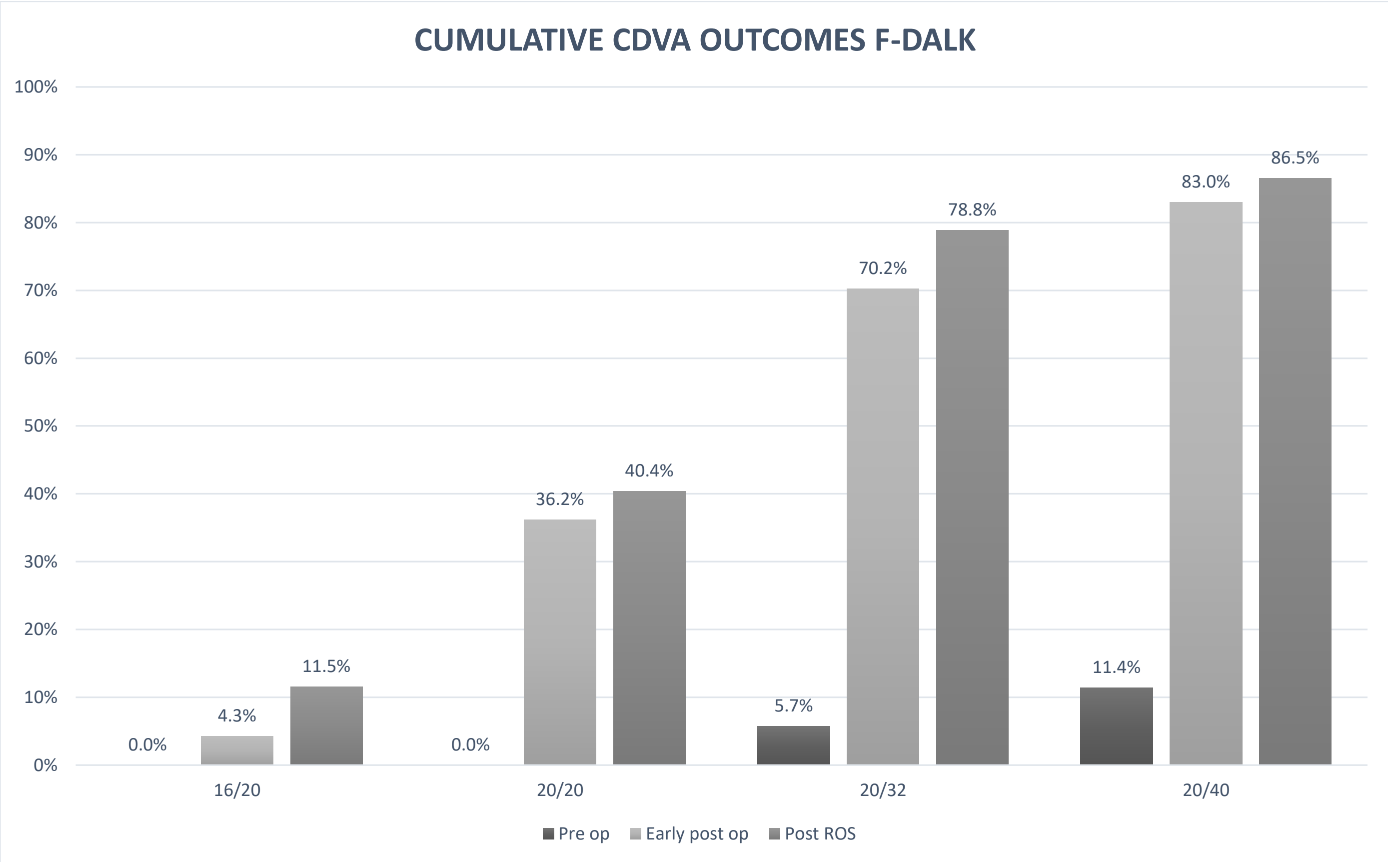

CUMULATIVE CDVA OUTCOMES F-DALK
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.0%
4.3%
11.5%
0.0%
36.2%
40.4%
5.7%
70.2%
78.8%
11.4%
83.0%
86.5%
16/20
20/20
20/32
20/40
Pre op
Early post op
Post ROS

Figure 3.

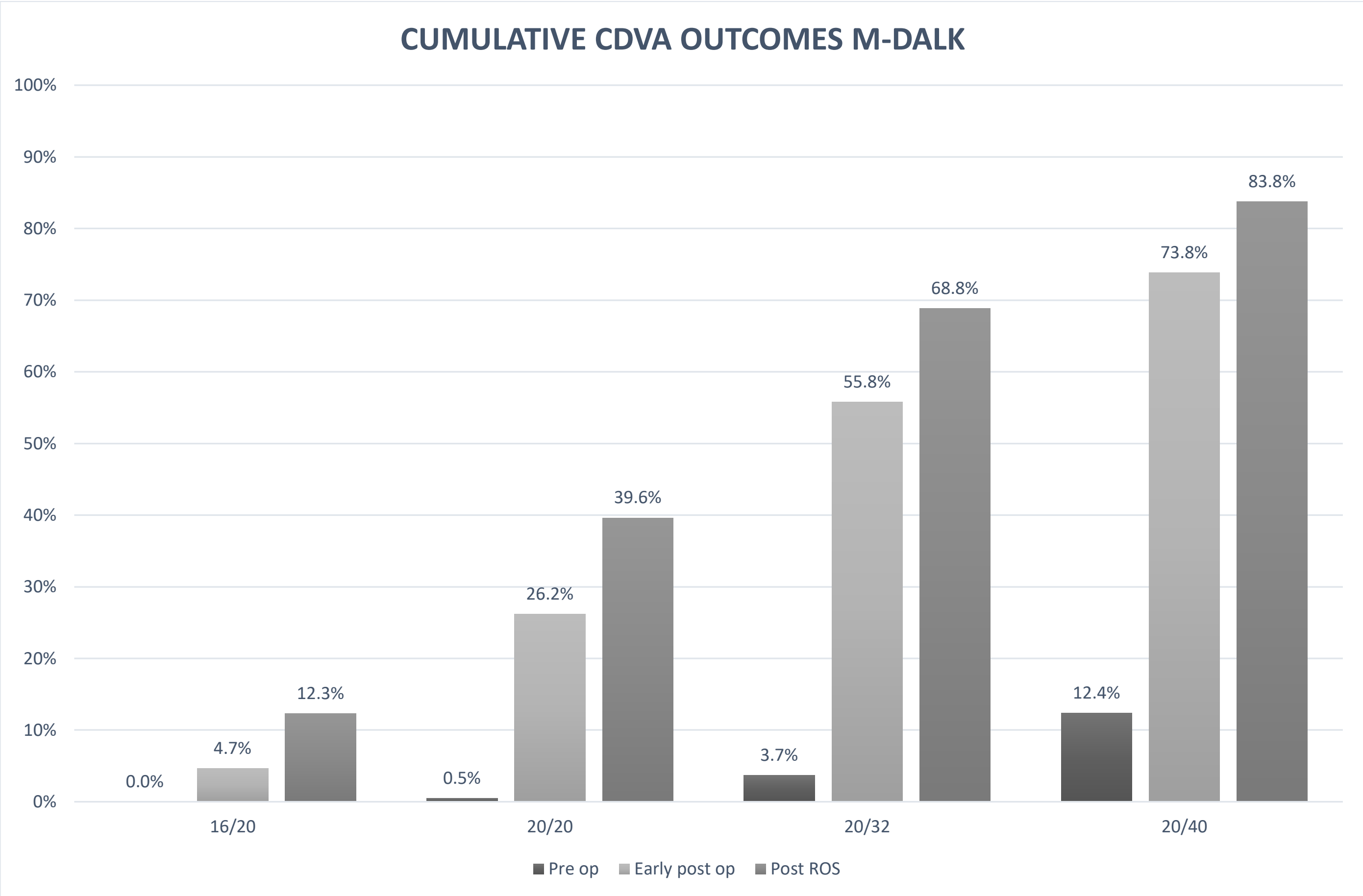

CUMULATIVE CDVA OUTCOMES M-DALK
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.0%
4.7%
12.3%
0.5%
26.2%
39.6%
3.7%
55.8%
68.8%
12.4%
73.8%
83.8%
16/20
20/20
20/32
20/40
Pre op
Early post op
Post ROS

Figure 4

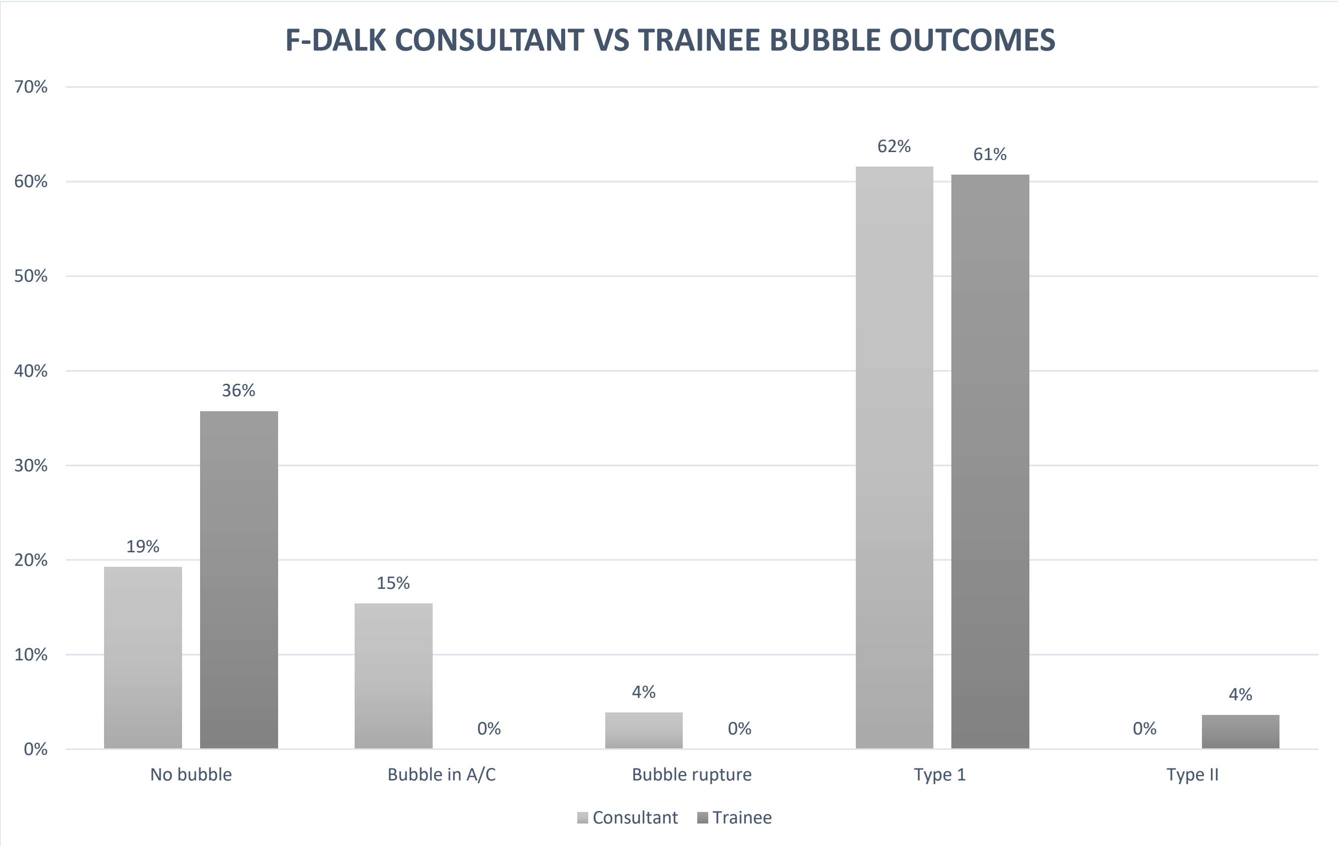

F-DALK CONSULTANT VS TRAINEE BUBBLE OUTCOMES
70%
60%
50%
40%
30%
20%
10%
0%
19%
36%
15%
0%
4%
0%
62%
61%
0%
4%
No bubble
Bubble in A/C
Bubble rupture
Type 1
Type II
Consultant
Trainee

Table 1 Pre-operative Data

Variable	Definition	F-DALK	M-DALK
Age	Age at time of surgery (years)	28±10.1	33.4±10.6
Gender	male/female	38/20	210/116
Pachymetry	Minimum corneal pachymetry (µm)	348±60.8	327±74.7
Disease stage	Keratoconus (Pentacam) Stage II	0 (0%)	8 (2.5%)
	Keratoconus (Pentacam) Stage III	8 (13.8%)	52 (15.9%)
	Keratoconus (Pentacam) Stage IV	48 (82.8%)	209 (64.1%)
	Not recorded	2 (3.4%)	57 (17.5%)
Co-pathology	Diagnosis other than keratoconus affecting final CDVA	2 (3.4%)	7 (2.2%)
Hydrops	Previous hydrops at preoperative examination	4 (7%)	16 (4.9%)
Atopy	Mild=eczema/asthma/hay fever/Olopatidinetreatment	8 (13.8%)	68 (20.9%)
	Severe = Ciclosporin treatment	3 (5.2%)	7 (2.2%)
CXL	Any form of collagen cross-linking before grafting	3 (5.2%)	6 (1.8%)
ICRS	Intracorneal ring segments implanted prior to DALK	2 (3.5%)	4 (1.2%)

DALK = deep anterior lamellar keratoplasty; CDVA = corrected distance visual acuity.

Commented [BA2]: Insert new row for not recorded here

Commented [kg3R2]: done

Table 2 Operative Details

		Femto DALK	Manual DALK
Surgeon grade	Consultant surgeon	26 (44.8%)	113 (31.7%)
	Surgeon in training	32 (55.2%)	213 (59.7%)
Graft diameter	Donor superficial diameter (mm)	9.17 ± 0.21	8.22 ± 0.25
	Host superficial diameter (mm)	8.91 ± 0.20	8.07 ± 0.24
DALK technique	Big-bubble	58 (100%)	326 (100%)
Donor DM	Removed	56 (96.6%)	122 (37.4%)
	Not removed	2 (3.4%)	147 (45.1%)
	Not recorded	*0 (0%)*	*57 (17.5%)*
Suture method	Continuous	4 (6.9%)	263 (80.7%)
	Interrupted	54 (93.1%)	63 (19.3%)

Commented [BA4]: Run through the tables and check the not recorded percentages are based on BB DALK cases rather than simply lifted from previous papers. You can take out all the not recorded rows where we have 0% in both columns (ie full accountability) see modified wording in the methods under stats.

Table 3 Intraoperative complications

		Femto DALK	Manual DALK
Perforation	Any perforation into A/C	15 (25.7%)	148 (45.4%)
Conversion to PK	Total conversion to PK	2 (3.4%)	80 (24.5%)
	Elective conversion to PK	0 (0%)	11 (3.4%)
	Perforation converted to PK	2 (13.3%)	69 (21.2%)

A/C = anterior chamber; PK = penetrating keratoplasty

Table 4 Post-operative complication

Complication	Definition	Femto DALK	Manual DALK
Double A/C	Fluid in interface between donor and host cornea at first postoperative review	3 (5.2%)	32 (9.8%)
Urrets Zavalier Syndrome	Fixed dilated pupil at first postoperative review	0 (0%)	0 (0%)
Atopic Sclerokeratitis	Host side inflammatory response in early postoperative period (often accompanied by suture loosening) requiring intensive topical steroids or systemic immunosuppression	0 (0%)	10 (3.1%)
Raised intraocular pressure	Any medical or surgical intervention for raised intraocular pressure	2 (3.4%)	50 (15.3%)
Infection	Any unscheduled treatment with anti-biotic, antiviral, or antifungal drugs	0 (0%)	4 (1.2%)
Graft rejection	Any unscheduled increase in topical steroids to treat: epithelial rejection line; stromal oedema; progressive stromal inflammation.	9 (15.5%)	61 (18.7%)
Graft Failure	Irreversible loss of graft clarity or repeat corneal transplantation	0 (0%)	12 (3.7%)

Table 5 Post-operative interventions

Intervention	Definition	Femto DALK	Manual DALK
Air injection	Any postoperative air injection for a double A/C	2 (3.4%)	14 (4.3%)
Wound revision	Any repeat or revision corneal sutureplacementin the operating room	11 (19%)	34 (10.4%)
Glaucoma surgery	Any glaucoma filtration surgery	1 (1.7%)	2 (0.6%)
Cataract surgery	Cataract surgery performed after transplantation	1 (1.7%)	9 (2.8%)
Refractive surgery	Incisional or excimer laser refractive surgery after suture removal	4 (6.9%)	16 (4.9%)
Repeat transplantation	Any revision corneal transplantation procedure with new donor material	0 (0%)	11 (3.4%)

Table 6

Summary of studies of Femtosecond assisted Deep Anterior Lamellar Keratoplasty (DALK) for Keratoconus (n>20) published since 2015. CDVA and absolute mean cylinder are as recorded after removal of corneal sutures

Study	Type	n	Laser model	Applanation	Diamter (Cut pattern)	Mean Cylinder	CDVA
Alio et al 2015 [17]	Single-surgeon	25	Intralase iFS	Flat	8mm (Mushroom)	5.43±NR	0.26±NR
Li et al 2016 [21]	Single-surgeon	94	Wavelight FS200	Flat	8.2mm (Button)	5.35±1.73	0.08±0.07
This study	Multi-surgeon	58	Intralase iFS	Flat	9.17 ± 0.21 (mushroom)	5.00±3.76D	0.16±0.20.
Espandar et al 2016 [18]	Single-surgeon	24	Femtec 520F	Curved	9.25mm (Decagonal cut)	1.82±0.67	0.26±0.16
Salouti et al 2019 [27]	Single-surgeon	109	Femtec 520F	Curved	9.3-9.5 (Mushroom or Decagonal)	1.43±1.08	0.09±0.09