**Title**: Rebubbling rate in preloaded versus surgeon prepared DSAEK

**Short title:** Preloaded versus surgeon prepared DSAEK

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**Disclosure**

None of the authors have any potential conflict of interest

**Abstract**

**Purpose**: To compare the clinical outcomes of eye bank preloaded Descemet stripping automated endothelial keratoplasty (DSAEK) grafts and surgeon prepared.

**Methods**: In this retrospective study, the data were obtained from two groups a) surgeon cut DSAEK where tissue was prepared by the surgeon immediately before surgery, and b) preloaded DSAEK tissue shipped to the surgeon after preparation by the eye bank. Standard DSAEK preparations using Moria microkeratome with single pass method were performed. For the tissues prepared by the eye banks, they were preloaded in an iGlide device and shipped in transport media. Standard DSAEK surgery using bimanual pull-through technique was performed for all the grafts. Air was used as a tamponade. Main outcome measures included best corrected visual acuity (BCVA) and rebubbling rate.

**Result**: Out of 107 eyes of 101 patients that underwent DSAEK surgery, 33 tissues were prepared by the surgeon (sc-DSAEK), while 74 were prepared by the eye bank (pl-DSAEK). sc-DSAEK showed a rebubbling rate of 9.1%, compared to the 16.2% for the preloaded DSAEK (p= 0.11). There was no statistical difference in postoperative BCVA between the two groups. Logistic regression analysis showed no association between detachment rate and cataract surgery, graft preparation method, graft diameter and reason for graft.

**Conclusion**: Preloaded grafts have similar rebubbling rate and visual acuity achieved compared with surgeon prepared grafts.

**Key words**: pre-loaded tissue; DSAEK; UT DSAEK; Eye Bank**;** Rebubbling; Detachment

**Introduction**

Descemet stripping automated endothelial keratoplasty (DSAEK) is a selective transplantation technique that replaces the dysfunctional corneal endothelium with a posterior lamellar healthy donor graft

1. This technique has several advantages including better post-operative rehabilitation, better visual outcomes and is a less invasive procedure compared to full thickness transplantation. Despite the obvious benefits of Descemet membrane endothelial keratoplasty (DMEK) in rehabilitation, best corrected vision and rejection episode, DSAEK is still the favored endothelial keratoplasty procedure in some regions2,

3. DSAEK is a standardized procedure with a shorter learning curve compared to DMEK and, therefore, has gained wider acceptance quickly for its relative simplicity, reproducibility and efficiency

4. However, the time and instruments required for preparing a DSAEK graft increase the overall cost of the procedure and sometimes tissue may be lost in preparation leading to cancelled surgery. To overcome this, eye banks started preparing ultra-thin DSAEK (UT-DSAEK) grafts, which they provide as pre-cut or preloaded lenticules that are ready for transplantation

5. These tissues are prepared by technicians with significant expertise in tissue handling, which reduces surgical time and tissue wastage due to irregular cuts or perforations made during preparation

6. Additionally, as the eye banks have the necessary instruments to check the thickness of the tissue and the endothelial health before loading the graft, pre-cut and preloaded tissues provide a fully validated and quality-controlled graft, a step that cannot be performed in the surgical theatre. In this study we compared the clinical outcomes and complications of patients treated with DSAEK grafts that were prepared either by the surgeon or by eye banks. Our primary outcomes were post-operative rebubbling rate and best corrected visual acuity (BCVA).

**Methods**

*Ethical statement and data collection*

The tissues for transplantation were obtained from Fondazione Banca degli Occhi del Veneto, (Venice, Italy) or NHS Blood and Transplant (Liverpool, UK) following a written consent from the donor’s next-of-kin. In this retrospective case series, all records from patients treated for an endothelial dysfunction (Fuchs’ endothelial corneal dystrophy (FECD) or bullous keratopathy (BK)) with a DSAEK between March 2017 and October 2019 were analyzed. The retrospective data collection was approved by the institutional review board (A0002786) at the Royal Liverpool University Hospital, Liverpool, UK.

The tissues were either shipped as full thickness to allow the surgeon to cut the DSAEK grafts or preloaded (as per the surgeon’s request) and shipped accordingly. All the tissues were preserved in transport media (supplemented with dextran) to regain the physiological thickness of the tissue before cutting. Data such as gender, age at the time of the surgery, primary diagnosis, donor details, time from harvesting to surgery,surgery details (graft diameter and combination with phacoemulsification), best corrected distance visual acuity, post-operative complications (such as air release and detachment rate) were recorded.

*Graft preparation and implantation method*

1. Surgeon cut DSAEK

All DSAEK grafts were prepared by one of 4 experienced corneal transplant surgeons. Briefly, donor corneoscleral disks were placed on an artificial anterior chamber, epithelium removed with sterile sponges, the height of the infusion bottle was set at 120 cm and the roller clamp was closed 10 cm before the artificial anterior chamber to increase artificial anterior chamber pressure. Corneal thickness was measured using ultrasound pachymetry. When the central donor corneal thickness was between 500 μm and 510 μm, an automated microkeratome (Moria, Antony, France) with a 350 μm head was used to remove the anterior lamellar cap. Manual dissection of the peripheral anterior stromal lamella was performed

7,8.

1. Eye Bank preloaded DSAEK

*Tissue cutting*: For the tissues prepared by the eye banks, the corneas were mounted on an artificial anterior chamber (Moria, Antony, France) with an intra-chamber pressure set at 50 mmHg, that was increased to 90-100 mm Hg before sectioning. The epithelium was peeled with sterile sponges and microkeratome (Moria, Antony, France) equipped with either a 300 or 350-micron blade was passed over the tissue in a horizontal sweeping fashion (depending on the original thickness measured using optical coherence tomography (OCT; Tomey Casia SS-1000, GmbH, Erlangen, Germany). This provided a tissue thickness of about 150 µm.

*Tissue loading:* Following the pre-cutting step, tissues were punched to a desired diameter (between 8.0-9.5 mm) using a contact lens as a base. The posterior lenticule was gently placed in the iGlide (Eurobio Scientific, France) prefilled with transport media. The cap was closed and the entire unit was preserved in the container filled with 50 mL of the media to ensure continuous enrichment of the cells. The preloaded DSAEK grafts were then shipped to Liverpool for transplantation.

All the grafts, regardless of their preparation method, were transplanted using standard DSAEK procedure using bimanual pull through technique with a 2.75 incision followed by air tamponade by one of 4 experienced corneal surgeons. Best corrected visual acuity (BCVA) and detachment rate were the main outcome measures.

*Statistical analysis*

The statistical analyses were performed using STATA 14.0 (StataCorp, College Station, TX) and a p-value of less than 0.05 was considered statistically significant. Quantitative variables were tested for normality using the Shapiro-Wilk test. Comparison of quantitative characteristics (age and preoperative and postoperative BCVA) between the two different group were performed with the Student t-test in case of normally distributed data and with two-sample Wilcoxon rank-sum (Mann-Whitney) test in case of non-normally distributed data. Qualitative parameters (gender, combined cataract surgery and detachment rate) were compared with a chi squared test. Pre-op and post-op BCVA in the same group were analyzed with matched-pairs Wilcoxon signed-rank test.

**Result**

We analyzed 107 eyes (60 male and 47 female) of 101 patients with a mean age of 70.8 ± 12.1 years. 33 eyes underwent surgeon-cut DSAEK (sc-DSAEK) and 74 had pre-loaded DSAEK. The average donor age was 60.4 ± 11.8 years and the average time from graft harvesting to surgery was 3.0 ± 0.8 days for preloaded tissue. Grafts had an average diameter of 9.2 ± 0.3 mm and a preoperative ECD of 2609 ± 158 cells/mm2. Reason for grafting was FECD (47/107, 44%) and BK (60/107, 56%). In 33 cases (30.8 %) the DSAEK surgery was combined with cataract surgery. Follow up time was 545 ± 301 days. All data are summarized in Table 1.

15/107 patients (14%) required a rebubbling procedure. 11/15, (73%) required only one rebubbling, while 4/15, (27%) required two consecutive rebubbling procedures. All grafts reattached after the rebubbling procedures.

Overall, sc-DSAEK showed a rebubbling rate of 9.1%, compared to the 16.2% for the pre-loaded DSAEK and the difference between groups was not statistically significant (p= 0.11).

No differences were noted in terms of multiple rebubbling between sc-DSAEK (6%) and pl-DSAEK (2.7%, p=0.38).

Overall the rebubbling rate was 15.5% in FECD patients and 13.7% in BK patients (p=0.80), and was 19.3% in combined DSAEK and cataract surgery and 12.5% in DSAEK only (p=0.37). In a logistic regression analysis, rebubbling rate was not significantly associated with combined cataract surgery (p=0.34), graft preparation method (p=0.13), graft diameter (p=0.78) and reason for graft (p=0.57).

Preoperative BCVA (1.20 ± 0.72 LogMAR) improved significantly after the surgery (0.54 ± 0.57 LogMAR, p<0.01). There was no statistical difference in postoperative BCVA between the sc-DSAEK (0.55 ± 0.57 LogMar) and pl-DSAEK groups (0.54 ± 0.57, p=0.58). The average time to reach the BCVA was 112 ± 102 days. There was no statistical difference in postoperative BCVA improvement between the two groups (p= 0.24). Postoperative BCVA was 0.37 ± 0.33 LogMAR in the FECD group, 0.72 ± 0.66 in the BK group (p=0.0007).

In a linear regression analysis, we found postoperative BCVA to be significantly associated with cataract surgery (p=0.02), while we found no significant association with reason for graft (p=0.85), graft preparation method (p=0.33), detachment procedure (p=0.19) and graft diameter (p=0.65).

**Discussion**

Over recent years endothelial keratoplasty has overtaken penetrating keratoplasty as the most common form of corneal transplantation in the USA and Europe9. Advances in eye banking expertise has increased the availability of preloaded UT-DSAEK tissue making EK surgery more appealing than ever. However, these advances and changes in eye bank tissue services are relatively recent and as a consequence there are few papers in the literature directly comparing outcomes of surgeon prepared versus eye bank prepared and preloaded DSAEK. We report the detachment and visual outcomes after DSAEK surgery where the grafts were prepared either by the surgeon or provided preloaded by the eye bank. In our primary outcome measures, we did not find any significant difference in the postoperative detachment or BCVA between the two groups.

Graft detachment is the most common complication of DSAEK surgery

10. Reported figures of detachment after DSAEK vary significantly from 0-82% reported by the American Academy of Ophthalmology, however, average rates stand at around 15%11. Detachment and re-bubbling attempts to attach the DSAEK lenticule are associated with endothelial cell loss

12 .

In our study, we demonstrated an overall rebubbling rate of 14% for our DSAEK grafts, similar to reported figures in the literature. We found that a significant detachment was observed following pl-DSAEK group in 16.2% of cases compared to 9.1% in the sc-DSAEK group, however, this difference was not statistically significant. Although we did not demonstrate a statistical significance the 1.8 x greater detachment rate in the pl-DSAEK group was a surprise to us. We speculate that this higher rate of detachment may have been secondary to changes in tissue properties from exposure of the stroma to dextran in the transport medium. In the pre-loaded DSAEK group, the lenticular stroma is exposed to the transport medium to a greater extent than surgeon cut and this may lead to deposition of the dextran, which may interact with the interfacial matrix on the surface. This may potentially interfere with attachment as has been postulated by Romano et al13. However, as we did not demonstrate significance, we are unable to conclude this finding. In the literature we found that the effect of transport and processing time, and stromal exposure to dextran on graft characteristics to be negligible. In a previous randomized controlled study by Price et al. it was demonstrated that surgeon cut and pre-cut tissue resulted in similar rates of detachment

14. Dempsey et al. reported in a 2011 study of 71 eyes with a 14% graft detachment rate that there were no significant influence in detachment rate accounting for the time to procurement of the tissue, time to processing, time in the eye bank and endothelial cell count between those DSAEKs that detached and those that were attached post-opertively

15. Parekh et al. demonstrated that UT-DSAEK tissue could be successfully preserved without significant change in graft characteristics such as thickness and endothelial cell density, however, reported a 23% graft detachment rate in a study of 39 eyes, higher than the aforementioned incidences of detachment

16.

In our sub-analysis we identified no additional risk of detachment in DSAEK triple procedures in both our sc-DSAEK and pl-DSAEK groups. This is in agreement with Terry et al. who demonstrated in a large case series of 315 eyes that there was no significant difference in DSAEK dislocation rates in DSAEK alone (4%) and DSAEK triple surgery (1.8%; P=0.327)

17.

In regards to visual outcomes we found no difference in the BCVA outcomes between sc-DSAEK and pl-DSAEK. This was despite the absence of validation of graft thickness or profile in the sc-DSAEK group. Dickman et al. demonstrated in a randomized clinical trial of 66 eyes of 66 patients with UT-DSAEK (mean central thickness of 101 µm) compared to DSAEK (mean central thickness of 209µm), that BCVA was significantly better with UT-DSAEK tissue (p=0.001-0.002) with no comparative increase in complications such as dislocation (p=0.9)

18 inferring a benefit to thinner tissue. As a consequence, we may have expected our pl-DSAEK group with validated UT tissue to demonstrate better BCVA post operatively as the eye banks ensured all DSAEK grafts were ultra thin. However our results suggest that use of a validated nomogram for surgeon DSAEK preparation as utilised at our centre may negate the need for additional and expensive validation procedures to improve and qualify graft quality and subsequent BCVA outcomes.

In our study we observed several limitations. Due to the retrospective nature of the study we did not randomize patients into groups so uneven numbers of PBK and Fuchs were observed in each group. In the preloaded DSAEK cases a higher number of PBK diagnosis were observed. This may be due to surgeon bias towards selecting preloaded grafts for PBK. Another limitation of our study was the absence of post operative endothelial cell counts which would have been of interest to calculate endothelial cell loss in the two groups. This resulted from the absence of specular microscopy at our unit during the period of the study. Finally, although our study demonstrated a trend towards more detachment in the pl-DSAEK group, it appears we were underpowered to demonstrate a statistically significant difference. We believe that the trend highlighted poses some questions regarding the cause and should form the basis of further analysis on this topic with larger studies if possible.

In conclusion, we found no statistically significant difference between pl-DSAEK and sc-DSAEK in regards to visual outcomes or detachment. Preloaded-DSAEK may potentially decrease surgeon time in the operating room and avoid loss of tissue during surgeon preparation and should be considered as a valid alternative to surgeon prepared tissue.

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