

# **Factors Associated with Early Graft Detachment in Primary Descemet Membrane Endothelial Keratoplasty**

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**Purpose:** To evaluate the risk factors for early graft detachment in Descemet Membrane Endothelial Keratoplasty (DMEK).

**Design:** Case-control Study.

**Methods:**

**Participants:** 173 donor corneas and 173 eyes of the patients following DMEK or DMEK in combination with phacoemulsification and IOL implantation were included.

**Intervention:** Pre-stripped DMEK grafts were transplanted using pull-through technique. At the end of surgery, the anterior chamber was filled with air, which was removed 3 hours later only if pupillary block was suspected. Re-bubbling was performed in all cases with graft detachment, independently of its extension, as documented by means of anterior segment optical coherence tomography. The donor characteristics were collected from the eye bank database and matched with the recipient database.

**Main Outcome Measures:** Donor and recipient characteristics affecting graft detachment using univariate and multivariate analysis.

**Results:** The combination of DMEK with cataract removal and IOL implantation (OR=5.31, 95% CI 2.03-13.86, P<0.002) and air fill of  $\leq 75\%$  of anterior chamber height at 2-3 hours postoperatively (OR=2.66, 95% CI 1.12-6.34, P=0.027) were found to be independent risk factors for postoperative graft detachment.

**Conclusions:** Cataract removal at the time of DMEK is a risk factor for early graft detachment and therefore sequential surgery may be preferred over combined surgery in an attempt at minimizing re-bubbling. Air level in the anterior chamber should be monitored and maintained above 75% in the early hours following surgery.

## **INTRODUCTION**

Despite the challenges of donor preparation and surgery, Descemet membrane endothelial keratoplasty (DMEK) is gaining popularity mainly because of the fast visual rehabilitation and optimal visual outcomes. Early postoperative graft detachment is the most common complication of DMEK and minimizing its incidence is one of the major challenges for all surgeons. Initially, complete graft detachment rates were reported in up to 30%<sup>1</sup>, and partial graft detachment rates in up to 63% of eyes undergoing DMEK.<sup>2-4</sup> However, with improved techniques and surgical experience, these rates have dropped to much lower values (4% in one case series).<sup>1,5,6</sup> The detached graft is re-attached with injection of air or sulfur hexafluoride (SF6) into the anterior chamber<sup>7</sup>, (termed anterior chamber re-bubbling). Anterior chamber re-bubbling can be performed at the slit-lamp<sup>8</sup> but may require extensive manipulation especially in case of total detachment and for this reason, most surgeons prefer to perform the procedure in theatre. In both cases, re-bubbling is associated with risks, inconvenience and costs.

Moreover, graft detachment has been associated with increased postoperative endothelial cell loss,<sup>6</sup> as well as increased risk of primary or late postoperative graft failure.<sup>1,5,9</sup> Several reports have addressed possible variations of the surgical technique aimed at minimizing the rate of graft detachment, such as selecting older donors,<sup>10</sup> varying the graft and descemetorhexis sizes,<sup>11</sup> changing the intraocular pressure in the first hours after surgery,<sup>12</sup> and using SF6 for tamponade, instead of air.<sup>13</sup> In contrast, studies evaluating possible risk factors for graft detachments are scant, small- numbered and report contradicting results.<sup>5</sup> In this work an institutional patient database was used in an attempt at identifying potential donor, recipient, intraoperative or postoperative factors associated with early postoperative graft detachment following primary DMEK.

## **METHODS**

### *Study Design*

This study is a case-control study including 173 eyes. The records of all consecutive DMEK procedures performed at Ospedali Privati Forlì (OPF), in Forlì (Italy) from November 2014 to July 2016 were reviewed. The study followed the tenets of the 2013 Declaration of Helsinki and approval from the ethical committee of OPF. The consent for donor cornea transplantations were

obtained from the donor's next of kin by the Veneto Eye Bank Foundation (FBOV) in Venice (Italy).

The study group consisted of 59 consecutive eyes that developed postoperative graft detachment and were treated with anterior chamber re-bubbling. The control cohort group consisted of 114 eyes that did not develop a detachment. In comparing the two groups, several variables were evaluated, including demographic characteristics, presence of ocular co-morbidities, indications for surgery, preoperative best-spectacle-corrected visual acuity (BSCVA), lens status, donor lenticule preparation technique and other procedures performed with DMEK such as cataract surgery or secondary lens implantation. In addition, the donor characteristics such as gender, age, post-mortem time, time of preservation and endothelial cell density (ECD) were also analyzed in this comparative analysis.

#### *Tissue Harvest, Preservation and Preparation at the Eye Bank*

All corneal tissues (n=173) were harvested from local hospitals in Northern Italy. The death to retrieval time was recorded. All tissues were preserved in tissue culture medium at FBOV at 31°C up to a maximum of three weeks, when the microbiological and serological analysis were performed. The ECD was counted using a reticule mounted in the eyepiece of an inverted microscope before preservation. The time of preservation was recorded. Before stripping, all tissues were preserved for at least 2 days in transport medium (tissue culture medium supplemented with 6% Dextran).

Microbiological analysis was completed before preparing a DMEK graft. The tissues showing positive serology or microbial growth were discarded. Each cornea (total n =173) was centred on the trephination block of a suction punch. Vacuum was created using a syringe and the cornea was secured onto the block. A 9.5 mm trephine (Moria, Antony, France) was used to make a superficial cut using a gentle tapping method. The endothelium was stained with trypan blue (0.4%) for about 20 seconds to determine the area of the cut. Excess peripheral membrane was removed using 120 mm long pointed acute forceps (J3683, Janach, Como, Italy). The endothelium was kept moist during the entire procedure using transport media (tissue culture media supplemented with 6% dextran T500) to create a film of fluid on the top of the endothelium. The membrane was stripped with a longitudinal movement using a 3-quadrant method, ensuring no torsions were generated during this phase to limit endothelial mortality. Once the tissue was stripped completely, it was

placed back on the corneal stroma.<sup>14</sup> All tissues were shipped to the surgeon (M.B.) as pre-stripped DMEK membranes on the corneal support in the transport medium.

### *Surgical Technique*

In all cases, anesthesia and akinesia were obtained by means of peribulbar injection of 10 ml of a 0.75% ropivacaine solution. Edematous epithelium affecting visualization of the intraocular structures was removed from a central area approximately 8 mm in diameter. Then, when necessary, bimanual phacoemulsification was performed. Eyes were dilated with two drops of Tropicamide 0.5% and one drop of Phenylephrine 10%. We did not aim for a fixed pupillary diameter. In all cases, a hydrophobic intraocular lens (IOL) (iSert 250; Hoya, Tokyo, Japan) was implanted into the capsular bag, expanded by the injection of viscoelastic device (IAL-F; Fidia Farmaceutici, Abano Terme, Italy), which was then removed carefully from the anterior chamber by prolonged irrigation and aspiration. The endothelium- Descemet complex was scored with a Price hook (Moria SA, Antony, France) and removed under air from the central 9 mm of the recipient cornea, possibly in a single piece. An inferior peripheral iridotomy was performed using vitreoretinal guillotine scissors under continuous irrigation from a specially designed anterior chamber maintainer (ACM; Moria SA) inserted at the 12- o'clock position.

The pre-stripped tissue was punched using an 8.25-mm Barron punch (Katena Products, Inc., Denville, NJ) and stained with trypan blue (VisionBlue, D.O.R.C., The Netherlands). As previously described by Busin et al. in detail,<sup>15</sup> the graft was then tri- folded with the endothelium inwards, transferred onto a sterile therapeutic soft contact lens (Sooft, Montegiorgio, Italy) and loaded into the funnel of a commercially available intraocular lens (IOL) cartridge (MDJ Company, La-Monniere-le-montel, France). The graft was finally delivered bimanually through a 3 mm clear-corneal incision sutured with 3 10-0 nylon single stitches (initial 61 cases) or through a sutureless scleral tunnel, 2 mm in length and 3 mm in width, as done in the following 112 cases, in an attempt at maximizing the speed of visual rehabilitation. Gentle tapping onto the cornea surface was used to facilitate unfolding of the lateral folds, which invariably occurred because of the natural tendency of the tissue to roll with the endothelium outward from its initial inward position. Then a 27-gauge cannula was inserted through the 12-o'clock side entry and advanced under the donor tissue to approximately the centre of the pupil before filling the anterior chamber with air and obtaining a proper attachment of the donor tissue onto the posterior surface of the recipient cornea.

### *Postoperative Evaluation*

After surgery, a pressure patch was applied and patients were instructed to lie on their backs for 2 hours before being checked at the slit lamp. If a pupillary block was present, a small amount of air was released from the main wound. Beginning the next morning, the postoperative medical treatment followed the regimen described in detail previously.<sup>15</sup> Two to 3 hours after surgery, as well as 1 day postoperatively, each eye was examined by means of a photographic slit-lamp to measure the air level in the anterior chamber and digital pictures were taken to document the data collected.

According to the amount of air in the anterior chamber, eyes were assigned to one of 3 sub-groups (air levels of 75%, 50%, and 25%), as shown in figure 1 left, center and right, respectively. Anterior segment optical coherence tomography (AS-OCT) (CASIA, Tomey, Tokyo, Japan) was also performed in each eye in order to detect any detached part of the graft. Re-bubbling was performed in all cases of graft detachment, as documented by means of AS-OCT, regardless of the extension of the detached area.

### *Statistical Analysis*

All data collected in the study was entered into an electronic database via Microsoft Excel 2007 (Microsoft Corp., Redmond, WA) and analysed with the Minitab Software, version 16 (Minitab Inc., State College, PA). Whenever appropriate, for the analysis of quantitative measurements the Student's t-test for normally distributed variables and the Kruskal-Wallis for nonparametric variables were used. Chi-Square or Fisher's exact test were utilized as indicated, for the analysis of categorical variables. Odds ratio was calculated for a continuous variable such as age by using a binary logistic regression analysis with graft detachment as the dependent variable and age as the independent variable.

We then performed binary logistic regression analysis. For this purpose we introduced, as independent, those variables that reached a significant level of less than 0.01 in univariate analysis in order to adjust for multiple comparisons. We used a backward stepwise logistic regression model in which the variables that remain in the final equation are not affected by the order of inclusion and/or exclusion.

The normit link function was chosen as it produced the best goodness of fit results. Variables that reached a significant level of less than 0.05 in multivariate analysis were considered significant.

Finally, to exclude potential correlation (multicollinearity) between variables that were found to affect the rate of graft detachment significantly, the variance inflation factor (VIF) was calculated.

## RESULTS

We evaluated 173 donor corneas and 173 recipient eyes undergoing DMEK during the study period. The donor and the recipient data are listed in Table 1. Indications and ocular co-morbidities are listed in Table 2. In 109 cases (63.01%) DMEK was performed in combination with phacoemulsification and IOL implantation. A single DMEK procedure was performed in 64 cases (36.99%). During the slit-lamp examination performed 2-3 hours postoperatively, air was removed from the anterior chamber in 2 eyes that had undergone combined cataract and DMEK surgery and in 1 eye that had undergone only DMEK. Anterior chamber air levels (in number of eyes) 2- 3 hours and 1 day after surgery are summarized in Figure 2.

Fifty-nine eyes (34.10%) showed early graft detachment and they all underwent re- bubbling. All grafts attached following re-bubbling and none of the cases was complicated by primary graft failure. Donor age ( $68.1\pm 10.8$  versus  $69.0\pm 11.2$ ;  $P=0.30$ ) and preoperative ECD counts ( $2572\pm 116$  versus  $2561\pm 115$ ;  $P=0.54$ ) were similar between eyes with graft detachment and those without. Table 3 summarizes the comparison between the two groups.

### *Univariate Analysis*

Briefly, there were no differences in terms of age ( $69\pm 11.2$  vs.  $68.1\pm 10.8$ ,  $OR=0.99$ , 95% CI 0.66-2.34,  $P=0.61$ ), gender, or donor characteristics between both groups. The graft detachment group had a significantly higher proportion of eyes with Fuchs (88.1% versus 61.4%;  $P<0.001$ ) and a significantly lower proportion of eyes with previous endothelial keratoplasty (3.4% versus 15.8%;  $P=0.008$ ). In addition, the graft detachment group had a significantly higher proportion of eyes that underwent removal of cataract and intraocular lens implantation (triple procedure) at the time of DMEK (86.4% versus 50.9%;  $P<0.001$ ). Immediate postoperatively (2-3 hours) air in the anterior chamber  $<75\%$  was associated significantly with graft detachment ( $OR=3.16$ , 95% CI 1.39-7.21,  $P=0.006$ ).

Two surgical techniques namely, sutured or sutureless (through scleral tunnel) did not show any significant difference in univariate analysis when correlated with occurrence of graft detachment with triple procedure or less amount of air in the anterior chamber.

### *Multivariate Analysis*

In multivariate analysis, only triple procedure (OR=5.31, 95% CI 2.03-13.86, P<0.002) and immediate postoperative (2-3 hours) air in the anterior chamber <75% (OR=2.66, 95% CI 1.12-6.34, P=0.027) remained significantly associated with graft detachment.

Also, in univariate analysis, 3.2% of the non-combined surgery patients had <75%, while 27.0% of the combined surgery patients had <75% at 3 hours (Chi square test, P=0.048). In order to double check that this correlation between the two factors did not lead to multicollinearity in the multivariate analysis, we calculated the variance inflation factor (VIF) and found that the VIF equalled 1 for both factors, meaning, that there was no multicollinearity between these variables. In other words, these two predictor variables were no longer significantly correlated to each other in multivariate analysis.

## **DISCUSSION**

Graft detachment is the most frequent postoperative complication of DMEK 5 and may involve only part or the entire donor tissue. Initial studies have distinguished only between partial and total graft detachments,<sup>9,16</sup> whereas more recently the extension of partial detachments has also been addressed in an attempt of predicting the possibility of spontaneous re-attachment.<sup>17</sup> In these studies several variations of the relatively new technique of DMEK were proposed with the purpose of minimizing the incidence of graft detachment. Instead, to date a thorough statistical analysis of pre-, intra-, and postoperative factors possibly affecting the rate of detachment has not been undertaken. To do so, all types of detachment must be considered, regardless of their tendency to progress or regress. The questions whether or not and when eventually re- bubbling has to be undertaken is beyond the scope of this analysis and may not be answered easily due to the different approach of different surgeons to this complication. The results of our study show that combining phacoemulsification and IOL implantation with DMEK is a major risk factor for early postoperative graft detachment. This data contradicts that of a large retrospective study by Chaurasia et al<sup>18</sup> who



compared 200 combined cases with 292 only-DMEK cases, and reported virtually identical re-bubbling rates. However, this study addressed mainly the management of graft detachment and not solely its occurrence, concluding that triple procedures were not associated with higher risk of complications than DMEK alone.<sup>18</sup> Instead, our study, although suffering from the limitations of being retrospective, based on a single center and including a relatively small number of eyes, indicates that triple procedures increase significantly the risk of graft detachment regardless of its extension. The fact that also the re-bubbling rate differs significantly between the two groups is probably only a consequence of our therapeutic approach of treating all the detachments by anterior chamber re-bubbling. The rationale for this approach is based both on the difficulty of treating at a later stage patients coming from long distances, including foreign countries, like most of those referred to our Institution, and on an attempt at maximizing as early as possible the amount of attached and therefore functioning endothelial cells. In fact, as detached portions of DMEK grafts become fibrotic with time and cannot be reattached,<sup>19</sup> it is conceivable that this loss of functioning donor tissue, although limited in extension, may affect the long-term graft survival. Nevertheless, independently of these considerations, the focus of our study was to identify risk factors for detachment and not to be able to predict the appropriate treatment of this complication.

There may be several additional explanations for our finding. It has been reported that in a combined surgery, achieving adequate miosis before DMEK is sometimes not possible. This factor together with the relative instability of a newly implanted IOL, as well as the possibility of residual viscoelastic device in the anterior chamber, can hinder proper attachment of the graft. Moreover, postoperative graft detachment may occur more often after triple procedures because of the increased patency of both main surgical wound and side entries, related to the additional manipulation required by phacoemulsification and IOL implantation, thus causing hypotony and insufficient air tamponade.<sup>20</sup> Finally, it has been noted that using SF<sub>6</sub> for tamponade instead of air<sup>13</sup> could reduce the graft detachment rate. We do not practice this technique and therefore our data are restricted to the use of air for re-bubbling.

The other variable correlating significantly with the rate of graft detachment was the level of air in the anterior chamber as measured 2 to 3 hours after DMEK. In particular, levels below 75% of air fill in the anterior chamber resulted into a significantly higher rate of graft detachment, as seen in figure 3 (top and bottom). Nearly 60% of eyes with low air levels 2 hours after DMEK showed the presence of graft detachment. Ćirković et al. reported in a series of 74 cases that an air fill of 80% of the anterior chamber was associated with significantly less need for re-bubbling than an air fill of 50%, an outcome that is in accordance with our results.<sup>21</sup> However, it must be noticed again that

these authors addressed the issue of re-bubbling rather than that of graft detachment, which, as previously explained, do not necessarily coincide.

No other factor/parameter analyzed in our study was found to influence significantly the rate of post-DMEK graft detachment. Finally, we did not find any specific indication or ocular co-morbidity associated with an increased risk for post-DMEK graft detachment. Notably, our study group included four cases of DMEK performed in patients with pre-existing tube-shunt or prior trabeculectomy, none of which was complicated by detachment. This is consistent with the recent data published by Aravena et al<sup>22</sup> demonstrating that eyes with prior glaucoma surgery had re-bubbling rates similar to those of their non-glaucomatous peers.

Previous failed keratoplasty was not a risk factor for post-DMEK graft detachment. However, our series included only 5 such cases and larger case series may contradict this conclusion. Interestingly, although we recently demonstrated a significantly higher incidence of DSAEK graft detachment in post-PK eyes,<sup>20</sup> the two eyes of our series that underwent DMEK after PK did not experience this complication. Perfect centering of the DMEK graft within the PK wound, may have eliminated the negative effect of wound irregularities on graft attachment.

In contrast to the univariate analysis, multivariate analysis showed no significant correlation between Fuchs' Endothelial dystrophy (FED) and graft detachment. By this it should be understood that while a specific patient with FED does have an increased risk for detachment compared to a non-FED patient, the increased risk is not directly related to the indication for surgery but rather to other variables associated with it, such as, the performance of a combined surgery.

The type of tissue preservation, (organ culture versus cold preservation), the relationship between descemetorhexis and graft sizes as well as the substance used for intraoperative tamponade (air versus gas) were not addressed in our study as our procedures were standardized for all eyes, including the use of tissues preserved exclusively in organ culture, a fixed diameter of both graft (8.25 mm) and descemetorhexis (9 mm) and the use of air for tamponade.

Our statistical analysis was not aimed at identifying the best approach to treat post- DMEK graft detachment. In fact, while total detachment requires quite obviously re- bubbling, which almost invariably succeeds with more or less extensive surgical manipulation, the management of partial detachment is more controversial. On one hand small detached areas may re-attach within weeks from surgery, but on the other hand, grafts detached for a prolonged period of time have shown fibrotic changes that do not allow late successful management.<sup>19</sup> As already mentioned above, our

choice to re-bubble all detachments, regardless of their extension, was aimed at avoiding this and did not have any detrimental effect on the final outcome of surgery.

In conclusion, our analysis demonstrates that phacoemulsification performed at the time of DMEK is a risk factor for early graft detachment, thus indicating that sequential surgery (i.e. cataract surgery initially and DMEK at a later time) may be instrumental in minimizing this complication. In making their decision, surgeons should weigh this finding against the disadvantages of repeat surgery, including the increased discomfort for the patient and risks of two separate procedures.

The amount of air persisting in the anterior chamber during the initial postoperative 2-3 hours is equally important for graft attachment. Therefore while taking maximal care to avoid the onset of pupillary block, surgeons should aim at maintaining an air level in the anterior chamber as high as possible during the first postoperative day.

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## FIGURE CAPTIONS

**FIGURE 1.** Different air-fluid levels following Descemet's membrane endothelial keratoplasty. Slit lamp images of the recipient eyes after Descemet's membrane endothelial keratoplasty showing air levels of 75% (left), 50% (center) and 25% (right). A small amount of air 2-3 hours postoperatively was associated in this study with increased risk for early graft detachment.

**FIGURE 2.** Distribution of postoperative air levels following Descemet's membrane endothelial keratoplasty. Graph indicating the distribution of air levels 2-3 hours and 24 hours following Descemet's membrane endothelial keratoplasty. Although at the end of the surgery all patients were left with complete air fill of the anterior chamber, 11/173 (6.3%) of them had less than 25% air fill 2-3 hours postoperatively. These patients were significantly more prone to develop early graft detachment.

**FIGURE 3.** Low postoperative air levels are associated with increased risk for graft detachment. Slit lamp and anterior segment optical coherence tomography images of two cases showing air levels <50% after 2 hours of Descemet's membrane endothelial keratoplasty (Left top and bottom), and <25% after 24 hours (Center top and bottom). The first one (Top right) was detached after 24 hours, while the second one (Bottom right) was perfectly attached.