IMPORTANCE It is essential to devise strategies that improve graft adhesion after Descemet membrane endothelial keratoplasty (DMEK) to reduce the rebubbling rate.

OBJECTIVE To evaluate the influence of the extent of descemetorhexis on graft adhesion properties after DMEK.

DESIGN, SETTING, AND PARTICIPANTS Single-surgeon, retrospective, observational case series conducted in the Department of Ophthalmology, University of Erlangen-Nuremberg, Germany, that reviewed the medical records of 200 consecutive patients undergoing DMEK. Fifty-three eyes of 51 patients undergoing DMEK for Fuchs endothelial dystrophy fulfilling the inclusion criteria were enrolled in this study. Based on intraoperative drawings, postoperative slitlamp examination, and photographs, eyes were divided into 2 groups. The diameter of the descemetorhexis was approximately 10 mm in group A (30 eyes), resulting in a peripheral 1-mm zone of denuded stroma between the graft and the host’s Descemet membrane, and approximately 6 mm in group B (23 eyes), resulting in a peripheral 1-mm zone of overlapping between the graft and the host’s Descemet membrane.

MAIN OUTCOMES AND MEASURES Graft detachment rate, extent of graft detachment (in clock hours of graft’s circumference), and rebubbling rate.

RESULTS Four days after DMEK, the graft detachment rate was 33.3% (10 of 30) in group A and 78.3% (18 of 23) in group B ($P = .002$). The mean (SD) extent of graft detachment was 0.6 (0.9) and 2.8 (2.5) clock hours in groups A and B, respectively ($P < .001$), 4 days after surgery. The rebubbling rate was 6.7% (2 of 30) and 30.4% (7 of 23) for groups A and B, respectively ($P = .03$).

CONCLUSIONS AND RELEVANCE A larger descemetorhexis in DMEK is correlated with better graft adhesion and lower rebubbling rates. Therefore, patients with a larger descemetorhexis require less intensive follow-up.
For almost a century, penetrating keratoplasty has been the criterion standard for treating endothelial disorders, such as Fuchs endothelial dystrophy. Recently, it has been replaced by lamellar surgery, which has superior outcomes and a shortened time needed for complete visual rehabilitation. Among the current strategies for lamellar replacement of the corneal endothelium, 2 approaches have gained special attention. Descemet stripping automated endothelial keratoplasty (DSEA), currently in widespread use, involves lamellar splitting of the donor tissue with a microkeratome, resulting in grafts of about 100 to 200 μm thickness. More recently, Descemet membrane endothelial keratoplasty (DMEK) has been described as a sole transplantation of the Descemet membrane (DM) and corneal endothelium. Despite visual outcome of unsurpassed quality, only a few corneal surgeons have adopted this technique. One of the major reasons is that postoperative detachment of the thinner grafts occurs more frequently in DMEK than detachment of thicker stromal grafts in DSEA. Especially in the setting of outpatient surgery, repeated injections of air into the anterior chamber, so-called rebubbling, are associated with frequent follow-up visits and cause inconvenience to patients and surgeons.

It is, therefore, necessary to investigate the reasons for graft detachment in DMEK and devise strategies that improve graft adhesion. As a first step toward this goal, we have investigated the hypothesis that extensive removal of the host’s DM (descemetorhexis), rendering a large denuded zone of bare stroma between the host’s DM and the graft, increases graft adhesion as opposed to a smaller descemetorhexis resulting in an overlap between the host’s DM and the graft.

Methods

Patients

The medical records of 200 consecutive patients undergoing DMEK for Fuchs endothelial dystrophy were reviewed. Twenty-one patients (10.5%) were excluded because of other ocular pathologic conditions (eg, macular degeneration, n = 11; glaucoma, n = 6; and epiretinal glialosis, n = 4). On the basis of intraoperative drawings and videos, as well as postoperative slitlamp examination and photographs, we excluded 126 patients (63.0%) from the analysis for not having a complete circular overlap or a complete circular denuded zone between the graft and the host’s DM. This was mostly due to a centered graft or uneven edges of irregular descemetorhexis. The remaining 53 eyes of 51 patients (25.5%) (female to male ratio of 31:20) had a well-centered graft and either a large (>9 mm) or a small (<7 mm) descemetorhexis, resulting in either no or complete overlap between the graft and the host’s DM. The sequence of appearance of such cases over time was evenly distributed. The mean (SD) age of patients was 69.8 (8.2) years (range, 52-88 years).

Fifty-three eyes of 51 patients were divided into 2 groups with respect to the presence of a circular peripheral zone of denuded stroma or a circular peripheral overlapping zone between the host’s DM and the graft (Figure 1). In group A (30 eyes), the diameter of the descemetorhexis was approximately 10 mm, resulting in a peripheral zone of denuded stroma between the host’s DM and the 8-mm graft, which measured approximately 1 mm. In group B (23 eyes), the diameter of the descemetorhexis was approximately 6 mm, resulting in a peripheral overlap between the host’s DM and the 8-mm graft, which measured approximately 1 mm.

All eyes received 8-mm grafts prepared from corneoscleral buttons that had been stored in Dulbecco modified Eagle medium containing streptomycin and penicillin (Biochrom) and fetal calf serum (Linaris) at 34°C (organ-cultured graft) in various German eye banks.

There was no statistically significant difference between groups A and B, respectively, concerning donor age (mean [SD], 73.8 [9.9] and 73.6 [11.1] years; P = .79), storage duration (350 [116] and 325 [115] hours; P = .44), and endothelial cell density of donor corneas (2501 [217] and 2603 [310] cells/mm²; P = .28).

Informed consent was obtained from the patients, and the study adhered to the tenets of the Declaration of Helsinki and all German federal or state laws. The study was approved by the institutional review board at Friedrich Alexander University, Erlangen, Germany, as a retrospective review of data.

Descemetorhexis Surgery: Air Injections (Rebubbling)

All operations were performed by one of us (F.E.K.) using a technique that has been described previously. One day before surgery, all patients received an Nd:YAG laser iridotomy to avoid pupillary block or Urrets-Zavalia syndrome. Donor preparation was performed immediately before transplantation. Donor preparation was successful in all DMEK cases, without any damage to the DMEK grafts.

During surgery, the epithelium was marked with a 9-mm and 7-mm marker to determine the size of the descemetorhexis. The intended size of the descemetorhexis was 8 mm. The patient’s DM was removed under air using an inverted hook (Price Hook; Moria). All eyes received an 8-mm graft. The graft was injected into the anterior chamber, carefully positioned centrally using short bursts of a balanced salt solution, and unfolded by injecting 1 small air bubble into the lumen of the roll. Special care was taken to place the graft exactly in the center of the descemetorhexis. After correct positioning of the graft, air was injected underneath the graft until the anterior chamber was completely filled with air and then left in place for 60 minutes. The correct position of the graft was checked again and documented by a drawing. On completion of the surgery, air was reduced to about 50% of the anterior chamber volume. Patients were instructed to preferably remain in a supine position for 48 hours after surgery.

In case of a postoperative intracameral air injection, the anterior chamber was completely refilled with air and then left in place for 60 minutes. Subsequently, air was reduced to about 50% of the anterior chamber volume.

All patients were treated with prednisolone acetate, 1% drops 5 times daily for 8 weeks (thereafter, it was decreased by 1 drop every month), ofloxacin, 0.3% drops 5 times daily for the first 2 weeks after surgery, and sodium chloride, 5% drops 5 times daily for the first 4 weeks after surgery.
Main Outcomes and Measures

Main outcome measures included graft detachment rate, extent of graft detachment (in clock hours), and rebubbling rate. Statistical evaluation was performed using SPSS version 18.0 for Windows (SPSS, Inc). The level of significance was set at α = 0.05. Because normal distribution in all groups of this study was not evident, nonparametric tests were used. Differences between groups were assessed by the Mann-Whitney and Fisher exact tests.

Results

Extent of Graft Detachment

Analyses were based on daily postoperative slitlamp examinations and drawings. Graft detachment was quantified by anterior-segment optical coherence tomography (AS-OCT) (Slit-lamp OCT; Heidelberg Engineering).
To analyze the graft detachment rate, we first defined graft detachment as significant if the radial extent of the nonadherent area was at least 1 mm (of an 8-mm graft). If the radial extent of graft detachment was less than 1 mm, the graft was considered attached. The extent of graft detachment was estimated as clock hours of the margin of the graft. The rate and extent of graft detachment were determined 4 days after surgery because the evaluation of a larger number of DMEK cases (n = 200; data not shown) had shown that air left in the anterior chamber on completion of the surgery is completely resorbed within 48 to 72 hours and that 90% of graft detachments are identified within this period. An additional assessment of graft detachment was performed 8 days after surgery, as well as 4 weeks postoperatively.

The graft detachment rate 4 days after surgery was 33.3% (10 of 30) in group A and 78.3% (18 of 23) in group B. It was significantly higher in group B ($P = .002$, Fisher exact test) (Figure 2).

The extent of graft detachment 4 days after surgery was a mean (SD) of 0.6 (0.9) and 2.8 (2.5) clock hours in groups A and B, respectively. It was significantly higher in group B ($P < .001$, Mann-Whitney test) (Figure 3).

Localization of Graft Detachment

On the basis of daily postoperative slitlamp examinations, AS-OCT, and drawings, we analyzed the localization of graft detachment. All graft detachments occurred in the periphery of the graft without affecting the visual axis. To assess localization of graft detachment, we divided the graft’s circumference into 4 quadrants: superior, inferior, nasal, and temporal. If the extent of graft detachment was greater than 1 quadrant, we chose the quadrant in which the highest elevation of the detachment was localized.

The localization of the graft detachment in eyes with significant graft detachment (n = 28, groups A and B) was as follows: inferior quadrant, 46.4% (13 of 28); nasal quadrant, 32.1% (9 of 28); superior quadrant, 10.7% (3 of 28); and temporal quadrant, 10.7% (3 of 28). The proportional distribution in each group was similar to the overall distribution. In group A (n = 10), 30.0% (3 of 10) of eyes showed graft detachment inferiorly, 30.0% (3 of 10) nasally, 20.0% (2 of 10) superiorly, and 20.0% (2 of 10) temporally. In group B (n = 18), 55.6% (10 of 18) of eyes showed graft detachment inferiorly, 33.3% (6 of 18) nasally, 5.6% (1 of 18) superiorly, and 5.6% (1 of 18) temporally (Figure 4).

Rebubbling Rate

Slitlamp examination was performed daily until 8 days after surgery. We made a decision for rebubbling because of graft detachment based on the extent and localization of the graft detachment as well as clinical evaluation. Furthermore, the decision was based on the presence of the following conditions: (1) detachment causing focal corneal edema that reached the visual axis or (2) detachment with a height of more than 1 thickness of the corneal stroma and an extent of more than 1 quadrant.

In group A (n = 30), 2 eyes (6.7%) required 1 air injection during the observation time. One eye received rebubbling 5 days and the other 6 days after surgery. No eyes in group A required a second intervention after the first rebubbling. In group B (n = 23), 7 eyes (30.4%) needed rebubbling. Six eyes received 1 air injection between days 4 and 7, and 1 eye received 2 air injections at days 4 and 14 after surgery. The rebubbling rate was significantly higher in group B ($P = .03$, Fisher exact test) (Figure 5). The overall rebubbling rate was 17.0% (9 of 53).

Eight days after surgery, 1 eye in group B showed significant graft detachment requiring a second rebubbling. A complete graft detachment was not observed during follow-up. All eyes (N = 53) were reexamined 4 weeks after surgery to ensure complete graft attachment. No eye showed any graft detachment 4 weeks postoperatively.

Discussion

Descemet membrane endothelial keratoplasty was introduced in 2006 after successful outcomes and widespread use
of Descemet stripping endothelial keratoplasty, which had been introduced between 2003 and 2004,5,6,15 Descemet stripping automated endothelial keratoplasty and Descemet stripping endothelial keratoplasty underwent several additional years of evolution and learning curves compared with DMEK. This may explain why widespread use of DMEK is still pending. While DMEK seems to render faster and more complete visual rehabilitation than DSAEK,7,16,17 it is associated with higher intraoperative complications related to donor preparation, graft implantation, and graft unfolding in the anterior chamber as well as postoperative complication rates, such as graft detachment. Standardized techniques for donor preparation and insertion, previously described by Dapena et al18 and Kruse et al,14 have tried to overcome intraoperative difficulties. However, there is still a lack of knowledge concerning graft adhesion, which is the primary factor determining the short-term success of DMEK.

One possible way to enhance graft adhesion and reduce the rebubbling rate could be the creation of a larger descemetorhexis during DMEK surgery. Removal of a larger portion of the host’s DM, resulting in more area of denuded stroma, avoids an overlap between the host’s DM and the DMEK graft. In this study, the graft detachment rate during the first postoperative week was 78.3% in group B, which is more than twice as high as that in group A (33.3%). Similar results were observed when comparing the extent of graft detachment, which during the first postoperative week in group B was more than 4 times as large as that in group A. On the last day of our daily follow-ups (8 days after surgery), only 1 eye showed significant graft detachment.

There seems to be a difference in graft adhesion depending on the presence of the host’s DM with endothelial cells in the donor-host interface (between the graft and the host’s stroma). Adhesion of the graft might be negatively influenced by interactions between the host’s DM and the graft. Previous data from our laboratory showed that the structural and biochemical properties of the anterior surface matrix of stripped DMEK grafts may determine graft adhesion to the recipient’s posterior corneal stroma.19 The interfacial matrix localized on the anterior surface of the graft contained various proteins, which could explain the adhesive properties of the graft to the posterior collagen lamellae of the corneal stroma, such as fibronectin, vitronectin, amyloid P, and osteonectin/secreted protein acidic and rich in cysteine, fibrillin 1, fibrin 1 to 3, and keratoepithelin. In particular, keratoepithelin was localized along the DM-stroma interface involving the anterior aspect of the DM and the posterior aspect of the corneal stroma. These adhesive proteins, which are pronounced along the interface, might facilitate the attachment of the graft. In contrast, the surface of endothelial cells on the remaining DM in the periphery of the cornea might not contain any domains that could support graft adhesion. Further studies investigating the anatomy and biochemistry of the DM are needed to further define the adhesive properties of DMEK grafts.

Graft detachment was more frequently localized inferiorly in groups A and B (30.0% and 55.6%, respectively) and in the whole sample of eyes (46.4%) as well. This finding is similar to a previous report describing that 58% of graft detachments after DMEK are localized in the inferior quadrant.20 This might be attributed to the fact that the inferior part of the graft has less contact with the air bubble once the air bubble becomes smaller, especially if the patient is not in a supine position.

Another parameter we analyzed was the rebubbling rate. Better adhesion of the DMEK graft is associated with less rebubbling. In our study, eyes in group B showed a higher rebubbling rate (30.4%) compared with eyes in group A (6.7%). The rebubbling rate in recent studies is reported to range between 3% and 62% after DMEK8,9 and between 2.5% and 16.1% after DSAEK or Descemect stripping endothelial keratoplasty.10-13

The decision for rebubbling is controversial. We preferred to perform rebubbling in the early postoperative period, mainly 6 days after surgery. Recently, Yeh et al21 analyzed the predictive value of AS-OCT in graft detachment after DMEK to determine the right time point for reintervention. Yeh et al described that the 1-hour and 1-week
AS-OCT scan should be the referential time points for predicting graft adhesion after DMEK. One of the conclusions in this study\(^2\) was that if the graft is completely attached 1 week after DMEK surgery, reintervention is usually not required. This finding is fully consistent with our results since all grafts that were attached on the eighth postoperative day remained attached at 4 weeks after surgery. In summary, the above-mentioned study\(^2\) suggests that rebubbling should not be performed too early in the postoperative period because most graft detachments show reattachment or spontaneous clearance.

In contrast to conventional knowledge, we did not clinically observe differences in peripheral edema between the 2 groups in the early postoperative period. During observation time (8 days postoperatively), edema was homogeneously distributed over the entire cornea in both groups. We routinely measure corneal thickness with the Pentacam software (Oculus). Peripheral corneal thickness measurements in our patients assessed at different time points within the first 8 days were not reproducible and therefore of limited use for further analysis. Four weeks after surgery, there were no significant differences in peripheral corneal thickness between groups A and B (data not shown).

When the endothelial barrier of the cornea is damaged, the aqueous humor is constantly entering the stroma, resulting in a loss of corneal transparency. When endothelial cells are lost in the adult human cornea, the defect must be covered by cellular reorganization, such as enlargement and migration. As mentioned earlier, we did not observe increased peripheral corneal edema in eyes with a zone of denuded stroma (group A). An explanation for this paradox phenomenon might be the ability of the endothelial cells, which are adjacent to the stromal gap, to spread and, therefore, take over the deswelling function of the missing endothelial cells. Our results suggest that an 8-mm graft with healthy endothelial cells is sufficient for restoring the clarity of the cornea, even in eyes with a small area of denuded stroma (group A). The ability of endothelial cells to migrate has been described by several authors and us.\(^22\) However, the time needed for endothelial migration is not known. Further studies are needed to investigate the time course of restoring corneal clarity over the denuded stroma areas.

An obvious limitation of our study is the fact that many patients rendered a partial but not complete overlap or denuded zone between the graft and the host’s DM. In many cases, we were not able to place the graft exactly in the center, especially in smaller eyes. Another reason for not achieving complete overlap or a complete denuded zone is an irregular descemetorhexis. Furthermore, a basic limitation of DMEK regarding placement of the graft is that the graft cannot be moved when attached by the air bubble without harming the endothelium. As a consequence, we suggest removing the host’s DM outside the 9-mm marking, resulting in a diameter of approximately 10 mm. Special care should be given to render a circular descemetorhexis.

In conclusion, a larger descemetorhexis in DMEK provided better graft adhesion and was, therefore, correlated with a lower rebubbling rate compared with a smaller descemetorhexis. Consequently, patients with a larger descemetorhexis required less intensive follow-up. Further studies are needed to investigate graft adhesion properties to make the DMEK procedure safer and more reproducible.

### ARTICLE INFORMATION

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### REFERENCES


OPHTHALMIC IMAGES

Pincushion Sign

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The “pincushion sign” is a sign of multiple previous intravitreal injections with a small-gauge needle. Treatment is often with an anti–vascular endothelial growth factor or steroid for conditions such as neovascular age-related macular degeneration, diabetic macular edema, or cystoid macular edema secondary to retinal vein occlusion. The dark punctuate appearance situated 3.5 to 4.0 mm behind the limbus represents visible pigment through the sclera (black arrowheads). It should be distinguished from the much larger naturally occurring vascular channels commonly seen (white arrowheads). It is not a universal sign of previous treatment, often only being seen in those with a thin sclera such as in myopia. It is important for ophthalmologists to be aware of this sign (1) as an indicator of previous treatment in those unable to communicate their history, (2) for the possibility of inadvertent zonular damage at the time of injection increasing the chance of complicated cataract surgery, and (3) when choosing an alternative site for surgery involving the sclera.