Ultrastructural findings in graft failure after Descemet membrane endothelial keratoplasty (DMEK) and new triple procedure

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Abstract

To investigate factors that influence graft failure after Descemet membrane endothelial keratoplasty (DMEK) based on transmission electron microscopy results.

Retrospective observational case series.

This single center study included 16 eyes of 16 patients with penetrating keratoplasty (n = 14) or repeat DMEK (n = 2) following graft failure after DMEK. The main outcome measures were ultrastructural changes in the explanted graft on transmission electron microscopy, best-corrected visual acuity, and central corneal thickness.

The mean preoperative and postoperative best-corrected visual acuity was 1.01 ± 0.54 logMAR and 0.56 ± 0.37 logMAR. The mean central corneal preoperative and postoperative thickness was 667 ± 187 μm and 511 ± 42 μm. Visual acuity and central corneal thickness improved significantly (P = .001/P = .003) after repeat surgery. Electron microscopy showed that 3 of 14 corneas showed upside down transplantation, and 3 corneas had pigmented cells or pigment granules at the Descemet–stroma interface. Further, 9 of 16 specimens showed a posterior collagenous layer deposited onto the Descemet membrane (average thickness 5.1 ± 6.2 μm; ranged 0.65–20 μm); this did not correlate significantly with the time between the original and repeat keratoplasty. Of 16 original grafts, 7 showed ultrastructural anomalies of the Descemet membrane, but one excised cornea showed no Descemet membrane pathologies.

The majority of eyes with graft failure after DMEK showed ultrastructural changes in the Descemet membrane. It is crucial to assess donor tissue quality and to conduct graft marking before surgery to avoid immediate or delayed graft failure after DMEK. Nevertheless, repeat keratoplasty provided significant improvement in central corneal thickness and visual acuity.

Abbreviations: ABL = anterior banded layer, BCVA = best-corrected visual acuity, CCT = central corneal thickness, DMEK = Descemet membrane endothelial keratoplasty, MAR = minimum angle of resolution, PCL = posterior nonbanded layer, PEX = pseudoexfoliation syndrome, PNBL = posterior nonbanded layer, YAG = yttrium aluminum garnet.

Keywords: Descemet membrane, DMEK, graft failure, transmission electron microscopy, ultrastructural findings

1. Introduction

Descemet membrane endothelial keratoplasty (DMEK) is a relatively new and promising technique and is one of the most common methods of corneal transplantation. The DMEK procedure was first described by Melles et al[1] and represents an advancement of previous methods of posterior lamellar keratoplasty without transfer of stromal tissue. Since its development, DMEK has become a safe and thus very popular method for curing endothelial pathologies, such as Fuchs’ endothelial dystrophy and bullous keratopathy, without stromal scars. The combined surgery of cataract extraction, intraocular lens implantation, and DMEK is usually called the new triple procedure, a term based on the term triple procedure that was used to describe the classic trio of cataract extraction, intraocular lens implantation, and penetrating keratoplasty.

In 2016, 57% of the corneal transplantations performed in Germany were posterior lamellar keratoplasties (90% DMEK).[2,3] The advantages of DMEK are its short surgical time, quick visual recovery, low risk of graft rejection, and little change in refraction.[3,4] Its disadvantages are the unpredictability of graft adhesion,[5,6] possible failure in graft preparation,[7,8] difficulties in graft unfolding (especially in young donors),[9,9] and damage to endothelial cells due to intraoperative iatrogenic maneuvers.

The purpose of this retrospective single-center study was to investigate factors that might influence graft failure after DMEK and the new triple procedure based on an ultrastructural analysis of explanted grafts.
2. Material and methods

This single-center observational study included 16 eyes of 16 patients (mean age, 70 ± 9 years; 7 women, 9 men). The patients underwent repeat DMEK (n = 2) or penetrating keratoplasty (n = 14) at the Department of Ophthalmology, Saarland University Medical Center UKS, Homburg/Saar because of graft failure after DMEK (n = 11) or after new triple procedure (n = 5). Of the 16 patients, 8 were referred to us by external ophthalmic surgeons. The excised DMEK grafts (n = 2) and host corneas (n = 14) were examined by transmission electron microscopy at the Department of Ophthalmology, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany.

The study complied with the tenets of the Declaration of Helsinki. The Institutional Review Board waived the need for approval. Written informed consent was obtained from all patients. The patients underwent complete ophthalmological examinations, including Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany).

The main outcome measures included an indication for repeat keratoplasty, pre- and postoperative best-corrected visual acuity, pre- and postoperative central corneal thickness, (CCT, Pentacam) and ultrastructural findings.

2.1. Transmission electron microscopy

The explanted DMEK grafts (n = 2) and corneal buttons (n = 14) were processed for electron microscopy as previously described and examined with a transmission electron microscope at the Department of Ophthalmology, Saarland University Medical Center UKS, Homburg/Saar because of graft failure after DMEK (n = 11) or after new triple procedure (n = 5). Of the 16 patients, 8 were referred to us by external ophthalmic surgeons. The excised DMEK grafts (n = 2) and host corneas (n = 14) were examined by transmission electron microscopy at the Department of Ophthalmology, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany.

The mean thickness of this PCL was 5.1 ± 6.2 μm (range 0.65–20 μm). There was no obvious correlation between the thickness of the PCL and the time to repeat surgery (r = .73) (Fig. 4).

Intrinsic ultrastructural abnormalities of the Descemet membrane were observed in 7 of 16 grafts; this included abnormal banded and fibrillary collagen inclusions within the normally non-banded posterior layer, which is indicative of pre-existing corneal endothelial dysfunction (Fig. 3B–D).[10] Abnormal fibrillary inclusions and posterior deposition of collagen fibers were also found in some of the specimens (Fig. 3B). Of the 7 specimens, 1 showed signs of beginning guttae formation, indicating the presence of early stages of Fuchs’ dystrophy in the donor (Fig. 3D). Only 1 of 16 specimens showed no abnormalities of the Descemet membrane.

4. Discussion

Donor graft preparation, a successful surgical procedure, and a lack of postoperative complications[10] are crucial for the success of DMEK. Complications in any surgical step can lead to primary or secondary graft failure.

If the 16 specimens that we analyzed, 3 showed upside down transplantation that lead to graft failure. We strongly recommend presurgical marking of the donor Descemet membrane to avoid this complication.[11,12] We think that the need for numerous failing re-bubbling procedures may be a strong sign of upside down transplantation.

Studies show that graft failure after DMEK occurs in 1.6% to 8% of patients.[13–15]

The majority of patients with primary or secondary graft failure show ultrastructural anomalies of the donor Descemet membrane, including intrinsic abnormal inclusions in the Descemet membrane and/or posterior collagenous layers deposited onto the membrane.[15,16] Abnormal inclusions within the Descemet membrane may reflect pre-existing subclinical endothelial dysfunction prior to...
| Patient | Age, years | Gender | Type of surgery | Indication for initial DMEK/new triple DMEK | Number of Re-Bubblings | BCVA preoperative (decimal) | BCVA postoperative (decimal) | Pachymetry preoperative (µm) | Pachymetry postoperative (µm) | Date of repeat surgery | Indication for surgery | Type of repeat surgery | Time to repeat surgery, months | Results of electron microscopy | Thickness of PCL (µm) |
|---------|------------|--------|-----------------|-------------------------------------------|------------------------|---------------------------|---------------------------|-----------------------------|-----------------------------|---------------------|----------------------|---------------------------|-----------------------------|---------------------------|------------------------|
| 1       | 63         | Female | New triple DMEK 02/2015 | "in home" | Fuchs' endothelial corneal dystrophy | 2                      | 0.4                        | 0.5                          | n.a                         | 534                         | 04/2015             | Primary graft failure | PnP | 2 | Upside-down transplantation | Degenerated endothelial cells, abnormal PCL | 0.85 |
| 2       | 80         | Male   | New triple DMEK 02/2015 | "in home" | Fuchs' endothelial corneal dystrophy | 3                      | 0.3                        | 0.8                          | 586                         | 564                         | 04/2015             | Primary graft failure | PnP | 9 | Primary graft failure | Secondary graft failure | 0.5 |
| 3       | 83         | Male   | DMEK 01/2016 | "elsewhere" | Bullous keratopathy | n.a                     | 0.16                       | 0.16                         | 682                         | 663                         | 12/2015             | Primary graft failure | PnP | 10 | Degenerated endothelial cells | Degenerated endothelial cells | 1.0 |
| 4       | 75         | Male   | New triple DMEK 02/2015 | "in home" | Posterior polymorphous corneal dystrophy | 1                      | 0.1                        | 0.5                          | n.a                         | n.a                         | 06/2016             | Immuneologic reaction (after 1 month) | PnP | 8 | Abnormal PCL, Pigmented cells in the Interface | Degenerated endothelial cells | 1.0 |
| 5       | 67         | Male   | New triple DMEK 02/2015 | "in home" | Fuchs' endothelial corneal dystrophy | 1                      | 0.5                        | 0.8                          | n.a                         | n.a                         | 12/2016             | Secondary graft failure | Repeat DMEK | 29 | Degenerated endothelial cells | Degenerated endothelial cells | 0.85 |
| 6       | 79         | Female | DMEK 01/2016 | "elsewhere" | Bullous keratopathy | 1                      | 0.01                       | 0.3                          | 1025                        | 515                         | 06/2016             | Primary graft failure | PnP | 18 | Abnormal PCL, degenerated endothelial cells | Degenerated endothelial cells | 0.85 |
| 7       | 68         | Female | New triple DMEK 02/2015 | "in home" | Fuchs' endothelial corneal dystrophy | 1                      | 0.04                       | 0.8                          | 1072                        | 549                         | 09/2015             | Primary graft failure | PnP | 9 | Thick abnormal PCL, degenerated endothelial cells | Normal Descemet's membrane | 3.75 |
| 8       | 67         | Male   | DMEK 04/2015 | "elsewhere" | Fuchs' endothelial corneal dystrophy | n.a                     | 0.2                        | 0.3                          | 549                         | 438                         | 01/2016             | Primary graft failure | PnP | 9 | Normal Descemet's membrane | Normal Descemet's membrane | 1.0 |
| 9       | 85         | Female | DMEK 06/2016 | "in home" | Pseudophakic bullous keratopathy | 3                      | 0.1                        | 0.1                          | 607                         | 542                         | 04/2016             | Primary graft failure | PnP | 10 | Degenerated endothelial cells | Degenerated endothelial cells | 1.0 |
| 10      | 70         | Male   | 1. new triple DMEK 02/2013 | 2. DMEK 10/2015 | Fuchs' endothelial corneal dystrophy | n.a                     | 0.3                        | 0.6                          | 674                         | 438                         | 04/2016             | Primary graft failure | PnP | 6 | Upside-down transplantation | Degenerated endothelial cells | 1.0 |
| 11      | 56         | Female | New triple DMEK 02/2015 | "in home" | Cornea plana, Axenfeld-Rieger syndrome | n.a                     | 0.05                       | 0.2                          | 665                         | 543                         | 08/2016             | Secondary graft failure | PnP | 25 | Thick abnormal PCL | Degenerated endothelial cells | 4.25 |
| 12      | 76         | Male   | DMEK 05/2016 | "in home" | Pseudophakic bullous keratopathy | n.a                     | 0.05                       | 0.5                          | 660                         | 511                         | 09/2017             | Primary graft failure | PnP | 8 | Thick abnormal PCL, Pigment in the Interface | Degenerated endothelial cells | 0.9 |
| 13      | 64         | Male   | DMEK 11/2015 | "in home" | Fuchs' endothelial corneal dystrophy | n.a                     | 0.01                       | 0.25                         | 473                         | 435                         | 01/2017             | Immuneologic reaction | Repeat DMEK | 13 | Thick Descemet's membrane | Degenerated endothelial cells | 0.9 |
| 14      | 81         | Male   | DMEK 11/2014 | "elsewhere" | Fuchs' endothelial corneal dystrophy | n.a                     | 0.3                        | 0.4                          | 461                         | 436                         | 02/2017             | Immuneologic reaction | PnP | 39 | Thin abnormal PCL | Degenerated endothelial cells | 1.9 |
| 15      | 52         | Male   | DMEK 07/2016 | "in home" | Bullous keratopathy, congenital glaucoma | n.a                     | 0.05                       | 0.1                          | 590                         | 590                         | 02/2017             | Primary graft failure | PnP | 7 | Thick abnormal PCL, Pigment granules | Upside-down transplantation | 1.0 |
| 16      | 76         | Female | DMEK 02/2013 | 2. Repeat DMEK 04/2015 | Fuchs' endothelial corneal dystrophy | n.a                     | 0.05                       | 0.1                          | 690                         | n.a                         | 01/2018             | Immuneologic reaction | PnP | 21 | Pigmented cells, degenerated endothelial cells | Degenerated endothelial cells | 1.0 |

BCVA = best-corrected visual acuity, DMEK = Descemet membrane endothelial keratoplasty, PCL = posterior collagenous layer, PEX = pseudoexfoliation syndrome, PKP = penetrating keratoplasty.
transplantation, whereas retrocorneal collagen deposits and fibroblast-like endothelial cells indicate peri- and postoperative endothelial damage.\textsuperscript{10}

Ultrastructural changes with abnormal banded and fibrillary collagen inclusions (like a duplicated anterior banded layer within the posterior nonbanded layer in Fig. 3B) within the

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**Figure 1.** Transmission electron micrographs of explanted corneal buttons after DMEK failure. (A, B) Inverted transplantation: the posterior nonbanded layer (PNBL) of the donor graft adjoins the host corneal stroma, whereas the anterior banded layer (ABL) of the Descemet membrane borders the anterior chamber or is overgrown by stromal keratocytes (Ke). (C, D) The accumulation of pigmented cells (C) or isolated pigment granules (D) in the Descemet-stromal interface (*). ABL = anterior banded, DMEK = Descemet membrane endothelial keratoplasty, PNBL = posterior nonbanded layer.

**Figure 2.** Histological findings in graft failure after DMEK. (A) Retrocorneal collagenous tissue (*) and (B) pigmented tissue (#) at the interface. The break in the Descemet membrane is an artifact. DMEK = Descemet membrane endothelial keratoplasty.
normally nonbanded posterior layer may also be a sign of incomplete removal of recipient’s Descemet membrane. Brockmann et al. [6] found that incomplete removal of the Descemet membrane from the recipient’s stroma can increase the detachment rate. They discovered an increased thickness of the anterior banded layer in patients with graft detachment and concluded that residual anterior banded layer fragments on the recipients’ stroma can create an anatomical border. [6]

Ultrastructural changes observed in a donor Descemet membrane may be preexisting or may be acquired during tissue harvesting, tissue storage, graft preparation, or surgery. Weller et al. [10] also concluded that ultrastructural anomalies can be signs of preoperative corneal endothelial dysfunction. The donor tissue we used did not show any noticeable problems during examination in our eye bank. However, the presence of early stages of pseudoexfoliation-associated keratopathy and cornea guttata may have gone undetected.

Of the 16 specimens, 9 showed an abnormal PCL. Weller et al. [10] postulated that a PCL may be indicative of intraoperative or postoperative trauma and is thought to be produced by damaged, fibroblast-like endothelial cells. In our study, there was no mathematical correlation between the thickness of the abnormal PCL (which ranged from 0.65 to 20 μm) and the time period until repeat surgery (which ranged from 2 to 39 months). However, this could be due to the small number of cases. In our case series, we could not evaluate potential risk factors during organ culture, graft preparation, or intraoperative manipulation in all patients, because 8 of the patients were referred to our department from other hospitals.

Figure 3. Transmission electron micrographs of explanted corneal buttons or Descemet membranes after DMEK failure. (A) Deposition of a posterior collagenous layer (PCL) onto Descemet’s membrane consisting of a normal anterior banded (ABL) and posterior non-banded layer (PNBL). (B) In addition to a PCL, abnormal banded material (#) can be seen within the PNBL. (C) Abnormal banded material inclusions (#) within the PNBL in the absence of a PCL. (D) Abnormal fibrillary inclusions (#) and guttae-like formations (*) of the PNBL. ABL = anterior banded, PCL = posterior collagenous layer, PNBL = posterior nonbanded layer.
Important indicators for graft preparation and unfolding include donor age, the presence of diabetes mellitus, previous phacoemulsification of the donor, and storage medium during organ culture.\cite{7,9,10} The storage medium may also influence the detachment rate.\cite{17} Heinzelmann et al.\cite{18} discussed storage in dextran as a risk factor for ultrastructural anomalies that could lead to primary graft failure after DMEK, especially in precut tissues, but the study only included 11 eyes. We also found that dextran in prestripped tissue had a negative impact on graft survival because there was a higher rate of repeat keratoplasty after DMEK when the tissues were stored in culture medium with dextran.\cite{19} On the other hand, Yoeruek and Bayyoud reported only a moderate loss of endothelial cells in precut tissue and safe donor graft preparation with or without dextran.\cite{20,21} Parekh et al.\cite{22} concluded that dextran should be used in precut tissues to prevent the loss of endothelial cells.

We think that the presence of pigment granules in the interface may decrease adhesion between the donor graft and recipient stroma. Pseudoexfoliation or pigment dispersion syndrome, both of which are associated with pigment liberation from the iris pigment epithelium, may cause pigment accumulation in the interface. However, further investigations in a larger study population are required. Peri-operative YAG iridotomy is another potential source of pigment, so we now perform YAG iridotomy at the 6 o’clock position many weeks before DMEK rather than the day before surgery.\cite{23} This may help avoid pigment dispersion during DMEK surgery.

To summarize, major causes of graft failure include previously undetected endothelial dysfunction or disease in the donor, endothelial damage during surgery, or surgical mistakes, such as inverted transplantation and damage to iris tissue, that results in the accumulation of pigmented cells or pigment granules in the Descemet–stroma interface, resulting in poor graft adhesion.\cite{10,16,24}

After repeat surgery, postoperative visual acuity improved significantly in all of our patients, and central cornea thickness decreased significantly. We conclude that repeat penetrating keratoplasty and repeat DMEK can lead to satisfactory functional results after failed DMEK and new triple procedure.

This study had some limitations, in that it was a retrospective study with a small number of cases. Prospective studies are warranted, including a larger case series, especially to investigate the culture conditions (e.g. role of dextran) to strengthen the interpretation of our results. A better understanding of graft failure may help in the development of preventive methods in the future.

**Author contributions**

**Conceptualization:** Ursula Schlötzer-Schrehardt, Berthold Seitz.

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**Methodology:** Achim Langenbucher, Berthold Seitz.

**Project administration:** Berthold Seitz.

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**Supervision:** Ursula Schlötzer-Schrehardt, Timo Eppig, Tobias Hager, Berthold Seitz.

**Validation:** Achim Langenbucher, Berthold Seitz.
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