Descemet Membrane Endothelial Keratoplasty for Graft Failure After Descemet Stripping Endothelial Keratoplasty: Clinical Results and Histopathologic Findings

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IMPORTANCE The management of graft failure is increasingly relevant with the spread and growing acceptance of endothelial keratoplasty.

OBJECTIVES To investigate the functional and anatomical results of secondary Descemet membrane endothelial keratoplasty (DMEK) for graft failure after Descemet stripping endothelial keratoplasty (DSEK) and to histologically analyze the stroma-to-stroma interface with respect to clinical implications.

DESIGN, SETTING, AND PARTICIPANTS In a single-surgeon prospective comparative case series at the Department of Ophthalmology, Charité-University Medicine Berlin, Berlin, Germany, 8 eyes (3.8%) of 210 consecutively performed DMEK procedures underwent a secondary DMEK for graft failure after DSEK from March 1, 2012, through February 28, 2013. Those cases were compared with the eyes of a reference collective (n = 30) and matched-pairs group (n = 8) after primary DMEK for Fuchs endothelial dystrophy.

INTERVENTION Descemet membrane endothelial keratoplasty.

MAIN OUTCOMES AND MEASURES Postoperative best-corrected visual acuity (BCVA) and central corneal thickness at 1, 3, 6, and 12 months. Intraoperatively obtained DSEK graft lenticels were investigated immunohistochemically.

RESULTS Patients with graft failure after DSEK had a mean (SD) age of 79.4 (7.2) years (range, 70-90 years). Preoperatively, the mean (SD) BCVA was 1.13 (0.50) logMAR (20/250 Snellen equivalents), and the mean (SD) central corneal thickness measured 704 (161) μm. Twelve months postoperatively, the mean (SD) corneal thickness decreased to 524 (27) μm after secondary and 516 (27) μm after primary DMEK (p = .57). A mean (SD) BCVA of 0.38 (0.36) logMAR (20/50 Snellen equivalents) was achieved after secondary DMEK compared with 0.15 (0.15) logMAR (20/28 Snellen equivalents) after primary DMEK. Histologically, failed DSEK graft lenticels presented condensations of collagen layers. Fibronectin and cytokeratin were accumulated along the stroma-to-stroma interface; vimentin was found in loosened graft stroma.

CONCLUSIONS AND RELEVANCE These data suggest that DMEK might be considered a feasible choice in patients with graft failure after DSEK. However, the visual restitution might be impeded because of preceded depositions of matrix proteins within the corneal stroma and the stroma-to-stroma interface, which are associated with corneal fibrosis. Thereby, fibrotic processes might be avoided by performing a secondary DMEK in an early phase of graft failure.
Endothelial keratoplasty has substantially enhanced the treatment of endothelial corneal dystrophies. Currently, Descemet stripping endothelial keratoplasty (DSEK)\(^1\)\(^-\)\(^4\) and Descemet membrane endothelial keratoplasty (DMEK)\(^3\)\(^-\)\(^4\) are considered the procedures of choice. When these 2 techniques are compared, visual results after DMEK are superior to those after DSEK.\(^1\)\(^-\)\(^6\) Therefore, the stroma-to-stroma interface and graft thickness are currently being discussed as possible limiting parameters for a complete functional restitution after DSEK.\(^7\)\(^-\)\(^8\) In this regard, the functional recovery of patients with poor visual acuity after DSEK might be enhanced by a secondary DMEK.\(^9\) However, individuals benefited to varying degrees from a secondary DMEK procedure.\(^10\)

With the increasing acceptance and spread of endothelial keratoplasty, the management of graft failure due to endothelial cell loss inevitably becomes of increasing clinical relevance.\(^11\) Currently, 5-year graft survival rates after DSEK were reported to be similar to those after penetrating keratoplasty.\(^12\)\(^-\)\(^13\) Patients with preexisting glaucoma and postoperative donor graft detachment are especially at high risk for graft failure.\(^14\)\(^-\)\(^16\) Common causes of primary graft failure after DSEK are an atrophic corneal endothelium, a residual host Descemet membrane, or improper donor trephination.\(^17\)\(^-\)\(^19\) Although additional DSEK in patients with graft failure after DSEK has been proposed as an effective therapeutic option, best-corrected visual acuity (BCVA) remained low and reached a mean of 20/63 Snellen equivalents.\(^20\) Therefore, this study has been designed to investigate the functional and anatomical outcome of a secondary DMEK after graft failure after DSEK in the same eye. Furthermore, causes that may explain a reduced visual outcome were analyzed to give clinical implications.

**Methods**

**Patients**

A total of 210 DMEK procedures were performed from March 1, 2012, through February 28, 2013; 8 eyes (3.8%) from 8 patients underwent a DMEK secondary to graft failure after DSEK. For statistical comparisons, a reference collective of 30 consecutive eyes of 30 patients who underwent a primary DMEK for Fuchs endothelial dystrophy (FED) from March 1 through July 31, 2012, served as controls. In addition, a matched-pairs analysis with patients who underwent primary DMEK for FED during the observational period was performed. Pairs were matched for the closest similarity of age, preoperative BCVA, and preoperative central corneal thickness to avoid selection bias. Consequently, 45 eyes of 45 white patients treated by DMEK were included in this prospective observational study. A detailed ophthalmologic examination, including documentation of the medical and ocular history, determination of the BCVA, slitlamp examination, and anterior segment optical coherence tomography (AS-OCT; Spectralis, Heidelberg Engineering GmbH), was performed. Postoperative evaluations were performed 1, 3, 6, and 12 months after surgery. Thereby, the BCVA was determined with the present refraction, and the central corneal thickness was measured using AS-OCT. Written informed consent was approved by the ethics committee of the Charité-University Medicine Berlin and obtained from all patients.

**Donor Tissue Preparation and Surgical Technique**

All DMEK procedures were performed by one experienced surgeon (N.T.) at the same department as previously described.\(^3\) Donor endothelial cell–Descemet membrane (EDM) complexes were stained with 0.06% trypan blue (VisionBlue; D.O.R.C. Dutch Ophthalmic Research Center [International] B.V.) and prepared from the organ-cultured donor cornea immediately before transplantation. The EDM complex was stripped from the corneal button mounted endothelial side up on a cutting block (Barron Vacuum Punch; Katena Products Inc) so that an 8.5-mm-diameter flap could be obtained. In recipient eyes undergoing a secondary DMEK, the DSEK graft was carefully removed from the recipient posterior stroma. The trypan blue–stained donor EDM flap was injected into the anterior chamber by a custom-made glass injector. By gentle manipulation of the flap with air and fluid, the graft was oriented endothelial side down and spread out on the iris. Afterward, air was injected underneath the graft so that the anterior chamber was completely filled with air and patients remained in a face-up position. After 50 minutes, an air–liquid exchange with balanced salt solution was performed, leaving the anterior chamber filled two-thirds with air. Intraoperatively, harvested DSEK lenticels were placed in formaldehyde, 4%, at 4°C overnight for fixation.

**Histologic and Immunohistochemical Analysis**

Obtained DSEK lenticels were cut into 2 portions for microscopy and handled with care to avoid postoperative mechanical manipulation. After fixation with paraformaldehyde, 4%, one portion of each sample was used for flat-mount analyses after 4,6-diamidino-2-phenylindole (DAPI) staining for 5 minutes. The DSEK lenticels were mounted on glass slides, which were examined using light microscopy, and endothelial cell density was calculated within a representative area (mean size, 200 × 200 μm) using the National Institutes of Health image analysis software (ImageJ, version 1.44 for Macintosh; http://rsb.info.nih.gov/ij/), as previously described.\(^21\)\(^-\)\(^23\) The other portion was paraffin embedded and microtome cross-sectioned to 10-μm sections. To ensure perpendicular sections, the tissues were placed into prefilled paraffin molds before being fully embedded. Cross-sections of all biopsy specimens were stained with hematoxylin-eosin and prepared for detailed immunohistochemical analysis.

Therefore, embedded sections were incubated at 60°C (60 minutes), deparaffinized with XyloL (40 minutes) followed by isopropanol (30 minutes), and rehydrated through a descending alcohol series (95%, 90%, 70%, and 50% alcohol for 5 minutes each) to hydrogen peroxide, 0.3%, in aqua (5 minutes) and Tris-buffered saline (pH 7.6 for 5 minutes). Antigen retrieval was performed with trypsin, 0.1%, for 60 minutes. Tissue sections were then permeabilized with Triton, 0.1% (10 minutes), and blocked with bovine serum albumin, 5%, in Tris-buffered saline for 60 minutes. Primary antibodies diluted in bovine serum albumin, 0.8%, in Tris-buffered saline against
cytokeratin (diluted 1:20, sc-57004; Santa Cruz Biotechnology), fibronectin (diluted 1:20, F0916; Sigma-Aldrich), and vimentin (diluted 1:20, sc-32322; Santa Cruz Biotechnology) were incubated overnight in a humidity chamber at 4°C. Fluorescence detection was attained with fluorescein isothiocyanate–conjugated secondary antibodies (diluted 1:100, F8771; Sigma-Aldrich) and DAPI nuclear counterstaining. Mounted slides were examined using light microscopy (Axio Imager.M2; Carl Zeiss AG).

### Statistical Analyses
Clinical data of patients undergoing primary and secondary DMEK for graft failure after DSEK were statistically compared. Normally distributed variables with equal variances were compared using the $t$ test; variables with unequal variances were compared using the Welch test for independent samples. Nominal variables were compared using the $\chi^2$ test. All data were analyzed with SPSS statistical software, version 20.0 (IBM Corporation).

### Table. Clinical Characteristics of Patients Undergoing Primary DMEK for Fuchs’ Endothelial Dystrophy and Secondary DMEK After Graft Failure Following DSEK

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Primary DMEK (n = 30)</th>
<th>Matched-Pairs DMEK (n = 8)</th>
<th>Secondary DMEK After DSEK (n = 8)</th>
<th>P Value</th>
<th>Matched-Pairs Secondary vs Primary DMEK</th>
<th>Matched-Pairs Secondary vs Primary DMEK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>71.3 (8.8)</td>
<td>78.9 (5.7)</td>
<td>79.4 (7.2)</td>
<td>.02</td>
<td>.50</td>
<td>.50</td>
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<tr>
<td>Range (95% CI), y</td>
<td>50-91 (68.0-74.6)</td>
<td>74-89 (74.1-83.7)</td>
<td>70-90 (73.3-85.5)</td>
<td></td>
<td></td>
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<td>Female, No. (%)</td>
<td>21 (70.0)</td>
<td>6 (75.0)</td>
<td>4 (50.0)</td>
<td>.29</td>
<td>.30</td>
<td>.30</td>
</tr>
<tr>
<td>Time from DSEK to DMEK, mo</td>
<td>21.4 (17.8)</td>
<td>4-58 (6.3-36.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Endothelial cell density, mean (SD), cells/mm²</td>
<td>1524 (446)</td>
<td>908 (143)</td>
<td></td>
<td>.01</td>
<td></td>
<td></td>
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<tr>
<td>BCVA, mean (SD), logMAR/Snellen equivalents</td>
<td>0.71 (0.39) [0.56-0.86]</td>
<td>1.08 (0.45) [0.70-1.45]</td>
<td>1.13 (0.50) [0.61-1.65]</td>
<td>.03</td>
<td>.82</td>
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<td>Central corneal thickness, mean (SD), μm</td>
<td>701 (87) [663-739]</td>
<td>756 (86) [649-863]</td>
<td>704 (161) [570-839]</td>
<td>.96</td>
<td>.53</td>
<td></td>
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<tr>
<td>Preoperative</td>
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<td></td>
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<tr>
<td>Postoperative, mo</td>
<td>0.34 (0.20) [0.25-0.42]</td>
<td>0.26 (0.15) [0.13-0.39]</td>
<td>0.57 (0.19) [0.37-0.76]</td>
<td>.02</td>
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<td>3</td>
<td>0.23 (0.25) [0.14-0.35]</td>
<td>0.26 (0.13) [0.15-0.37]</td>
<td>0.53 (0.30) [0.21-0.84]</td>
<td>.02</td>
<td>.04</td>
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<td>6</td>
<td>0.20 (0.15) [0.14-0.26]</td>
<td>0.18 (0.14) [0.14-0.30]</td>
<td>0.40 (0.19) [0.17-0.63]</td>
<td>.01</td>
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<td>12</td>
<td>0.15 (0.15) [0.09-0.21]</td>
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Abbreviations: BCVA, best-corrected visual acuity; DMEK, Descemet membrane endothelial keratoplasty; DSEK, Descemet stripping endothelial keratoplasty; NM, not measurable.

* Because of limited visual potential due to relevant maculopathies, 2 eyes were excluded from the analysis.
Results

Clinical Data
Eight eyes underwent DMEK secondary to graft failure after initial DSEK, and 37 eyes underwent primary DMEK for FED. All procedures were uneventful, and 17 cases (37.8%) of graft detachment were successfully treated with a secondary air injection into the anterior chamber (rebubbling). As indicated in the Table, the mean (SD) patient age after DSEK graft failure was 79.4 (7.2) years (range, 70-90 years) compared with 71.3 (8.8) years (range, 50-91 years) in patients with primary DMEK ($P = .02$). The mean (SD) time between initial DSEK and secondary DMEK was 21.4 (17.8) months (range, 4-58 months). The underlying pathologic mechanism was FED in 7 cases (87.5%) and polymorphous endothelial dystrophy in 1 case (12.5%). Preoperatively, 2 of 8 DSEK lenticels were partially separated from the posterior corneal stroma. At the time of secondary intervention, the mean (SD) DSEK graft thickness measured 195 (99) μm, whereas the mean (SD) total corneal thickness was 900 (209) μm.

Preoperative characteristics and postoperative courses at 1, 3, 6, and 12 months are summarized in Figure 1 and the Table. Comparing the BCVA between patients undergoing secondary DMEK for graft failure after DSEK and patients with primary DMEK for FED, the mean (SD) preoperative BCVA was 1.13 (0.50) logMAR (20/250 Snellen equivalents) and 0.71 (0.39) logMAR (20/100 Snellen equivalents), which improved 12 months postoperatively to 0.38 (0.36) logMAR (20/50 Snellen equivalents) and 0.15 (0.15) logMAR (20/28 Snellen equivalents), respectively. Thereby, the BCVAs were different between the 2 groups preoperatively and postoperatively at 1, 3, 6, and 12 months ($P < .05$). Because of differences in the baseline characteristics, especially preoperative BCVA and age, a matched-pairs analysis was performed to compare postoperative outcomes of patients after secondary DMEK for graft failure and primary DMEK for FED. The postoperative BCVAs at 1, 3, and 6 months were different between the studied groups ($P = .006$, $P = .04$, and $P = .04$, respectively). However, no difference was found 12 months postoperatively ($P = .11$). Because of limited visual potential due to age-related macular degeneration and relevant macular pucker, 2 patients were excluded from this analysis. Irregular astigmatism as a vision-limiting parameter$^{24}$ was not observed within the study groups.

Analyzing the postoperative course of the mean central corneal thicknesses and its reduction from the baseline between studied groups, no relevant differences were observed (Figure 2 and eTable in the Supplement). The mean (SD) corneal thickness decreased from 704 (161) μm and 701 (87) μm preoperatively to 524 (27) μm and 516 (27) μm 12 months postoperatively in patients after secondary and primary DMEK, respectively. Correspondingly, the corneal thickness decreased from 756 (86) μm preoperatively to 535 (47) μm after 12 months in the matched-pairs primary DMEK group.

Morphologic Data
The endothelial cell density was reduced in patients with graft failure after DSEK compared with patients with FED, measuring 908 (143) cells/mm$^2$ and 1524 (446) cells/mm$^2$, respectively ($P = .01$). In this context, DAPI nuclear staining contrasts vital and avital endothelial cells so that the effective functional endothelial cell density might be lower. Histologic investigations of harvested DSEK graft lenticels revealed a condensation of stromal collagen layer along the stroma-stroma interface. Immunohistochemically, accumulations of vimentin were observed within the corneal stroma, whereas...
fibronectin and cytokeratin were found on the stromal cleavage plane (Figure 3). Those condensations and depositions of matrix proteins within the corneal stroma and the stroma-to-stroma interface were stable across all investigated DSEK lenticels and are likely to correspond to preoperatively observed hyperreflective layers in AS-OCT A-scans. Whereas the host stroma cannot be investigated histologically, a retained hyperreflective layer was observed in postoperative AS-OCT A-scans between the donor EDM and the host’s corneal stroma, which was not found in cases of primary DMEK (eFigure 1 and eFigure 2 in the Supplement).

**Discussion**

The aim of this study was to investigate functional and anatomical results after secondary DMEK for graft failure after DSEK compared with primary DMEK for FED. Although previous publications revealed a BCVA of 0.5 logMAR (20/63 Snellen equivalents) after secondary DSEK, in our patients a slightly better mean BCVA of 0.38 logMAR (20/50 Snellen equivalents) was obtained after secondary DMEK. In contrast, the mean BCVA after primary DMEK was 0.15 logMAR (20/28 Snellen equivalents).
len equivalents), which is in accordance with previously published results.\textsuperscript{25-28} Compared with primary DMEK, the postoperative BCVAs at 1, 3, 6, and 12 months were reduced after secondary DMEK for graft failure. However, the BCVA was not different at 12 months in the matched-pairs analysis because of the small sample size (n = 6). Because the anatomical outcome with respect to the reduction of the corneal edema was comparable between primary and secondary DMEK, we assume alterations of the corneal configuration to be causal for the impeded visual restitution.

Therefore, explanted DSEK lenticels were investigated histologically and immunohistochemically. In light microscopy, considerable condensations of stromal collagen layers were observed along the stroma-to-stoma plane. Immunohistochemically, accumulations of cytokeratin and fibronectin were found on the stroma-to-stoma interface, whereas deposits of vimentin were observed within loosened corneal stroma. Corresponding to our histologic observations, hyperreflective deposits were depicted within the stroma-to-graft interface in preoperative and postoperative AS-OCT images. Postoperatively, this hyperreflective layer remained adjacent to the attached donor EDM graft (eFigure 1 in the Supplement). On this account, we assume that histologically observed protein deposits, which were concentrated within the corneal stroma and along the stroma-to-graft interface, might cause this hyperreflectivity.

Depositions of vimentin and fibronectin in graft failure are especially related to corneal fibrosis.\textsuperscript{29,30} Although vimentin is a nonspecific ubiquitous protein found in structural elements, fibronectin is a provisional matrix protein that is of particular importance in various stages of wound healing. Therefore, those proteins act as scaffolders to support the organization of more specific and durable proteins, such as collagen type I and glycosaminoglycans. Because epithelial ingrowth was reported as a cause for graft failure, anticytokeratin staining was performed.\textsuperscript{31} Hereby, cytokeratin is associated with intermediate filaments in cells, primarily epithelial cells. Even though we observed subtle accumulations of cytokeratin in the interface plane, no evident epithelial cell ingrowth was observed.

Previously, Ham et al\textsuperscript{9} reported that functional results after secondary DMEK in patients with poor visual acuity after DSEK who did not experience endothelial failure were comparable to primary DMEK. However, our functional results after DMEK for graft failure were considerably reduced compared with primary DMEK. We attribute those observations to fibrotic changes in the host cornea due to persistent corneal edema after graft failure. Corneal fibrosis induces structural reorganizations and depositions of matrix proteins. Likewise, Arnalich-Montiel et al\textsuperscript{32} observed increased corneal haze and corneal light scattering within the full-thickness central cornea after secondary DSEK after failed DMEK compared with primary DSEK. Definite causes for poor visual outcome after DMEK and DSEK are still under debate. On this account, corneal fibrosis due to graft failure can occur after both DMEK and DSEK, which might reduce vision by the following mechanisms: interface reflectivity, interfacial remnants, graft thickness irregularities, donor stromal contraction, anterior surface irregularities, and elevated anterior and posterior corneal higher-order aberrations.\textsuperscript{10,24,33-37}

We are aware that our study has several limitations. Although the number of secondary DMEKs after graft failure is currently small, the matter is of increasing relevance with further spread of endothelial keratoplasty. For this reason, the total numbers evaluated are relatively small and the CIs are fairly large; likewise, comparisons could not control for unknown confounders in the absence of randomization, and observations on morphologic alterations are primarily qualitative. Nevertheless, our data suggest that DMEK might be considered a feasible choice in patients with graft failure after DSEK. However, the visual restitution might be impeded because of preceding depositions of matrix proteins within the corneal stroma and the stroma-to-stroma interface that are associated with corneal fibrosis. Fibrotic processes might be anticipated by performing a secondary DMEK in an early phase of graft failure.

Conclusions

We believe that our findings expand the current understanding of the stroma-to-graft interface and the corneal configuration in endothelial keratoplasty, especially in cases of graft failure, and the suggestions may be used to refine current techniques. Thereby, our conclusions are in accordance with investigations by Baydoun et al\textsuperscript{38} and Price and Price,\textsuperscript{36} suggesting an additional DMEK in the early postoperative phase after complicated primary DMEK to avoid secondary stromal changes induced by persistent corneal edema. Furthermore, therapeutic strategies to inhibit corneal fibrosis are needed and therefore should be the focus of future research.

Disclosure of Potential Conflicts of Interest and none were reported.

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Original Investigation Research

Additional Contributions: Mrs. Karin Oberlaender provided excellent technical assistance.

REFERENCES