Histologic Analysis of Descemet Stripping in Posterior Lamellar Keratoplasty

Ludwig M. Heindl, MD; Carmen Hofmann-Rummelt, MTA; Ursula Schlötzer-Schrehardt, PhD; Friedrich E. Kruse, MD; Claus Cursiefen, MD

Objective: To investigate how precise Descemet stripping works in posterior lamellar keratoplasty (Descemet stripping automated endothelial keratoplasty [DSAEK]) for the treatment of corneal endothelial disorders.

Methods: In a prospective, single-center, nonrandomized consecutive series, 20 Descemet membrane specimens obtained after Descemet stripping in DSAEK using a Price hook were examined using histologic analysis and transmission electron microscopy for the presence of residual stroma, thickness of the Descemet membrane, endothelial cell count, and presence of guttae or a posterior collagenous layer. Pathologic findings were correlated with the underlying clinical disease.

Results: Light and electron microscopy revealed no evidence of adherent rests of corneal stroma in all 20 specimens after Descemet stripping. The mean (SD) total thickness of the Descemet membrane was 21.5 (4.5) µm in peripheral localization and 17.6 (3.8) µm in central localization. The anterior banded layer measured a mean (SD) of 3.0 (0.8) µm thick; the posterior nonbanded layer, 16.7 (5.2) µm thick. The mean (SD) endothelial cell count was 1.7 (1.4) cells per high-power field. Guttae were seen in 15 specimens (75%), and a posterior collagenous layer was found in 3 (15%).

Conclusion: Descemet stripping in DSAEK using the Price hook achieves complete and specific removal of the Descemet membrane without adherent stroma in different underlying endothelial pathologic abnormalities.


POSTERIOR LAMELLAR KERATOPLASTY is a new and exciting surgical alternative1-13 to standard full-thickness penetrating keratoplasty for the treatment of corneal endothelial disorders. Approximately 40% of all penetrating keratoplasties are performed for diseases of the corneal endothelium.14 Selective replacement of the dysfunctional posterior portion of the cornea offers distinct advantages compared with penetrating keratoplasty, including faster visual rehabilitation,3-13 improved surface topography with reduction of postsurgical astigmatism,3-13 reduced risk of expulsive hemorrhage in a “closed-system” procedure,3-13 protection of corneal innervation for the prevention of neurotrophic keratopathy,5,6,11 reduced immunologic rejection against the grafted endothelium6,11 due to the reduced amount of foreign surface antigens on the recipient cornea, and the presence of the recipient cornea’s own anti-inflammatory and antiangiogenic corneal epithelium.15

In Descemet stripping automated endothelial keratoplasty (DSAEK), a donor posterior lamellar disc consisting of the posterior stroma, Descemet membrane, and endothelium is transplanted after dissecting the donor tissue using a microkera-

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METHODS

Light and electron microscopic analyses were performed on 20 Descemet membrane specimens obtained after descemetorhexis in a consecutive series of the first 19 patients (20 eyes) who had undergone DSAEK for Fuchs endothelial dystrophy, pseudophakic bullous keratopathy, and pseudoexfoliation syndrome keratopathy between July 1, 2006, and April 15, 2007, at the Department of Ophthalmology, University Eye Hospital, University Erlangen-Nürnberg, Erlangen-Nürnberg, Germany.
SURGICAL TECHNIQUE

According to Melles et al and Price and Price, donor tissue was prepared before surgery by placing a donor corneoscleral shell onto an artificial anterior chamber (ALTK System; Moria, Doylestown, Pennsylvania). An anterior lamellar cap was removed from the posterior portion using a microkeratome (CBm; Moria) with a 350-µm head. The remaining posterior portion of the donor cornea was transferred endothelially side up to a Hanna punch block (Moria) and trephined. Using general anesthesia, the recipient corneal surface was marked with a 9-mm circular marker (Moria). An 8-mm nasal scleral tunnel incision was made in addition to 2 paracenteses. Via one paracentesis, an infusion trocar was inserted. A DSAEK Price hook (Moria) was used to score the Descemet membrane along the perimeter of the epithelial reference mark and to strip off the Descemet membrane with attached dysfunctional endothelium centripetally in the scored area (Figure 1). After full Descemet stripping, the Descemet membrane with endothelium was removed from the anterior chamber using a forceps and was sent for histopathologic evaluation. There was no use of trypan blue or air injection into the anterior chamber before Descemet membrane removal. The donor posterior lamellar disc was placed endothelially side down on a viscoelastic (Acri.Hylon; Acri.Tec, Hennigsdorf, Germany) covered glideboard, gently grasped using a retinal forceps, and inserted through the 8-mm nasal scleral tunnel incision into the eye. Once the donor tissue was well centered, an air bubble was injected into the anterior chamber. After removal of the anterior chamber infusion trocar, both paracenteses and scleral tunnel incisions were sutured as a precaution. A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously). A preoperative YAG laser iridotomy was performed (or described previously).

The anterior chamber maintainer is seen at the 1-o'clock position.

HISTOPATHOLOGIC ANALYSIS

All Descemet membrane specimens were fixed in 4% buffered formaldehyde, dehydrated, and embedded in paraffin. Sections cut at 4 µm were stained with hematoxylin-eosin and periodic acid–Schiff and analyzed using a microscope (Axioskop; Zeiss, Oberkochen, Germany) following digital documentation. Main outcome measures included the presence, localization, and thickness of residual stroma, the mean peripheral and central thickness of the Descemet membrane, the presence of guttae, the mean endothelial cell count, and the presence of a posterior collagenous layer. Mean thickness of the Descemet membrane and mean endothelial cell count were determined in 5 randomly selected high-power fields (HPFs) per slide (original magnification ×400).

ELECTRON MICROSCOPY

For transmission electron microscopy, specimens from all 20 recipient eyes were postfixed in 2% buffered osmium tetroxide, dehydrated in graded alcohol concentrations, and embedded in epoxy resin according to standard protocols, as described previously. Semi thin (1-µm) sections for orientation were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate–lead citrate and were examined using a transmission electron microscope (EM 906E; Zeiss). Electron microscopy was used for detection and measurement of adherent rests of corneal stroma. In addition to the total thickness of the Descemet membrane specimen, the anterior banded layer, posterior nonbanded layer, and if present, posterior collagenous layer were measured for thickness in 5 randomly selected corneal segments per slide at a magnification of ×2000.

RESULTS

The 20 Descemet membrane specimens in this prospective, nonrandomized series were derived from 19 consecutive patients (10 women and 9 men) with a mean (SD) age at surgery of 71 (8) years (range, 53-82 years). Bilateral DSAEK was performed in 1 patient within 4 months. Seven of the surgical eyes (35%) were right eyes. The clinical diagnoses included Fuchs endothelial dystrophy in 14 eyes (70%), pseudophakic bullous keratopathy in 5 (25%), and pseudoexfoliation syndrome keratopathy in 1 (5%). Light microscopy revealed no evidence of adherent rests of corneal stroma in all 20 specimens after Descemet stripping (Figure 2).
ness of the Descemet membrane was 21.5 (4.5) µm (range, 14.0-30.5 µm) in peripheral localization and 17.6 (3.8) µm (range, 11.0-25.0 µm) in central localization. Central guttae were found in 15 Descemet membranes (75%). The mean (SD) endothelial cell count was 1.7 (1.2) cells/HPF (range, 0-5.0 cells/HPF). A collagenous layer posterior to the Descemet membrane was seen in 3 specimens (15%) (Table 1).

By transmission electron microscopy, no stromal adherences were detected in all 20 Descemet stripping specimens (eFigure; available at: http://www.archophthalmol.com). Mean (SD) total specimen thickness was 20.3 (5.2) µm (range, 12.4-34.4 µm). The anterior banded layer measured a mean (SD) of 3.0 (0.8) µm thick (range, 2.2-5.6 µm thick); the posterior abnormally banded layer, 16.7 (5.2) µm thick (range, 5.7-29.2 µm thick). The mean (SD) posterior collagenous layer had a thickness of 6.1 (3.7) µm (range, 3.9-10.4 µm) (Table 2).

The present study analyzes, for the first time to our knowledge, the accuracy of manual Descemet stripping using a Price hook in DSAEK by means of light and electron microscopic techniques. In this prospective, nonrandomized consecutive series, complete and specific removal of the Descemet membrane with its endothelial cell layer without adherent stroma was achieved in 100% of the 20 recipient eyes.

These findings coincide with the results of the first description of the descemetorhexis technique for preparation of a recipient stromal bed in posterior lamellar keratoplasty by Melles et al1 in 2004. After visualization of the Descemet membrane using a reflective glide placed on the iris, the Descemet membrane was carefully stripped off the posterior stroma by loosening the membrane at the 6-o’clock position and pulling it toward the incision at the 12-o’clock position using a custom-made scraper.1 Microscopic examination showed isolated Descemet membranes without stromal tissue elements in all 3 patients with Fuchs endothelial dystrophy.1

Because we used a different surgical technique (without air instillation in the anterior chamber and no use of trypan blue), a different surgical manipulation of the Descemet membrane (Price hook), and a larger series of patients with different underlying diseases (Fuchs endothelial dystrophy, pseudophakic bullous keratopathy, and pseudoexfoliation syndrome keratopathy), this study supports the concept that in routine DSAEK, the Descemet membrane is removed specifically without adherent stromal tissues. This has 2 important clinical implications. First, it supports the concept that the superior visual results achieved in patients having undergone DSAEK compared with other lamellar keratoplasty techniques is due to reduced interface problems. In posterior lamellar keratoplasty, complete removal of the Descemet membrane without stromal damage may minimize the risk of interface haze.1 Second, our observation that in a larger series of patients with different endothelial pathologic ab-

### Table 1. Clinicohistopathologic Correlation of 20 Descemet Membrane Specimens After Descemet Stripping in DSAEK

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fuchs Endothelial Dystrophy (n = 14)</th>
<th>Pseudophakic Bullous Keratopathy (n = 5)</th>
<th>Pseudoexfoliation Syndrome Keratopathy (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral thickness of Descemet membrane, mean (SD), µm</td>
<td>21.9 (4.0)</td>
<td>21.9 (5.4)</td>
<td>14.0</td>
</tr>
<tr>
<td>Central thickness of Descemet membrane, mean (SD), µm</td>
<td>18.0 (3.4)</td>
<td>17.8 (4.4)</td>
<td>11.0</td>
</tr>
<tr>
<td>Presence of guttae, No. (%)</td>
<td>14 (100)</td>
<td>1 (20)</td>
<td>0</td>
</tr>
<tr>
<td>Endothelial cell count, mean (SD), cells/HPF</td>
<td>1.7 (1.2)</td>
<td>2.2 (1.7)</td>
<td>0</td>
</tr>
<tr>
<td>Presence of posterior collagenous layer, No. (%)</td>
<td>2 (14)</td>
<td>0</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>

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<tr>
<th>Characteristic</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Peripheral thickness of Descemet membrane, mean (SD), µm</td>
<td>20.6 (4.1)</td>
<td>21.0 (7.8)</td>
<td>12.4</td>
</tr>
<tr>
<td>Central thickness of Descemet membrane, mean (SD), µm</td>
<td>2.8 (0.5)</td>
<td>3.5 (1.3)</td>
<td>2.9</td>
</tr>
<tr>
<td>Thickness of posterior nonbanded layer, mean (SD), µm</td>
<td>17.1 (3.9)</td>
<td>17.9 (6.8)</td>
<td>5.7</td>
</tr>
<tr>
<td>Presence of posterior collagenous layer, No. (%)</td>
<td>2 (14)</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Thickness of posterior collagenous layer, mean (SD), µm</td>
<td>7.2 (4.5)</td>
<td>NA</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Abbreviations: DSAEK, Descemet stripping automated endothelial keratoplasty; HPF, high-power field (original magnification ×400).

a There were no stromal adherences among any of the Descemet membrane specimens.
b No standard deviations are given because there was only 1 case.

### Table 2. Ultrastructural Findings in 20 Descemet Membrane Specimens After Descemet Stripping in DSAEK

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fuchs Endothelial Dystrophy (n = 14)</th>
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<th>Pseudoexfoliation Syndrome Keratopathy (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total specimen thickness, mean (SD), µm</td>
<td>20.3 (3.2)</td>
<td>21.0 (7.8)</td>
<td>12.4</td>
</tr>
<tr>
<td>Thickness of anterior banded layer, mean (SD), µm</td>
<td>2.8 (0.5)</td>
<td>3.5 (1.3)</td>
<td>2.9</td>
</tr>
<tr>
<td>Thickness of posterior nonbanded layer, mean (SD), µm</td>
<td>17.1 (3.9)</td>
<td>17.9 (6.8)</td>
<td>5.7</td>
</tr>
<tr>
<td>Presence of posterior collagenous layer, No. (%)</td>
<td>2 (14)</td>
<td>0</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Thickness of posterior collagenous layer, mean (SD), µm</td>
<td>7.2 (4.5)</td>
<td>NA</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Abbreviations: DSAEK, Descemet stripping automated endothelial keratoplasty; NA, not available.
a There were no stromal adherences among any of the Descemet membrane specimens.
b No standard deviations are given because there was only 1 case.
normalities, specific and isolated removal of the Descemet membrane is possible also supports the concept that it is possible to solely transplant the Descemet membrane in patients with endothelial pathologic abnormalities. Melles et al\textsuperscript{13} recently showed the general feasibility of that concept (ie, Descemet membrane endothelial keratoplasty).

In conclusion, Descemet stripping using a Price hook in routine DSAEK is a precise, controlled technique to achieve complete and specific removal of the Descemet membrane without adherent stroma.

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Additional Information: The eFigure is available at http://www.archophthalmol.com.

REFERENCES