Purpose: To investigate the effect of intensified postoperative topical steroid therapy after Descemet membrane endothelial keratoplasty combined with cataract surgery (Triple-DMEK) on postoperative endothelial cell density (ECD).

Methods: This comparative clinical study with historical controls was conducted at a tertiary hospital, specialized in corneal surgery. Patients undergoing DMEK or Triple-DMEK are included prospectively in the Cologne DMEK Database. Until April 2014, first week’s postoperative standard therapy for lamellar keratoplasties was prednisolone acetate eye drops 1% applied 5× daily. After April 2014, first week’s postoperative standard therapy changed to prednisolone acetate eye drops 1% applied hourly. We compared 75 consecutive eyes before (group 1) with 75 consecutive eyes after (group 2). Patients received ECD analysis 3 and 6 months after surgery.

Results: ECD of grafts in group 1 before transplantation and 3 months and 6 months after surgery was 2697 ± 218, 1765 ± 349, and 1703 ± 432 cells/mm², respectively. ECD of grafts in group 2 was 2696 ± 267, 1737 ± 450, and 1694 ± 482 cells/mm², respectively. Over 3 and 6 months, ECD in group 1 decreased by 35% ± 13% and 38% ± 18%, respectively. In group 2, ECD decreased by 36% ± 16% and 38% ± 16%, respectively. Neither absolute numbers nor decrease in ECD differed significantly between groups 1 and 2 at any time point (all P > 0.60).

Conclusions: Intensified early postoperative topical steroid therapy during the first postoperative week does not stabilize ECD, nor does it have a toxic effect on endothelial cells during a follow-up of 6 months.

Key Words: cornea, lamellar keratoplasty, endothelial cell density, steroid therapy

Descemet membrane endothelial keratoplasty (DMEK) has become the leading surgery for endothelial dysfunction worldwide besides Descemet stripping automated endothelial keratoplasty (DSEAK). In cases of accompanying cataracts, a triple procedure combined with cataract surgery is a safe approach (Triple-DMEK). The postoperative endothelial cell density (ECD) is crucial for the survival of the graft and subsequent long-term success of the surgery. Trauma during preparation and implantation of DMEK lamellae accounts for a loss of around 30% of endothelial cells during the first postoperative year. This loss occurs mainly within the first 3 months after surgery. Afterward, cell decay seems to decelerate but does not come to a complete halt. Acute immunoreactions after lamellar keratoplasty are scarce. However, an ongoing subclinical immunoreaction could be responsible for a steady loss of endothelial cells. The effect of topical steroids on ECD is discussed controversially. After penetrating keratoplasty, patients seem to benefit from prolonged topical steroid therapy. After cataract surgery, some results suggest a benefit of stronger topical steroids, whereas newer observations after DMEK surgery do not. In vitro experiments, steroids had a stabilizing effect on endothelial cells.

In our clinic, we changed the postoperative therapy regimen from a topical steroid application 5 times/d (low dose) to an hourly regimen (high dose) for the first postoperative week. As recently published, this procedure significantly reduces postoperative macular edema after Triple-DMEK and DMEK. In this study, we investigated the potential effect of this intensified early postoperative topical steroid application on the trauma-related endothelial cell loss, as well as on further cell loss, possibly due to subclinical immunoreaction.
MATERIALS AND METHODS
This single-center comparative clinical study with historical controls was conducted at the Department of Ophthalmology, University of Cologne, Germany, a tertiary hospital specialized for corneal surgery (>80% lamellar transplant surgeries, approximately 60% DMEK, and 40% Triple-DMEK). The protocol followed the tenets of the Helsinki protocol. All patients were included prospectively in the Cologne DMEK Database: Long-Term Safety and Outcome. Standard intervention and follow-up procedure in the Cologne DMEK Database are outlined in paragraphs 2 and 3. The ethics committee of the University of Cologne, Germany, approved collection and analysis of data within the Cologne DMEK Database (file number 14-373). All patients gave informed consent for storage and analysis of their data. The inclusion criterion was the need for Triple-DMEK surgery. A single surgeon (C.C.) conducted all surgeries including all preparation steps according to a standardized technique. Therefore, surgeon effects are excluded. Surgeries were performed between December 2012 and January 2015.

We performed surgery, as described before. In short, under a balanced salt solution, we obtained a standardized 8-mm graft in a stepwise manner with the donor cornea fixed to a Hanna punch block (Moria, Doylestown, PA). The endothelium–Descemet membrane graft was transferred into a culture medium (Dulbecco’s modified Eagle medium; Biochrom, Berlin, Germany) for immediate transplantation. We performed all preparation steps directly before transplantation surgery. Before grafting Descemet membrane into the recipient, we performed cataract surgery according to the following specifications. The lens was extracted using the phaco chop technique after clear corneal tunnel incision and capsulorhexis. A preloaded one-piece acrylic lens was implanted into the capsular bag under viscoelastic substance cover after polishing of the posterior lens capsule. All lenses were implanted into the capsular bag. Viscoelastics were removed by irrigation and aspiration. The corneal tunnels did not require suturing in any case. The central 9 mm of Descemet membrane in the host cornea was peeled under complete air filling of the anterior chamber. The donor graft was stained with trypan blue solution 0.06% (VisionBlue; Dutch Ophthalmic Research Corp, Rotterdam, the Netherlands) and injected into the fluid-filled anterior chamber with a conventional lens injector cartridge, endothelial side outward. The graft was rotated 90 degrees by blunt strikes to the corneal surface, unfolded by a sterile air-bubble injection on top of the graft and then pressed against the corneal stroma by complete filling of the anterior chamber with sterile air. If necessary, Descemet folds were removed by a LASIK roller (BD Visitec, Abingdon, United Kingdom). The anterior chamber was filled with air for up to 90% of the anterior chamber volume. Patients had to maintain a postoperative supine position for 24 hours. The air dissolved spontaneously during approximately 5 days postoperatively.

On the day before surgery (approximately 16 hours preoperatively), all 150 patients received a YAG-laser iridotomy in the 6 o’clock position in miosis (1 drop pilocarpine 2%), which was enlarged intraoperatively by a 20-gauge cutter. From our clinical experience, this procedure results in less pronounced iris bleeding than iridectomy with the 20-gauge cutter alone. Preoperatively, as well as at every follow-up visit, Snellen best-corrected visual acuity (in the following given as logMAR), ECD analysis (EM 3000; Tomey, Nagaya, Japan), macular spectral domain optical coherence tomography (Spectralis HRA+OCT; Heidelberg Engineering GmbH, Dossenheim, Germany), slit-lamp OCT (Heidelberg Engineering GmbH), and Scheimpflug corneal topography as well as thickness analysis (PentaCam; Oculus, Wetzlar, Germany) were performed. We performed the ECD analysis, as described before. An experienced ophthalmic technician manually aligned the center of the pupil, followed by automated refined alignment. A maximum of 15 endothelial images were obtained, from which a high-quality image was selected. The manufacturers’ software identified and counted the endothelial cells. All images were controlled by an experienced ophthalmologist (R.H.) and manually corrected, where necessary. Follow-up visits were performed at 3 months and 6 months postoperatively. We performed additional visits in any case of a questionable postoperative result according to the patients’ ophthalmologist. In 2 cases, we contacted the patients’ ophthalmologist, who performed the follow-up visits including spectral domain optical coherence tomography.

Before the first of April 2014, all patients receiving lamellar keratoplasty (DMEK/DSAEK and the respective triple procedures) were treated with topical steroids (prednisolone acetate 1%) 5 times daily for the first postoperative month. Steroids were then tapered 1 drop per month (the last drop to be carried on for at least 1 year). Based on published results and on our own clinical experience, we changed our routine therapy regimen to hourly topical steroids for the first postoperative week, starting from the first of April 2014. These were applied until 11-o’clock in the evening. One week later, topical steroids were reduced to 5 times daily for the rest of the first month and thereafter again tapered 1 drop per month (the last drop to be carried on for at least 1 year, i.e., the old scheme used before). All other surgical and medical treatments were unchanged. We analyzed the last 75 consecutive eyes of patients before and the first consecutive 75 eyes of patients after the change of the therapy regimen. No patient was excluded from the analysis.

Statistical analysis was performed using commercially available software (GraphPad-Prism, La Jolla, CA). Significance levels were calculated using the Fisher exact test to compare frequencies of possible confounding factors, as well as the Mann–Whitney test to compare the mean ECD, age, best-corrected visual acuity, and central corneal thickness. Significance levels of $P < 0.05$ were deemed significant.

RESULTS
Baseline Characteristics
We analyzed 150 eyes of 131 patients (54 men and 77 women). We included both eyes of 19 patients (10 men and 9 women). We compared the last 75 consecutive eyes of patients before the change of the therapy regimen (group 1: low-dose topical steroids) with the first 75 consecutive eyes of patients after the change of the therapy regimen (group 2:...
high-dose topical steroids). Of the 19 patients with both eyes analyzed, 17 patients had one eye in group 1 and one eye in group 2. Two patients had both their eyes in group 1. Possible confounding factors influencing corneal integrity were comparable in both groups (Table 1). Corneal disorders leading to a Triple-DMEK procedure were Fuchs endothelial dystrophy in 147 cases and keratopathy due to pseudoexfoliation in 3 cases.

### Postoperative Characteristics

In 50 cases (67%) in group 1 and 39 cases (52%) in group 2, rebubbling was necessary. None of the patients needed repositioning of the membrane. Two pairs of corneas in group 1 were from one donor each. In 3 cases of group 1 and 2 cases of group 2, partial removal of the anterior-chamber air fill became necessary because of insufficient iridectomy and angle closure mechanism with pronounced intraocular pressure (IOP) elevation (>40 mm Hg).\(^{19}\) We observed no dislocation of the intraocular lens into the anterior chamber. Two patients in group 2 suffered from elevated IOP (>25 mm Hg) 6 weeks and 5 months after surgery, which were putatively related to the use of topical steroid eye drops. One of the patients had a known history of open-angle glaucoma. Both cases were in treatment group 2. In both cases, topical steroids were changed to rimexolone 1% (Vexol; Alcon, Freiburg, Germany). IOP was controlled under temporary topical antiglaucomatous agents and normalized within 2 weeks after adaption of therapy in both cases.

### Development of Endothelial Cell Density

The mean preoperative ECD of the transplanted grafts in group 1 was 2697 ± 218 cells/mm\(^2\) (2280–3300). Over the first 3 months, it decreased by 35% ± 13% (6–72) to a mean of 1765 ± 349 cells/mm\(^2\) (728–2554). Decrease of cell density from the transplanted grafts 6 months after surgery was 38% ± 18% to a mean of 1703 ± 432 (700–2569) cells/mm\(^2\) (Fig. 1).

The mean ECD of grafts in group 2 was 2269 ± 267 cells/mm\(^2\) (2263–3800). Over the first 3 months, it decreased by 36% ± 16% (9–72) to a mean of 1737 ± 450 cells/mm\(^2\) (777–2898). Decrease of cell density from the transplanted grafts 6 months after surgery was 38% ± 16% (8–74) to a mean of 1694 ± 482 (684–3046) cells/mm\(^2\) (Fig. 1).

### TABLE 1. Possible Confounding Factors Influencing the Endothelial Cell Density After Posterior Lamellar Keratoplasty Combined With Cataract Surgery (Triple-DMEK) at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 75) (“Low-Dose”: Topical Steroids 5×/d)</th>
<th>Group 2 (n = 75) (“High-Dose”: Topical Steroids Hourly)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>70 ± 8 (51–89)</td>
<td>68 ± 10 (24–85)</td>
<td>0.376</td>
</tr>
<tr>
<td>Best-corrected visual acuity preoperatively</td>
<td>0.51 ± 0.22 logMAR (20/60 Snellen) (range 1.30–0.22)</td>
<td>0.51 ± 0.38 logMAR (20/60 Snellen) (range 2.00–0.22)</td>
<td>0.157</td>
</tr>
<tr>
<td>Central corneal thickness preoperatively</td>
<td>631 ± 82 µm (513–1038)</td>
<td>642 ± 87 µm (515–987)</td>
<td>0.666</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>n = 7</td>
<td>n = 4</td>
<td>0.533</td>
</tr>
<tr>
<td>Preoperative open-angle glaucoma</td>
<td>All: n = 2</td>
<td>All: n = 6</td>
<td>0.276</td>
</tr>
<tr>
<td>Accompanying pseudoexfoliation</td>
<td>n = 1</td>
<td>n = 2</td>
<td></td>
</tr>
<tr>
<td>Postoperative steroid response</td>
<td>n = 1</td>
<td>n = 1</td>
<td></td>
</tr>
<tr>
<td>Graft cell density, cells/mm(^2)</td>
<td>2697 ± 218 (2280–3300)</td>
<td>2269 ± 267 (2263–3800)</td>
<td>0.391</td>
</tr>
<tr>
<td>Donor age, yrs</td>
<td>67 ± 11 (39–89)</td>
<td>67 ± 11 (41–88)</td>
<td>0.717</td>
</tr>
<tr>
<td>Graft culture time, h</td>
<td>15 ± 6 (7–34)</td>
<td>16 ± 7 (4–34)</td>
<td>0.831</td>
</tr>
</tbody>
</table>

One patient suffered from postoperative steroid response without known history of open-angle glaucoma.

<FIGURE 1. ECD of grafts preoperatively (baseline), as well as 3 and 6 months postoperatively in group 1 (topical steroids 5× daily) and group 2 (hourly topical steroids). ECD does not differ at any time point between groups 1 and 2.>
The mean ECD after 3 and 6 months did not differ significantly between groups 1 and 2 \(P = 0.932\) (3 mo) and \(P = 0.847\) (6 mo)]. The decrease from preoperative ECD of the transplanted grafts to months 3 and 6 did not also differ between groups 1 and 2 \(P = 0.636\) (3 mo), and \(P = 0.761\) (6 mo)].

**DISCUSSION**

Graft survival and consecutive visual rehabilitation after posterior lamellar keratoplasty crucially depend on ECD.\(^5\)\(^,\)\(^6\)\(^,\)\(^7\) Opposed to penetrating keratoplasty, posterior lamellar keratoplasty poses greater trauma to the graft endothelium. This trauma accounts for a pronounced loss of endothelial cells shortly after lamellar keratoplasty, mainly occurring during the first 3 months after surgery.\(^4\)\(^,\)\(^5\) The combination of lamellar keratoplasty with cataract surgery (triple procedure) did not seem to pose significantly greater trauma to endothelial cells and did not affect ECD.\(^3\)\(^,\)\(^20\) A subclinical immune reaction could be responsible for the minimal, however present, decay of endothelial cells in the longer follow-up.\(^6\)\(^,\)\(^10\) We analyzed the effect of intensified topical steroid therapy on the median-term ECD [group 1: topical steroid application 5 times per day, as opposed to hourly application for the first postoperative week (group 2)].

We did not find any effect of postoperative intensified topical steroids on the trauma-related endothelial cell loss during the first 3 months after surgery. Neither the absolute numbers of endothelial cells per square millimeter nor the decrease of cells between groups 1 and 2 (low- vs. high-dose topical steroids) differed. The decrease of cells after surgery was 35% during the first 3 months in both groups. These results are in accordance with previously published analyses.\(^5\)\(^,\)\(^6\)\(^,\)\(^21\) In vitro corticosteroids had a stabilizing effect on endothelial cells.\(^13\) However, the effect of the trauma during surgery and preparation of the graft seem to outweigh this stabilizing effect, as we did not observe any endothelial cell preservation by the intensified steroid therapy.

A low-grade immunoreaction with slow decay of endothelial cells occurred in observations over a longer follow-up of up to 8 years.\(^6\) From months 3 to 6 of our observation, cell density decreased by a further 3% and did not differ in both groups. If a low-grade immunoreaction was responsible for this further cell loss, intensified topical steroids shortly after surgery did not attenuate it. However, our short observation period may not be enough to show any beneficial long-term effects. Moreover, the underlying immunoreaction may recur after discontinuation of the intensified steroid therapy after the first postoperative week. It is necessary to conduct longer observations to address this question in the future.

As we recently published, hourly topical steroids for the first week after surgery have a protective effect against postoperative cystoid macular edema.\(^14\) Very high concentrations of steroids in vitro had a toxic effect on endothelial cells.\(^13\) So the question could arise, if the intensified steroid therapy, desired to prevent cystoid macular edema, may have a toxic effect on the graft endothelium as well. The hourly application in our study however seems to be safe regarding corneal ECD.

We conclude that hourly topical steroids during the first postoperative week after Triple-DMEK can be safely administered and do not compromise ECD up to 6 months after surgery. Preparation of lamellae and intraoperative trauma which are intrinsic to all posterior lamellar keratoplasties (DMEK and Triple-DMEK) causes loss of endothelial cells, which can not be counteracted by the simple measure of intensified topical steroids.

**REFERENCES**


