Corneal Endothelial Cell Loss in Femtosecond Laser-assisted Descemet’s Stripping Automated Endothelial Keratoplasty: A 12-month Follow-up Study

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Abstract

Background: Descemet’s stripping automated endothelial keratoplasty (DSAEK) surgery offers a more standardized approach and reliable method to create corneal grafts with an instrument such as a microkeratome. With the development of Descemet’s membrane endothelial keratoplasty, an excellent clinical outcome is seen in the treatment of corneal endothelial dysfunctions, which indicates that thinner corneal graft results in better clinical outcome. With the recent development of the femtosecond laser, ultrathin corneal graft preparation has become possible. This study aimed to report corneal graft endothelial cell loss (ECL) in a large series of cases undergoing DSAEK with femtosecond laser-assisted corneal graft preparation within a 12-month period.

Methods: This study was designed as a prospective, noncomparative, interventional case series. Totally 126 consecutive eyes with endothelial failure of 120 patients, who had corneal endothelial decompensation and underwent femtosecond-assisted DSAEK using the VisuMax femtosecond laser system, were included in the study. Central endothelial cell density (ECD) was recorded postoperatively at 2 weeks (n = 126), 1 month (n = 126), 3 months (n = 110), 6 months (n = 101), and 12 months (n = 71) and then compared with the preoperative eye bank measurements. Pre- and postoperative central ECDs were evaluated using Heidelberg retina tomography-III confocal microscopy. ECL was calculated for each postoperative time point. Graft thickness was examined using anterior segment-optical coherence tomography.

Results: Mean preoperative cell count was 3383 ± 350 cells/mm². Mean postoperative cell counts were 2382 ± 707 cells/mm², 2179 ± 685 cells/mm², 2074 ± 668 cells/mm², 1884 ± 662 cells/mm², and 1723 ± 624 cells/mm² at 2 weeks, 1, 3, 6, and 12 months, respectively; these represented the ECL of 29.7 ± 19.7%, 35.4 ± 19.5%, 38.6 ± 19.8%, 44.3 ± 18.9%, and 48.9 ± 18.4% at the each corresponding time point. The mean corneal graft thickness after surgery was 142 ± 48 µm, 118 ± 41 µm, 108 ± 37 µm, 100 ± 32 µm, and 99 ± 32 µm at each corresponding study visit, respectively. There was no correlation between corneal graft thickness and corneal ECL (R = 0.039).

Conclusions: Corneal ECL remained relatively stable up to 12 months after femtosecond laser-assisted ultrathin DSAEK in a large case series. No correlation between cell loss and corneal graft thickness was found, which indicated that corneal graft preparation by the femtosecond laser was safe. ECL was faster within the first 6 months and relatively stable thereafter.

Key words: Corneal Transplantation; Descemet's Stripping Endothelial Keratoplasty; Femtoseconds

Introduction

Descemet’s stripping automated endothelial keratoplasty (DSAEK) has undergone rapid development in the last 10 years. Selective lamellar replacement of damaged recipient cornea with healthy donor corneal tissue has several advantages over the classic full-thickness penetrating keratoplasty (PK). With the development of Descemet’s membrane endothelial keratoplasty (DMEK), an excellent clinical outcome is seen in the treatment of corneal endothelial dysfunctions.₁⁻³ However, successful management of graft preparation is not easily achieved...

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and endothelial cell loss (ECL) is also significant. Due to the requirement of specialized surgical skills for graft preparation and intraoperative management, only a fraction of ophthalmologists have access to DMEK surgery. Furthermore, in complicated situations such as glaucoma drainage implants, aphakia, or presence of iris-fixated lenses, DMEK surgery has an increased risk of complications. In contrast, DSAEK surgery offers a more standardized approach and reliable methods to create corneal grafts with an instrument such as a microkeratome. With the recent development of the femtosecond laser, ultrathin corneal graft preparation has become possible.14,5

Femtosecond lasers have been widely used in refractive surgery for years. Recently, ophthalmologists have applied them in corneal transplantation. According to the published literature, the femtosecond laser created corneal flaps with better predictability than mechanical microkeratomes.6–10 Other studies have shown that the femtosecond laser could be used for the preparation of the endothelial graft for femtosecond laser-assisted DSAEK.11–14 With the assistance of the femtosecond laser, surgeons can produce ultrathin corneal endothelial grafts, which will possibly result in better clinical outcomes. The published literatures have demonstrated graft uniformity, technical feasibility, and also endothelial cell death in vitro. A delayed detrimental effect of the femtosecond laser on endothelial cells in ultrathin graft preparation cannot be ruled out by the existing data. Due to lack of long-term follow-up and small case numbers, safety evaluation of femtosecond laser-assisted DSAEK was incomplete. A long-term in vivo study for the safety assessment of femtosecond laser-assisted corneal graft preparation seems necessary. This study examined the corneal graft ECL in a large series of cases undergoing DSAEK with femtosecond laser-assisted corneal graft preparation during a 12-month period.

Methods

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Peking University 3rd Hospital. Informed written consent was obtained from all patients prior to their enrollment in this study.

Participants

The 126 consecutive eyes with endothelial failure of 120 patients, who had corneal endothelial decompensation and underwent femtosecond-assisted DSAEK using the VisuMax femtosecond laser system in the Department of Ophthalmology, Peking University 3rd Hospital, were included in the study. General data, corneal diseases, combined ocular diseases, and previous ocular surgical history were obtained from each patient. A detailed examination was performed that included a slit-lamp examination, confocal microscopy using the Heidelberg retina tomography with the cornea module (HRT III, Heidelberg Engineering, Heidelberg, Germany), anterior segment-optical coherence tomography (AS-OCT, Visante, Carl Zeiss Meditec, Dublin, CA, USA), and conventional photography. DSAEK with femtosecond laser-assisted corneal endothelial graft preparation using the VisuMax femtosecond laser system was performed in all eyes by the same surgeon. Central endothelial cell density (ECD) was recorded postoperatively at 2 weeks (n = 126), 1 month (n = 126), 3 months (n = 110), 6 months (n = 101), and 12 months (n = 71) by the same examination technician. ECD measurements were compared with the preoperative eye bank measurements.

Corneal graft preparation

For corneal endothelial graft preparation, all surgeries were performed with a 500 kHz femtosecond laser (VisuMax, Carl Zeiss Meditec, Jena, Germany). The donor cornea was mounted on an artificial anterior chamber (Moria, Antony, France). The femtosecond laser was programmed to use a lamellar cut with a cutting angle of 90° and a targeted diameter of 8 mm. The femtosecond laser parameters were as follows: corneal radius 7.7–7.8, pachymetry 540–550 µm, and treatment pack size was “medium.” The graft parameters were as follows: the targeted diameter was 8.0 mm, thickness was 480 µm, and the side cut angle was 90°.

Descemet’s stripping automated endothelial keratoplasty with suture pull-through technique

A superior 4-mm corneoscleral tunnel was created. With the aid of Healon GV (Abbott Laboratories Inc., Abbott Park, IL, USA), Descemet’s membrane and endothelium were stripped from the recipient’s cornea with a diameter of 8 mm. The donor lenticule was folded into a “taco” shape with endothelial cells enclosed. The “taco” was then placed into the Busin Glide and pulled through with forceps. An anchoring 10/0 prolene stitch was placed on the donor disc at the 6 o’clock position. After the anchoring stitch was passed through the auxiliary incision, exiting at the limbus at the 6 o’clock position, Healon GV in the anterior chamber was removed with balanced saline solution (BSS, Alcon, Fort Worth, TX, USA). The Busin glide was brought to the main limbal incision at the 12 o’clock position. The donor lenticule disc was inserted by pulling the stitch and the disc into the anterior chamber under low-flow irrigation from an anterior chamber maintainer at 3 o’clock. The donor lenticule was unfolded by increasing irrigation. The anchoring prolene stitch was then cut. A lenticule-sized bubble was injected to attach the graft.15

Assessment of corneal lenticule: Heidelberg retina tomography-III, optical coherence tomography, and slit-lamp examination

The corneal endothelium of the lenticule was assessed by AS-OCT and HRT-III confocal microscopy. Pre- and postoperative central ECDs were prospectively evaluated by EYE bank Specular microscope Group I, Class A (HAI EB-3000 xyz, HAI laboratories, Inc., USA), data were supplied from the same technician at our eye bank.
Postoperative central ECDs were prospectively evaluated by HRT-III confocal microscopy, and ECL was calculated for each postoperative time point. Graft thickness was also examined by AS-OCT. All examinations were performed by the same experienced technician at 2 weeks, 1 month, 3 months, 6 months, and 12 months after DSAEK.

Statistical analysis
Statistical analysis was performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). The results were shown as mean ± standard deviation (SD). Person’s correlation coefficient was used to detect the relationship between the corneal graft thickness and the corneal ECL. A P < 0.05 was considered statistically significant.

RESULTS

DSAEK was performed on 126 eyes (74 female eyes and 52 male eyes). The mean age of all patients was 58.6 ± 17.7 years. Preoperative characteristics and surgical intervention are summarized in Table 1. Indications for surgery were as follows: 76 eyes with postoperative bullous keratopathy (60.3%); 33 eyes with Fuchs’ corneal endothelial dystrophy (26.2%); 3 eyes suffering from postoperative descemet’s membrane detachment (2.3%); 5 eyes with iridocorneal endothelial syndrome (3.9%); and 9 eyes with Descemet membrane detachment postoperatively (7.1%). Single DSAEK procedure was conducted in 43 eyes (34.1%) and 83 eyes (65.9%) received DSAEK combined with phacoemulsification, intraocular lens (IOL) implantation, anterior vitrectomy, IOL suspension, or anterior chamber IOL removal.

Central ECD was recorded postoperatively at 2 weeks (n = 126), 1 month (n = 126), 3 months (n = 110), 6 months (n = 101), and 12 months (n = 71). Image quality was insufficient to obtain an endothelial cell count in 36 eyes at 2 weeks because of insufficient graft clarity and stromal swelling. The mean preoperative cell count was 3383 ± 350 cells/mm². The mean postoperative corneal endothelial cell counts were 2382 ± 707 cells/mm² (range: 919–3714 cells/mm²), 2179 ± 685 cells/mm² (range: 834–3964 cells/mm²), 2074 ± 688 cells/mm² (range: 757–3789 cells/mm²), 1884 ± 662 cells/mm² (range: 725–3619 cells/mm²), and 1723 ± 624 cells/mm² (range: 602–3001 cells/mm²) at 2 weeks, 1 month, 3 months, 6 months, and 12 months, respectively. These represented the ECL of 29.7 ± 19.7% (2.0–74.0%), 35.4 ± 19.5% (0.2–76.0%), 38.6 ± 19.8% (0.1–78.0%), 44.3 ± 18.9% (3.0–78.0%), and 48.9 ± 18.4% (7.0–80.0%) from eye bank measurements at 2 weeks, 1 month, 3 months, 6 months, and 12 months, respectively [Table 2].

The mean corneal graft thickness after the surgery was 142 ± 48 µm (range: 53–219 µm) at 2 weeks, 118 ± 41 µm (range: 42–214 µm) at 1 month, 108 ± 37 µm (range: 38–206 µm) at 3 months, 100 ± 32 µm (range: 43–189 µm) at 6 months, and 99 ± 32 µm (range: 49–210 µm) at 12 months [Table 2]. All surgical procedures were uneventful. Favorable corneal transparency was obtained in all cases unless

### Table 1: Preoperative characteristics and surgical intervention combined with DSEK of all patients in this study, n (%)

| Characteristics                              | Total eyes (n = 126) | DSEK (n = 43) | Combined surgeries (n = 83) |
|----------------------------------------------|---------------------|---------------|---------------------------|
| Postoperative bullous keratopathy            | 76 (60.3)           | 21 (16.7)     | 24 (19.4)                 |
| Pseudophagic bullous keratopathy             | 33 (26.2)           | 17 (13.4)     | 0 (0.0)                   |
| Aphakia                                      | 15 (11.9)           | 0 (0.0)       | 0 (0.0)                   |
| Glaucoma                                     | 14 (11.1)           | 2 (1.6)       | 0 (0.0)                   |
| After vitrectomy (watering eye)              | 7 (5.5)             | 0 (0.0)       | 0 (0.0)                   |
| Failed PKP                                   | 7 (5.5)             | 6 (4.7)       | 0 (0.0)                   |
| Fuchs’ endothelial dystrophy                 | 33 (26.2)           | 13 (10.3)     | 16 (12.7)                 |
| Descemet membrane detachment postoperatively | 3 (2.4)             | 3 (2.4)       | 0 (0.0)                   |
| Virus-infected endothelial dysfunction       | 9 (7.1)             | 8 (6.3)       | 1 (0.8)                   |
| Iridocorneal endothelial syndrome            | 5 (3.9)             | 3 (2.4)       | 2 (1.6)                   |

DSEK: Descemet stripping endothelial keratoplasty; PEA: Phacoemulsification; IOL: Intraocular lens; ACIOL: Anterior chamber intraocular lens; PKP: Penetrating keratoplasty.

### Table 2: ECD, ECL rate, and corneal lenticule thickness at different postoperative time points in this study

| Time points | Number of eyes | ECD (cells/mm²) | ECL rate (%) | Corneal lenticule thickness (µm) |
|-------------|----------------|-----------------|--------------|---------------------------------|
| 2 weeks     | 126            | 2382 ± 707 (919–3714) | 29.7 ± 19.7 (2.0–74.0) | 142 ± 48 (53–219) |
| 1 month     | 126            | 2179 ± 685 (834–3964) | 35.4 ± 19.5 (0.2–76.0) | 118 ± 41 (42–214) |
| 3 months    | 110            | 2074 ± 688 (757–3789) | 38.6 ± 19.8 (0.1–78.0) | 108 ± 37 (38–206) |
| 6 months    | 101            | 1884 ± 662 (725–3619) | 44.3 ± 18.9 (3.0–78.0) | 100 ± 32 (43–189) |
| 12 months   | 71             | 1723 ± 624 (602–3001) | 48.9 ± 18.4 (7.0–80.0) | 99 ± 32 (49–210) |

The data are shown as mean ± SD (range). ECD: Endothelial cell density; ECL: Endothelial cell loss; SD: Standard deviation.
postoperative complications occurred during follow-up period [Figure 1 and Table 3]. We did not observe a correlation between the corneal graft thickness and the corneal ECL ($R = 0.039, P = 0.913$).

Postoperative complications included graft dislocation ($n = 2$), viscoelastic residual stress causing a microcleft between graft and stroma ($n = 7$), peripheral anterior synechiae formation to the graft edge ($n = 31$), graft rejection ($n = 2$), increased intraocular pressure ($n = 25$), and viral infections ($n = 5$). Three patients suffered from uncontrolled glaucoma and had ciliary body photocoagulation at 3 months, so the ECL was not analyzed in these three patients [Table 3]. In all the other 22 eyes with an increased postoperative intraocular pressure, pressure regulation was achieved by medication or switching the steroid medication to calcineurin inhibitors without the need for glaucoma surgery during the 12-month follow-up period [Supplementary Table 1].

**DISCUSSION**

One of the major concerns after successful corneal transplantation is the corneal ECL, because it is directly correlated with corneal graft survival. ECL in PK has been reported to vary between 24.0% and 39.8% at 12 months of follow-up.\cite{16,17} There are various reasons for the corneal ECL in DSAEK. Mechanical trauma during folding of the graft to a taco configuration, long-standing contact with the air bubble, and trauma during insertion through a small incision are the common reasons.\cite{18,19} ECL after small-incision DSAEK has been reported between 34% and 61% at 12-month follow-up.\cite{2,20-23} Moreover, with the modification of the lenticule inserting procedure, ECL was reported between 23.5% and 25.3% at 6- and 12-month follow-up.\cite{24,25}

Femtosecond lasers dissect the corneal tissue by laser pulse-induced microphoto disruption. Damage to the corneal...
endothelial cells due to the applied energy or resulting shockwaves during the preparation procedure cannot be ruled out. Minimizing corneal ECL during preoperative femtosecond laser-assisted graft preparation is vital. There are many studies regarding femtosecond lasers and the ECL in vitro and in vivo. In vitro studies have proved that femtosecond laser might lead to ECL, the ECL has been reported as 4% after 150 to 200 µm thick endothelial graft for side preparation and 4.3% versus 7.7% ECL after 30 kHz laser lamellar cutting compared to 15 kHz laser for horizontal lamellar cuts at corneal depth of 400 µm and 9.5 mm diameter in another study. These studies provided evidence that the femtosecond laser affected corneal endothelial cells. Currently, there are few in vivo studies. According to the study of Cheng et al., the femtosecond-assisted Descemet’s stripping endothelial keratoplasty (DSEK) was not very favorable. In the study of Cheng et al., the corneal ECL was significantly higher in the femtosecond laser-assisted endothelium keratoplasty (FLEK) group compared with the PK group over time, ECL in the FLEK group at 12 months was reported as 65 ± 12%. The authors discussed the reason for the faster ECL: the multiple procedures could possibly have influenced the postoperative ECD such as surgical technique for different surgeons and folding to the taco configuration.

ECL in our study was lower than that in the study of Cheng et al. Other studies have reported ECL after small-incision DSEK and DSAEK between 34% and 61% at 12-month follow-up. However, Terry et al. reported ECL of 22.9–34% at 6 months and 23.2–32% at 12 months in a large amount of cases. The other convincing study by Price and Price showed that the ECL rate was 34% at 6 months and 35% at 12 months. However, the corneal ECL by femtosecond laser-assisted DSAEK in this study was higher than that of the previously published data of DSAEK with a microkeratome mentioned above. There were only 34.1% single DSAEK procedures included in our study, 65.9% of cases represented combined surgeries with phacoemulsification, IOL implantation, anterior vitrectomy, IOL suspension, and anterior chamber IOL removal. The added complexity of the additional procedures possibly resulted in longer time of surgery and longer periods of postoperative inflammation compared to lamellar corneal grafting alone. For this reason, the ECL rate might be higher than the published data. We have performed thousands of DSAEK procedures in the past 7 years. However, we do not routinely perform DMEK surgery, so the thinner graft could lead to longer intraoperative management. Furthermore, it is known that the anterior chamber is shallower in Asian eyes. This leads to additional difficulties in corneal graft management during the surgery, which may also influence ECL rates.

The intended corneal graft thickness in the VisuMax software (VisuMax, Cari Zeiss Meditec, Jena, Germany) was set to 70 µm for all corneal graft preparations. We did not examine the thickness and curvature of all individual donor corneas. We chose to not examine each donor cornea to minimize the risk of contamination and also because of limited availability of examination equipment in the eye bank. We set up the data according to the mean thickness and curvature of the normal cornea. The final corneal graft thickness was 99 µm, which was similar to the targeted thickness, indicating that it was not necessary to examine the corneal thickness and curvature before graft preparation. We considered this important fact to reduce the complexity and the amount of measurements needed for femtosecond laser-assisted graft preparation.

The final mean corneal graft thickness of this study was 99 ± 32 µm (range: 49–210 µm) at 12 months, which was quite close to the targeted thickness. It was possible that damage might occur at the corneal endothelial cells due to the applied energy or due to shockwave absorption during the preparation procedure. If that was the case, thinner grafts would probably be at an increased risk of ECL. We did not observe a correlation between the corneal thickness and the ECL. This indicated that the femtosecond laser-assisted graft preparation might have little influence on the ECL rate during a 12-month period. Although long-term studies are still needed, this is an encouraging result.

During the 12 months of follow-up period, we observed seven cases of microbleeds (clear space) between the recipient cornea and the posterior donor lenticule. Anshu et al. have reported similar cases of clear space formation separating graft and host cornea detected after DSEK combined with cataract surgery; and the cause for this was attributed to viscoelastic residuals. Irrigation/aspiration of the graft–host interface was performed in some of the cases. In this study, no extra management was performed and the clear space spontaneously disappeared 1–4 weeks later.

This study has confirmed that mean cell loss remained relatively stable up to at least 12 months after femtosecond laser-assisted ultrathin DSAEK in a large case series. We found no correlation between cell loss and corneal graft thickness, which indicated that corneal graft preparation by the femtosecond laser was safe. However, the corneal ECL rate was higher than some of the published data of microtome-assisted DSAEK. In published large series, the DSAEK ECLs were ranged from 22.9% to 34% at 6 months and 23.3% to 35% at 12 months; DMEK ECLs were ranged from 32% to 41% at 6 months [Supplementary Table 1]. With confounding effects as shallow anterior chambers in Asian eyes and combined procedures in this study, we cannot rule out a possible influence of femtosecond laser-assisted graft preparation on endothelium cell damage. Future studies with longer follow-up and continuous observation are necessary.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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**Conflicts of interest**

There are no conflicts of interest.

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### Supplementary Table 1: Current studies about endothelial cell loss at 6 and 12 months with large case series in DSAEK and DMEK

| Authors     | Years | Number of eyes | Procedure | Preoperative EC | 6 months | 12 months |
|-------------|-------|----------------|-----------|----------------|----------|-----------|
|             |       |                |           |                | ECD (cells/mm²) | ECL rate (%) | ECD (cells/mm²) | ECL rate (%) |
| Price et al. | 2008  | 263            | DSAEK     | 3100 ± 250     | 2000 ± 540 | 34 ± 18    | 1900 ± 480     | 35          |
| Terry et al. | 2008  | 80             | DSAEK     | 1908 ± 354     | 1908 ± 354 | 34 ± 12    | 1856 ± 371     | 35 ± 13      |
| Terry et al. | 2009  | 125            | DSAEK     | 1955           | 1955      | 32         | 1979 (n = 89)  | 32          |
| Terry et al. | 2011  | 154            | DSAEK     | 2635           | 2011      | 22.9       | 2009          | 23.3        |
| Ang et al.  | 2012  | 141            | DSAEK     | 2635           | 2635      |            | 30 ± 22       |             |
| Kruse et al.| 2011  | 34             | DMEK      | 2575 ± 260     | 2575 ± 260| 41         | 22.9          |             |
| Price et al. | 2014  | 673            | DMEK      | 26 (1 month)   | 26 (1 month)| 32 ± 20    | 32 (1 month)   | 32 ± 20      |
| Price et al. | 2014  | 38             | DMEK      | 26 (1 month)   | 26 (1 month)| 32 ± 20    | 32 (1 month)   | 32 ± 20      |

EC: Endothelial cell; ECD: Endothelial cell density; ECL: Endothelial cell loss; DSAEK: Descemet’s stripping automated endothelial keratoplasty; DMEK: Descemet’s membrane endothelial keratoplasty.