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Severe Corneal Edema Increases ECL From Grafts After DSAEK—Corneal Edema and ECL After DSAEK

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Objectives: To determine the relationship between the preoperative degree of corneal edema in the recipient and the endothelial cell density in grafts after Descemet stripping automated endothelial keratoplasty (DSAEK).

Methods: This retrospective case series enrolled 111 eyes of 107 patients who underwent DSAEK. The preoperative and postoperative central corneal thickness (CCT) was measured by anterior-segment optical coherence tomography. Eyes were divided into three groups according to the preoperative recipient CCT: group A (mild edema): 550 μm<CCT≤750 μm, n=38; group B (moderate edema): 750 μm<CCT≤900 μm, n=51; and group C (severe edema): 900 μm<CCT≤1,500 μm, n=22. The endothelial cell loss (ECL) was calculated by in vivo confocal microscopy and compared at 1, 6, 12, and 24 months after surgery.

Results: The recipient CCT (all groups combined) was 805.99±132.70 μm preoperatively and decreased to 656.31±105.02 μm at 1 month, decreased to 626.08±81.40 μm at 6 months, and remained stable between 12 (*P=0.144*) and 24 months (*P=0.485*) postoperatively. The mean ECL was 27.34±15.43%, 33.56±17.13%, 39.18±16.71%, and 45.87±14.27% at 1, 6, 12, and 24 months, respectively. The percentage of ECL in group C was higher than that in the other 2 groups through the 24-month follow-up. The difference in ECL between groups A and C was significant at 24 months (group A: 42.45±14.47%; group C: 52.49±10.65%; *P=0.019*).

Conclusions: The degree of corneal edema in the recipient was associated with implant ECL. Compared with mild and moderate corneal edema, the severe corneal edema may cause greater ECL after DSAEK.

Key Words: DSAEK—Endothelial cell loss—Degree of corneal edema.

(*Eye & Contact Lens* 2022;48: 250–255)

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The authors have no funding or conflicts of interest to disclose.

This research project was supported by the National Natural Science Foundation of China (Grant No. 81970768). The funding organization had no role in the design or conduct of this research.

The data analyzed in this study are available from the corresponding author upon reasonable request.

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Accepted September 22, 2021.

DOI: 10.1097/ICL.0000000000000857

Endothelial keratoplasty (EK), such as Descemet stripping automated EK (DSAEK) and Descemet membrane EK (DMEK), has become the most frequently performed technique for corneal endothelial dysfunction.[1,2] EK offers several advantages over penetrating keratoplasty (PKP) in rapid vision recovery, a lower incidence of graft rejection, minimal surgically induced astigmatism, and fewer surgical complications.[3] Compared with DSAEK, DMEK has been reported to offer quick and consistent visual recovery and improved outcomes.[4] In Western countries, Fuchs endothelial corneal dystrophy (FECD) is a common cause of corneal blindness, and the number of DMEK procedures performed has increased in patients with FECD.[5] In contrast, bullous keratopathy (BK) is the leading reason for endothelial dysfunction in Asians.[6] However, DMEK is especially difficult to perform in the eyes of Asians because of the narrow palpebral fissure, small deep-set eyes, relatively shallow chamber, and dark iris.[7] Therefore, DSAEK is the preferred treatment choice in Chinese patients.

Despite the advantages mentioned above, one potential drawback after DSAEK is chronic loss of endothelial cell density (ECD) over time, which is one of the leading causes of graft failure. Price et al.[8] reported that by 10 years postoperatively, the amount of endothelial cell loss (ECL) was comparable for both DSAEK and PKP. Risk factors for ECL after DSAEK include donor age, graft size, donor preoperative endothelial cell count, donor tissue storage time, and graft detachment, among others.[9–11]

The shortage of donor corneas is a significant issue worldwide, especially in China. Patients have to wait a long time for a healthy donor cornea, and consequently, corneal edema tends to reach the severe stage before surgery. Furthermore, the severity of corneal edema was found to be associated with elevated levels of inflammatory cytokines in the aqueous humor (AqH),[12] and a combination of proinflammatory cytokines was shown to synergistically induce the apoptosis of corneal endothelial cells in vitro.[13] Thus, we hypothesized that severe preoperative corneal edema may influence the ECD after DSAEK. However, as mentioned above, most previous studies have focused on the analysis of donors and have primarily evaluated the influence of donor factors on the ECD after DSAEK. Whether the preoperative degree of corneal edema in the recipient would affect the ECD after DSAEK has not been reported. To investigate this issue, we evaluated the association between the preoperative degree of corneal edema in the recipient (clinically assessed as the central corneal thickness [CCT]) and the loss of corneal endothelial cells in the implant in a group of patients treated with DSAEK.

METHODS

This study was a single-center, retrospective, noncomparative interventional case series comprising consecutive eyes that

underwent DSAEK between January 2013 and December 2017 at Peking University Third Hospital with a minimum follow-up of 24 months. Participants who demonstrated corneal endothelial decompensation from pseudophakic BK (PBK) or FECD and clear grafts at the last follow-up were recruited into this trial. The presence of other ocular comorbidities was a criterion for patient exclusion. Donor tissues with an ECD greater than 2000 cells/mm,^{2} any age between 14 and 60 years old, and death-to-transplantation time of up to 11 days were accepted. In addition, eligible patients and donors who were diagnosed with diabetes were excluded from the analysis.

This research followed the tenets of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the Ophthalmic Research Center, which is affiliated with Peking University Third Hospital, Beijing, China. An informed consent form was signed by the patients after the purpose of the study was explained.

Donor Preparation and Examination

All donor corneas with a qualitative grade of very good or excellent were procured from the Eye Bank of Peking University Third Hospital. The donor data obtained from the Eye Bank included age and sex, cause of death, death-to-preservation time (hours), and storage time (days). Contact ultrasonic pachymetry (A/B scan, Sonomed, Inc, Lake Success, NY) was used to measure the CCT. After collection from the cadaver, the whole globe was preserved at 4°C in K-Sol medium (Cilco, Huntington, WV). Donor endothelial images were obtained with an Eye Bank specular microscope (HAI EB-3000XYZ; HAI Laboratories, Lexington, MA) before surgery. The preoperative endothelial cell count was obtained using computer-assisted morphometry. Quantitative morphometric analysis was performed using the center method, and at least 100 cells were counted from projected endothelial images of each cornea.

Surgical Procedure

Descemet stripping automated EK was performed by the same surgeon under general or retrobulbar anesthesia using a method similar to that described by Price and Price.[10] In all cases, the donor tissue was prepared with a Carriazo Barraquer microkeratome (Moria, Inc, Doylestown, PA) by the surgeon. The intended thickness of the anterior corneal lamella was 400 or 450 μm, and the graft diameter was 8.0 mm. After stripping of Descemet's membrane and scraping of the recipient bed with a Terry Scraper (Bausch & Lomb Surgical, St. Louis, MO), the lamellar donor tissue was folded and inserted over a glide into the anterior chamber (AC) through a 5-mm scleral tunnel incision, and the AC was filled with air to aid attachment of the graft to the host cornea.

Pachymetry of CCT

The preoperative recipient CCT and postoperative CCT were measured by anterior-segment optical coherence tomography (AS-OCT, Visante; Carl Zeiss Meditec, Inc, Dublin, CA). All subjects were positioned in a headrest with their attention directed to an internal fixation light. The same examiner actively monitored that all patients' eyes were well centered and aligned. The CCT was determined by obtaining a horizontal cross-sectional image with good central reflective light representing the anterior corneal vertex. EK graft and total corneal thickness were determined manually using the LASIK flap tool software. The operator adjusted the software system to position the vertex at the center of the AS-OCT image. More than three horizontal scans were performed, and the scan with the best quality was selected for measurements. The thickness was measured using the software of the Visante AS-OCT system. The preoperative recipient CCT and postoperative CCT were measured with the caliper position at zero and recorded as the distance from the surface epithelium to the endothelium. The central graft thickness was the distance between the high-light reflective plane (i.e., the graft–host interface) and the endothelium.

Eyes were divided into three groups based on the preoperative recipient CCT, and these groups were named according to the edema severity, as follow: group A (mild edema): 550 μm$<$CCT$\leq$750 μm; group B (moderate edema): 750 μm$<$CCT$\leq$900 μm; and group C (severe edema): 900 μm$<$CCT$\leq$1,500 μm.

Postoperative Endothelial Cell Imaging and Counting

Postoperatively, specular images of the graft's central corneal endothelium were obtained by in vivo confocal microscopy (HRT3/RCM; Heidelberg Engineering, Dossenheim, Germany). A clear image of the endothelial layer was selected for endothelial evaluation. The number of endothelial cells was counted manually and postoperative ECD was derived as the number of cells/mm^2 using the proprietary software within the corneal confocal microscope. At least 50 cells from the endothelial images for each cornea were counted. The postoperative ECD and percentage of corneal ECL were recorded at 1, 6, 12, and 24 months. Analysis of endothelial polymorphism and polymegathism was not performed in this study.

Statistical Analysis

Statistical analyses were performed with SPSS Statistics for Windows, version 21.0 (IBM Corp, Armonk, NY). Data are expressed as the mean$\pm$SD and range. The normal distribution of continuous variables was verified using the Kolmogorov–Smirnov test and a Q-Q plot. The preoperative and postoperative total and each group CCT were compared by Friedman and Wilcoxon tests. Baseline characteristics and the percentage of ECL after DSAEK were compared between groups using the Kruskal–Wallis test along with Bonferroni correction. A P value less than 0.05 was considered significant.

RESULTS

In total, 111 eyes of 107 patients (49 female subjects) were included in this study; the mean age at surgery was 55.16$\pm$17.23 (range, 16–83) years. The participants experienced corneal endothelial decompensation from PBK (90.1%) or FECD (9.9%). Demographic and clinical characteristics are illustrated in Table 1. The mean donor age was 47.8$\pm$18.6 years (range, 16–58 years). The mean death-to-preservation time was 6.8$\pm$3.4 hr (range, 0–12 hr), and the mean death-to-surgery duration was 5.6$\pm$2.7 days (range, 1–11 days). The donor trephine size was 8.0 mm. The preoperative donor ECD was 2,597.63$\pm$380.83 cells/mm^2 (range, 2,178–3,732 cells/mm^2). The mean recipient age and preoperative donor ECD of the three groups were evaluated and compared.

TABLE 1. *Recipient Characteristics of 111 Eyes Treated With DSAEK*

Characteristics	Number
Recipient mean age (years)	55.16±17.23 (range, 16–83)
Recipient sex	Female, 49 (45.79%); male, 58 (54.21%)
Recipient CCT (preoperative) (μm)	805.99±132.70 (range, 550–1,480)
Diagnosis, number of eyes (%)	100 (90.1%)
PBK group A	33 (29.7%)
Group B	46 (41.5%)
Group C	21 (18.9%)
FECD group A	11 (9.9%)
Group B	5 (4.5%)
Group C	5 (4.5%)
	1 (0.9%)

CCT, central corneal thickness; DSAEK, Descemet stripping automated endothelial keratoplasty surgery; FECD, Fuchs endothelial corneal dystrophy; PBK, pseudophakic bullous keratopathy.

There were no statistically significant differences among the three groups, as shown in Table 2.

No complications occurred intraoperatively. After surgery, graft nonattachment was observed in one eye (0.9%) and resolved spontaneously. Nine eyes (8.1%) demonstrated a high intraocular pressure, which resolved with the use of antiglaucoma eye drops and steroid reduction.

The mean preoperative recipient CCT of all groups combined was 805.99±132.70 (range, 550–1,480) μm, decreased to 656.31±105.02 (range, 468–975) μm at 1 month, decreased to 626.08±81.40 (range, 446–933) μm at 6 months, and remained stable between 12 (628.51±78.91, *P=0.144*) and 24 months (627.61±80.41, *P=0.485*) postoperatively. The CCT of each group decreased at 1 month and 6 months after DSAEK ($P<0.001$) and remained stable between 12 and 24 months. The details were shown in Table 3.

After DSAEK, there was a progressive decrease in the implant ECD over time. The mean percentage of ECL in all groups combined (111 eyes) was 27.34±15.43% at 1 month, increased to 33.56±17.13% at 6 months, and continued to increase to 39.18±16.71% at 12 months; at 24 months, it rose to 45.87±14.27%.

The percentage of ECL in group C was 31.10±16.26% at 1 month, 36.56±13.22% at 6 months, 44.21±12.02% at 12 months, and 52.49±10.65% at 24 months. These data were higher than those in group A (22.11±13.03% at 1 month; 30.69±16.43% at 6 months; 34.17±17.12% at 12 months; 42.45±14.47% at 24 months) and group B (29.62±15.97% at 1 month; 34.42±19.02% at 6 months; 40.73±17.43% at 12 months; 45.57±14.74% at 24 months). The difference in ECL was not significant among the three groups at 1 month (*P=0.082*), 6 months (*P=0.431*), or 12 months (*P=0.092*). However, there was a significant difference between groups A and C at 24 months after surgery (*P=0.019*), which is described in Table 4.

As PBK has an increased risk for the ECL, we compared the ECL between different diagnoses (FECD vs. PBK) within the group. The results showed no significant difference between FECD and PBK among the within-group at each postoperative visit. The details are shown in Table 5.

DISCUSSION

The corneal endothelium preserves corneal transparency by regulating the outflow of AqH to the stroma through its barrier and pump mechanisms. The normal function of the corneal endothelium is a prime determinant of corneal thickness, and factors that impair the ability of the endothelium disrupt this balance with resultant corneal edema and an increase in thickness.[14] The decrease in CCT after DSAEK may mean that the properly functioning endothelial graft led to stromal deturgescence and maintained the stability of the recipient cornea. In the current study, the preoperative mean CCT significantly decreased at one month and stabilized approximately six months postoperatively. These results are similar to those of previous studies, which showed that the total corneal thickness and graft thickness stabilized by 3 to 6 months after DSAEK.[15,16] A study by Ahmed et al. showed that the total CCT increased after DSAEK, and it was concluded that this abnormally high cornea thickness was because of the additive procedure of DSAEK.[17] However, we observed a significantly decreased CCT after DSAEK, which we believe could be caused by differences in the preoperative degree of corneal edema. In our investigation, the mean preoperative CCT was 805.99±132.70 μm, and the total CCT in the study by Ahmed et al. was 610±132.70 μm.

To improve graft survival after DSAEK, it is essential to provide a great number of healthy endothelial cells and maintain the long-term functions of these endothelial cells. If high ECL occurs, it could lead to graft failure, which usually requires repeat surgery. Many articles have investigated donor factors that may influence ECL after DSAEK.[18,19] However, the influence of the preoperative degree of recipient corneal edema on postoperative ECL remains unclear. To investigate this issue, we divided all eyes into three groups based on the preoperative recipient CCT. We found a progressive decrease in implant ECD over time and an increase in ECL through 24 months of follow-up. In addition, we noticed that the ECL in group C (severe edema) was higher than in the other two groups through 24 months.

The higher ECL in group C at one month may be related to more surgical manipulation than the other groups. In group C (severe edema), severe corneal edema may have limited visualization of the AC with standard microscope illumination. This limited visualization may preclude surgical procedures because of the inability to see and manipulate the endothelial graft intraoperatively. Difficulty with inserting, unfolding, or positioning DSAEK grafts would necessitate more manipulation, which may be why the

TABLE 2. *Comparison of the Three Groups with Respect to Mean Recipient Age and Preoperative Donor ECD*

	Mean Recipient Age (years) (Mean±SD)	Preoperative Donor ECD (cells/mm^2) (Mean±SD)
Group a (n=38)	54.71±16.98 (range, 16–83)	2,636.13±366.50 (range, 2,212–3,690)
Group B (n=51)	55.65±16.61 (range, 16–83)	2,601.06±429.47 (range, 2,178–3,732)
Group C (n=22)	54.82±19.70 (range, 20–83)	2,523.18±274.47 (range, 2,188–3,214)
P	0.955	0.560

ECD, endothelial cell density.

TABLE 3. *Preoperative and Postoperative CCT of Each Group and the Corresponding Comparisons at Each Postoperative Visit*

	Group A (n=38)		Group B (n=51)		Group C (n=22)	
	CCT(Mean±SD)	*P*	CCT (Mean±SD)	*P*	CCT (Mean±SD)	*P*
Preoperative	687.05±51.05 (range, 550–750)		810.90±43.35 (range, 760–895)		1,000.05±134.41 (range, 908–1,480)	
1 month[a]	624.11±100.03 (range, 485–975)	<0.001	672.71±108.27 (range, 468–905)	<0.001	674.82±96.11 (range, 548–884)	<0.001
6 months[b]	597.63±89.78 (range, 446–933)	0.001	644.51±79.32 (range, 494–816)	0.008	632.5±56.54 (range, 555–744)	0.048
12 months[c]	597.92±83.44 (range, 444–921)	0.248	648.41±79.05 (range, 489–815)	0.551	635.23±52.59 (range, 553–759)	0.338
24 months[d]	596.53±87.13 (range, 459–933)	0.994	645.90±79.03 (range, 483–813)	0.961	638.91±54.08 (range, 553–768)	0.130

[a]Preoperative vs 1 month in group A, $P<0.001$; Preoperative vs 1 month in group B, $P<0.001$; Preoperative vs 1 month in group C, $P<0.001$.

[b]Postoperative 1 vs 6 months in group A, $P=0.001$; Postoperative 1 vs 6 months in group B, $P=0.008$; Postoperative 1 vs 6 months in group C, $P=0.048$.

[c]Postoperative 6 vs 12 months in group A, $P=0.248$; Postoperative 6 vs 12 months in group B, $P=0.551$; Postoperative 6 vs 12 months in group C, *P=0.338*.

[d]Postoperative 6 vs 24 months in group A, $P=0.994$; Postoperative 6 vs 24 months in group B, $P=0.961$; Postoperative 6 vs 24 months in group C, $P=0.130$.

CCT, central corneal thickness.

highest ECL in group C (severe edema) occurred one month after the operation. Previous reports have shown that the greatest ECL occurs by one month after DSAEK[20] and have suggested surgical and very early postoperative trauma as significant factors influencing ECL after DSAEK.[21,22]

However, implant ECL in group C (severe edema) remained highest at 6 and 12 months, with a significant difference between group A (mild edema) and group C (severe edema) at 24 months. In the present study, all procedures were performed by a single experienced surgeon using a uniform DSAEK technique without serious operative complications. There were no significant differences in donor ECD, graft size, or graft thickness among the three groups. Therefore, the difference in ECL in the implant among the groups indicated that in addition to the endothelial damage caused by the initial surgical trauma, there may be other potential factors affecting postoperative ECL.

Corneal edema is widely believed to be caused by the loss of pump function in the corneal endothelium and excessive AqH entry into the corneal stroma. AqH is present between the corneal endothelium and the iris and has a unique composition including proteins, glutathione, glucose, and other biologically active substances. Under normal conditions, AqH provides an immunosuppressive microenvironment and contains immunosuppressive neuropeptides. These neuropeptides suppress the activation and differentiation of T cells and inhibit effector functions of activated macrophages, helping to maintain both homeostasis in the AC and the blood–aqueous barrier (BAB).[23] Increased levels of inflammatory factors in the AqH indicate breakdown of the BAB, which might lead to a decrease in the ECD. It has previously been reported that preexisting iris damage is a clinical factor of graft failure and rapid ECL after DSAEK because of breakdown of the BAB.[24] Furthermore, the preoperative levels of AqH cytokines, such as MCP-1, IL-17A, and sICAM-1, were found to be significantly correlated with reductions in the ECD after DSAEK and PKP.[25,26] However, the exact mechanism is poorly understood. Yagi-Yaguchi et al.[27] speculated that the chronic elevation of cytokine levels in the AqH may increase intracellular oxidative stress in corneal endothelial cells and lead to reductions in the ECD. And a recent study reported that the severity of corneal edema was correlated with the

TABLE 4. *ECD and Percentage of ECL at Each Postoperative Visit and the Corresponding Comparisons of ECL Among the Three Groups*

	1 Month Postoperatively (Mean±SD)		6 Months Postoperatively (Mean±SD)		12 Months Postoperatively (Mean±SD)		24 Months Postoperatively (Mean±SD)	
	ECD (cells/mm^2)	ECL%	ECD (cells/mm^2)	ECL%	ECD (cells/Mm^2)	ECL%	ECD (cells/mm^2)	ECL%
Group A (n=38)	2055.95±450.51 (range, 1,020–3,266)	22.11±13.03 (range, 1.22–53.89)	1826.21±501.89 (range, 966–2,854)	30.69±16.43 (range, 0.17–64.33)	1722.66±452.33 (range, 889–2,666)	34.17±17.12 (range, 2.61–60.32)	1,515.11±423.47 (range, 805–2,299)	42.45±14.47 (range, 12.53–70.56)
Group B (n=51)	1836.9±541.08 (range, 1,041–3,204)	29.62±15.97 (range, 0.34–59.51)	1723.86±622.28 (range, 761–3,141)	34.42±19.02 (range, 3.35–66.87)	1,559.45±583.86 (range, 700–2,911)	40.73±17.43 (range, 3.84–74.25)	1,430.92±509.14 (range, 792–2,966)	45.57±14.74 (range, 7.40–65.60)
Group C (n=22)	1749±490.65 (range, 1,083–2,660)	31.10±16.26 (range, 2.43–56.69)	1,596.09±342.11 (range, 806–2086)	36.56±13.22 (range, 14.16–64.35)	1,410.36±352.65 (range, 959–2,173)	44.21±12.02 (range, 10.14–62.76)	1,202.77±325.74 (range, 840–2005)	52.49±10.65 (range, 31.09–62.80)
P	0.082[a]		0.431[b]		0.092[c]		0.019[d]	

[a]Comparisons of the percentage of ECL among the three groups at 1 month postoperatively.

[b]Comparisons of the percentage of ECL among the three groups at 6 months postoperatively.

[c]Comparisons of the percentage of ECL among the three groups at 12 months postoperatively.

[d]Comparison of the percentage of ECL between groups A and C at 24 months postoperatively.

ECD, endothelial cell density; ECL, endothelial cell loss.

TABLE 5. *Percentage of ECL of Different Diagnoses (FECD vs. PBK) at Each Postoperative Visit and Corresponding Comparisons Among Within Group*

	1 Month Postoperatively (Mean±SD)		6 Months Postoperatively (Mean±SD)		12 Months Postoperatively (Mean±SD)		24 Months Postoperatively (Mean±SD)	
	ECL% (PBK)	ECL% (FECD)	ECL% (PBK)	ECL% (FECD)	ECL% (PBK)	ECL% (FECD)	ECL% (PBK)	ECL% (FECD)
Group A (n=38)	19.86±13.41 (range, 1.22–53.89)	28.34±6.52 (range, 24.35–41.11)	30.24±16.19 (range, 1.66–64.33)	28.27±20.04 (range, 3.94–56.41)	37.10±17.60 (range, 2.61–60.32)	37.95±13.85 (range, 21.08–59.88)	40.76±14.92 (range, 12.53–70.56)	47.67±9.73 (range, 36.36–63.67)
P^a	0.084		0.900		0.675		0.310	
Group B (n=51)	29.14±15.14 (range, 3.36–58.24)	33.96±24.14 (range, 5.51–59.51)	34.91±18.85 (range, 7.84–66.87)	29.90±22.21 (range, 3.35–62.16)	41.06±17.74 (range, 3.84–74.25)	37.75±15.64 (range, 14.21–58.00)	45.64±14.63 (range, 7.40–64.51)	44.93±17.52 (range, 24.58–65.60)
P^b	0.633		0.656		0.569		1.000	
Group C (n=22)	30.10±15.95 (range, 2.43–56.69)	52.10 (range, 52.10–52.10)	35.24±11.96 (range, 14.16–61.28)	64.35 (range, 64.35–64.35)	43.72±12.09 (range, 10.14–62.76)	54.44 (range, 54.44–54.44)	52.10±10.74 (range, 31.09–62.80)	60.81 (range, 60.81–60.81)
P^c	0.182		0.091		0.455		0.545	

[a]Percentage of ECL of group A, PBK vs FECD at 1 month, P=0.084; at 6 months, P=0.900; at 12 months, P=0.675; at 24 months, P=0.310.

[b]Percentage of ECL of group B, PBK vs FECD at 1 month, P=0.633; at 6 months, P=0.656; at 12 months, P=0.569; at 24 months, P=1.000.

[c]Percentage of ECL of group C, PBK vs FECD at 1 month, P=0.182; at 6 months, P=0.091; at 12 months, P=0.455; at 24 months, P=0.545.

PBK, pseudophakic bullous keratopathy; FECD, Fuchs endothelial corneal dystrophy, ECL, endothelial cell loss.

AqH levels of protein and specific inflammatory cytokines (IL-13, and sICAM-1).[12] Thus, we postulate the high levels of proinflammatory cytokine in the AqH may be one of the reasons for the greater ECL in group C (severe edema) after DSAEK.

The cornea is the most innervated tissue in the body, and corneal nerves play a role in maintaining endothelial homeostasis.[28] It has been shown that reductions in corneal innervation are associated with ECD loss.[29] Lambiase et al.[30] reported a decline in the ECD in surgically induced neurotrophic keratitis. Koh[31] found that autocrine signaling by the neuropeptide vasoactive intestinal peptide is critical in regulating the apoptosis and survival of endothelial cells. However, previous studies have suggested that extended stromal edema could result in irreversible pathological changes, such as abnormal subepithelial fibrosis and abnormal innervation.[32,33] Furthermore, some of these changes, including abnormal innervation, may persist for a long time and cannot be resolved even after restoring endothelial function by EK.[34,35] This may be another reason for the highest ECL occurring in group C (severe edema) in the present study.

In addition, we noticed the loss of ECL in group B was less than that in the other groups during 1 to 6 months and 12 to 24 months postoperatively. We could not determine the reason for the lower ECL loss in group B during these periods. Further prospective randomized controlled studies are needed to clarify this issue.

This study has several limitations. First, we did not assess the levels of inflammatory factors in the AqH and did not compare histological changes in the edematous cornea, especially histological changes in the sub-basal corneal nerves, although such histological changes have been demonstrated in previous investigations. In the current study, we observed the endothelial cells using in vivo confocal microscopy, which can also be used to evaluate corneal nerve density. In the future, proteomic analysis of the AqH and histological examination of the cornea (including in vivo confocal microscopy) may enable identification of the cause of ECD loss after EK. Second, this was a retrospective study; however, we did discover that the percentage of ECL in group C (severe edema) was higher than that in the other two groups through the 24-month follow-up. Third, this study had a small sample size and short follow-up time. Thus, a prospective study involving a larger cohort of patients is needed. Besides, we only observed the grafts that were clear at 2 years. Eyes that experienced severe ECL were excluded from the analyses because of corneal opacity. Therefore, some bias may be present in the analytical results.

In conclusion, the current study demonstrates that compared with mild and moderate degrees of corneal edema, severe corneal edema was associated with greater ECL after DSAEK. High preoperative levels of AqH cytokines and histological changes in the sub-basal corneal nerves may be factors of this difference. To the best of our knowledge, this is the first study to observe the correlation between the preoperative degree of recipient corneal edema and implant ECL after DSAEK. Our results suggest that a preoperative CCT less than 900 μm is a better indication for DSAEK than a CCT greater than 900 μm caused by stromal edema.

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