Donor Corneal Endothelial Cell Maturity and Its Impact on Graft Survival in Glaucoma Patients Undergoing Corneal Transplantation

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• PURPOSE: To examine corneal graft survival via corneal endothelial cell density (ECD) and corneal endothelial cell loss (ECL) at 5 years post-transplantation in the eyes of patients with and without a history of undergoing glaucoma surgery according to the maturity of the donor corneal endothelial cells.

• DESIGN: Prospective cohort study.

• METHODS: This prospective cohort study included 17 patients with glaucoma and 51 patients without glaucoma who underwent Descemet's stripping automated endothelial keratoplasty or penetrating keratoplasty at the Baptist Eye Institute, Kyoto, Japan, between October 2014 and October 2016. Human corneal endothelial cells were cultured from residual peripheral donor cornea tissue, and the maturity of the cells was evaluated by cell surface markers (ie, $CD166^{+}$, $CD44^{-/dull}$, $CD24^{-}$, and $CD105^{-}$) using fluorescence-activated cell sorting. Kaplan–Meier analysis or the chi-square test was used to assess the rate of successful corneal graft survival post-transplantation.

• RESULTS: At 36 months postoperatively, the mean ECD and ECL in the glaucoma-bleb eyes were 1197 ± 352 cells/mm^2 and 55.5% ± 13.9% in the high-maturity group and 853 ± 430 cells/mm^2 and 67.7% ± 18.1% in the low-maturity group, respectively. Kaplan–Meier analysis revealed that at 5 years postoperatively, the overall rate of survival was 45%, that is, 100% in the high-maturity group and 25% in the low-maturity group ($P < .05$).

• CONCLUSIONS: The findings in this prospective cohort study revealed that the use of donor corneal grafts containing mature-differentiated corneal endothelial cells could maintain the survival of the transplanted graft for a long-term period, even in patients with a history of undergoing glaucoma surgery. (Am J Ophthalmol 2024;262:1–9. © 2024 Elsevier Inc. All rights reserved.)

Glaucoma, an irreversible ocular disorder that ultimately leads to blindness, is becoming increasingly prevalent with the aging global population, thus leading to a higher number of patients requiring glaucoma surgery each year. Although new surgical strategies for the treatment of glaucoma, such as the implantation of glaucoma drainage devices, have been found to improve the required decrease of intraocular pressure (IOP),[1] the findings of long-term longitudinal studies have revealed potential corneal endothelial loss.[2-6] Moreover, bullous keratopathy (BK), a late-stage complication that can occur after glaucoma surgeries such as trabeculectomy and glaucoma drainage device implantation, has been reported in 1% to 5% of cases.[3,7,8]

It has been reported that corneal endothelial cell density (ECD) decreases over time after corneal transplantation strategies such as penetrating keratoplasty (PK), Descemet stripping automated endothelial keratoplasty (DSAEK), and Descemet membrane endothelial keratoplasty,[9] and even more rapidly in patients who have previously undergone glaucoma surgery, even without allograft rejection.[10-12] The findings in our recent retrospective study revealed that the 5-year cumulative rate of corneal graft survival in glaucoma patients after trabeculectomy is less than 50%, significantly lower compared with the rate for Fuchs endothelial corneal dystrophy (FECD) (100%) and non-FECD (90%).[13] Moreover, elevation of IOP and the development of glaucoma are commonly observed after corneal transplantation,[14] and repeat keratoplasty may increase the risk of subsequent glaucoma. Thus, although it is crucial to maintain long-term graft survival in patients with a history of undergoing glaucoma surgery, a standardized benchmark solution has yet to be proposed.

Previously, our research findings demonstrated the maturity of donor cultured corneal endothelial cells (CECs) referred to the state of differentiation and functional capability of corneal endothelial cells.[15-17] For instance, we found that the CD44-positive CECs, which were supposed to be

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lower mature-differentiated human CECs (HCECs), that is, cell-state transition cells, exhibited a distinct metabolic profile, possibly resulting in flattened and transformed cell-like shapes and lower expression of ion transporters and water transporters.[17,18] We also demonstrated that the lower maturity of donor CECs was associated with significantly reduced donor-graft ECD at 36 months postoperatively.[19] We found that donor corneas with a higher number of mature-differentiated HCECs were associated with higher postoperative ECD in the midterm period after successful corneal transplantation compared with donor corneas with fewer mature-differentiated HCECs.[19]

In the present study, we investigated postoperative ECD and corneal endothelial cell loss (ECL) in patients with and without a history of previously undergoing glaucoma surgery, stratified by the donor CEC maturity, and examined the potential impact of donor CEC maturity on graft survival at 5 years postoperatively.

METHODS

• **PATIENTS:** This prospective cohort study involved consecutive patients with BK with or without glaucoma who underwent DSAEK or PK between October 2014 and October 2016 at the Baptist Eye Institute, Kyoto, Japan. The protocols of this study were approved by the Institute's Institutional Review Board (Approval #ERB-C 1006) and in accordance with the tenets set forth in the Declaration of Helsinki. Written informed consent was obtained from all patients before their involvement in the study. Clinical trial registration was obtained from UMIN UMIN000024892 (http://www.umin.ac.jp/english/). The original indications for DSAEK or PK included 17 eyes that had previously undergone glaucoma surgery (trabeculectomy) followed by BK (glaucoma-bleb eyes) and 51 eyes with no history of undergoing glaucoma surgery (control eyes). All the patients with previous glaucoma surgery included in the study had undergone trabeculectomy, and none had tube shunt surgery. Of those 51 eyes, there were 21 eyes with corneal stromal opacity, 15 eyes with pseudophakic BK, 6 eyes with FECD, and 4 eyes with keratoconus. In addition, there were 5 eyes with other types of BK (ie, 1 eye with iridocorneal endothelial syndrome, 1 eye with pseudoexfoliation syndrome, and 3 eyes with BK of an unknown cause).

• **CULTURE OF DONOR CECS:** Donor CECs were obtained from the peripheral area of the donor corneal transplant tissue remaining and then cultured in accordance with our previously reported method.[20] Briefly, the corneal endothelium layer was carefully stripped from the Descemet's membrane of the posterior corneal tissue and then digested at 37°C with 1 mg/mL collagenase A (Roche Diagnostics) for 2 hours. The isolated CECs were then seeded onto a culture dish coated with type I collagen and cultured according to the previously published protocol,[20] with some modifications.[15] The culture medium consisted of Opti-MEM I (Thermo Fisher Scientific), supplemented with 8% fetal bovine serum, 5 ng/mL epidermal growth factor (Thermo Fisher Scientific), 20 μg/mL ascorbic acid (Sigma-Aldrich), 200 mg/L calcium chloride (Sigma-Aldrich), 0.08% chondroitin sulfate (Fujifilm Wako Pure Chemical Corporation), 10 μM Y-27632 (Fujifilm Wako Pure Chemical Corporation), 10 μM SB203580 (Cayman Chemical), and 50 μg/mL gentamicin. The cells were then incubated at 37°C in a humidified atmosphere containing 5% CO2. After reaching confluency at 5 weeks, the cultured cells were passaged at the cell density of 800 cells/mm^2. The cultured donor CECs at passage (P)1 confluence were used for the subsequent flow cytometry analysis, because the number of those cells at P0 confluence was too small to be used for analysis.

• **FLOW CYTOMETRY ANALYSIS OF THE CULTURED DONOR HCECS:** For flow cytometry analysis, the cultured donor HCECs were collected and washed with phosphate-buffered saline to remove any debris or dead cells. The cells were suspended at a concentration of 4×10^6 cells/mL in a fluorescence-activated cell sorting buffer (phosphate-buffered saline containing 1% bovine serum albumin and 0.05% NaN_3). The single-cell suspension was incubated at 4°C for 2 hours with the antibodies of interest, including appropriate isotype controls to account for nonspecific binding of the antibodies. After washing the cultured HCECs with fluorescence-activated cell sorting buffer, a FACSCanto II Flow Cytometry Analyzer System (BD Biosciences) was used to analyze the cells and evaluate the content of mature-differentiated HCECs (ie, cell surface markers CD166$^+$, CD44$^{-/dull}$, CD24$^-$, and CD105$^-$). The antibodies were the following: E-conjugated anti-human CD166 mAb, PerCP-Cy 5.5 conjugated anti-human CD24 mAb, PE-Cy 7-conjugated anti-human CD44 mAb (all from BD Biosciences), and APC-conjugated anti-human CD105 monoclonal antibody (Thermo Fisher Scientific).

• **SURGICAL TECHNIQUE:** All patients were surgically treated under general anesthesia (or retrobulbar anesthesia in cases with a history of respiratory, heart, or kidney problems). The donor corneas used in this study were obtained from CorneaGen Eye Bank. The techniques used for all DSAEK and PK surgeries were as previously described.[21,22] All DSAEK flaps were prepared by CorneaGen before being shipped to Japan. For DSAEK, the Descemet's membrane at the central posterior cornea was removed, leaving the posterior stroma intact. The DSAEK flap was cut with a Barron Vacuum Donor Cornea Punch (Katena Products, Inc) or a Moria One Corneal Vacuum Punch (MORIA, Inc) inserted through a 4-mm limbo-cornea incision into the anterior chamber using a Busin glide and positioned over the posterior stroma. An air bubble was injected to fill the

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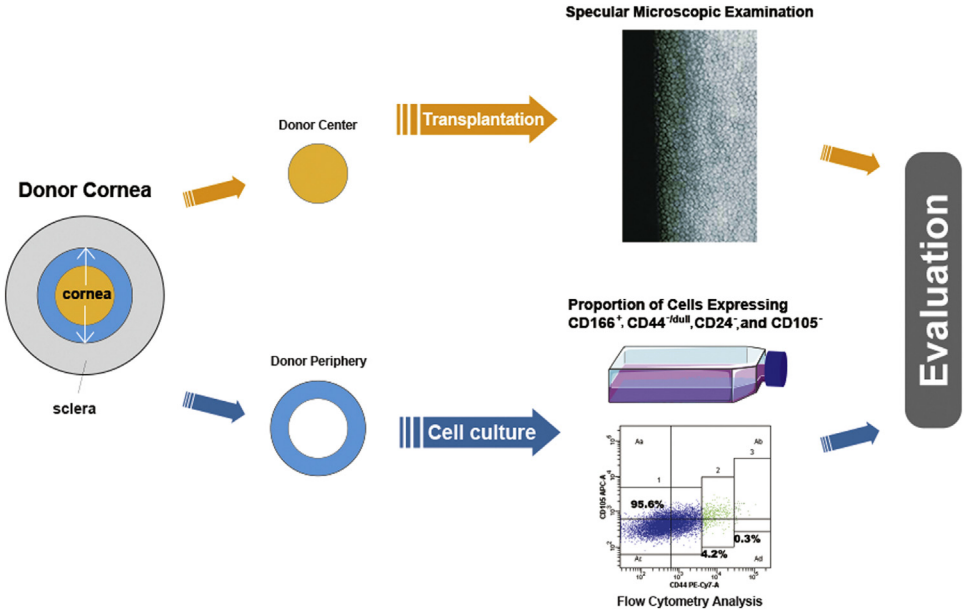

FIGURE 1. Schematic of biological evaluation of donor corneas and transplanted corneas. The relationship between the postoperative corneal endothelial cell density (ECD) and the maturity of cultured corneal endothelial cells (CECs) from the peripheral donor cornea was investigated.

anterior chamber and maintain pressure against the transplanted donor corneal graft. For PK, a circular incision in the host cornea was made using a Hessburg-Barron Vacuum Trephine (Katena Products) or a Moria One Single-Use Adjustable Vacuum Trephine (MORIA). The host cornea was surgically replaced with the donor corneal graft 0.25 mm oversized or the same size cut with a vacuum punch (Katena Products or MORIA). The donor graft was sutured to the host cornea with 8 interrupted sutures, followed by 1 continuous suture with 10-0 nylon. Cataract extraction was performed by phacoemulsification and aspiration, followed by intraocular lens (IOL) implantation or transscleral suture of the IOLs with 10-0 polypropylene before DSAEK or PK, if needed.

• **POSTOPERATIVE MANAGEMENT:** Postsurgery, the patients were closely monitored for any signs of complications, such as high IOP, infection, or allograft rejection. The postoperative medication regimen included topical application of 0.3% gatifloxacin and 0.1% betamethasone eye drops 4 times daily for the first 6 months and then tapered to 0.1% fluorometholone eye drops 2 to 4 times daily. All participants continued topical steroid treatment during follow-up. Systemic steroid treatments were administered immediately before surgery (ie, 125 mg of methylprednisolone), with 4 mg betamethasone administered for the first 2 days postoperatively, followed by 1 mg betamethasone for another 5 days postoperatively, as we previously reported.[21]

• **CLINICAL DATA COLLECTION:** Patient data were collected from electronic medical records, including demographic information, medical history, surgical details, and postoperative outcomes. Preoperative characteristics included age, sex, diagnosis, and a history of glaucoma surgery, and postoperative outcomes included ECD, ECL, and graft survival. In each patient, ECD was measured via the use of a noncontact specular microscope (EM-3000; TOMEY Corp). The specular microscopic images were captured in high-quality images, and the ECD was calculated automatically through an internal program. In cases where the images were insufficient for analysis, they were immediately recaptured on the same day of seeing patients. The primary outcome was transparent graft survival, defined as the absence of graft failure or retransplantation 5 years postoperatively. The exploratory outcomes included ECD and ECL at various postoperative time points until 36 months when all the grafts remained transparent (Figure 1).

To investigate the donor characteristics, we examined the donor's age, sex, trephination size, cause of death (ie, acute deaths: heart disease, cerebrovascular disorders, acute respiratory failure [eg, asphyxia], and chronic deaths: malignant tumors, chronic liver diseases, and other), duration of donor cell preservation, and postmortem days of the donor cornea. The biological quality of the donor cornea was evaluated by HCEC culture at 5 weeks after the P1 culture and was determined according to the expression pattern of surface markers (ie, $CD166^{+}$, $CD44^{-/dull}$, $CD24^{-}$, and $CD105^{-}$)[15] as previously described[19] (Figure 1); high-maturity group: >70% content of mature-differentiated HCECs; middle-maturity group: 10% to 70% content of mature-differentiated HCECs; and low-maturity group: <10% content of mature-differentiated HCECs.

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• **STATISTICAL ANALYSIS:** Statistical analyses were performed using Prism 9 version 9.2.0 (283) (GraphPad Software) statistical analysis software. Normality assumption for samples was examined with the Kolmogorov–Smirnov test. Differences in recipient characteristics, donor characteristics corresponding to each recipient, and recipient and postoperative ECDs among groups were analyzed using the Kruskal–Wallis test or chi-square test. The correlation between the donor age and the percentage of high-maturity HCECs was assessed with Pearson correlation coefficient.

The ECD and ECL at 36 months postoperatively was analyzed with the Mann–Whitney test according to the maturity of donor CECs. In addition, according to the donor maturity, the statistical significance was analyzed with a log-rank test in Kaplan–Meier graft survival curves and in cases in which ECD was maintained at 1000 cells/mm^2 or above. A P value of $< .05$ was considered statistically significant.

RESULTS

• **DEMOGRAPHIC DATA:** We initially compared the glaucoma-bleb eyes with the control eyes to investigate whether there were any differences in patient background and the donor factors that were assessed (ie, age, gender, donor ECD, trephination size, donor CEC preservation time, postmortem days of the donor cornea, and cause death). No significant difference in any of the factors was found between the 2 groups. Recipient characteristics such as patient age, gender, and surgical technique used for corneal transplantation were examined, and no significant differences were found between the 2 groups (Table 1). Moreover, we did not observe any patients who experienced allograft rejection and required additional glaucoma surgery due to uncontrolled IOP during follow-up. We also investigated the relationship between donor age and the percentage of high-maturity HCECs. However, there was no significant association ($P = 0.38$) (Figure S1).

• **POSTOPERATIVE ECD AND ECL IN THE EYES WITH OR WITHOUT PREVIOUS GLAUCOMA SURGERY:** We evaluated ECD and ECL in patients with or without previous glaucoma surgery. Because all of the patients included in this study had a clear corneal graft for up to 36 months postoperatively, ECD and ECL at 36 months postoperatively were analyzed. At 36 months postoperatively, ECD in the control eyes was 1422 ± 597 cells/mm^2, whereas ECD in the glaucoma-bleb eyes was 1036 ± 403 cells/mm^2, which was significantly lower ($P < .05$) (Figure S2). Next, ECL at 36 months postoperatively was examined and found to be 48.6% ± 21.3% in the control eyes and 61.8% ± 15.8% in the glaucoma-bleb eyes. Although not statistically significant ($P = .05$), ECL was greater in the glaucoma-bleb eyes (Figure S2).

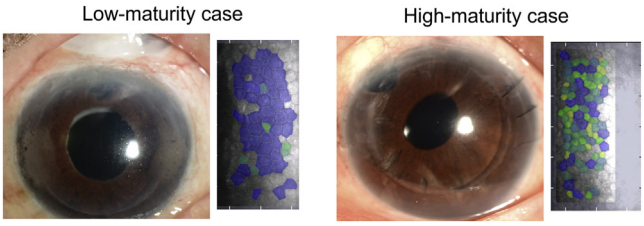

FIGURE 2. Slit-lamp and specular microscopy images of cases with previous glaucoma surgery in the low- and high-maturity groups. Slit-lamp and specular microscopy images of the glaucoma-bleb eyes at 36 months postoperatively in the low-maturity group (left) and the high-maturity group (right). The representative cases showed clear donor corneas in both groups, yet the mean ECD at 36 months postoperatively was 728 cells/mm^2 in the low-maturity group and 1577 cells/mm^2 in the high-maturity group.

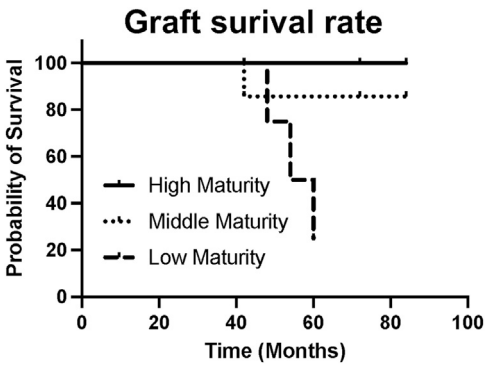

FIGURE 3. Kaplan–Meier graft survival curve in patients with previous glaucoma surgery according to the maturity of donor CECs. The overall graft survival curves were plotted for glaucoma-bleb eyes based on the maturity of the donor CECs.

• **POSTOPERATIVE GRAFT SURVIVAL IN THE DONOR CORNEAS WITH CONTENTS OF THE DIFFERENT MATURITY OF CECS IN THE GLAUCOMA-BLEB EYES:** We previously reported that the donors with a high proportion of lower-maturity HCECs displayed a significantly lower postoperative ECD, even after successful corneal transplantation.[19] At 36 months postoperatively, all the transplanted corneas remained transparent, even in the eyes with glaucoma-bleb, but ECD showed differences among the maturity level of donor CECs (Figure 2). Thus, we plotted graft survival curves for eyes with glaucoma-blebs to examine the graft survival rate according to the maturity of CECs. Glaucoma-bleb eyes included 5 eyes in the high-maturity group, 7 eyes in the middle-maturity group, and 5 eyes in the low-maturity group. Extending the follow-up period up to 84 months postoperatively, the overall graft survival rate at 5 years postoperatively was 50%, with the high-maturity group showing a 100% survival rate and the low-maturity group showing a 25% survival rate (log-rank test, $P < .05$) (Figure 3). Cases after only DSAEK showed

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TABLE 1. Characteristics of Donor and Recipient

	Control	Glaucoma-Bleb	*P* Value
Donor			
Age, mean (SD), y	61.6 (9.3)	59.6 (9.6)	.429
Range	23-74	30-71	
Female, No. (%)	26 (51)	10 (59)	.780
ECD, mean (SD), cells/mm^2	2774 (299)	2759 (231)	.755
Range	2503-3543	2510-3207	
Cause of death (acute/chronic)	31/20	14/3	.142
Death to preservation mean (SD), min	763 (353)	841 (312)	.351
Range	210-1430	499-1310	
Postmortem days mean (SD)	6.1 (1.0)	6.1 (0.9)	.821
Range	4-9	5-8	
Trephination sizes (SD), mm	7.90 (0.23)	8.00 (0.41)	.730
Range	7.25-8.5	7.0-9.0	
Recipient			
Age, mean (SD), y	66.7 (14.3)	72.4 (10.4)	.163
Range	26-94	51-86	
Female, No. (%)	26 (51)	10 (59)	.780
Surgical procedure, No. (%)			
DSAEK/DSAEK + IOL	24 (47)	12 (71)	.160
PK/PK + IOL	27 (53)	5 (29)	.160
Total	51	17	

DSAEK = Descemet's stripping automated endothelial keratoplasty; ECD = endothelial cell density; IOL = intraocular lens; PK = penetrating keratoplasty.

TABLE 2. Endothelial Cell Density Over Time After Successful Corneal Transplantation in Patients With or Without Previous Glaucoma Surgery

	Control			ECD, *P* Value	Glaucoma-Bleb			ECD, *P* Value
	High Maturity	Middle Maturity	Low Maturity		High Maturity	Middle Maturity	Low Maturity	
ECD								
Baseline	2688 (201)	2838 (358)	2734 (240)	.517	2710 (199)	2810 (262)	2736 (250)	0.667
12 mo	2050 (326)	2085 (545)	1706 (553)	.119	1999 (198)	1693 (384)	1725 (572)	0.515
24 mo	2071 (343)	1896 (545)	1238 (518)	<.001	1591 (352)	1295 (458)	1195 (626)	0.721
36 mo	1774 (353)	1528 (624)	931 (387)	<.001	1197 (351)	1052 (421)	853 (430)	0.495
Total	12	25	14		5	7	5	

ECD = endothelial cell density.
Statistical analysis for multiple comparison was performed with Kruskal–Wallis test.

a similar trend (2 eyes in high-maturity group, 6 eyes in middle-maturity group, and 4 eyes in low-maturity group), although there was no significant difference because of low statistical power (log-rank test, $P = .11$) (Figure S3).

• **POSTOPERATIVE ECD AND ECL ACCORDING TO THE MATURITY OF DONOR CECS:** To investigate the differences in maturity level of CECs, we analyzed postoperative ECD and ECL at 36 months postoperatively in both the glaucoma-bleb eyes and the controls. ECD over the time period after corneal transplantation is summarized in Table 2. Control eyes included 12 eyes in the high-maturity group, 25 eyes in the middle-maturity group, and 14 eyes in the low-maturity group (Table 2). In the glaucoma-bleb eyes, the mean ECD and ECL, respectively, was 1197 ± 352 cells/mm^2 and 55.5% ± 13.9% in the high-maturity group, 1052 ± 421 cells/mm^2 and 62.0% ± 16.0% in the middle-maturity group, and 853 ± 430 cells/mm^2 and 67.7% ± 18.1% in the low-maturity group. In the control eyes, the mean ECD and ECL, respectively, was 1774 ± 353 cells/mm^2 and 33.4% ± 15.4% in the high-maturity group, 1528 ± 629 cells/mm^2 and 46.2% ± 21.0% in the middle-maturity group, and 931 ± 387 cells/mm^2 and 66.0% ± 13.8% in the low-maturity group (Figures 4

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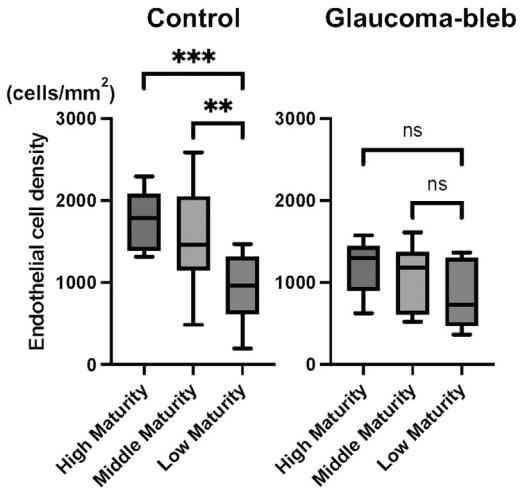

FIGURE 4. ECD at 36 months after corneal transplantation according to the maturity of donor CECs. ECD at 36 months postoperatively in the control eyes (left) and the glaucoma-bleb eyes (right) based on the high-maturity (control; n = 12, bleb; n = 5), middle-maturity (control; n = 25, bleb; n = 7), and low-maturity (control; n = 14, bleb; n = 5) donor CECs. The upper and lower edges of each box represent the interquartile range (25th-75th percentile). The upper bar indicates the maximum value, and the lower bar indicates the minimum value. ***P < .001, **P < .01. ns = not significant.

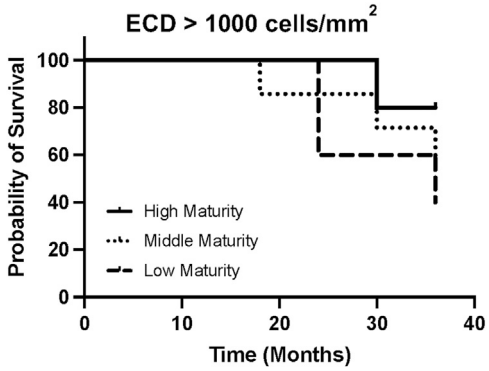

FIGURE 5. Kaplan–Meier survival curve graph of the cases in which an ECD of 1000 cells/mm^2 or greater was maintained. The overall survival curves were plotted for glaucoma-bleb eyes based on an ECD threshold of 1000 cells/mm^2, and the eyes were grouped according to the maturity of the donor CECs. In all cases, graft clarity was maintained for up to 36 months after corneal transplantation. ECD = endothelial cell density.

and S4). With donors that contained low-maturity CECs, the control eyes showed significantly lower ECD than the high-maturity and middle-maturity groups (P < .001 and P < .01). No significant differences were found among the different maturity groups in the glaucoma-bleb eyes; however, ECD seemed to be lower in the low-maturity group than the high-maturity group in the control group (Figure 4), which was similar to DSAEK eyes (Figure S5). ECL displayed the same trend as ECD, showing significantly higher ECL in the low-maturity group compared with the high-maturity and middle-maturity groups in the control eyes (P < .001 and P < .05), but no significant differences in the glaucoma-bleb eyes among the maturity groups (Figure S4).

• **POSTOPERATIVE SURVIVAL RATE OF ABOVE 1000 CELLS/MM^2 IN THE GLAUCOMA-BLEB EYES:** Because graft clarity was maintained in all cases up to 36 months postoperatively, we further investigated the survival rate of glaucoma-bleb eyes with an ECD threshold of 1000 cells/mm^2 among the different maturity groups. At the 36-month postoperative time point, the high-maturity group demonstrated a ECD of >1000 cells/mm^2 in 80% (4 of 5 eyes) of the grafts, whereas the low-maturity group demonstrated a ECD of >1000 cells/mm^2 in only 40% (2 of 5 eyes) of the grafts (log-rank test, P = 0.33) (Figure 5). The analysis of glaucoma-bleb eyes after only DSAEK showed 2 (100%) of 2 eyes in the high-maturity group achieved an ECD of >1000 cells/mm^2, but 4 (66.7%) of 6 eyes in the middle-maturity group and 2 (50.0%) of 4 eyes in the low-maturity group (Figure S6).

DISCUSSION

After corneal transplantation, the ECD progressively decreases, especially in glaucoma-bleb eyes, which typically have a poor prognosis and exhibit a more rapid decrease of ECD. Consistent with the findings in the previous reports,[12,19] the findings in the present prospective study revealed that at 36 months postoperatively, ECD was significantly lower and ECL was significantly greater in the glaucoma-bleb eyes. Although no significant difference in postoperative ECD was observed in the low- and middle-maturity groups, it was lower in the glaucoma-bleb eyes compared with the control eyes. Moreover, at 36 months postoperatively, an ECD of >1000 cells/mm^2 was maintained in a higher proportion of grafts in the high-maturity group. In addition, at the maximum postoperative follow-up period of 84 months, long-term graft survival was observed in the high-maturity group, whereas early graft failure was observed in the low-maturity group.

We are currently in the process of refining a new therapeutic modality for the treatment of BK that involves the injection of cultured HCECs and have reported the clinical outcomes of the first 11 cases that have undergone the therapy at 2 and 5 years postoperatively.[20,23] In line with findings of Ueno and colleagues,[24] we compared the first 11 cases with the subsequent 7 cases, which contained different proportions of mature-differentiated HCECs be-

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tween the 2 groups. Our findings revealed that even at 3 years postoperatively, the mean ECD in the subsequent 7 cases, with a higher proportion of mature-differentiated HCECs, was maintained at 3083 cells/mm^2, which was significantly higher than that of the first 11 cases (ie, 1349 cells/mm^2). This finding suggests that the content of the mature-differentiated cells has a distinct biological effect on the HCECs, thus suggesting that the donors with a higher proportion of mature-differentiated HCECs in our study contributed to the better clinical outcomes in the glaucoma-bleb eyes.

Previous studies have extensively investigated the recipient factors (ie, the "soil factors") that affect ECD or ECL after corneal transplantation, and multiple studies have reported on the postoperative outcomes in glaucoma-bleb eyes.[11,13,25-30] Moreover, it has been reported that in eyes with iris damage, there is a rapid decrease in ECD due to elevated cytokine levels in the aqueous humor.[31-34] In glaucoma-bleb eyes, iris damage is commonly observed, and changes in anterior chamber flow, as well as iris alterations, may possibly contribute to the decrease in ECD postsurgery. However, and as the findings in our study demonstrate, even in the patients with unfavorable factors in the anterior chamber, such as a history of undergoing glaucoma surgery, the rate of graft survival may be extended if the donor corneas contain a higher proportion of mature-differentiated HCECs. Of note, the findings in the 2011 Corneal Donor Study by Lass and colleagues[35] revealed that female donors were associated with a more favorable ECD postsurgery. However, the findings in this present study did not reveal any correlation between female donors and higher maturity content in the donor corneas.

As to whether or not the mature cells differ functionally, the findings in the previous reports by Hamuro and colleagues[17] revealed that low mature-differentiated HCECs exhibit a distinct intracellular metabolism characterized by changes in microRNA profiles,[36,37] including anaerobic glycolysis.[17] Moreover, those cells exhibit upregulation of genes associated with epithelial-mesenchymal transition, cellular senescence, and fibrosis under glucose starvation[17] and downregulation of proteins associated with ion transporters and water transporters,[18] thus indicating potential functional differences. It is important to note that although highly mature HCECs are functionally competent and exhibit advanced differentiation characteristics, they are different from "senescent" HCECs. Senescent cells have undergone irreversible growth arrest and may have diminished functionality. These findings suggest that donor corneas with a higher proportion of mature-differentiated HCECs may have fewer senescent cells, whereas donor corneas with a lower proportion of mature-differentiated HCECs may have a higher number of senescent cells, as indicated by the differential gene expression patterns (ie, the "seeds factors"). Furthermore, our previous reports showed that mature-differentiated HCECs function normally with mitochondria-dependent oxidative phosphorylation.[18,38] Additionally, we previously showed that the "spring constant" of the CECs, which can be calculated from phase-contrast images, enabled the quality assessment of in vitro cell sources for cell-injection therapy.[39] This suggests the potential of endothelial specular microscopy as a tool to assess CEC maturity.

We demonstrated the importance of the CEC maturity in our previous report.[19] In the present study exploring the specific role of endothelial cell maturity in graft survival, particularly in patients with prior glaucoma surgery, donor corneas with a higher proportion of mature-differentiated CECs showed a favorable graft survival rate (Figure 3). However, it should be noted that this present study did have some limitations. First, the number of glaucoma-bleb eyes was relatively small (ie, only 17 of the 68 eyes). We analyzed DSAEK cases alone to minimize the surgical bias, but statistical limitations among the maturity groups still remain (ie, lack of statistical power). Thus, further investigation with a number of cases is needed to make a precise conclusion. Second, the assessment of donor cornea maturity was based on interpretation of results from donor cell culture experiments, which may not be as ideal as directly analyzing donor corneas without cell culture, and the amount of remaining peripheral corneal endothelium that could be used for analysis was limited. Nevertheless, and as we previously reported, mature cultured HCECs significantly influence rapid recovery of corneal thickness and long-term maintenance of high ECD post-HCEC injection therapy,[24] thus adding support to the validity of the findings in this present study. Third, the inclusion of eyes with implanted glaucoma drainage devices in addition to trabeculectomy in the evaluation of previous glaucoma surgery is warranted in future studies. Last, the time-consuming nature of cell culture–based evaluation, which takes approximately 1 month, highlights the need for identifying new biomarkers for early determination of donor cornea maturity to facilitate translation of study results into clinical practice, which allow eye banks to adopt to enhance the viability and success of corneal transplantation. Thus, we view the findings in this current study to be a crucial initial step toward achieving that goal.

In conclusion, the rates of longer-term graft failure remain elevated in patients with a history of undergoing glaucoma surgery. However, and as the findings in this present study demonstrate and the "seeds and soil theory" conceptually supports, donor corneas with a higher proportion of mature-differentiated CECs could result in improved surgical outcomes even in glaucoma-bleb eyes.

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