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Full Length Research Paper

Endothelial cell loss and graft survival after Descemet stripping automated endothelial keratoplasty (DSAEK): 6-year follow-up

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Purpose: **To evaluate graft survival and central endothelial cell density (ECD) loss after Descemet stripping automated endothelial keratoplasty (DSAEK) during a six-year follow-up and to identify factors related with higher postoperative ECD loss.** ***Methods:*** **This prospective, non-comparative, observational case series study included 91 consecutive cases of DSAEK by a surgeon in 80 subjects (mean age 73.6±11.2 years, range 35-95). ECD measurements were available in 52 eyes at 6 months, and 15 eyes of 15 patients at 6 years. Preoperative ECD was obtained from the eye bank charts; postoperative central ECD was assessed using non-contact specular microscope during 6 years. Pre- and post-operative data were compared using paired *t*-test. Multivariate analysis of variance was used to identify factors associated with donor ECD loss at 6 months postoperatively.** ***Results:*** **Of the 91 eyes undergoing DSAEK procedure, 19.8% of eyes experienced primary graft failure and 5.5% late graft failure. The overall graft survival during a six-year follow-up was 74.7%. The donor ECD progressively decreased after surgery. The mean percentage of ECD loss from before to after surgery increased form 49.9±15.1% 6 months (n=52 eyes) to 72.3±8.8% 6 years postoperatively (n=15 eyes). Predictors of the postoperative ECD loss included: donor ECD (p=0.02); donor endothelial cell polymorphism degree (p=0.004); and donor death-to-use time (p=0.02).**

Keywords: Descemet stripping automated endothelial keratoplasty (DSAEK), non-contact specular microscopy; graft survival, central endothelial cell density, corneal graft.

INTRODUCTION

Descemet stripping automated endothelial keratoplasty (DSAEK) was introduced in 2006 (Gorovoy, 2006; Price and Price, 2006), and since then, it has become the preferred surgical method for treating endothelial dysfunction (Patel, 2012). The DSAEK technique involves mechanical stripping of diseased host endothelium and Descemet's membrane and replacement with a donor graft of endothelium, Descemet's membrane, and a thin layer of posterior stroma harvested with an automated microkeratome (Gorovoy, 2006; Price and Price, 2006). The advantages of DSAEK over penetrating keratoplasty (PK) include faster postoperative visual recovery with minimal refractive changes, lower rate of graft rejection,

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limited damage to the corneal surface and innervation and better tectonic stability (Anshu et al., 2012; Mau, 2009).

Until now, the greatest concern with DSAEK had been the intra-operative endothelial damage, graft dislocation and early graft failure induced by endothelial injury (Price and Price, 2006; Koenig et al., 2007; Price and Price, 2008; Terry et al., 2008). Consistent with higher DSAEK donor tissue manipulation (Price and Price, 2008; Terry et al., 2008; Terry et al., 2007), the endothelial cell density (ECD) loss determined 6 months after DSAEK is substantially greater than that recorded after modern PK surgery (Price and Price, 2008; Terry et al., 2008; Busin et al., 2008), although the gap between the two techniques seems to reduce at 1 year after surgery (Price and Price, 2008; Terry et al., 2008; Bahar et al., 2008), and tends to disappear 2-3 years postoperativelty (Price and Price, 2008; Terry et al., 2008; Price and, Price, 2009; Price et al., 2013). There is limiting data in literature based on the long-term survival of the donor graft after DSAEK, however, recent studies have suggested that the rate of cell loss between 3 and 5 years remains lower with DSAEK than with PK, resulting in lower cumulative 5-year cell loss with DSAEK (Price et al., 2011; Ratanasit and Gorovoy, 2011).

The purpose of the present study was to evaluate the long-term survival of endothelial cells after DSAEK and to identify factors associated with higher postoperative ECD loss.

METHODS

This prospective, non-comparative, observational case series study included 91 consecutive cases of DSAEK performed by a single surgeon in 80 consecutive subjects (mean age of 73.6±11.2 years, range 35-95) affected by corneal endothelial dysfunction. One eye was considered in 69 patients; both eyes were considered in 11 patients that underwent surgery for each eye at different time points (minimum period of 6 months between surgeries). The study was in compliance with the tenets of the Helsinki's Declaration, and informed consent was obtained from all participants prior to testing. The study was in compliance with the Institutional Review Boards (IRBs) and HIPAA requirements, and was approved by the IRB of the Azienda Ospedaliero-Universitaria "S. Maria della Misericordia", Udine, Italy. Inclusion criteria were: good image quality with non-contact specular microscopy (NCSM); and willingness to provide informed written consent. Exclusion criteria included: insufficient NCSM image quality; postoperative follow-up less than 6 months.

All surgeries were performed by a single surgeon (PB) at the Department of Ophthalmology of the S. Maria della Misericordia Hospital, Udine, Italy, between September 2007 and October 2013, and they were the first surgical DSAEK cases for the surgeon. Donor corneas in the form of a sclera-corneal button stored in organ culture at 31°C were provided by the "Fondazione Banca degli Occhi del Veneto" (Venezia-Mestre, Italy) Eye Bank.

DSAEK technique used in the present study has extensively been reported elsewhere (Price and Price, 2006). Briefly, the donor lamellar graft dissection was performed with a hand-driven microkeratome using the Moria automated lamellar therapeutic keratoplasty (ALTK) microkeratome (model Evolution 3E) equipped with a 350-micron-deep blade and associated artificial anterior chamber (Moria, Antony, France). After dissection, the anterior corneal cup was discarded. At the beginning of surgery, the posterior corneal lamellar tissue was transferred to a punching system and was punched from the endothelial side using an 8.5-mm Hanna punch trephine (Moria, Antony, France). The donor corneal lenticule remained resting on the donor punching block covered by Optisol solution until use. After a peribulbar anesthesia, a clear corneal temporal incision was made in the host with a 2.75-mm keratome. Two paracentesis were made at the 7- and 10-o'clock positions. A paracentesis was made 2 hours clockwise from the corneal incision to allow the positioning of an anterior chamber (AC) maintenance cannula. The host endothelium and the Descemet's membrane were scored in a circular pattern for a diameter of 8.5 mm, using a reverse-bent Price-Sinskey hook (Asico, Westmont, IL). The Descemet's membrane and the endothelium were stripped using a Sinskey hook and spread on the anterior surface of the recipient cornea to make sure a sufficient area had been removed. The clear corneal incision was widened to approximately 4.2 mm. The donor corneal lenticule was placed on a Busin-glide (Moria USA, Doylestown, Pennsylvania, USA) (endothelial side up) and inserted into the AC using the Price forceps. Unfolding and positioning of the donor lamella were performed using air inserted in the AC with a 30-gauge cannula. After the AC was filled with air for 7-10 minutes, a portion of the air was removed and replaced with balanced salt solution (BSS). The cornea was then massaged with a roller to help remove fluid from the graft interface. The corneal incisions were closed with 10-0 nylon sutures.

After surgery, all patients underwent patching overnight and were instructed to lie supine for at least 6 hours to help press the donor tissue up against the recipient cornea. Post-operative local therapy included 0.1% dexamethasone sodium phosphate and ofloxacin (Alcon Laboratories, Inc. Fort Worth, TX, USA) eye drops, which were administered 4 times daily for 1 week. The antibiotic drops were discontinued 1 week after surgery, and dexamethasone eye drops were tapered for 12 months in all groups.

Each participant underwent post-operative examinations at 6 months, 1, 2,3,4,5 and 6 years. The examination at follow-up included: complete

Table 1. Pre-, intra-, and post-operative data of the recipient eyes.

Findings	Total group		Subgroup	
	n=91 eyes	%	n=52 eyes	%
Preoperative diagnosis				
Fuchs' endothelial dystrophy	37	40.7	27	51.9
Pseudophakic bullous keratopathy	25	27.5	14	26.9
Pseudophakic bullous with IOL in the AC	2	2.2	0	0
Aphakic bullous keratopathy	1	1.1	0	0
Bullous keratopathy in phakic IOL in the AC	4	4.4	3	5.8
Endothelial dysfunction after recidivant keratouveitis	1	1.1	0	0
Endothelial dysfunction after ocular trauma	2	2.2	1	1.9
Endothelial dysfunction after glaucoma surgery	3	3.3	1	1.9
Failed DSAEK	11	12.1	5	9.6
Failed PK	5	5.5	1	1.9
Preoperative lens status				
Phakic	50	54.9	28	53.8
Pseudophakic	41	45.1	24	46.2

ophthalmologic examination, including best corrected visual acuity (BCVA) evaluation, slit-lamp anterior segment examination by a corneal specialist (MLS, FM, PB), and fundus biomicroscopy with a 90-diopter lens; and ECD evaluation with a Tomey noncontact specular microscope (NCSM) EM-3000 (Tomey, Japan). In performing NCSM with the Tomey EM-3000, the subject was positioned on the chin and forehead rest, and asked to fixate on a red target. Upon proper alignment on the center of the cornea, a bright central specular image of the central CEL was obtained. The process was repeated if the endothelial image displayed on the monitor was not in focus. The device is equipped with an auto-focus, digital image capturing system and automated image-analysis software for ECD and central corneal thickness (CCT) assessment. The optical magnification of the device is x190 and the display resolution is 1.14 µm. With regards to the data collection with this instrument, up to 300 cells per image are counted in a fixed area of 0.135 mm^2 (0.25 x 0.54 mm). A proprietary automated cell contour recognition algorithm based on contrast differences and area-based counting technique are used to asses ECD, with internal calibration for magnification.

The same ophthalmic technician performed all postoperative testing of patients, using the same specular microscope each time. The NCSM measurement was repeated 3 times, with a time interval of at least 5 minutes between measurements, and the mean of the three measurements was used for the analysis.

The pre-operative ECD data were obtained from the eye bank clinical chart using specular microscopy before cutting the tissue; post-cut specular microscopy was not performed. The eye bank usually images the donor endothelium within 1 to 2 days of the donor death and provide the donor ECD value and the degree of the donor endothelial cells polymorphism, ranging from 0 (absence of polymorphism) to 2 (moderate polymorphism).

The main outcomes of the study were: 1) graft survival, and 2) postoperative ECD loss. Primary graft failure was defined as failure of the endothelium to function within 2 weeks post-operatively; late graft failure was defined as a gradual decompensation of the endothelium, with irreversible loss of optical clarity and visual acuity for a minimum of 3 consecutive months, which may manifest months to years after surgery (Thompson et al., 2003).

Pre- and post-operative data were compared using the paired *t*-test; inter-groups comparisons were performed using the unpaired *t*-test. The Bonferroni correction was used for multiple comparisons. Multivariate analysis of variance was used to identify the pre-, intra- and post-operative variables associated with postoperative donor ECD loss. The statistical analysis was performed using SPSS 20.0 (Chicago, IL) and the Minitab Statistical Software. Statistical significance was defined as $p<0.05$.

RESULTS

The pre-, intra-, and post-operative data of the 91 recipient eyes are listed in Table 1. Of the 91 eyes: 7 eyes (7.7%) were lacking data for all follow-up examinations; 18 eyes (19.8%) experienced primary graft failure; and, 14 eyes (15.4%) had unanalyzable endothelial NCSM images despite clear grafts. Complete ECD measurements during a six-year follow-up were available in a subgroup of 52 eyes of 46 patients (mean age of 73.1±10.2 years, range 42-89 years, male/female=17/29). Out of these 52 eyes, 5 eyes experienced late graft failure during the follow-up, occurring between 12 and 36 months after surgery. The overall graft survival during a six-year follow-up was

Table 1 continue

Associated ocular phatologies				
Senile cataract	44	48.4	20	38.5
Primary or secondary glaucoma	20	22.0	11	21.2
Filtering bleb after trabeculectomy	10	11.0	2	3.8
Age-related macular degeneration	11	12.1	8	15.4
Keratoconjunctivitis sicca	7	7.7	5	9.6
Degenerative myopia	7	7.7	4	7.7
Cystoid macular oedema	3	3.3	2	3.8
Macular pucker	2	2.2	0	0
Amblyopia	2	2.2	2	3.8
Aphakia	1	1.1	0	0
Keratoconus	1	1.1	1	1.9
Post-herpetic leucoma	1	1.1	1	1.9
Irido-corneal sinechiae	1	1.1	0	0
Surgical procedures				
DSAEK	51	56.0	24	46.2
DSAEK + cataract phacoemulsification + IOL	24	26.4	20	38.5
DSAEK + phakic IOL extraction	4	4.4	3	5.8
DSAEK donor lenticule replacement	11	1.1	5	9.6
DSAEK + scleral fixation IOL	1	1.1	0	0
Introperative complications				
Donor tissue perforation	1	1.1	0	0
Postoperative complications				
Ocular hypertension	24	26.4	13	25.0
Angle-closure glaucoma	3	3.3	2	3.8
Chronic epitheliopathy	9	9.9	5	9.6
Lenticule peripheral wrinkles	7	7.7	6	11.5
Interfacies opacities	7	7.7	6	11.5
Donor lenticule abscess	1	1.1	0	0
Partial graft decentration	6	6.6	3	5.8
Partial peripheral graft detachment	4	4.4	2	3.8
Total graft detachment	11	12.1	6	11.5
Without donor reposition after rebubbling	5	5.5	0	0
With donor reposition after rebubbling	6	6.6	6	11.5
Graft rejection	4	4.4	3	5.8
Primary graft failure	18	19.8	0	0
With donor detachment persistent after rebubbling	5	5.5	0	0
Without donor detachment	13	14.3	0	0
Late graft failure	5	5.5	5	5.5
After graft rejection episode	1	1.1	1	1.9
In absence of documented graft rejection	4	4.4	4	7.7
Postoperative surgical procedures				
Successfull rebubbling with donor reposition	6	6.6	6	11.5
Unsuccessfull rebubbling without donor reposition	5	5.5	0	0
Regrafted with DSAEK	12	13.2	4	7.7
Regrafted with PK	8	8.8	1	1.9

IOL=intraocular lens; AC=anterior chamber; DSAEK=Descemet's stripping automated keratoplasty; PK=penetrating keratoplasty.

Table 2. Donor Variables (52 donor leticules)

Variables	mean ± SD (range)
Donor age (years)	62.0 ± 9.6 (38 - 74)
Donor sex (M/F)	40/12
Donor death-to-preservation time (hours)	11.5 ± 6.5 (2 - 25)
Donor death-to-use time (days)	17.1 ± 5.7 (6 - 32)
Donor tissue quality (score)	0.75 ± 0.68 (0 - 2)

SD= standard deviation; M=male; F=female

Table 3. Pre- and post-operative endothelial cell density (ECD) and ECD loss in comparison with pre-operative data.

	ECD (cell/mm^2)	ECD loss (%)
(n. of eyes)	mean ± SD 95% CI	mean ± SD 95% CI
pre-operatively	2562.2 ± 152.4	-
(n=52)	2300 - 2800	-
6 months post-op	1239.9 ± 406.7	49.9 ± 15.1
(n=52)	590.8 - 1952.4	27.6 - 78.1
1 year post-op	1223.8 ± 430.8	50.6 ± 15.5
(n=51)	583.8 - 1963.3	29.0 - 77.6
2 years post-op	1086.2 ± 367.3	55.3 ± 13.1
(n=45)	558.7 - 1742.2	34.7 - 79.1
3 years post-op	1023.2 ± 357.9	57.8 ± 12.4
(n=37)	525.8 - 1574.7	42.7 - 79.4
4 years post-op	844.3 ± 333.4	64.6 ± 12.3
(n=30)	552.7 - 1505.3	45.1 - 80.6
5 years post-op	700.8 ± 196.4	69.9 ± 9.4
(n=21)	494.3 - 976.8	61.7 - 81.2
6 years post-op	641.5 ± 187.6	72.3 ± 8.8
(n=15)	507.8 - 833	69.3 - 80.9

SD = standard deviation; CI = Confidence Interval

74.7%. The recipient variables of these 52 eyes and the donor variables and are reported in Tables 1 and 2, respectively.

Compared with the pre-operative data, the mean ECD significantly decreased 6 months after surgery (p=0.0001) (Table 3). ECD tended to reduce progressively post-operatively, which appeared to be statistically significant between 1 and 2 years (p=0.007), between 3 and 4 years (p=0.019) and between 4 and 5 years (p=0.016). The ECD loss progressively increased postoperatively (Table 3). The increase was significant between 1 and 2 years (p=0.008), between 3 and 4 years (p=0.018) and between 4 and 5 years (p=0.017).

When the first group of consecutive 45 cases were compared with the later group of 46 consecutive cases, both the rate of primary graft failure (20% vs 17.4%) and the ECD loss at all time points were not statistically different (chi-square test=0.67; unpaired *t*-test >0.05; data not shown).

Table 4 shows the independent variables used in the multiple stepwise linear regression analysis versus the postoperative ECD loss considered as dependent variable at the different follow-up time points. At 6 months post-operatively, the statistically significant variables included: donor ECD (inverse relationship, p=0.02, adjusted R^2=0.23); donor endothelial polymorphism degree (p=0.004, adjusted R^2=0.36); and donor death-to-use time (p=0.02, adjusted R^2=0.12). The relationship amongst dependent and independent variables at 1-6 years after surgery were not statistically significant.

Table 4. Independent variables used in the multiple stepwise linear regression analysis versus the postoperative recipient ECD loss

Donor age (years)
Donor endothelial cell density (cells/mm^2)
Donor death-to-preservation time (hours)
Donor death-to-use time (days)
Donor endothelial quality (3 quality scores)
Recipient age (years)
Recipient endothelial dysfunction type (7 types)
Recipient lens status (2 types)
Combined surgical procedure (yes/no)
Postoperative ocular hypertension (yes/no)
Graft decentration (yes/no)
Partial peripheral graft detachment (yes/no)
Graft dislocation (requiring air bubble injection)(yes/no)
Graft rejection (yes/no)

ECD=endothelial cell density

CONCLUSIONS

The ECD of the normal cornea declines with age at a rate of approximately 0.6% per year, which is mainly due to the limited or non- proliferative capacity of the normal adult human corneal endothelial cells (Bourne et al., 1997). The physiological ECD loss is greatly accelerated after penetrating or endothelial keratoplasty (Price and Price, 2008; Terry et al., 2008; Bahar et al., 2008) and can impact graft survival, considering that the post-operative corneal clarity of the donor graft is dependent on the integrity and number of healthy endothelial cells, which should be at least 400 cells/mm^2 (Bourne, 2001). The loss of endothelial cells after PK or DSAEK mainly occurs immediately after surgery, however, continues for numerous years after transplantation. The ECD decline rate tends to increase after graft, suggesting that the host ocular environment may also compromise long-term endothelial cell survival after initial surgical insult of the anterior segment. The exact cause of post-operative cell loss is unknown, however, has been attributed to several factors which include: donor or preservation factors; surgical stress; cellular redistribution and interactions between the donor and the recipient; chronic subclinical immune rejection; persistent low-grade inflammation; accelerated cellular aging; and, glaucoma (Bourne, 2001).

The endothelial cell loss has been shown to be higher after DSAEK than after PK (Price and Price, 2008; Terry et al., 2008; Busin et al., 2008), in the early postoperative period, as expected considering the higher manipulation of the donor lenticule during DSAEK surgery (Price and Price, 2008; Terry et al., 2008; Terry et al., 2007). This difference between the two techniques, however, seems to disappear in time (Price and Price, 2008; Terry et al., 2008; Price and, Price, 2009; Price et al., 2013; Price et al., 2011; Ratanasit and Gorovoy, 2011). The limited quality of the donor graft and/or insult during DSAEK surgery can result in low postoperative ECD count, primary graft failure or donor detachment, considering that a functioning endothelial pump is necessary to promote the adherence with the recipient stroma (Terry et al., 2008).

Of the 91 eyes undergoing DSAEK procedure in the present study, 19.8% of eyes experienced primary graft failure, 5.5% late graft failure and 12.1% donor detachment, 54.5% of which were successfully treated with a rebubbling procedure. The overall graft survival was 74.7% during a six-year follow-up.

The donor dislocation rate in our cohort (12.1%) was comparable to the mean reported rate of 14% after DSAEK, however, there is a large variation amongst studies in literature (Price and Price, 2006; Koenig et al., 2007; Bahar et al., 2008; Koenig and Covert, 2007). The primary graft failure rate in our study (19.8%) appeared higher than that reported by other authors, which ranges from approximately 3 to 12% (Koenig et al., 2007; Bahar et al., 2008; Koenig and Covert, 2007).

The post-DSAEK mean ECD loss found in our study was approximately 50% at 6 months; it significantly and progressively increased postoperatively, reaching 72% at 6 years post-operatively. At this time point, approximately half of the donor lenticules experienced a cell loss of ≥84%, and were found to have an ECD <663 cells/mm^2. The ECD loss reported in our cohort was comparable (Price and Price, 2006; Koenig et al., 2007) or higher (Gorovoy, 2006; Price and Price, 2008; Terry et al., 2008; Busin et al., 2008; Terry et al., 2011; Ang et al., 2012) than that reported by previous authors. Our results, however, are in disagreement with other studies (Price and Price, 2008; Terry et al., 2008), showing that the bulk of ECD loss after DSAEK appeared at the first post-

operative examination typically after 6 months, with minimal subsequent increase at the second follow-up at 1 year, considering that the ECD loss in our patients appeared to increase progressively during the follow-up.

The amount of ECD loss after DSAEK reported in the literature is very different in the various studies; moreover, there are only a few studies currently available that report long-term follow-ups. The mean post-DSAEK ECD loss reported in literature varies, and ranges from: 20% to 50% at 6 months (Koenig et al., 2007; Terry et al., 2008; Busin et al., 2008; Terry et al., 2011). 23% to 36% at 1 year (Price and Price, 2008; Terry et al., 2008; Terry et al., 2007; Busin et al., 2008; Bahar et al., 2008; Price et al., 2013; Terry et al., 2011; Ang et al., 2012); 26% to 41% at 2 years (Price and Price, 2008; Terry et al., 2007; Busin et al., 2008; Price et al., 2013; Terry et al., 2011; Ang et al., 2012); 39% to 48% at 3 years (Price et al., 2013; Ang et al., 2012); and, 51% to 53% at 5 years after surgery (Price et al., 2011; Ratanasit and Gorovoy, 2011).

Several factors have been associated with the rate of primary graft failure, donor detachment and postoperative ECD, including: donor age (Price and Price, 2008); type and duration of the donor tissue storage (Rose et al., 2008); donor preoperative count (Price and Price, 2008; Price et al., 2011) and size (Busin et al., 2008); recipient endothelial dysfunction type (Terry et al., 2008; Price et al., 2013; Price et al., 2011); surgeon experience; donor preparation; incision size (Terry et al., 2008; Price et al., 2013); forceps or injectors type, insertion and unfolding technique (Patel, 2012; Busin et al., 2008; Price and Price, 2006); combined surgical procedure (Terry et al., 2008); graft edge morphology; recipient bed morphology; duration of exposure to air; postoperative elevated IOP; and, technique used to evaluate the pre- and postoperative ECD. Moreover, in cases of graft dislocation, a re-bubbling procedure is normally required, which may reduce the ECD of the donor graft and increase the risk of late endothelial failure (Price and Price, 2008).

The high rate of primary graft failure and postoperative ECD loss found in our study when compared to others reported in literature could theoretically be due to several variables, which include: donor tissue characteristics; recipients inclusion criteria; and, surgical experience. The mean pre-operative ECD in our series ranged from 2300 to 2900 cells/mm^2, which was significantly lower than that required by other surgeons (Hesham and Schultze, 2015). Eyes with pre-existing ocular pathologies that could potentially influence the endothelial cells survival and are known to cause significant ECD loss were not excluded from our analysis; these conditions include glaucoma, filtering blebs, IOL in the anterior chamber, or eyes with dislocation or rejection events. The experience of the surgeon could have deeply influenced our results, considering that our series included the initial learning curve period. The absence of significant differences for the rate of primary graft failure and the ECD loss between the first 45 cases and the next 46 cases could be related to the limiting number of patients included in the our study. A prospective study is currently underway to calculate the primary failure rate and 6-months ECD loss in DSAEK cases done by the same surgeon after this series of cohort.

The only predictors of amount of postoperative ECD loss found in our study were donor factors: ECD; donor endothelial polymorphism degree; and the donor death-to-use time. The adjusted R^2 values reported in our analysis indicate that a significant percentage of variance in the postoperative ECD loss (approximately 36%) can be attributed to donor variables. Intrinsic biological and physiological recipient factors and surgical technique seem to influence graft ECD less than the evaluated preoperative donor tissue characteristics. It is important to note that the donor characteristics were associated with the postoperative ECD loss only at 6 months post-operatively. These data suggest that the host ocular environment may play an important role in the donor endothelial cell survival rate in the late part of the follow-up. The relationship between donor characteristics and postoperative ECD is not fully understood yet. The relationship between baseline donor ECD and postoperative ECD loss was found to be significant by some authors (Price and Price, 2008; Price et al., 2011), yet not significant by others (Hesham and Schultze, 2015; Terry et al., 2008). The influence of storage time reported in literature is variable; some found this factor to be significant (Rose et al., 2008), while others not (Price and Price, 2008; Terry et al., 2011; Hesham and Schultze, 2015; Terry et al., 2008). The association between donor characteristics and increased endothelial cell loss needs further clarification, in order to support the value of donor-specific requests to ensure higher donor ECD survival, especially considering the small cohort of patients included in our analysis.

Our study adds to current literature in this field, especially considering the data based on the long-term results of DSAEK surgery performed by a single surgeon with a single surgical technique. There are, however, several limitations to our study. The case series was limited in number, and the initial learning curve period of the surgeon was included. Our exclusion criteria were not stringent, thus recipient characteristics that could negatively influence postoperative ECD survival were included in the study. It is important to note that any study based on the assessment of ECD loss pre- and postoperatively may include data that may not necessarily reflect a true numerical decrease due to several confounding factors, including: the different counting methods used in the eye bank and post-operative clinical setting; observer bias that may influence and vary with every counting method; the different part of the cornea used to obtain the ECD, etc.

In conclusion, we found the ECD loss to be substantial

during the six-year follow-up after successful DSAEK. In the first 6 months after surgery, the ECD loss appeared associated with donor characteristics, including ECD, polymorphism degree and death-to-use time. Further studies based on a larger cohort of patients and longer follow-up are needed to determine the clinical importance of these associations and the impact these factors have on graft survival.

REFERENCES

Ang M, Mehta JS, Lim F, Bose S, Htoon HM, Tan D (2012). Endothelial cell loss and graft survival after Descemet's stripping automated endothelial keratoplasty and penetrating keratoplasty. Ophthalmology 119:2239-2244.

Anshu A, Price MO, Tan DTH, Price FW Jr (2012). Endothelial keratoplasty: a revolution in evolution. Surv. Ophthalmology. 57:236-252.

Bahar SB, Kaiserman I, McAllum P, Slomovis A, Rootman D (2008). Comparison of posterior lamellar keratoplasty techniques to penetrating keratoplasty. Ophthalmology. 115:1525-33.

Bourne WM (2001). Cellular changes in transplanted human corneas. Cornea. 20:560-569.

Bourne WM, Nelson LR, Hodge DO (1997). Central corneal endothealial cell changes over a ten-year period. Invest Ophthalmology. Vis. Sci.;38:779-782.

Busin M, Bhatt PR, Scorcia V (2008). A modified technique for Descemet membrane stripping automated endothelial keratoplasty to minimize endothelial cell loss. Arch. Ophthalmology. 126:1133-1137.

Gorovoy MS (2006). Descemet-stripping automated endothelial keratoplasty. Cornea. 25:886-889.

Hesham N, Schultze RL (2015). Impact of donor characteristics on 2-year Descemet stripping automated endothelial keratoplasty outcomes in patients with Fuchs endothelial dystrophy. Cornea. 34:6-10.

Koenig SB, Covert DJ (2007). Early results of small-incision Descemet's stripping and automated endothelial keratoplasty. Ophthalmology. 114:221-226.

Koenig SB, Covert DJ, Dupps WJ, Meisler DM (2007). Visual acuity, refractive error, and endothelial cell density six months after Descemet stripping and automated endothelial keratoplasty (DSAEK). Cornea. 26:670-674.

Mau K (2009). What DSAEK is going on? An alternative to penetrating keratoplasty for endothelial dysfunction. Optometry. 80:513-523.

Patel SV (2012). Graft survival and endothelial outcomes in the new era of endothelial keratoplasty. Exp. Eye Res. 95:40-47.

Price FW, Price MO (2006). Descemet's stripping with endothelial keratoplasty in 200 eyes: early challenges and techniques to enhance donor adherence. J. Cataract Refract Surg. 32:411-418.

Price FW, Price MO (2009). Does endothelial survival differ between DSEK and standard PK? Ophthalmology. 116:367-368.

Price MO, Fairchild KM, Price DA, Price FW Jr (2011). Descemet's stripping endothelial keratoplasty: five-year graft survival and endothelial loss. Ophthalmology. 118:725-729.

Price MO, Gorovoy M, Price FW, Benetz BA, Menegay HJ, Lass JH (2013). Descemet's stripping automated endothelial keratoplasty. Three-year graft and endothelial cell survival compared with penetrating keratoplasty. Ophthalmology. 120:246-251.

Price MO, Price FW (2006). Descemet's stripping with endothelial keratoplasty: comparative outcomes with microkeratome-dissected and manually dissected donor tissue. Ophthalmology. 113:1936-1942.

Price MO, Price FW Jr (2008). Endothelial cell density after Descemet's stripping endothelial keratoplasty: influencing factors and 2-year trend. Ophthalmology. 115:857-865.

Ratanasit A, Gorovoy MS (2011). Long-term results of Descemet's stripping automated endothealial keratoplasty. Cornea. 30:1414-1418.

Rose L, Briceno CA, Stark WJ, Gloria DG, Jun AS (2008). Assessment of eye bank-prepared posterior lamellar corneal tissue for endothelial keratoplasty. Ophthalmology. 115:279-286.

Terry MA, Chen ES, Shamie N, Hoar KL, Friend DJ (2008). Endothelial cell loss after Descemet's stripping endothelial keratoplasty in a large prospective series. Ophthalmology. 115:488-96.

Terry MA, Shamie N, Chen ES, Hoar KL, Phillips PM, Friend DJ (2008). Endothelial keratoplasty: the influence of postoperative donor endothelial cell density on dislocation, primary graft failure, and 1-year cell counts. Cornea. 27:1131-1171.

Terry MA, Shamie N, Straiko MD, Friend DJ, Davis-Boozer D (2011). Endothealial keratoplasty. The relationship between donor tissue storage time and donor endothelial survival. Ophthalmology. 118:36-40.

Terry MA, Wall JM, Hoar KL, Ousley PJ (2007). A prospective study of endothelial cell loss during the 2 years after deep lamellar endothelial keratoplasty. Ophthalmology. 114:631-639.

Thompson RW Jr, Price MO, Bowers PJ, Price FW Jr (2003). Long-term graft survival after penetrating keratoplasty. Ophthalmology. 110:1396-1402.