Clinical outcomes and graft survival after Descemet membrane endothelial keratoplasty

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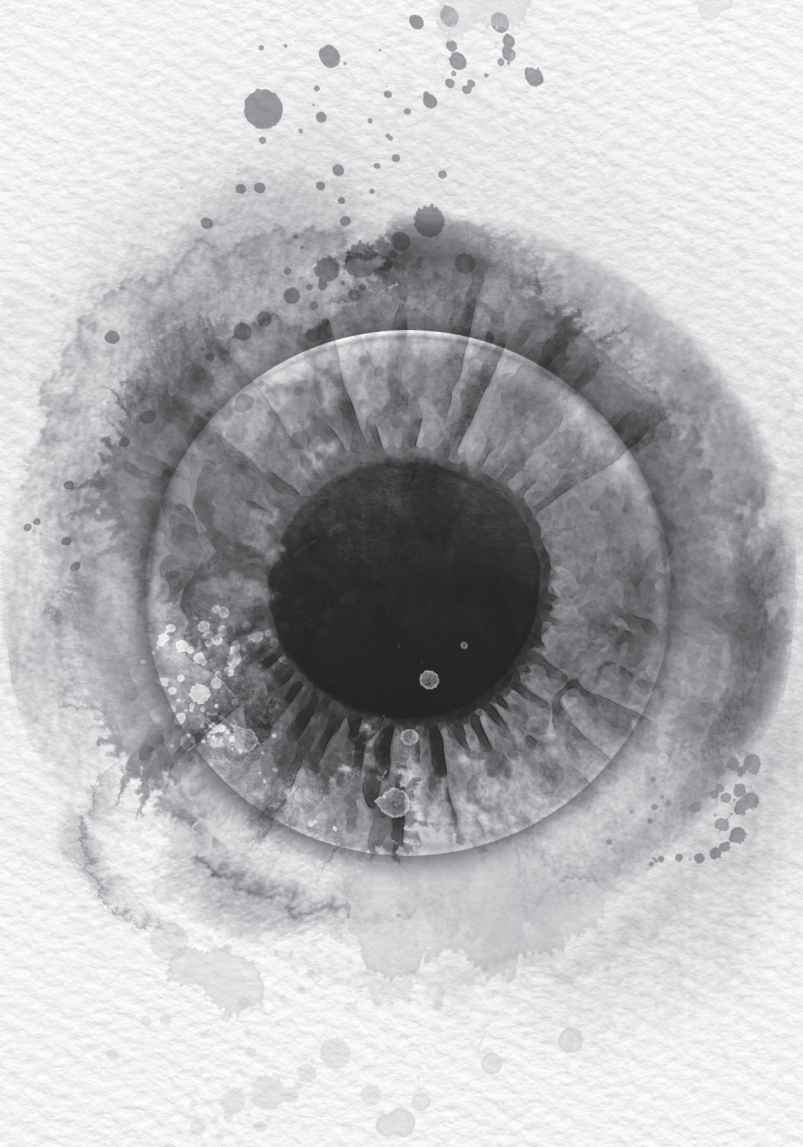

Chapter 4

Effect of Six-Month Postoperative Endothelial Cell Density on Graft Survival after Descemet Membrane Endothelial Keratoplasty

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Ophthalmology. 2021;128(12):1689-1698.

ABSTRACT

Objective: To analyze if endothelial cell density (ECD) at 6-months affects long-term ECD outcome and graft survival 5-years after Descemet membrane endothelial keratoplasty (DMEK) in eyes with Fuchs endothelial corneal dystrophy (FECD).

Participants: A total of 585 DMEK eyes (443 patients) with surgery indication FECD were included. The study group was divided into 4 groups based on 6-month ECD quartiles: Group 1 (n=146) with 313-1245 cells/mm^2, Group 2 (n=148) with 1246-1610 cells/mm^2, Group 3 (n=145) with 1611-1938 cells/mm^2 and Group 4 (n=146) with 1939-2760 cells/mm^2. Group 1 was further split into Subgroup 1a (n=36) with 6-month ECD of ≤828 cells/mm^2, Subgroup 1b (n=37) with 829-1023 cells/mm^2, Subgroup 1c (n=37) with 1024-1140 cells/mm^2 and Subgroup 1d (n=36) 1141-1245 cells/mm^2.

Results: Mean preoperative donor ECD of the overall group decreased from 2543 (±185) cells/mm^2 preoperatively to 1584 (±479) cells/mm^2 at 6-months postoperatively (-38 (±18) %). For Group 1, ECD decreased from 951 (±233) cells/mm^2 (n=146) at 6-months to 735 (±216) cells/mm^2 (n=99) at 5-years postoperatively. For Group 1 graft survival probability was 0.95 [95% CI, 0.91-0.99] at 5-years postoperatively, which was significantly lower than for Groups 2-4 (P=0.001). Five-year graft survival in Subgroup 1a (6-month ECD ≤828 cells/mm^2) was 0.79 [95% CI, 0.67-0.94], which was significantly lower than in Subgroups 1b - 1d (P=0.001). Preoperative ECD did not influence graft survival (P=0.393), while higher 6-month ECD values were associated with lower rates of graft failure (hazard ratio 0.994 [95% CI, 0.99-1.00], (P=0.001)).

Conclusions: Six-months ECD is associated with DMEK graft survival. High early cell loss after DMEK negatively affects long-term ECD outcome and graft survival. Grafts in the subgroup with ECD ≤828 cells/mm^2 at 6 months are at higher risk of failure within 5-years after DMEK. To ensure sufficiently high ECD at 6-months postoperatively preoperative graft quality assessment should be optimized and cellular stress induced to the graft be minimized. Additionally, developing therapeutical options for the treatment of eyes in which low postoperative ECD could not be prevented, may further improve DMEK graft longevity.

INTRODUCTION

Descemet Membrane Endothelial Keratoplasty (DMEK) has become a widely accepted treatment option for endothelial disorders,[1] that provides excellent short- and long-term clinical outcomes.[2-6] Endothelial cell density (ECD) at 6-months postoperatively, one of the benchmark parameters when evaluating graft performance, has been reported to decline by 25-40% after DMEK with a high degree of variability between eyes.[3-6]

For penetrating keratoplasty (PK) grafts it has been shown in the Specular Microscopy Ancillary Study that lower 6-month ECD was associated with secondary graft failure, i.e., late endothelial graft failure, while preoperative ECD was not.[7] More recently, for Descemet stripping automated endothelial keratoplasty (DSAEK) grafts Patel and colleagues also found an association of lower 6-month ECD with late endothelial failure.[8] For DMEK, several studies analyzed potential risk factors associated with low 6-month ECD, however, the effect of early endothelial cell (EC) loss and its impact on long-term ECD and late endothelial failure on a larger DMEK group is not yet known.[9,10] Therefore, the aim of this study is to analyze how EC loss at 6-month postoperatively may affect long-term ECD outcome and graft survival up to 5-years after DMEK.

METHODS

Patient data

Out of 750 consecutive eyes that underwent DMEK between October 2007 and March 2015, 585 eyes that had endothelial cell density (ECD) counts available at 6-months postoperatively were included in this retrospective study (**Table 1**). In total, 165 eyes (22%) were excluded, either for surgery indication other than FECD (n=101), or based on criteria used by Patel et al.[8] such as uncontrolled preoperative intraocular pressure >25mm/Hg (n=1), re-transplantation withing 6-months (n=10), missing 6-month ECD (n=32), graft failure following an allograft rejection (n=4) and eyes with technical failures (i.e., eyes with persistent large graft detachment that required re-transplantation) or with persistent graft detachments of >1/3 of the graft surface area (n=17) (**Supplemental Table 1**). The

mean patient age was 68.5±11 years (**Table 1**). All patients signed an informed consent prior to surgery for research participation and the study adhered to the Declaration of Helsinki. Due to the retrospective nature of the study an Institutional Review board approval was not required.

Table 1. Descemet Membrane Endothelial Keratoplasty Patient and Donor Demographics.

Characteristic	Total group (n=585)	Groups used in ECD analysis*				
		Group 1 (n=146) (6-month ECD ≤1245 cells/mm²)	Group 2 (n=148) (6-month ECD 1246-1610 cells/mm²)	Group 3 (n=145) (6-month ECD 1611-1938 cells/mm²)	Group 4 (n=146) (6-month ECD ≥1939 cells/mm²)	P-value
Number of eyes / patients	585 / 443⁺	146 / 137⁺	148 / 142⁺	145 / 135⁺	146 / 131⁺	-
Patient gender (males / females)	44.5% / 55.5% (197 / 246)	38% / 62% (52 / 85)	53.5% / 46.5% (76 / 66)	43% / 57% (58 / 77)	43.5% / 56.5% (57 /74)	-
Mean patient age ± SD (in years)	68.5 ± 11 (range, 37 – 96)	68.1 ± 11 (range, 37 – 91)	69.8 ±11 (range, 41 – 96)	67.7 ± 10 (range, 42 – 93)	68.3 ± 11 (range, 41 – 90)	0.377
Lens status						0.580
Pseudophakic	74.4% (n=435)	72.6% (n=106)	77% (n=114)	71.7% (n=104)	76% (n=111)	
Phakic	25.5% (n=149)	27.4% (n=40)	22.3% (n=33)	28.3% (n=41)	24% (n=35)	
Aphakic	0.1% (n=1)		0.7% (n=1)	-		
Mean follow-up time after DMEK ± SD (in months)	51.7 ± 16 (range, 6 – 60)	52 ± 15 (range, 6 – 60)	50.6 ± 17 (range, 6 – 60)	52.8 ±14 (range, 6 – 60)	51.3 ± 16 (range, 6 – 60)	0.655
Donor gender (males / females)	61% / 39% (357 / 228)	57.5% / 42.5% (84 / 62)	64.9% / 35.1% (96 / 52)	56.6% / 43.4% (82 / 63)	65.1% / 34.9% (95 / 51)	0.274
Mean donor age ± SD (in years)	66.5 ± 10 (range, 40 – 86)	65.1 ± 11 (range, 40 – 85)	67.3 ± 10 (range, 41 – 86)	67.3 ± 9.7 (range, 46 – 86)	66.3 ± 9 (range, 44 – 85)	0.218
Donor Cause of Death						0.813
Cardiovascular	46.3% (n=271)	49.3% (n=72)	50% (n=74)	42.8% (n=62)	43.2% (n=63)	
Cancer	26.5% (n=155)	22.6% (n=33)	25.7% (n=38)	28.3% (n=41)	29.5% (n=43)	
Respiratory	19.5% (n=114)	19.2% (n=28)	16.9% (n=25)	21.4% (n=31)	20.5% (n=30)	
Trauma	2.6% (n=15)	4.1% (n=6)	2.7% (n=4)	2.8% (n=4)	0.7% (n=1)	
Other	5.1% (n=30)	4.8% (n=7)	4.7% (n=7)	4.8% (n=7)	6.2% (n=9)	

* Eyes were divided into 4 groups based on the 6-month ECD quartiles; not all groups are equally sized because threshold ECD values were observed in >1 eye (e.g. Group 2 had 4 eyes with 6-month ECD of 1610 cells/mm²)

⁺Some bilateral DMEK eyes were not in the same ECD group, therefore the sum of patients from the four groups is higher than the number of patients in the total group

DMEK: Descemet membrane endothelial keratoplasty; ECD: Endothelial cell density; n: Number; SD: Standard deviation

Supplemental Data Table 1. Demographics of Descemet membrane endothelial keratoplasty eyes that were excluded from the study.

Parameters	Excluded eyes (n=165)
Number of eyes / patients	165 / 136
Gender (males / females)	48.5% / 51.5% (66 / 70)
Mean patient age ± SD (years)	65.9 ± 15 (range, 20 – 94)
Indication for DMEK	
FECD	38.8% (n=64)
BK	41.8% (n=69)
Failed previous keratoplasty	18.2% (n=30)
Other (trauma)	1.2% (n=2)
Lens status	
Pseudophakic	77.6% (n=128)
Phakic	21.2% (n=35)
Aphakic	1.2 % (n=2)
Mean follow-up time after DMEK ± SD (months)	32 ± 23 (range, 1w – 60m)
Donor gender (males / females)	61.2% / 38.8% (101 / 64)
Donor age ± SD (years)	65.5 ± 10 (38 – 85)
Donor Cause of Death	
Cardiovascular	49.1% (n=81)
Cancer	24.2% (n=40)
Respiratory	17.6% (n=29)
Trauma	4.8% (n=8)
Other	3.6% (n=6)
Not available	0.6% (n=1)
Reason for exclusion	
Indication other than FECD	61.2% (n=101)
Preoperative IOP >25 mm/Hg	0.6% (n=1)
Re-transplantation within 6m postop	
Re-DSEK	3.6% (n=6)
Re-DMEK	2.5% (n=4)
LTFU	
Own ophthalmologist	7.3% (n=12)
Passed away	1.8% (n=3)
6-month ECD not available*	9.7% (n=16)
6-month ECD not possible	0.6% (n=1)
Graft failure following allograft rejection	2.4% (n=4)
Detachment of >1/3 graft surface area	10.3% (n=17)

*Patient did not attend scheduled 6-month follow-up for various reasons (e.g. illness; check-up with own ophthalmologist; etc) but returned for later follow-up visits

BK : Bullous Keratopathy; DMEK : Descemet Membrane Endothelial Keratoplasty; DSEK : Descemet Stripping Endothelial Keratoplasty; ECD : Endothelial cell density; FECD : Fuchs Endothelial Corneal Dystrophy; IOP : Intraocular Pressure; LTFU : Lost to follow-up; m : months; n : Number; SD : Standard Deviation

Graft preparation and surgery

DMEK graft preparation was performed at Amnitrans EyeBank Rotterdam as previously described.[11,12] Grafts were stored free-floating in organ-culture medium (CorneaMax; Eurobio, Courtaboeuf, France) until the time of the transplantation. DMEK surgery was carried out in the Melles Cornea Clinic Rotterdam by 4 experienced corneal surgeons as a single procedure, i.e., DMEK was not combined with cataract surgery, using the standardized "no-touch" technique, described in detail earlier.[13] The postoperative medication included chloramphenicol 0.5% 6 times daily during the first week and 2 times daily during the second week; ketorolac tromethamine 0.4% and dexamethasone 0.1% 4 times daily for 4 weeks. At the 1-month follow-up dexamethasone was switched to fluorometholone 0.1% 4 times daily followed by a routine tapering regimen; after 1-year patients were advised to continue using fluorometholone once a day or every other day indefinitely.

Data collection

Patients were examined prior to the surgery, 6-months, and 12-months after DMEK, and annually thereafter up to 5-years postoperatively. Outcome measures included ECD, graft survival probability and postoperative complications.

Donor ECD was measured preoperatively *in-vitro* by the eye bank using an inverted light microscope (Axiovert 40; Zeiss), while postoperative ECD was collected using a Topcon SP300p non-contact specular microscope (Topcon Medical Europe BV, Capelle a/d IJssel, the Netherlands). ECD analysis was performed using the ECD analysis program with automatic cell border recognition of the commercial specular microscope software (ImageNet software, Topcon Medical Europe). Automatically delineated cell borders of all endothelial images of the central corneal window were checked and when incorrectly assigned, the cell borders were manually re-assigned by a trained technician. For each ECD measurement an average of 3 images was used. All analyzed images were saved with the overlying cell border lines that were used for the analysis and in case of discrepancies in ECD between the three analyzed images, these images were checked by a second experienced reader for accuracy. For all images, the largest possible area of the image was

analyzed. For the purpose of the study, eyes were divided into 4 groups based on the 6-month ECD quartiles (**Table 1**).

Statistical analysis

Kaplan-Meier analysis was used to determine graft survival probabilities. Differences in survival between the groups were compared using log-rank test. Survival time was calculated as the time between DMEK surgery and the last available follow-up time-point of an eye with a clear cornea, or as the time between DMEK and endothelial graft failure. Endothelial graft failure, also termed secondary failure, was defined as an irreversible corneal clouding after a period of postoperative corneal clearance requiring re-transplantation or corneal decompensation with persistent edema, that was present for longer than 3 months, after initial clearance. Eyes with graft failure following allograft rejection were not included in the study.

For statistical analysis on ECD data, distributions of the variables were evaluated by histograms, and by skewness and kurtosis with the standard errors. Continuous variables between the 4 groups were compared by one-way ANOVA. Homogeneity of variance was assessed with the Levene's test, if the assumption of the homogeneity was met a Bonferroni post hoc test was used, while in other cases Games-Howell was used. Categorical variables were compared by Chi-square test. Changes in ECD through time were assessed by linear mixed model to compensate for unbalanced data, adjusting for preoperative ECD. Cox proportional hazard model was fitted to assess the preoperative and the 6-month ECD association with graft survival. Proportional hazard assumption was assessed via the Schoenfeld residuals.

All data analyses were performed using SPSS 26.00 (IBM, Armonk, New York) statistical software, Excel for Window (Microsoft, Redmond, Washington) and R statistical software v.1.3.1093 (Vienna, Austria). P-values <0.05 were considered statistically significant.

RESULTS

Demographics

The study group (n=585) was divided into 4 groups according to the 6-month ECD quartiles. Group 1 (n=146), representing 25% of eyes with the lowest 6-month ECD of 313-1245 cells/mm^2, Group 2 (n=148) with values between 1246-1610 cells/mm^2, Group 3 (n=145) with 1611-1938 cells/mm^2 and Group 4 (n=146) representing 25% of eyes with the highest ECDs of 1939-2760 cells/mm^2. Donor or patient baseline demographics were similar in all groups (**Table 1**).

Endothelial cell density and endothelial cell loss

Mean preoperative donor ECD of the overall study group was 2543 (±185) cells/mm^2 and declined to on average 1584 (±479) cells/mm^2 at 6-months postoperatively, which corresponds to a decrease in ECD of 38 (±18) %. Pre- and postoperative ECD differed significantly between all groups at all timepoints (all P<0.05) except for the preoperative ECD values of Group 1 and 2 (P=0.583) (**Table 2)**. Preoperative ECD was correlated to 6-month ECD for Group 4 (P=0.001), but not for Groups 1-3 (P>0.05).

For Group 1, ECD decreased from 951 (±233) cells/mm^2 at 6-months to 906 (±221) cells/mm^2 at 1 year, and 735 (± 216) cells/mm^2 at 5-years postoperatively (**Table 2, Figure 1**). The absolute ECD values between consecutive follow-up timepoints differed significantly for Groups 1-4 (all P=0.001). Post hoc analysis showed that from 2 years onwards the EC loss in percentage between consecutive follow-up time-points was similar between Groups 2-4 (P>0.5). From 3 years onwards the EC loss in percentage was lowest in Group 1 **(Table 2)**.

At 5 years postoperatively, 18 eyes had an ECD lower than 500 cells/mm^2 (range, 303 – 497 cells/mm^2) with 11 eyes (61%) from Group 1, 6 eyes (33%) from Group 2 and 1 eye (6%) from Group 3. At the same timepoint 12 eyes of Group 4 had an ECD above 2000 cells/mm^2 (range, 2016 – 2465 cells/mm^2).

Table 2. Endothelial Cell Density after Descemet Membrane Endothelial Keratoplasty of the Total Cohort and Groups 1-4.

	Mean ECD ± SD (cells/mm²)										Between Groups 1-4
Timepoint	**Total group (n=585)**		**Group 1 (n=146) (6-month ECD ≤1245 cells/mm²)**		**Group 2 (n=148) (6-month ECD 1246-1610 cells/mm²)**		**Group 3 (n=145) (6-month ECD 1611-1938 cells/mm²)**		**Group 4 (n=146) (6-month ECD ≥1939 cells/mm²)**		**P-value**
Baseline	2543 ± 185	(n=585)	2466 ± 188#	(n=146)	2499 ± 159#	(n=148)	2567 ± 172	(n=145)	2640 ± 171	(n=146)	**0.001**
6 months	1584 ± 479	(n=585)	951 ± 233	(n=146)	1436 ± 105	(n=148)	1771 ± 98	(n=145)	2179 ± 180	(n=146)	**0.001**
EC Loss in %[0]	-38 ± 18	(n=585)	-61.2 ± 10	(n=146)	-42.3 ± 6	(n=148)	-30.9 ± 6	(n=145)	-17.4 ± 7	(n=164)	**0.001**
1 year	1524 ± 477	(n=560)	906 ± 221	(n=139)	1366 ± 157	(n=139)	1717 ± 141	(n=141)	2097 ± 208	(n=141)	**0.001**
EC Loss in %*	-4.4 ± 8	(n=559)	-5.9 ± 11#	(n=138)	-5 ± 8#	(n=139)	-3 ± 6#	(n=141)	-3.9 ± 5#	(n=141)	**0.022**
2 years	1425 ± 477	(n=518)	836 ± 231	(n=126)	1265 ± 220	(n=130)	1601 ± 211	(n=132)	1978 ± 245	(n=130)	**0.001**
EC Loss in %*	-7.1 ± 10	(n=515)	-7.5 ± 11#	(n=126)	-8 ± 10#	(n=129)	-6.9 ± 9#	(n=131)	-6 ± 7#	(n=129)	0.287
3 years	1326 ± 478	(n=378)	792 ± 225	(n=118)	1161 ± 288	(n=118)	1483 ± 280	(n=122)	1848 ± 307	(n=121)	**0.001**
EC Loss in %*	-7.3 ± 9	(n=471)	-5.9 ± 9#	(n=114)	-8.7 ± 11#	(n=117)	-7.6 ± 9#	(n=120)	-7.1 ± 8#	(n=120)	0.197
4 years	1227 ± 466	(n=452)	759 ± 217	(n=118)	1090 ± 305	(n=110)	1376 ± 321	(n=113)	1709 ± 363	(n=111)	**0.001**
EC Loss in %*	-6.9 ± 11	(n=440)	-3.5 ± 14	(n=111)	-8.2 ± 10#	(n=108)	-7.8 ± 9#	(n=111)	-8.1 ± 9#	(n=110)	**0.002**
5 years	1155 ± 456	(n=409)	735 ± 216	(n=99)	1019 ± 320	(n=104)	1281 ± 360	(n=103)	1569 ± 420	(n=103)	**0.001**
EC Loss in %*	-7.1 ± 12	(n=399)	-3 ± 13#	(n=99)	-7 ± 12#	(n=102)	-8.5 ± 10#	(n=98)	-9.7 ± 10#	(n=100)	**0.001**

[0]Compared to baseline

*Compared to previous follow-up

#*Post hoc* differences between the consecutive ECD of these groups at this timepoint were not significant (P>0.5)

Bold: significant P-value

EC: Endothelial Cell; ECD: Endothelial Cell Density; n: Number

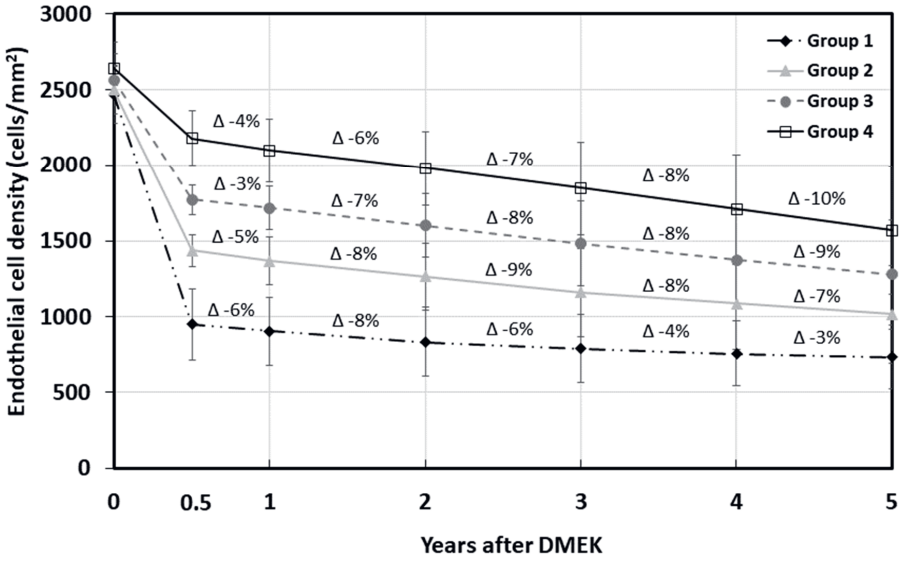


Figure 1. Endothelial cell loss after Descemet membrane endothelial keratoplasty for groups 1-4. Mean endothelial cell density (ECD) values are displayed with vertical standard deviation bars and delta, representing the percentage of EC loss between the two timepoints.

Intraoperative difficulties, postoperative complications, and re-interventions

Intraoperative difficulties were recorded in 66 eyes (11.3%). Difficult graft unfolding was the most common (7.4%, n=43), followed by positive vitreous pressure (3.1%, n=18) and iris root hemorrhage (0.8%, n=5). In the total group, eyes with difficult graft unfolding had lower 6-month ECD values, compared to eyes with no intraoperative difficulties (P=0.001).

At 6 months postoperatively, out of 585 eyes, 66 eyes (10.8%) showed a minor graft detachment (equal or less than 1/3 of the graft surface area). During the entire study period, allograft rejection occurred in 10 eyes (1.7%) (**Table 3**).

Within 5-years, endothelial graft failure was diagnosed in 8 eyes (1.4%) on average 29.1 (± 16) months after DMEK. Most endothelial failures occurred in Group 1 (n=7), while none were observed in Group 3 or 4. In total, 7 eyes (1.2%) underwent re-transplantation on average 31.3 (± 19) months after DMEK **(Table 3)**.

Group 1 had the highest rates of minor detachments (20.5%, P=0.001), graft failures (4.8%, P=0.001) and re-transplantations (4.1%, P=0.002) when compared to the other groups. Group 1 and Group 2 comprised the most re-bubbling procedures (6.2% and 7.4%, respectively, P=0.004), while Group 4 had the least postoperative complications.

Table 3. Intraoperative Events and Clinical Outcomes after Descemet Membrane Endothelial Keratoplasty of the Total Cohort and Groups 1-4.

	Total group (n=585)	Group 1 (n=146) (6-month ECD ≤1245 cells/mm²)	Group 2 (n=148) (6-month ECD 1246-1610 cells/mm²)	Group 3 (n=145) (6-month ECD 1611-1938 cells/mm²)	Group 4 (n=146) (6-month ECD ≥1939 cells/mm²)	P-value
Intraoperative difficulties	11.3% (n=66)	15.7% (n=23)	14.2% (n=21)	9.6% (n=14)	5.5% (n=8)	0.152
Difficult graft unfolding	7.4% (n=43)	11.6% (n=17)	8.1% (n=12)	5.5% (n=8)	4.1% (n=6)	
Positive vitreous pressure	3.1% (n=18)	4.1% (n=6)	2.7% (n=4)	4.1% (n=6)	1.4% (n=2)	
Iris root hemorrhage	0.8% (n=5)	-	3.4% (n=5)	-	-	
Graft detachment*						
Minor (≤1/3 graft surface area)	10.3% (n=66)	20.5% (n=30)	10.1% (n=15)	9% (n=13)	5.5% (n=8)	**0.001**
Allograft rejection	1.7% (n=10)	2.1% (n=3)	3.4% (n=5)	0.7% (n=1)	0.7% (n=1)	0.224
Graft failure						
Secondary#	1.4% (n=8)	4.8% (n=7)	0.7% (n=1)	-	-	**0.001**
Mean time ±SD (range) in months	29.1 ± 16 (10 – 57)	27.1 ± 16 (10 – 57)	43	-	-	0.381
Re-bubbling	3.9% (n=23)	6.2% (n=9)	7.4% (n=11)	1.4% (n=2)	0.7% (n=1)	**0.004**
Mean time ±SD (range) in weeks	3.3 ± 2 (1 – 11)	3.3 ± 2 (1 – 6)	3.5 ± 3 (1 – 11)	2.5 ± 1 (2 – 3)	3	0.973
Re-transplantation†	1.2% (n=7)	4.1% (n=6)	0.7% (n=1)	-	-	**0.002**
Mean time ±SD (range) in months	31.3 ± 19 (12 – 61)	27.5 ± 18 (12 – 61)	54		-	0.228

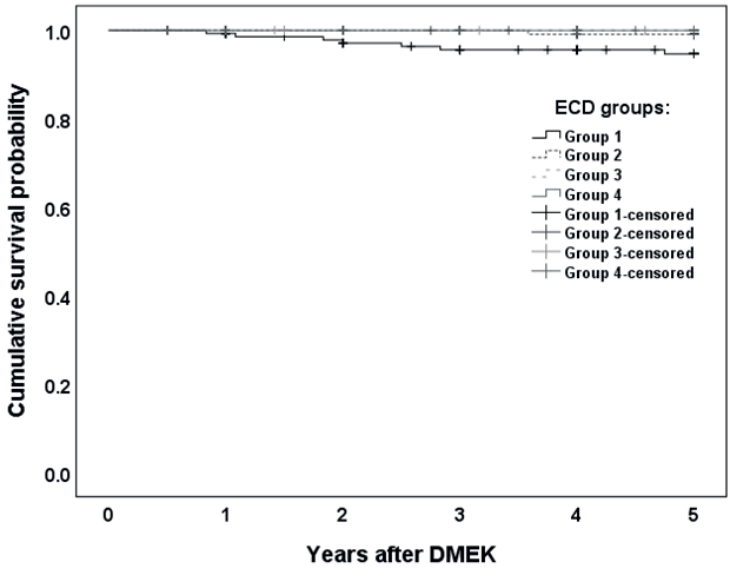
*: Includes the graft detachments as observed at the 6-month follow-up
#: Secondary graft failure refers to an attached graft with (signs of) corneal clearance, followed by corneal decompensation
†: One eye diagnosed with graft failure had not undergone re-surgery within the study period

ECD: Endothelial Cell Density; SD: Standard Deviation; n: Number; m: Months

Significant P-values are presented in **bold**

Graft survival

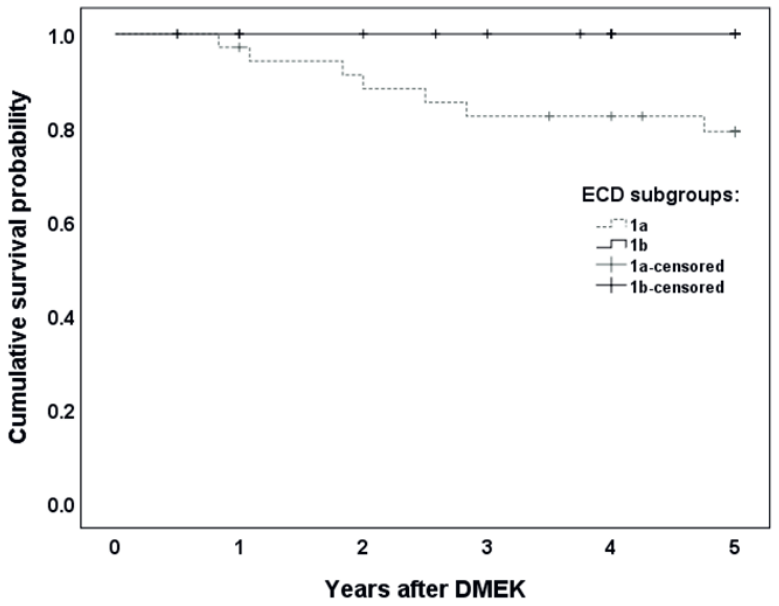
Group 1 had the lowest graft survival probabilities compared to Groups 2-4 (P=0.001). For Group 1, survival rates at 1 year decreased from 0.99 [95% Confidence Interval (CI), 0.98-1.00] to 0.95 [95% CI, 0.91-0.99] at 5-years postoperatively. For the same timepoints, survival probabilities for Group 2 were 1.0 and 0.99 [95% CI, 0.97-1.00], while for Group 3 and Group 4 those probabilities remained 1.0, for both groups (**Figure 2**).



	Years after DMEK		0	1	2	3	4	5
Group 1: (6m ECD ≤1245 cells/mm²)	Cumulative survival probability at FU	Estimate	-	0.99	0.97	0.96	0.96	0.95
		SE	-	0.01	0.01	0.02	0.02	0.02
	Cumulative events		0	1	4	6	6	7
	Remaining cases		146	138	130	120	114	100
Group 2: (6m ECD =1246-1610 cells/mm²)	Cumulative survival probability at FU	Estimate	-	1	1	1	0.99	0.99
		SE	-	-	-	-	0.01	0.01
	Cumulative events		0	0	0	0	1	1
	Remaining cases		148	143	133	120	112	107
Group 3: (6m ECD =1611-1938 cells/mm²)	Cumulative survival probability at FU	Estimate	-	1	1	1	1	1
		SE	-	-	-	-	-	-
	Cumulative events		0	0	0	0	0	0
	Remaining cases		145	142	135	126	120	107
Group 4: (6m ECD >1938 cells/mm²)	Cumulative survival probability at FU	Estimate	-	1	1	1	1	1
		SE	-	-	-	-	-	-
	Cumulative events		0	0	0	0	0	0
	Remaining cases		146	142	132	124	113	106

Figure 2. Kaplan-Meier curve of cumulative graft survival probabilities after Descemet membrane endothelial keratoplasty for groups 1 – 4. Survival probabilities and number of eyes at risk per follow-up moments are presented in the table beside the graph.

Group 1 was further divided into Subgroup 1a (n=36) (6-month ECD ≤828 cells/mm^2), 1b (n=37) (6-month ECD 829 – 1023 cells/mm^2), 1c (n=37) (6-month ECD 1024-1140 cells/mm^2) and 1d (n=36) (6-month ECD 1141-1245 cells/mm^2). Graft survival of Subgroup 1a was 0.97 [95% CI, 0.92-1.00] at 1-year and decreased to 0.79 [95% CI, 0.67-0.94] at 5-years after DMEK, which was lower than the graft survival of Subgroups 1b - 1d at both 1- and 5-years after DMEK (1b – 1d: 1.0 [95% CI, 1.00], at both 1- and 5-years) (P=0.001) (**Figure 3**). Demographics of eyes from Subgroup 1a that did not fail despite low ECD at 6 months postoperatively are summarized in **Supplemental Table 2**.



	Years after DMEK		0	1	2	3	4	5
Subgroup 1a: (6m ECD ≤828 cells/mm²)	Cumulative survival probability at FU	Estimate	-	0.97	0.88	0.82	0.82	0.79
		SE	-	0.03	0.06	0.07	0.07	0.07
	Cumulative events		0	1	4	6	6	7
	Remaining cases		36	33	30	28	26	23
Subgroup 1b: (6m ECD =829–1023 cells/mm²)	Cumulative survival probability at FU	Estimate	-	1	1	1	1	1
		SE	-	-	-	-	-	-
	Cumulative events		0	0	0	0	0	0
	Remaining cases		37	34	33	31	29	24
Subgroup 1c: (6m ECD =1024–1140 cells/mm²)	Cumulative survival probability at FU	Estimate	-	1	1	1	1	1
		SE	-	-	-	-	-	-
	Cumulative events		0	0	0	0	0	0
	Remaining cases		37	35	34	31	28	23
Subgroup 1d: (6m ECD =1141–1245 cells/mm²)	Cumulative survival probability at FU	Estimate	-	1	1	1	1	1
		SE	-	-	-	-	-	-
	Cumulative events		0	0	0	0	0	0
	Remaining cases		36	35	34	32	31	30

Figure 3. Kaplan-Meier curve of cumulative graft survival probabilities after Descemet membrane endothelial keratoplasty for Subgroups 1a – 1d. Survival probabilities and number of eyes at risk per follow-up moments are presented in the table beside the graph.

Supplemental Table 2. Demographics of eyes from Subgroup 1a without graft failure.

Characteristics	Eyes (n=29)
Number of eyes / patients	29 / 29
Patient gender (males / females)	24% / 76% (7 / 22)
Mean patient age ± SD (in years)	68.9 ± 10 (49 – 83)
Lens status	
Pseudophakic	79.3% (n=23)
Phakic	20.7% (n=6)
Donor gender (males / females)	55% / 45% (16 / 13)
Mean donor age ± SD (in years)	62.4 ± 12 (43 – 84)
Donor Cause of Death	
Cardiovascular	51.7% (n=15)
Cancer	20.7% (n=6)
Respiratory	17.2% (n=5)
Other	10.3% (n=3)
Graft detachment <1/3 graft surface area	34.5% (n=10)
Allograft rejection	3.5% (n=1)
Re-bubbling	20.7% (n=6)
Mean time ± SD (range) in weeks	2.9 ± 2 (1 – 6)

N: Number; SD: Standard Deviation

The effect of preoperative ECD on graft survival was assessed by dividing the study group into quartiles based on absolute preoperative ECD values and comparing the 5-year survival probabilities. Group A (n=151) represented the lowest preoperative ECD of ≤2430 cells/mm^2, Group B (n=144) had values between 2431-2500 cells/mm^2, Group C (n=154) had 2501-2680 cells/mm^2, and Group D (n=137) represented eyes with ECD of ≥2681 cells/mm^2. Additionally, by dividing the group according to the decrease in ECD in percentage, compared to preoperative values, where Group A^1 (n=138) had eyes with a decrease in ECD of >51%, Group B^1 (n=147) with decrease between 38-50%, Group C^1 (n=146) with 26-37% and Group D^1 (n=154) with ≤25% decrease. No difference in graft survival was observed between the

absolute preoperative ECD Groups A-D (P=0.802), while Group A[1] had a lower survival compared to Group B[1] (0.94 [95% CI, 0.90-0.99] vs 0.99 [95% CI, 0.98-1.00] (P=0.027).

Preoperative ECD and intraoperative difficulties were not associated with graft survival (P=0.400 and P=0.784, respectively), while higher 6-month ECD values were associated with lower rates of graft failure (hazard ratio 0.994 [95% CI, 0.99-1.00], (P=0.001)). The average hazard rate indicates that with every 500 cells/mm^2 decrease in 6-month ECD, the risk of graft failure increases by 3 times (hazard ratio 3.00 [95% CI, 1.5-4], (P=0.001)).

DISCUSSION

This study evaluates the impact of 6-month ECD on 5-year ECD outcomes and graft survival after DMEK in eyes with FECD. A low 6-month ECD had a negative effect on 5-year ECD and DMEK graft survival. This effect was most pronounced in grafts with a 6-month ECD of ≤828 cells/mm^2, whereas surprisingly almost all grafts with a 6-month ECD of >828 cells/mm^2 showed good long-term outcomes.

Our results on low 6-month ECD being predictive for late endothelial failure are in line with previous studies on DSAEK and PK eyes.[7,8] Lass et al. found that PK eyes with a 6-month ECD of <1700 cells/mm^2 had a 4-6 times higher 5-year graft failure rate than eyes with >1700 cells/mm^2. For DSAEK eyes, Patel et al. identified a lower threshold of <1200 cells/mm^2. The latter finding is comparable to our DMEK results with eyes in Group 1 (6-month ECD ≤1245 cells/mm^2) having a significantly higher 5-year graft failure probability than eyes in the other groups (6-month ECD >1245 cells/mm^2, only 1 failure in Group 2). Furthermore, Subgroup analysis showed that DMEK eyes with an even lower 6-month ECD threshold of ≤828 cells/mm^2 were prone to graft failure (5-year graft survival probability of 79%), while the other Subgroups (ECD >828 cells/mm^2) did not have a single endothelial failure. This may support the hypothesis of a lower ECD limit in DMEK eyes needed to maintain the corneal clarity. However, this limit may vary between corneas and may depend on additional factors such as the state of the peripheral endothelial cells.

If 6-month ECD is predictive for late endothelial graft failure after DMEK, it may be important to assess measures to prevent early endothelial cell loss and to enhance

DMEK graft longevity. In an earlier study, we could show that main risk factors associated with higher endothelial cell loss at 6-months postoperatively were (minor) graft detachment, cardiovascular donor death cause, recipients' advanced stage of FECD and the occurrence of postoperative complications, such as allograft rejection and high intraocular pressure.[9] Other studies also evaluated the effect of graft storage medium, preoperative donor ECD, and intraoperative complications on postoperative ECD but without consistent outcomes.[6,9,10,14-20] Inconsistent results between the studies on the effect of intraoperative complications on ECD may be caused by different definitions of intraoperative complications and may also depend on surgical experience. In this study we observed an effect of difficult graft unfolding on 6-month ECD, but no correlation with graft survival, which may point to an only moderate EC loss caused by intraoperative difficulties as suggested previously.[9]

An explanation for an early EC loss was provided by Miron et al., who suggested that the postoperative ECD decrease that can be already observed as early as 1 day and 1 week after DMEK, may be attributed to the overestimation of the viable endothelial cell population on the graft in the eye bank which would also result in an overestimation of preoperative ECD.[21] In a follow-up in-vitro study it was shown that for some DMEK grafts a decrease in endothelial cell viability can occur within hours after graft preparation in the eye bank.[22] This suggests that there is a high degree in variability of how well endothelial cells respond to the stress of the graft preparation and supports the hypothesis that the preoperative ECD may be overestimated for some grafts, leading to apparent early endothelial cell loss. A similar hypothesis was provided by Patel et al. for the degree in variability of early cell loss after DSAEK which the authors suggested to be partly caused by different cellular tolerance levels to surgical manipulation during DSAEK surgery.[8] Based on their analysis, Miron et al. suggested that for pre-stripped eye bank grafts an additional step for checking tissue quality after graft preparation may help to identify DMEK grafts with low cellular tolerance to the stress of graft preparation, as ECD provided at the first graft evaluation may be overestimated.[22] It is therefore important to identify the underlying causes of cellular stress intolerance to develop adequate test methods for eye banks. This may ensure that only grafts with good tolerance to surgical manipulation and therefore with higher postoperative ECD

counts, are transplanted. Nevertheless, this possibility to identify 'low-performing' grafts before surgery, would only apply to pre-stripped eye bank grafts and not to grafts prepared by surgeons directly before surgery. Analysis of cytokine levels in the donor aqueous humor may, on the other hand, be a potential screening option for all donor corneas, as for example, elevated preoperative monocyte chemotactic protein - 1 levels were shown to be associated with postoperative cell loss after DSAEK and PK and might therefore also play a role in donor eyes.[23,24]

Though some grafts with early low ECD can survive and maintain corneal clarity in the longer term, as also observed in PK,[7,25] DSAEK,[26] and other DMEK studies,[2] it may be important to consider, for example, pharmaceutical therapies before corneal decompensation for eyes with low ECD. As such, the more frequent application of (potent) topical steroids or the use of topical ROCK inhibitor could be a potential treatment option for maintaining long-term corneal clarity. Studies on the off-label use of ROCK inhibitor ripasudil have shown to promote endothelial wound-healing and stabilize ECD in FECD eyes that underwent ´Descemetorhexis only´.[27,28] However, clinical studies in DMEK eyes supporting its efficiency are still lacking.

Due to the retrospective design of the study, the main limitation was missing data and attrition caused by loss to follow-up. However, this limitation was addressed by using linear mixed models, which adjusts for imbalanced data.

In conclusion, this study showed that low 6-month ECD has a negative effect on the 5-year ECD outcome and graft survival after DMEK. However, the negative effect of low ECD is predominantly observed in grafts with ECD ≤828 cells/mm^2 at 6-months, whereas grafts with higher 6-month ECD present with good long-term outcomes. Therefore, preventing high EC loss by optimizing preoperative graft quality assessment, minimizing cellular stress induced to the graft, and developing therapeutical options for the treatment of eyes with low postoperative ECD may contribute to further improve DMEK graft longevity.

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