# Elucidation of the migratory behaviour of the corneal endothelium

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# *Part I*

# *DMEK graft analysis before and after transplantation*

# CHAPTER 2

*In vivo* endothelial cell density decline in the early postoperative phase after Descemet membrane endothelial keratoplasty (DMEK)

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## ABSTRACT

**Purpose:** To evaluate endothelial cell density (ECD) in the first 6 months after Descemet membrane endothelial keratoplasty (DMEK) by eliminating method error as a confounding variable.

**Methods:** From 24 DMEK eyes operated for Fuchs endothelial corneal dystrophy, from which specular microscopy images could be taken at 1 day and 6 months postoperatively, ECD values were compared between these 2 time points.

**Results:** Using the 1-day ECD measurement as baseline, mean ECD decreased from 1913 (±326) cells/mm$^2$ to 1524 (±393) cells/mm$^2$ at 6 months, a decline of -18 (±19)%. With the 1-week ECD as baseline [1658 (±395) cells/mm$^2$], the decline at 6 months was -6 (±19)% and when using preoperative ECD as baseline [2521 (±122) cells/mm$^2$], the decline was -39 (±16)% at 6 months.

**Conclusions:** After DMEK, ECD shows an *in vivo* decline of 18% from 1 day to 6 months postoperatively, with a sharp 13% drop in the first week, and a slower decrease thereafter. The remaining difference of 20% from preoperative ECD values may be attributed to a measurement error in the eye bank with an overestimation of the graft's viable endothelial cell population and/or intraoperative trauma to the graft.

## INTRODUCTION

Descemet membrane endothelial keratoplasty (DMEK) is currently the most selective endothelial keratoplasty technique, by which only the diseased Descemet membrane (DM) and endothelium are replaced by a healthy donor.[1,2] With growing experience, DMEK may increasingly be preferred over Descemet stripping endothelial keratoplasty/ Descemet stripping automated endothelial keratoplasty owing to better visual outcomes,[3,4] a lower risk of interface haze, and a reduced chance of allograft rejection.[5,6]

Endothelial cell density (ECD) has been found to show a postoperative decrease comparable with earlier endothelial keratoplasty techniques, that is, 30% to 40% within the first 6 months after surgery followed by an annual decrease of 7% to 9% thereafter.[7–9] A postoperative ECD decrease for all endothelial keratoplasty techniques is usually reported for 6-month follow-up,[8,10,11] and it is therefore not known whether the perceived drop in ECD relates to the measurement error (light microscopy in the eye banks versus *in vivo* specular microscopy after surgery), intraoperative trauma to the graft, or a drop in central ECD in the first months after surgery.

Because DMEK often provides enough corneal deturgescence within the first 24 hours to enable specular microscopy, the purpose of our study was to use the 1-day ECD (instead of preoperative values) as baseline to evaluate the *in vivo* change in ECD within the early postoperative phase and to determine at which time points any change in *in vivo* ECD might occur.

## METHODS

Of 46 consecutive DMEK surgeries performed for Fuchs endothelial corneal dystrophy, successful ECD images could be taken for 24 eyes of 24 patients on the first postoperative day, and these eyes were included in the study (**Figure 1**). For these 24 eyes, 13 patients (54%) were women and 11 were men with a mean age of 69 (±11) years (range 42–94 years). Six eyes (25%) were phakic and 18 (75%) pseudophakic (**Table 1**). All patients signed an institutional review board-approved informed consent form for research participation, and the study was conducted according to the Declaration of Helsinki.

### Donor tissue protocol

The procedure for harvesting a DMEK graft has been previously described in detail.[12,13] Briefly, corneoscleral buttons were excised from donor globes obtained less than 36 hours postmortem and stored in organ culture medium at 15 to 31°C (CorneaMax, Eurobio, Courtaboeuf, France). After on average 1 week of culture, endothelial cell morphology and viability were evaluated again, and a 9.5 mm-diameter Descemet sheet with its endothelium was carefully stripped from the posterior stroma. Each "Descemet-roll" was then stored in organ culture medium until the time of transplantation (**Table 1**).[12] Preoperative donor ECD was assessed in vitro in the eye bank (Axiovert 40 inverted light microscope, Zeiss, Göttingen, Germany) after provoked swelling and staining with 0.04% trypan blue (Hippocratech, Rotterdam, The Netherlands)[12,13] and determined by manual counting according to the fixed-frame method.

### Surgical protocol

All surgeries were performed according to the previously described DMEK technique.[2] After performing "descemetorhexis" under air,[14] a 3.0-mm tunnel incision was made for the insertion of the graft. The "Descemet-roll" was inserted endothelial side down (donor DM facing the recipient posterior stroma) into the recipient anterior chamber and then unfolded over the iris and positioned against the recipient posterior

stroma.[2] The anterior chamber was left completely filled with air for 60 minutes, followed by air-liquid exchange to pressurize the eye while leaving 30% to 50% air fill in the anterior chamber. Patients were instructed to remain supine for 48 to 72 hours after surgery. Postoperative medication included 0.5% chloramphenicol, 5 mg/mL ketorolac, and 0.1% dexamethasone eye drops for 4 weeks followed by a routine steroid tapering (fluorometholone) regimen over the course of a year.

## Measurements and statistics

Routine follow-up examinations were performed at 1 day, 1 week, and at 1, 3, and 6 months after surgery. *In vivo* postoperative ECD was evaluated using noncontact specular microscopy (Topcon Medical Europe BV, Capelle a/d IJssel, The Netherlands). ECD analysis was performed by multiple trained technicians. For all endothelial images of the central corneal window, the automatically delineated cell borders (ImageNet software, Topcon Medical Europe BV) were carefully checked and in case they were not correctly assigned by the program, a "manual correction" was applied to reassign the cell borders. For every analysis, the largest possible part of the image was used, and measurements of 3 central images were averaged per follow-up time.

A paired t test was performed for ECD data comparison between preoperative and postoperative follow-up measurements. $P < .05$ was considered statistically significant.

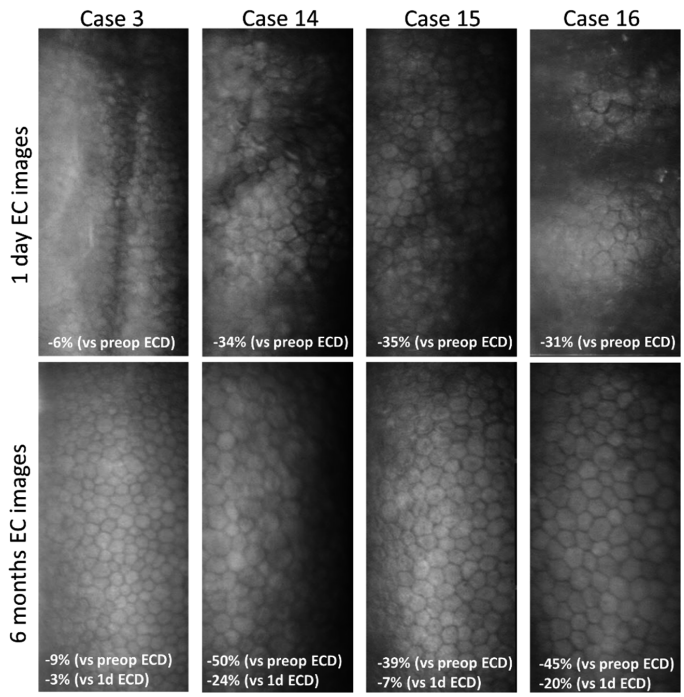


**Figure 1| Specular microscopy images taken at 1 day and 6 months DMEK**. One-day (upper row) and 6 months (bottom row) postoperative specular microscopy images are displayed for 4 cases. ECD decrease compared with preoperative donor ECD and with the 1 day ECD count are listed for each case.

**Table 1.** Demographics of DMEK Eyes

| Patient and Donor Information | Group with successful 1d ECD count |
|---|---|
| **Patient data** | |
| No. of eyes (patients) | 24 (24) |
| Gender | |
| Female | 13 (54%) |
| Male | 11 (46%) |
| Mean age (±SD) (range), yrs. | 69 (±11), (42–94) |
| Preoperative lens status | |
| Pseudophakic | 18 (75%) |
| Phakic | 6 (25%) |
| Mean preoperative pachymetry (±SD), µm | 661 (±56) |
| **Donor data** | |
| Gender | |
| Female | 7 (29%) |
| Male | 17 (71%) |
| Mean age (±SD) (range), yrs. | 69 (±10), (46–82) |
| Mean storage time (±SD) (range), days | 14 (±3), (8–20) |
| Mean time between last ECD evaluation and surgery (±SD) (range), days | 9 (±3), (6–14) |
| Cause of death | |
| Cardio/Stroke | 13 (54%) |
| Trauma | 2 (8%) |
| Respiratory | 5 (21%) |
| Cancer | 2 (8%) |
| Other | 2 (8%) |
| Mean donor ECD (±SD), cells/mm$^2$ | 2521 (±122) |

## RESULTS

Average central ECD decreased from 2521 (±122) cells/mm$^2$ preoperatively to 1913 (±326) cells/mm$^2$ at 1 day, to 1658 (±395) cells/mm$^2$ at 1 week, to 1629 (±367) cells/mm$^2$ at 1 month, to 1592 (±369) cells/mm$^2$ at 3 months, and to 1524 (±393) cells/mm$^2$ at 6 months. This corresponded to an ECD decrease of -39 (±16)% at 6 months compared with the preoperative value (**Table 2**, **Figure 2**).

When using the 1-day ECD measurement as a baseline value, mean ECD decreased by -13 (±14)% at 1 week, by -14 (±17)% at 1 month, by -15 (±19)% at 3 months, and by -18 (±19)% at 6 months. Using the 1-week ECD value as baseline, the ECD decrease was 0 (±16)% at 1 month, -3 (±19)% at 3 months, and -6 (±19)% at 6 months.

When comparing the average ECD between the consecutive follow-up time points, the initial decline between preoperative/1 day was -24 (±12)%, between 1 day/1 week -13 (±14%), between 1 week/1 month 0 (±16)%, between 1 month/3 months -3 (±10)%, and between 3 months/6 months -3 (±5)% ($P > 0.05$ for all paired time point comparisons after 1 week).

Although the average ECD decrease between 1 day/6 months was -18% (median 15%) within the study group, there was a large variation in the ECD decrease for this time interval ranging from +7% to -78% (**Table 2**).

**Table 2.** ECD and ECD decrease (ΔECD) after DMEK

| Patient data | | | ECD, cells/mm² | | | | ΔECD | | |
|---|---|---|---|---|---|---|---|---|---|
| Case | Age, yrs. | Lens Status | Preop. | 1 d FU | 1 w FU | 6 mo FU | 6 mo FU vs. Preop., % | 6 mo FU vs. 1 d FU, % | 6 mo FU vs. 1 w FU, % |
| 1 | 94 | Pseudophakic | 2400 | 1768 | NA | 1432 | -40% | -19% | NA |
| 2 | 70 | Pseudophakic | 2700 | 2293 | 1734 | 1889 | -30% | -18% | +9% |
| 3 | 73 | Pseudophakic | 2600 | 2438 | 2440 | 2355 | -9% | -3% | -3% |
| 4 | 87 | Pseudophakic | 2600 | 2065 | 2327 | 1911 | -26% | -7% | -18% |
| 5 | 62 | Pseudophakic | 2700 | 2396 | 1457 | 536 | -80% | -78% | -63% |
| 6 | 79 | Pseudophakic | 2300 | 1870 | 1010 | 881 | -62% | -53% | -13% |
| 7 | 72 | Pseudophakic | 2600 | 1957 | 1413 | 1089 | -58% | -44% | -23% |
| 8 | 80 | Pseudophakic | 2400 | 2003 | 1852 | 2143 | -11% | +7% | +16% |
| 9 | 68 | Phakic | 2400 | 2120 | 1945 | 1789 | -25% | -16% | -8% |
| 10 | 68 | Pseudophakic | 2400 | 1265 | 942 | 1395 | -42% | +10% | +48% |
| 11 | 49 | Phakic | 2500 | 1956 | 1333 | 1220 | -51% | -38% | -8% |
| 12 | 71 | Pseudophakic | 2500 | 1237 | 1242 | 1222 | -51% | -1% | -2% |
| 13 | 42 | Phakic | 2600 | 1799 | 1882 | 1638 | -37% | -9% | -13% |
| 14 | 54 | Pseudophakic | 2500 | 1645 | 1409 | 1253 | -50% | -24% | -10% |
| 15 | 73 | Pseudophakic | 2500 | 1630 | 1629 | 1518 | -39% | -7% | -7% |
| 16 | 74 | Pseudophakic | 2300 | 1588 | 1410 | 1267 | -45% | -20% | -10% |
| 17 | 63 | Pseudophakic | 2400 | 2206 | 2097 | 1633 | -32% | -26% | -22% |
| 18 | 67 | Pseudophakic | 2800 | 2380 | 2225 | 2020 | -28% | -15% | -9% |
| 19 | 51 | Phakic | 2600 | 2163 | 1768 | 1821 | -30% | -16% | +3% |
| 20 | 76 | Pseudophakic | 2500 | 1822 | 1355 | 1182 | -53% | -35% | -13% |
| 21 | 69 | Phakic | 2500 | 1607 | 1452 | 1396 | -44% | -13% | -4% |
| 22 | 66 | Phakic | 2600 | 1828 | 1793 | 1846 | -29% | +1% | +3% |
| 23 | 78 | Pseudophakic | 2600 | 1644 | 1369 | 1515 | -42% | -8% | +11% |
| 24 | 67 | Pseudophakic | 2500 | 2240 | 2059 | 1978 | -21% | -12% | -4% |
| Average | | | 2521 | 1913 | 1658 | 1524 | -39% | -18% | -6% |
| Standard Deviation | | | 122 | 326 | 395 | 393 | ±16% | ±19% | ±19% |
| Median | | | 2500 | 1913 | 1629 | 1516 | -40% | -15% | -8% |

ECD, endothelial cell density; FU, Follow-up; NA, not available; Preop, preoperative; ΔECD, ECD decrease.

## DISCUSSION

Commonly, ECD decrease is considered one of the main outcome parameters in the evaluation of corneal transplantation procedures, both as a measure of efficacy and for predicting long-term graft survival.[15–17] For both Descemet stripping endothelial keratoplasty/Descemet stripping automated endothelial keratoplasty and DMEK, multiple studies have described an approximate 30% to 40% drop in ECD at 6 months after surgery, compared with preoperative values. So far, it has been unknown whether the ECD at 6 months reflects solely surgical trauma to the graft[3,17,18] or *in vivo* cell loss or redistribution. Also, it has been unknown at what time point any *in vivo* decrease in ECD might occur, and whether it reflects a gradual decrease or a sudden drop. In a small case series, we previously found a significant decrease in ECD within the first month after DMEK.[8] This finding triggered the current study that aimed to overcome the lack of reliable measurements in the early postoperative phase, using 1-day postoperative specular microscopy readings as baseline for *in vivo* ECD analysis.

Interestingly, our study showed that a -18% *in vivo* drop in ECD after DMEK occurred within the first 6 months after surgery and particularly within the first week after surgery. This finding may shed a different light on various causes that are hypothesized for the drop in ECD after DMEK, including endothelial cell migration and/or redistribution, after surgical inflammation or a subclinical immunological response and would indicate that approximately half of the apparent drop in ECD at 6 months occurs *in vivo*, that is, after transplantation.

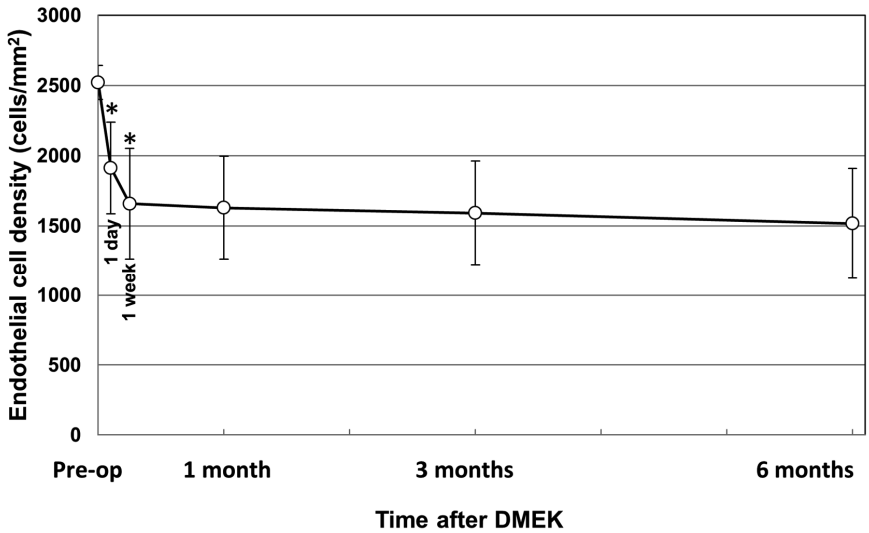


**Figure 2| Preoperative and postoperative ECD after DMEK.** ECD was measured preoperatively, and at 1 day, 1 week , 1 month, 3 months, and 6 months postoperatively. The highest ECD drop was observed at 1 day after surgery. Error bars represent SD. The t test was used for paired consecutive follow-up time points: *: $P \leq .05$.

On average, *in vivo* ECD dropped from day 1 to 1 week postoperatively by -13%, whereas the decrease between 1 week and 6 months was only -6%, indicating that different mechanisms may cause the *in vivo* ECD decrease. Endothelial cell migration and/or redistribution may contribute to the ECD drop within the first postoperative week, whereas the lower ECD decrease after 1 week may be caused by a subclinical immune reaction that had also been suggested to cause an ECD decrease in the longer term.[11,15,19,20] However, application of higher-dose steroids in the first postoperative week does not seem to influence postoperative ECD as shown in a recent study by Hoerster et al.[21]

When approximately half of the observed ECD decrease at 6 months occurs *in vivo*, the remaining decline in ECD as observed at 1 day postoperatively may be attributed to intraoperative trauma to the graft and/or a measurement error in the eye bank with an overestimation of the graft's viable endothelial cell population.[22–25] The latter has been addressed in a study by Pipparelli et al., which showed for endothelial grafts pre-dissected by eye banks that the actual pool of viable endothelial cells on the graft is commonly overestimated.[22] The same group showed in another study with paired organ cultured donor corneas, in which 1 cornea was used for penetrating keratoplasty (PK) and the contralateral cornea was used to determine the number of viable endothelial cells *in vitro*, that the number of viable cells counted in vitro was virtually similar to the ECD measured 5 days after PK.[23] Assuming that the number of viable cells is similar between eyes of the same pair, this suggests that the observed -30% drop in ECD at 5 days after PK was caused by a substantial overestimation of the number of viable endothelial cells on the graft. This is further substantiated by a recent study by Bhogal et al. in which global endothelial cell viability of DMEK grafts was assessed after preparation, and it was concluded that an early postoperative ECD reduction of up to -25% may be expected from tissue preparation alone.[25]

In conclusion, our study demonstrated that approximately half of the observed ECD decrease at 6 months after DMEK is an *in vivo* decline from 1 day to 6 months postoperatively, with a sharp -13% drop in the first week, and a slower decrease thereafter. The remaining decrease between preoperative and 1 day postoperative ECD values may be attributed to a measurement error in the eye bank with an overestimation of the graft's viable endothelial cell population and/or intraoperative trauma to the graft.

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