ASSESSMENT OF ENDOTHELIAL CELL DENSITY AND CORNEAL THICKNESS IN CORNEAL GRAFTS AN AVERAGE OF 5 YEARS AFTER PENETRATING KERATOPLASTY

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Abstract

Background: Corneal transparency is a useful indicator for corneal function. Our aim was to investigate central corneal endothelial cells and corneal thickness in transplanted corneas at an average of 5.4 years after penetrating keratoplasty.

Patients and methods: The study involved 68 penetrating keratoplasty patients with at least a one-year-follow-up. Postoperatively, central corneal endothelial layer was observed using contact specular microscope (Tomey, EM 1200). Central endothelial cell density, corneal thickness and the coefficient of variation of endothelial cell size were statistically analysed.

Results: The postoperative follow-up time was ranging from 12 months to 23 years. Endothelial cell density (ECD) was 1501±249 cell/mm^2. Average cell size was 673.6±98.3 μm^2, the coefficient of variation of cell size was 0.61±0.11. No difference in ECD was detected between diagnostic groups. Corneal thickness was 0.56±0.06 mm. Correlation between ECD and postoperative time was not significant (r=0.02; p=0.85).

Conclusion: Our study concluded that ECD showed a higher rate of decrease after penetrating keratoplasty with no relation to preoperative diagnosis.

Keywords: endothelial cell loss, graft survival, penetrating keratoplasty, specular microscope

Background

There are several sorts of keratoplasty techniques, but ever since the first successful full-thickness corneal transplantation, also called penetrating keratoplasty (PKP) by Eduard Zirm in 1906 (Moffatt SL 2005), it is regarded the most frequently performed tissue transplantation in humans.

The long lasting optical clarity of the cornea after penetrating keratoplasty is considerably determined by the number, the morphology and the function of donor endothelial cells. The morphology of corneal endothelial cells can be evaluated among others by specular microscopy, a widely-used and precise method. In our study, the morphology of the donors' central corneal endothelial cell has been traced for an average of 5.4 years after penetrating keratoplasty in order to assess the longevity of the transplanted grafts. Relatively few reports have been made to study at least 5 years graft survival including detailed donor endothelial data as is present in our study (Linn et al., 1981; Price et al., 1993; Vail et al 1993; Ing et al., 1998; Kus et al., 1999; Patel et al., 2000; Bourne, 2001; Zadok et al., 2005).

Patients and methods

68 patients after PKP were to investigate at least one year after penetrating keratoplasty. Causative diagnosis were keratoconus (n=36), bullous keratopathy (n=14), corneal leucoma (n=9), herpetic keratitis (n=7), acanthamoeba keratitis (n=1), or corneal dystrophy (n=1).

29 of the donor corneas were preserved in Optisol corneal storage medium (Chiron Ophthalmics, Irvine, California), 39 were stored in moist chamber. The donor corneal grafts

were between 6.5 and 7.5 mm in diameter. The recipients' corneas were between 6.0 and 7.0 mm in diameter. The grafts were sutured using a 10-0 nylon running suture.

The parameters of donor endothelial cells were detected at an average of 67.8 months postoperatively (ranging from 12 months to 23 years). Patients with history of contact lens wear were excluded from this study. At follow-up visits, after using tetracaine hydrochloride eyedrop (manufacturer) as topical anaesthesia, central corneal endothelial photographs were taken of 3 different central areas of the recommended sample size of 50-100 cells (2-4) with contact specular microscope (EM 1200, Tomey, Tennenlohe, Germany). Mean central endothelial cell density (ECD) and the coefficient of variation of endothelial cell size describing polymegethism were calculated with the built-in image analysis software (version 1.5.1) of the device. Regarding corneal thickness, the normalized magnification conversion table provided by the manufacturer was used to ensure an accurate cell density. The annual endothelial cell loss was calculated from the pre- and postoperative cell density. All data were assessed in relation to the preoperative diagnosis and the type of preservation. The protocol was in full compliance with Good Clinical Practices, the Declaration of Helsinki (1996), and the guidelines of the Medical and Health Science Centre of the University of Debrecen. Written informed consent was obtained from all patients for the publication of this paper.

Statistical analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago, Illinois, USA) and data were described in terms of mean ± standard deviation (SD), coefficient of variation (standard deviation/mean) and range. Differences between groups were recorded using the paired test of Wilcoxon, and a p value of 0.05 was considered as the level of significance. Associations between groups were established with Spearman’s correlation of rho (r).

Results

At all follow-up visits, each transplanted cornea was clear at slit-lamp examinations. At examination time recipient patients' mean age was 42.4±17.1 years. Mean age of donors was 66.2±14.3 years. The average postoperative follow-up time was 67.8±74.1 months (ranging from 12 to 276 months).

Endothelial parameters were counted in 3 different central donor corneal areas of 80.3±34 cell/mm^2 each. At examination time, the overall ECD was 1501±249 cell/mm^2 (ranging from 1100 to 2225 cell/mm^2). (Fig. 1.) In the keratoconus group, it was 1483±244 cell/mm^2, in bullous keratopathy group 1528±279 cell/mm^2, in herpetic keratitis group 1573±263 cell/mm^2, in leucoma group 1509±334 cell/mm^2. No difference in ECD was detected between keratoconus group and bullous keratopathy (p=0.58), herpetic keratitis (p=0.42) or leucoma (p=0.82) groups. 5.4 years after PKP average ECD was 1545±237 cell/mm^2 in the preserved group, 1467±256 cell/mm^2 in the moist chamber group (p=0.2). Average cell size was 665.4±118.8 mm^2 (ranging from 447.5 to 915.5 mm^2), the coefficient of variation of cell size was 0.61±0.11 (ranging from 0.37 to 0.85). Corneal thickness was 0.56±0.06 mm (ranging from 0.45 to 0.73 mm). Correlation between ECD and postoperative time was r=0.02 (p=0.85). At examination time, there was no significant correlation between patient's age and ECD (r=0.11, p=0.35). The rate of endothelial cell loss was 15.8 %/year in the first 2 years in the preserved group.

The coefficient of variation of cell size was 0.61±0.11 (ranging from 0.37 to 0.85). Mean endothelial cell size was 673.6±98.3 μm^2 (ranging from 447.5 to 915.5 mm^2). Corneal thickness was 0.56±0.06 mm (ranging from 0.45 to 0.73 mm) and was measured with contact specular microscope. No significant correlation was found between endothelial cell density and recipient (r=0.11; p=0.35) or donor age (r=0.04; p=0.83).

Discussion

Penetrating keratoplasty (PKP) has become the most frequently employed allograft transplantation surgery (Laibson and Rapuano, 1996). Pseudophakic bullous keratopathy, Fuch's dystrophy, keratoconus, corneal scarring, and aphakic bullous keratopathy (Price et al., 1993; Ing et al., 1998; Patel et al., 2000) are considered to be main indications for PKP.

Different studies on PKP have reported that the one-year survival rate of donor cornea is up to 90 %, at 5 years it is 88 %, at 10 years it is 80 %, and in 2007 Australian graft report it is 60 % (Williams et al., 1997, 2008; Thompson et al., 2003). Graft clarity rate can reach the extent of 97% after 4 years in low-risk group (Kirkness et al., 1990). In transplanted corneas, endothelium can keep its function in some cases even for 30 years (Bigar, 1982; Mishama, 1982; Armitage et al., 2003; Muraine et al., 2003), with an initial cell density above 2500 cells/mm^2. Minimal cell density at the time of penetrating keratoplasty is reported to range between 2000 and 2500 cells/mm^2 and the minimal, critical cell density limit for corneal decompensation is 250-500 cells/mm^2 (Hoffer, 1979; Olsen and Eriksen, 1980; Bigar, 1982; Waring et al., 1982; Yee et al., 1985; Mishama, 1992). Donor corneas with initial cell densities under 2000 cells/mm^2 could reach this critical cell density in less than 20 years (Bourne, 2001). In normal eyes the half- time for the slow component of ECD loss due to ageing is 224 years (Armitage et al., 2003), which is decreasing to 21-26 years after intraocular surgeries (Armitage et al., 2003).

In our study, corneal endothelium of 68 eyes with an optically clear donor cornea at slit lamp examinations were evaluated after an average of 5.4 postoperative years with a minimum cell density of 1100 cell/mm^2. We observed no higher endothelial cell loss associated with higher initial endothelial cell number.

Human corneal endothelial cells were first examined and photographed in vivo with a specular microscope in 1968 (Maurice, 1968). Age-related and postoperative changes, such as increasing pleomorphism, polymegethism, decreasing cell density in corneal endothelial layer and increasing corneal thickness have already been observed and well described in several studies (Laule et al., 1978; Yee et al., 1985; Carlson et al., 1988; Møller-Pedersen, 1997; Ing et al., 1998; Iwashita, 1998; Patel et al., 2000). Bourne reported that the markedly enlarged endothelial cells in long-term corneal transplants have a reduced ability to keep cornea clear (Bourne, 1995). Møller-Pedersen described higher rate of cell loss in younger groups (Møller-Pedersen, 1997). His data about annual loss of 2.9% up to 14 years and 0.3% after 14 years postoperatively is lower than those in our study.

Several studies report a rate of 0.3-0.6 % endothelial cell loss per year in normal corneas (Hoffer et al., 1980; Linn et al., 1981; Yee et al., 1985; Carlson et al., 1988; Bourne et al., 1997; Rao et al., 2000). The rate of endothelial cell loss is increased after intraocular surgery. (Linn et al., 1981; Liesegang et al., 1984; Ambrose et al., 1991) After cataract surgery, the rate of ECD loss increases up to 2.5%/year during the first 10 years (Bourne et al., 1994). ECD decreasing is also known after posterior lamellar keratoplasty (Patel et al., 2000; Bourne, 2001). Endothelial cell loss was more rapid after penetrating keratoplasty than that of postcataract patients in 5-10 postoperative years, although donor corneas obviously lose endothelial cells during preservation and transplantation procedure. Decreasing is generally observed in studies (Bourne, 1980; Abbot et al., 1983; Obata et al., 1992; Bourne et al., 1994), but there is one reported case finding with no endothelial cell decrease after PKP (Kus et al., 1999). Relatively few attempts have been made to study at least 5 years graft survival with at least 500 eyes (Redmond et al., 1992; Vail et al., 1993; Ing et al., 1998; Patel et al., 2000).

Endothelial cell loss after surgeries like penetrating keratoplasty is mostly described with biexponential model with two periods: a rapid period in the first postoperative year and a slow one that persists for years (Bourne, 1980; Zacks et al., 1990; Obata et al., 1992; Redmond et al., 1992; Bourne et al., 1994; Moller-Petersen, 1997; Langenbucher et al., 2000; Bourne, 2001; Böhringer et al., 2001, 2002; Armitage et al., 2003). In the first two postoperative years after penetrating keratoplasty an overall endothelial cell loss of 33% has been reported (Culbertson et al., 1982), similarly to our data of 15.8%/year in that period. After these two initial years, endothelial cell density continues to decrease with a 3-7 times higher rate than normally for up to 20 years after surgery (Abbot et al., 1983; Obata et al., 1992; Patel et al., 2000; Bourne, 2001; Böhringer et al., 2002). 10 years after keratoplasty, density resuction is at an average of 50-65% (Linn et al., 1981; Nishimura et al., 1999). After penetrating keratoplasty, the rapid period of endothelial cell loss is longer than after cataract surgery, but after 4 years this difference becomes negligible (Armitage et al., 2003). Another model for describing cell loss called monoexponential model underestimates early cell loss and overestimates long-term cell loss when applied for long-term data interpretation (Redmond et al., 1992).

Endothelial cell density decreases with age so donor age can be a significant risk factor for late endothelial donor failure (Bourne et al., 1997). Only donor corneas with an endothelial cell density of at least 2000 cells/mm^2 were suitable for penetrating keratoplasty in our study. One of the researchers observed higher endothelial cell loss in grafts for keratoconus (Patel et al., 2005), others reported higher ECD loss after penetrating keratoplasty for Fuchs' dystrophy or corneal oedema (Reinhard et al., 2002; Langenbucher et al., 2002). Opposing studies, similarly to our data, found no significant correlation between endothelial cell loss and recipient or donor age or preoperative diagnosis, even in long term period (Linn

et al., 1981; Abbot et al., 1983; Kus et al., 1999; Nishimura et al., 1999; Inoue et al., 2002; Zadok et al., 2005).

In one of his publications, Kus reported an endothelial cell density of 808±194 cells/mm^2 with a mean thickness of 608±75 μm correlated with neither thickness nor with follow-up interval (Kus et al., 1999). Coefficient of variation of cell area was approximately 0.29 in the first 5 years in Ing's study, which was smaller than corresponding data in our study (Ing et al., 1998). The corneal thickness was between 0.54 and 0.57 mm in the first 5 postoperative years (Ing et al., 1998), which is similar to our data.

In conclusion, the process of endothelial cell loss is highly accelerated after penetrating keratoplasty. There are relatively few studies with more than 5 years postoperative donor endothelial data as in our study (Linn et al., 1981; Vail et al., 1993; Ing et al., 1998; Kus et al., 1999; Patel et al., 2000; Bourne, 2001; Zadok et al., 2005). The rate of loss was similar in different preoperative causative lesions, and did not differ in consideration of preoperative donor endothelial cell number, donor or recipient's age.

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Author(s)	Mean postoperative time	ECD loss in %	ECD at postoperative time (cells/mm^2)
Linn et al. ()	1-10 years	50%/10 years	502-1708

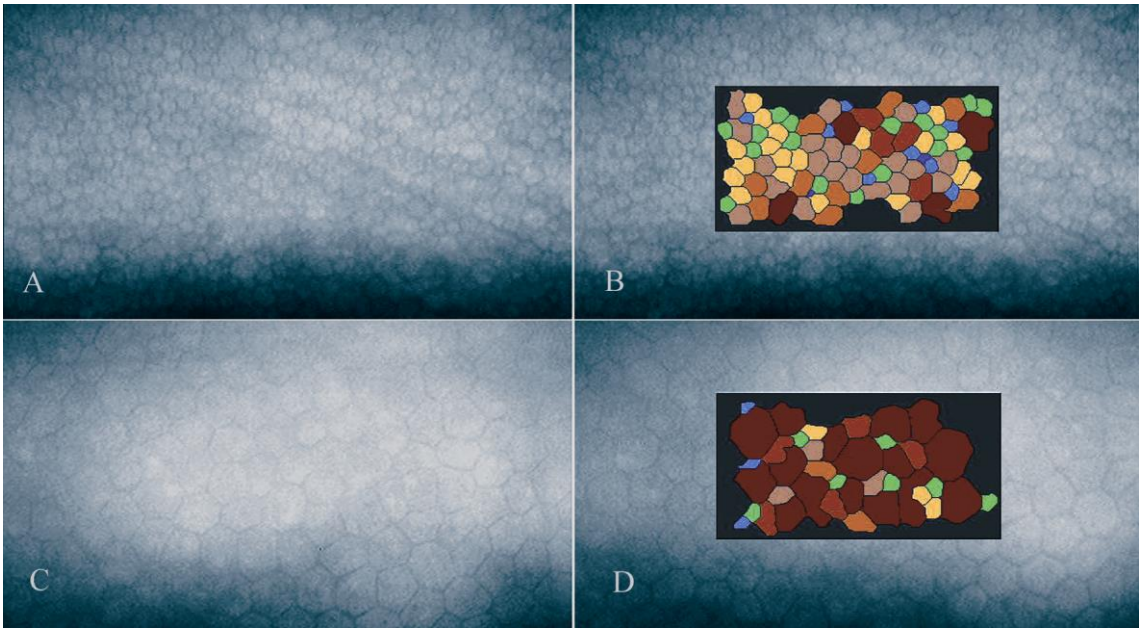
Table legend

Table 1.: Donor corneal endothelial cell loss after penetrating keratoplasty in various studies (ECD: endothelial cell density).

Linn et al. ()	20 years		502-1708
Abott et al. ()	17.4 years		684
Bourne et al. ()	3 years	52±19% compared to the initial number	1418±600
Bourne et al. ()	5 years	59±17% compared to the initial number	1214±533
Ing et al. ()	1 year	34±22% compared to the initial number	1958±718
Ing et al. ()	3 years	53±19% compared to the initial number	1376±86
Ing et al. ()	10 years	67±17% compared to the initial number	960±470
Kus et al. ()	22±6 years		808 ± 194
Langenbucher et al. ()	2 years	9.5%/year	1751±605
Bourne et al. ()	3 years	53±19%	1376±586
Bourne et al. ()	5 years	59±17%	1191±523
Bourne et al. ()	15 years	72±10%	850±237
Inoue et al. ()	10 years	12.1±16.3	998±343
Langenbucher et al. ()	2 years	2.9±28.0%/year	1617±553
Patel et al. ()	15 years	71±12% compared to the initial number	872±348
Zadok et al. ()	13.3 years		695±113.6

Figure legend

Figure 1. Endothelial image analysis made by the contact specular microscope
A. and B.: number of cells counted: 86 cells, endothelial cell desity: 2300 cells per square millimeter, average cell size: 431 μm^2, coeffitient variation of the cell area: 0,46.
C. and D.: number of cells counted: 54 cells, endothelial cell desity: 1600 cells per square millimeter, average cell size: 616 μm^2, coeffitient variation of the cell area: 0,71.


A
B
C
D