Saudi Journal of Ophthalmology (2019) 33, 7–11

Original Article

Five-year endothelial cell count post penetrating keratoplasty using internationally-transported corneal donor tissue

Ashbala Khattak *; Fouad anNakhli

Abstract

Aim: To study the five-year endothelial cell loss in patients having undergone penetrating keratoplasty (PKP) and who received corneal donor tissue from international eye banks.
Methods: This retrospective clinical study reviewed outcomes in 41 patients who underwent PKP at a tertiary eye center from February 2008 to July 2011. Standard PKP surgical technique was used for all patients, and graft tissue was supplied in all cases by eye banks in the United States of America. At five years after surgery, measurements were taken of endothelial cell density (ECD), coefficient of variation, hexagonality, donor's age, recipient's age, Death-to-preservation (DP), and preservation time (PT).
Results: The recipients and donors median age was 30.0 years (24.0–35.5 years) and 59.0 years (53.0–61.0 years) respectively; the median DP and PT were 8.0hours.
(6.0–10.0hours) and 10.0 days (9.0–11.5 days) respectively. At baseline, the ECD was 2398(2325–2525). At five years after surgery, all of the grafts were found to have survived; the median ECD was 1035 cell/mm^2 (693–1346 cell/mm^2); the mean coefficient of variation was 35.2 ± 9.8%; and the mean hexagonality was 63.7 ± 24.3%. The overall ECD loss was 56% (95% CI: 50–62%); the ECD loss was 51.3% and 61.2% in corneas from donors younger and older than 60 years respectively (p = 0.056); likewise, the ECD loss was 52.6% and 61.0% in corneas with PT shorter and longer than 10 days respectively (p = 0.289). Although the difference in both cases was not statistically significant, it was clinically important (about 10%).
Conclusion: The ECD after five years in patients undergoing internationally-transported corneal tissue grafts incurred 56% loss; the donor's age and the PT were positively associated with ECD loss.

Keywords: Penetrating keratoplasty, Corneal endothelial cell loss, Corneal endothelial polymegathism, Corneal endothelial pleomorphism

https://doi.org/10.1016/j.sjopt.2018.10.005

Introduction

The clarity of a corneal graft several years after transplantation is mostly dependent on the endothelial cell density. Following surgery, endothelial cells are progressively lost over time, at a higher rate than average cell loss.[1,2] The underlying reasons for this observed cell loss several years after surgery are poorly understood, although factors which have been shown to be connected with this loss include pre-operative donor endothelial cell density (ECD), death-to-preservation time (DP) of the donor tissue, preservation time (PT), the donor's age, storage media, surgical trauma, glaucoma, rejection and accelerated aging.[3,4] A graft for transplantation is often selected based on the ECD and the

Received 14 February 2018; received in revised form 6 August 2018; accepted 14 October 2018; available online 22 October 2018.

Dhahran Eyes Specialist Hospital, Dhahran, Eastern Province, P.O. Box: 39455, Zip: 31942, Saudi Arabia

* Corresponding author.
e-mail address: ashbalakhattak@gmail.com (A. Khattak).

Peer review under responsibility of Saudi Ophthalmological Society, King Saud University

Production and hosting by Elsevier

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age of the donor. The Cornea Donor Investigator Group has published figures for five- and ten-year endothelial cell loss and factors associated with cell loss after penetrating keratoplasty, and has found that younger donor age (<65) and higher baseline ECD are connected with a lower rate of cell loss.[1,5]

In countries where surgeons are dependent on graft tissue shipped from eye banks thousands of miles away, graft tissue is typically unable to be individually selected for a particular patient, and the tissue will remain in storage for as long as it takes to transport it internationally to the hospital where it will be used.[6] This retrospective study reviews the cell loss outcomes in patients five years after receiving penetrating keratoplasty involving internationally-shipped graft tissue, and assesses other factors which may be involved in this observed cell loss.

Methods

The study was conducted at a tertiary care eye hospital; it was approved by the hospital's Institutional Review Committee and adhered to the Declaration of Helsinki. Forty-one eyes from thirty-four patients who underwent penetrating keratoplasty from February 2008 to July 2011 were included in the study. Indications for the keratoplasty were keratoconus (31 patients), macular dystrophy (6 patients), pseudophakic bullous keratopathy (2 patients), and corneal scars (2 patients). Patients with pre-operative glaucoma or uveitis were excluded from the study. All patients received graft tissue provided by eye banks in the United States of America. All donor corneas were sent for microbiology routinely; none of the cultures were positive for any bacteria or fungus in all the samples. Data recorded from the donor tissue included endothelial cell density, donor age, DP, and PT. Data recorded post-surgery included five-year endothelial cell count, cell variance, cell size, post-surgery trauma, endothelial rejection, glaucoma, and intraocular pressure spike or post transplant intraocular surgery. Intraocular pressure spike was defined as intraocular pressure > 24 mmHg.

Specular Microscopy

Device and acquisition

All postoperative images were acquired via SP-3000P Topcon Specular Microscopy (Topcon Corporation, Tokyo, Japan). The imaging area captured by the device is 0.25 × 0.50 mm, and the capturing magnification is 150×, as shown in Fig. 1. Although the device has the capability to capture images at the two, six, ten and twelve o'clock positions, all images for this study were captured at the centre of the cornea. The patient was asked to blink twice and fixate a central green light, with focusing, fine alignment, flash, and camera triggering being carried out automatically by the imaging device.

Image quality

For the purpose of this study, images were classified as being of either analysable or unanalysable quality. For analysable images, the following definitions were used: excellent quality images were defined as having all cell borders, boundaries, and centers distinct except for peripheral edges, with at least 50 contiguous cells from a single fixed frame; good quality images were defined as having distinct borders, boundaries, and centers with at least 30 contiguous cells from a single fixed frame; and fair quality images were defined as having at least 15 contiguous cells from a single fixed frame. Unanalysable images were those in which most cells showed indistinct borders, boundaries, and centres. For this research, all images obtained were of analysable quality, with most being classified as good quality images.

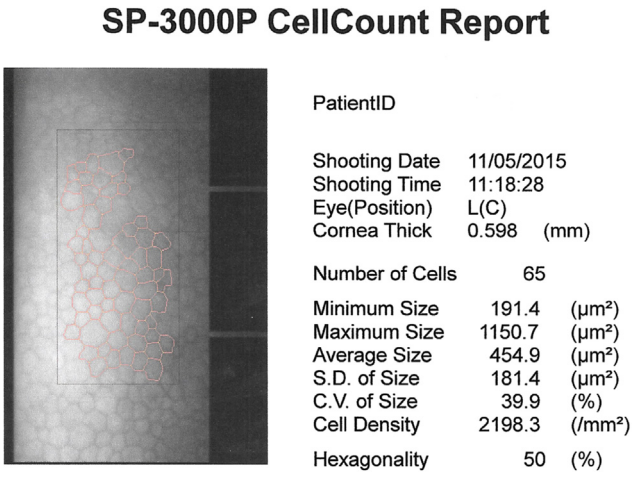

Fig. 1. Specular microscopy report.

ECD determination

Reference measurements for ECD for each donor and the time at which the sample was preserved were noted by the eye bank. Values for the coefficient of variation and hexagonality of baseline donor endothelial cells were not available from the eye bank. The five-year ECD was determined via single fixed-frame imaging using IMAGEnet Cell Analysis software equipped with the device. The software generates a report (Fig. 1) with all endothelial cell parameters including number of cells, ECD, minimum cell size, maximum cell size, average cell size, standard deviation (SD), coefficient of variation (C.V.) which is directly related to polymegathesim or variation in size, and the percentage of hexagonality which is inversely related to pleomorphism or variation in shape. All postoperative images were acquired by experienced optometrists using the technique described above.

Surgical procedure

All penetrating keratoplasty procedures were performed under general anesthesia, and all patients received graft tissue from USA eye banks. The recipient bed was prepared by appropriate centration and sizing of the recipient cornea. Hessburg-Barron vacuum trephine (Katena Products Inc, Denville, NJ) was used to trephinate the recipient cornea. A graft oversize was punched from the donor cornea using a donor punch (Katena Products Inc, Denville, NJ) of 0.25 mm in keratoconus patients and 0.5 mm in patients with a diagnosis other than keratoconus. The recipient corneal dissection was completed using corneal scissors, and the donor tissue was sutured to the host bed with 10–0 nylon sutures. Sixteen

interrupted sutures were placed to secure the donor cornea in position. All knots were rotated and sub-conjunctival injections of Dexamethasone and Gentamicin were given. No cataract extraction or another combined procedure were performed.

Postoperative management

All patients were seen at one day post-surgery, at one week, at one month and then subsequently every one to two months. All patients received antibiotic and steroid eye drops. Antibiotic eye drops were stopped after two weeks; all patients received frequent steroid eye drops in the first few weeks i.e. every two hours, and these were then slowly tapered over a period of one year and stopped at this time if the patient was clinically stable. The dosage of the steroid eye drops was modified if the intraocular pressure (IOP) increased in any of patients.

These patients were also followed up post-keratoplasty using topography (Pentacam or Atlas) to monitor postoperative corneal astigmatism. Sutures were removed after twelve months if graft-host junction healing was deemed complete, although in some patients selective suture removal was carried out as early as four months. Antibiotic and steroid eye drops were used for one week following suture removal, and topography and manifest refraction were acquired at 6–8 weeks following suture removal.

Statistical analysis

The normality of the data was assessed by the Shapiro-Wilk test. Paired t-test was performed to compare baseline donor and five-year endothelium parameters. Unpaired t-test was performed to compare levels of factors. Non-normally distributed data were tested with Mann–Whitney U test. Percentage difference of 10% was considered clinically significant. P value less than 0.05 was considered statistically significant. Statistical analysis was implemented using IBM SPSS Statistics for Windows (V.22, Armonk, NY: IBM Corp).

Results

Forty-one eyes from thirty four patients underwent penetrating keratoplasty by the first author; of whom eighteen were males and sixteen females. The median age of recipients was 30.0 years (24.0–35.5 years), whereas the median age of donors was 59.0 years (53.0–61.0 years). The median DP was 8.0hours (6.0–10.0 h), while the PT was 10.0 days (9.0–11.5 days). Table 1 shows the statistics values for the parameters of corneal endothelium study. Indications for keratoplasty were 31 eyes (75.6%) with keratoconus, 6eyes (14.6%) with macular dystrophy, 2eyes (4.9%) with decompensation, 1eye (2.4%) with scar, and 1eye (2.4%) with microbial keratitis. Prior to keratoplasty, thirty-eight eyes were phakic and three eyes were pseudophakic.

Table 1. Parameters of corneal specular microscopy study.

Parameter	Statistic
Recipient's age (year)	30.0(24.0–35.5)*
Donor's age (year)	59.0(53.0–61.0)*
DP (hour)	8.0(6.0–10.0)*
PT (day)	10.0(9.0–11.5)*
Baseline donor ECD (cell/mm^2)	2398(2325–2525)*
Five year ECD (cell/mm$^{2)}$	1035(693–1346)*
Five year CV (%)	35.24 ± 1.52**
Five year hexagonality (%)	63.68 ± 3.79**

ECD denotes endothelial cell density; DP, Death-to-preservation; PT, Preservation time; CV, coefficient of variation.
* Median (interquartile range).
** Mean ± standard deviation.

The mean preoperative baseline ECD was 2466.3 ± 204.3 cell/mm^2 while mean five-year postoperative ECD was 1082.4 ± 435.5 cell/mm^2. The baseline ECD had small correlation with 5 years ECD (Spearman's rho = 0.091 with p = 0.571). The mean ECD difference between baseline and 5 years was 1384.0 ± 477.0 cell at $p < 0.0001$; which was an ECD loss of 56% (95% CI: 50–62%). After five year postoperatively, the mean cell area was 1056.4 ± 397.21 μm^2; the mean coefficient of variation was 35 ± 10%; the mean hexagonality was 64 ± 24%. Table 2 compares ECD loss and 5 years ECD between sublevels of donor's age, recipient's age, gender, DP, and PT. The cutoffs for sublevels were selected in such a way both levels have similar sample size. Corneas from younger donor or corneas with shorter PT had 10% less ECD loss than corneas from older donor or corneas with longer PT. Corneas from donors older than 60 years had ECD loss of 61.2% compared to ECD loss of 51.3% in corneas from donors younger than 60 years. Also corneas with PT longer than 10 days had ECD loss of 61.0% compared to endothelial loss of 52.6% in corneas with PT shorter than 10 days.

Secondary surgeries/complications

There were no intraoperative complications, and all keratoplasty were uneventful. The graft survival was 100% at five years; of the 41 eyes, only a single episode of rejection occurred to two eyes (4.9%) and treated with medication successfully without recurrence. Eight eyes (19.5%) had IOP spike (IOP > 24 mmHg) and controlled successfully with medication. Twelve eyes (29.3%) had astigmatic keratotomy for high cylinder. One eye (2.4%) had phototherapeutic keratectomy for sub-epithelial scar from viral keratoconjunctivitis. One eye (2.4%) had Nd:YAG (neodymium-doped yttrium aluminium garnet) laser for posterior sub-capsular opacification. Otherwise, neither uveitis, nor glaucoma, nor trauma were observed at any time during the five-year follow-up period.

Discussion

The development of Optisol GS over two decades ago has meant that it is now practical for graft tissue to be preserved for as long as two weeks, allowing the transport and use of donor tissue across international and intercontinental borders and greatly facilitating the treatment of reversible blindness worldwide.[7,8] This study investigated five-year outcomes of corneal transplantation in patients who received donor tissue from eye banks in the USA. Surgeons are typically reluctant to use donor corneas that have remained in storage media for longer than 7 days if other options are available; however, there was an average interval of 10 days PT in all of our patients.[1,7]

The patients in this study had post-transplantation outcomes that were generally successful. All of the grafts survived over five years. Two patients underwent rejection

Table 2. Factors associated with corneal endothelial cells changes five years after keratoplasty.

Factor	Level	N	ECD loss (%)	5 years ECD (cell/mm^2)
Donor's age (year)	<60	22	51.3	1186 ± 457
	≥60	19	61.2	962 ± 386
	P-value		0.056	0.105
Recipient's age (year)	≤30	23	54.6	1124 ± 442
	>30	18	57.5	1030 ± 434
	P-value		0.546	0.401
Gender	Males	24	54.3	1124 ± 449
	Females	17	58.1	1022 ± 422
	P-value		0.597	0.327
Death-to-preservation (hour)	≤8	22	54.7	1099 ± 423
	>8	19	57.3	1063 ± 461
	P-value		0.652	0.799
Preservation time (day)	≤10	25	52.6	1176 ± 473
	>10	16	61.0	935 ± 333
	P-value		0.289	0.084

ECD, endothelial cell density.

episodes that were treated and reversed early enough to be successful. It is common to see elevated intraocular pressure post keratoplasty.[9,10] In this research, eight patients experienced intraocular pressure elevation which was medically controlled.

The endothelial cell loss at five years was shown to be 56 % (CI: 50–62%), and this five-year loss is comparable to other similar studies carried out elsewhere in the world in patients with keratoconus.[11,12] Figures published by the Cornea Donor Investigator Group showed five-year endothelial cell loss to be 69–75%; this study was a large, multicentre, randomised controlled study using 347 subjects, and unlike the current research, the primary indication for surgery was pseudophakic/aphakic corneal edema, followed by Fuch's corneal edema, with 3% of the patients having other diagnoses.[1,5] In addition, the age group of patients involved in the current study was much younger than the group used in Cornea Donor study, and keratoconus (KCN) was the main indication for penetrating keratoplasty. Several published studies have found similar rates of cell loss in patients with KCN.[12] Research carried out by Obata et al. has indicated that the rate of cell loss is dependent on the diagnosis of the recipient, with keratoconus patients showing the lowest rate of cell loss after one year of observation.[13] Similarly, Gupta et al, Langenbucher et al, and Hyun Soo found that the endothelial cell loss after PKP was the least in keratoconus.[14–16] Our study shows that a higher patient age and a longer PT both have an effect on endothelial cell density. Advanced donor age and longer PT were both associated with lower five-year ECD. This finding is consistent with earlier research by Böhringer et al, in which advanced age, DP, and to a less degree PT were found negatively associated with loss of endothelial cells.[17] Our study baseline donor ECD showed very low correlation (0.091) with five year ECD, with $p = 0.571$; Bertelmann et al showed similar results in their published research.[18] For the international transport of graft tissue, longer preservation times are unavoidable; and for this cohort of patients, where most of the subjects are younger than 40 years of age, outcomes are generally successful. However, clinical experience suggests that for an older group of patients, these longer preservation times may negatively affect the graft surface and cause abnormal surface healing patterns, requiring a longer postoperative recovery period.

Endothelial morphometric parameters of Coefficient of Variation (CV), which is directly related to polymegathesim, and Hexagonality (HEX), which is inversely related to pleomorphism, have shown the prospect of being more sensitive than ECD in the detection of endothelial dysfunction, although The Cornea Donor Investigator Group investigated this hypothesis and found that these parameters show significant fluctuation and may not add to the predictive value of ECD.[19] Nevertheless, it was suggested in earlier research by Feizi et al that pleomorphism was associated with donor age ($p = 0.01$).[20] Other studies have found increased polymegathesim and pleomorphism 10 years post keratoplasty.[21] This research was limited by unavailability of baseline polymegathesim and pleomorphism which prevented their analysis. Though our younger patients tended to show 10% less ECD loss, suggesting that a relatively young and healthy intraocular nutritive environment may better support the newly implanted graft tissue; however, it's an assumption and needs to be explored with further studies. Moreover, the present research found that patients who received grafts with longer PT showed 10% more ECD loss, indicating more instability and variation.

This research involves patients receiving graft tissue from international eye banks with accordingly longer tissue preservation times. It analyses and compares these outcomes with similar studies done elsewhere in the world. This research is limited, however, by the small size of the patient sample and the preponderance of keratoconus as an indication for surgery. More studies involving larger sample sizes are required in order to further evaluate the final outcomes in patients receiving grafts from international eye banks. There is also a need for a wider patient sample which includes older patients and a larger range of diagnoses in order to allow better understanding and prediction of outcomes in such cases.

Conflicts of interest

The authors declared that there is no conflict of interest.

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