Visual acuity and endothelial cell density with respect graft thickness in **Descemet's** to the stripping automated endothelial keratoplasty: one year results

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Abstract

• AIM: To evaluate the visual acuity and endothelial cell density according to the thickness in Descemet's stripping automated endothelial keratoplasty (DSAEK) one year after surgery.

• METHODS: DSAEK patients' data were reviewed. Thirty seven eyes of 37 patients who underwent DSAEK for pseudophakic bullous keratopathy (PBK) were included in this study. Graft thickness was measured with optical coherence tomography (OCT) 12mo after DSAEK. Eyes were divided into 3 groups based on the graft thickness: thick (>200 μ m), medium-thick (150-200 μ m) and thin (<150 µm). Best corrected visual acuity (BCVA), endothelial cells density (ECD) and complications were assessed and comparisons were done between groups.

• RESULTS: Median thickness of postoperative grafts was 188 (range 73-317 μ m). There was no significant difference in age, sex, preoperative BCVA, or follow-up period between DSAEK groups. At postoperative 12mo, mean BCVA was 0.28±0.10 in thick graft group, 0.52±0.08 in medium-thick graft group, and 0.72±0.06 in thin graft group. Thin grafts showed better postoperative BCVA as compared with the medium-thick and thick grafts (P= 0.001). Thick graft group had 1637.44 ±88.19 -mm², medium thick graft had 1764.50±34.28-mm² and thin graft group had 1845.30 ±65.62 -mm². Thin graft group had better ECD at 12mo after surgery (P=0.001).

• CONCLUSION: Thin grafts after DSAEK ensure better rehabilitation. Eyes with visual thin grafts had significantly lesser loss of ECD compared to eyes with medium-thick and thick grafts one year after surgery.

• KEYWORDS: Descemet's endothelial stripping keratoplasty; graft thickness; visual acuity; corneal endothelial cell density

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INTRODUCTION

P enetrating keratoplasty (PK) has been very popular for the treatment of many corneal diseases for over a century. However, disadvantages of PK like endothelial rejection results in searching new surgical techniques [1,2]. Unlike PK where the entire cornea replaced in order to decrease the risks, these techniques are intended to replace, either just the endothelium or the diseased anterior portion of the cornea. For this purpose partial thickness posterior lamellar surgery was developed as an alternative^[3,4].

In lamellar surgeries, endothelial keratoplasty has gained increasing popularity for the treatment of endothelial diseases. According to the Eye Bank Association of America 2009 statistical report 30.50% of all keratoplasties were endothelial (18 221 of 42 606 tissues used in America)^[5]. With Descemet's stripping automated endothelial keratoplasty (DSAEK), stromal dissection on the donor cornea is done with an automated keratome. In DSAEK, creating thinner grafts have been assumed to be better for visual outcomes and anatomic success however the creation of thinner donor tissue is technically difficult. Theoretically, thinner grafts are seen to be more efficient because inserting the same tissue taken instead of more tissue is better for anatomic success in spite of lacking clear evidence since now. While folding and inserting the thin donor tissue through the corneal tunnel incision, more endothelial damage and tissue wrinkling are possible complications because of lacking stromal support^[6]. Because of lack of strong evidence to support the use of thin

graphs, the goal of this study was to determine whether graft thickness influences visual acuity, endothelial cell loss and complications or not^[7-11].

SUBJECTS AND METHODS

All DSAEK surgery patients' data were reviewed in Cornea Department of Haydarpasa Numune Education and Research Hospital and thirty-seven eyes of 37 patients (17 men, 20 women) with pseudophakic bullous keratopathy (PBK) who underwent DSAEK were included in this retrospective study. The study was conducted in accordance with the tenets of the Declaration of Helsinki and informed consent was obtained from each participant. All the patients had no history of ocular surgery or disease other than cataract surgery. They had no active or previous ocular or systemic disease likely to affect corneal wound healing.

The donor corneal lenticules were obtained from the Haydarpasa Numune Education and Research Hospital Eye Bank which were under the age of 50, had higher than 2500-mm² and remained covered by Optisol-GS solution (Bausch&Lomb, St. Louis, MO, USA) until use. All donor coneal endothelial cell counts were done by same eye bank technician by donor specular microscope (Keratoanalyzer, EKA-98, Konan Medical, U.S.A Inc.).

Surgical Procedure All surgeries were performed by one surgeon (Acar BT) under monitored local anesthesia. A supero-temporal side-port incision was made in the clear cornea using the 20-gauge microvitreoretinal blade, followed by air injection into the anterior chamber. A scoring hook for DSAEK (Janach, Como, Italy) was used to incise and 'T' shaped DSAEK stripper (Janach, Como, Italy) to remove the host endothelium and Descemet's membrane along the circumference corresponding to the epithelial trephine mark. Trypan-blue was injected into the anterior chamber to carefully check for any remnants of Descemet's membrane within the borders of the epithelial trephine mark. A temporal 5.0 mm and nasal 3.0 mm incisions were made in the clear cornea.

The donor lenticule was prepared from the donor cornea and its scleral rim by using a Moria ALTK microkeratome with and a Moria artificial anterior chamber maintainer (Moria, Antony, France) filled with balanced salt solution (BSS; Alcon, Fort Worth, TX, USA). Prior to the microkeratome cut, the thickness of the donor cornea was measured and either 300 or 350 μ m head was used depending on the central corneal thickness (CCT). For CCT values up to 520 μ m the 300 μ m microkeratome head and for thicker donor corneas the 350 μ m head was used. Graft was punched from the endothelial side by using Barron-Hessburg trephine (Katena, Danville, NJ, USA). The endothelial surface of the donor lenticule was coated with small amount of viscoelastic and gently placed into a Busin glide (Moria, Antony, France), endothelial side up. After placement of anterior chamber

maintainer, the Busin glide was inserted through the 5.0 mm temporal incision and the folded donor corneal lenticule was gently grasped at the leading stromal edge and placed into the anterior chamber, using the 23-gauge Busin micrograsper (Moria, Antony, France). A 5 mL syringe of balanced salt solution with a 30-gauge cannula was used to unfold the donor corneal lenticule. Following the removal of the anterior chamber maintainer, the anterior chamber was filled with 100% air to moderate tactile tension by using a cannula for 30min. The incisions were sutured using 10/0 nylon. At the end of 30min, approximately 1/3 of air bubble was partially removed and replaced with BSS. Postoperatively, patients received topical antibiotics 1 drop 4 times a day and topical prednisolone phosphate 1% one drop 6 times a day. After 1mo, the topical prednisolone phosphate 1% was gradually tapered over a 3mo period and maintained at twice daily dosing.

All patients underwent a detailed ophthalmologic examination including best corrected visual acuity (BCVA), slit lamp biomicroscopy, optical coherence tomography (OCT), fundus examination and B-scan ultrasound and specular microscopy.

Patients (n=37) were divided by the postoperative thickness of lamellar graft in three subgroups: thick lamella was >200 μ m (*n*=13), medium-thick lamella was 150-200 μ m (n=9) and thin lamella was <150 μ m (n=15). The thickness of a lamellar graft was evaluated with non-contact OCT (Optovue Inc., Fremont, CA, USA), BCVA was measured at standard Snellen chart in same room and at same distance, postoperative refractive error was estimated by subjective and objective refraction by retinoscopy, autorefractometer and corneal topography at the postoperative 12mo. Corneal endothelial cell analysis was performed with a noncontact specular microscope (EM-2000, Tomey, Germany). In each analysis, endothelial cell counts were obtained by the same technician using the variable-frame analysis method, in which the borders of the largest possible known area of cells are outlined, preferably incorporating a minimum of 100 contiguous cells. The cells in this area are counted and the number of cells within the given area is used to calculate the endothelial cells density (ECD). We evaluated the influence of lamellar thickness on the visual recovery and endothelial cell density.

Statistical Analysis Statistical analysis was performed using the number cruncher statistical system (NCSS) 2007 & power analysis and sample size (PASS) 2008 Statistical Software (Utah, USA). Besides descriptive statistics (mean \pm standard deviation, median, frequency, ratio) Kruskal-Wallis test was used between group comparisons not a normal distribution of parameters. If significant difference between the groups was found, Mann-Whitney U test was used to evaluate the group that led the difference. Changes between the preoperative and postoperative values in the same group were compared using

Graft thickness after Descemet's stripping automated endothelial keratoplasty

Table 1Preoperative and preoperative and preopera	postoperative visual acui	ties with respect to the gr	aft thickness $\overline{x} \pm s$	(median)
Variables –	Graft thickness			
	>200 µm (<i>n</i> =13)	150-200 μm (<i>n</i> =9)	<150 µm (<i>n</i> =15)	Г
Preop. VA	0.02±0.02 (0.02)	0.03±0.04 (0.03)	0.05±0.03 (0.04)	0.091
Postop. VA	0.28±0.10 (0.30)	0.52±0.08 (0.50)	0.72±0.06 (0.70)	0.001 ^b
^{2}P	0.008^{b}	0.028^{a}	0.005 ^b	
Difference (preoppostop.)	0.26±0.09 (0.27)	0.49±0.06 (0.49)	0.67±0.04 (0.66)	0.001 ^b

VA: Visual acuity. ¹Kruskal Wallis test; ²Wilcoxon signed ranks test. ^aP<0.05, ^bP<0.01.

the Wilcoxon signed rank test. A P value <0.05 was considered as the indication of statistical significance. Correlations were tested using the Pearson correlation test.

RESULTS

This study included a total of 37 PBK patients with no previous ocular disease before surgery, who were treated with DSAEK. These patients were divided into 3 subgroups according to the postoperative thickness of grafted lamella: thick grafts (thickness>200 µm; range: 220-317 µm), medium-thick grafts (150 μ m \leq thickness \leq 200 μ m; range 167-195 µm) and thin grafts (thickness<150 µm; range: 73-146 µm). OCT pictures representative of each DSAEK group is shown in Figure 1. Median thickness of postoperative grafts was 188.32 µm (range 73-317 µm) at 12mo postoperatively. There was no statistically significant difference in age, sex, preoperative BCVA or follow-up period between groups. The mean age of patients was 72.3± 8.2y (range 56-82) in the thick graft group, $74.2 \pm 5.8y$ (62-79) in the medium-thick graft group and 75.0 ± 4.9 y (67-81) in the thin graft group. There was no significant difference in age between the three groups (P=0.452). Data were compared in regards to visual outcome, endothelial cell density and complications at 12mo postoperative.

There were 13 (35.15%) thick grafts which had thickness of $>200 \ \mu\text{m}, 9 \ (24.35\%)$ medium thickness grafts which had thickness of 150-200 µm and 15 (40.50%) thin grafts which had thickness of <150 µm at 12mo visit. Thin DSAEK grafts showed statistically significant better postoperative BCVA as compared with the medium-thick and thick grafts. In thin graft group 3 patient had 0.8, 10 patients had 0.7 and 2 patient had 0.63 BCVA. In medium thick group 2 patients had 0.63, 3 patients had 0.5 and 4 patient had 0.4 BCVA. Thick grafts never reached BCVA of thin DSAEK grafts. All eyes in thin group had BCVA ≥ 0.6 at 12mo after surgery. In contrast, only 55% of medium-thick and none of the thick DSAEK grafts reached BCVA ≥ 0.5 at 12mo after surgery. At 12mo visit mean BCVA was 0.28 ±0.10 in thick graft group, 0.52 ± 0.08 in medium thickness graft group, and 0.72 ± 0.06 in thin graft group. The difference between preoperative and postoperative BCVA were 0.26 ± 0.09 , 0.49 ± 0.06 , 0.67 ± 0.04 in order of thick grafts to thin grafts. Mean visual acuity of thin group was statistically better than other 2 groups (P=0.001; P=0.001). In addition, mean visual



Figure 1 DSAEK grafts A: Thick graft; B: Medium-thick graft; C: Thin graft.



Figure 2 Correlation of BCVA at 12mo and graft thickness Negative correlation between 12mo graft thickness and visual acuity (vision falls with increasing thickness) at the level of 96% was found which was a statistically significant correlation(r=-0.960, P<0.001).

acuity of median-thick group was statistically better than thick group (P=0.002; P<0.01). Preoperative and postoperative visual acuities according to the graft thickness are given in Table 1 and Figure 2.

Negative correlation between 12mo graft thickness and visual

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Table 2 i reoperative (grate) and postoperative endottenar een densites with different timekiess of grate $x \ge 5$ (including)							
Variables	Graft thickness			¹ D			
	>200 µm (<i>n</i> =13)	150-200 μm (<i>n</i> =9)	<150 µm (<i>n</i> =15)	- <i>Г</i>			
Graft ECD	2883.78±109.29 (2865.0)	2879.83±139.17 (2912.0)	2873.80±85.09 (2876.0)	0.747			
Postop ECD	1637.44±88.19 (1645.0)	1764.50±34.28 (1772.0)	1845.30±65.62 (1863.0)	0.001 ^b			
^{2}P	0.008^{b}	0.028 ^a	0.005 ^b				
Difference (preoppostop.)	1246.33±79.85 (1235.0)	1115.33±107.80 (1132.0)	1028.50±108.23 (1025.5)	0.001 ^b			

Table 2 Preoperative (graft) and postoperative endothelial cell densities with different thickness of graft $\bar{x} \pm s$ (median)

ECD: Endothelial cell density; ¹Kruskal Wallis test; ²Wilcoxon signed ranks test. ^aP<0.05, ^bP<0.01.

acuity (vision falls with increasing thickness) at the level of 96% was found which was a statistically significant correlation (r=-0.960, P<0.001). Moreover, mean cylindrical refraction change of the patients was (1.00 ± 0.75) D and mean hypermetropic shift of the cases were $(+1.50\pm1.00)$ D. The difference of astigmatism between groups was not statistically significant (P=0.501). There was no correlation between graft thickness and astigmatism (r=0.26, P=0.125). Hypermetropia difference between thin and thick graft, medium and thick graft groups was statistically significant (P=0.010, P=0.035). However, hypermetropia difference between thin and medium graft groups was not statistically significant (P = 0.601). Moreover, positive correlation between graft thickness and hypermetropia at the level of 65% was found which was a statistically significant correlation (1=0.65, P<0.001; Figure 3).

Thin graft group had better ECD at 12mo after surgery. Mean ECD values at 12mo of surgery were 1637.44 ± 88.19 -mm² (preop. 2883.78 ±109.29, P=0.008) in thick graft group, 1764.50±34.28-mm² (preop. 2879.83±139.17, P=0.028) in medium thick graft, and 1845.30 ± 65.62-mm² (preop. 2873.80 ±85.09, P=0.005) in thin graft group. Endothelial cell densities with respect to the thickness of the graft in 3 groups are given in Figure 4. Negative correlation between 12mo graft thickness and endothelial cell density (ECD decreases with increasing thickness) at the level of 89% was found which was a statistically significant correlation (*r*=-0.890, P<0.001). Preoperative and postoperative ECD of three groups are given in Table 2.

There was statistically significant difference of mean ECD at 12mo between three groups (P < 0.01). Mean ECD in thin group was greater than other two groups and this difference was statistically significant (P = 0.001, P = 0.013). Additionally, mean ECD in medium-thick group was greater than thick group and results were statistically significant (P=0.007, P < 0.01).

Four eyes of thick graft group (31%) had total graft dislocation at 1st postoperative day while 2 eyes had partial dislocation in thin (13%) and 2 eyes had total graft dislocation in medium thick graft (22%) group. In thin grafted group partial dislocation was treated by paracentesis of interface fluid successfully. Total graft dislocations diagnosed by biomicroscopy and OCT were treated with injecting air to



Figure 3 Correlation of hypermetropia at 12mo and graft thickness Positive correlation between graft thickness and hypermetropia at the level of 65% was found which was a statistically significant correlation (r=0.65, P<0.001).



Figure 4 Correlation of endothelial cell density at 12mo and graft thickness Negative correlation between 12mo graft thickness and endothelial cell density (ECD decreases with increasing thickness) at the level of 89% was found which was a statistically significant correlation (r = -0.890, P < 0.001).

anterior chamber. In all patients, graft reattachments were achieved without any complications.

DISCUSSION

Endothelial keratoplasty is the selective replacement of diseased endothelium with a healthy one and it becomes preferred surgery for endothelial diseases. It has some advantages over PK, including; not an open sky surgery,

fewer sutures (prevent suture related problems), smooth anterior surface, earlier visual recovery and lesser induced astigmatism^[7]. Beside these advantages endothelial cell loss remains as a problem. Terry et al [8] reported the endothelial cell loss has been 34% at the 6mo and 35% at the 12mo postoperative period. In other study Price and Price reported that endothelial cell loss had been 34% at 6mo, 36% at 12mo and 41% at 24mo postoperative period ^[9]. In our study, endothelial loss was 38% at 12mo which is consistent with other studies. In the literature endothelial cell loss differs between 21%-61% at 1y postoperatively ^[7]. Endothelial cell loss is one of the major problems because of the tissue manipulation in endothelial keratoplasty. Here by, this wide range of endothelial cell loss in different studies derived from different surgical techniques and different surgical skills of the surgeons.

Endothelial keratoplasty do not touch the anterior layers of cornea so the topography is not changed by surgery significantly. Moreover increase in induced astigmatism is limited. McCauley *et al* ^[10] reported that the mean change in refractive cylinder at 6mo was (0.1 ± 1.1) D which was not statistically significant difference. In our study we found that mean astigmatism change was (1.00 ± 0.75) D. However hypermetropic shift is more marked than astigmatism. Mean hypermetropic shift in the literature is $0.5 \cdot 1.5$ ^[10]. Our results are compatible with the literature that we found that hypermetropic shift of the cases were $(+1.50 \pm 1.00)$ D. In DSAEK surgery more tissue is added to the host cornea than removed. Tissue added is the estimated factor causing hypermetropic shift^[12].

Endothelial keratoplasty results in good visual acuity rates in different studies. Ratanasit and Gorovoy ^[13] reported the long term results of the DSAEK and they concluded that long term results were excellent. In the study 75% of eyes had BCVA of 20/20 to 20/40, 10% of eyes had BCVA between 20/60-20/100. Ham et al [14] also reported that in Descemet's membrane endothelial keratoplasty (DMEK) approximately 70% of eyes reached 20/40 or better at 6mo of surgery. In another study Guerra et al [15] compared two different endothelial keratoplasty techniques. They compared 15 patients' eyes which were treated with DSAEK in one eye and DMEK in the other eye. At 12mo DMEK group had 20/24 visual acuity, DSAEK group had 20/32 visual acuity. Eighty-five percent of the patients had better visual acuity in the eye treated with DMEK. Despite these results, the technical difficulty performing DMEK, and promising results of the DSAEK surgery its popularity will not decrease at least some time. Moreover, DMEK surgery' better results can encourage to create thinner grafts in DSAEK surgery.

How about the effect of thin grafts on postoperative visual acuity? In this study all thin DSEAK graft had BCVA ≥ 0.6 , however only 67% of medium thick had BCVA ≥ 0.5 and

none of the thick DSAEK grafts reached BCVA ≥ 0.5 at 12mo after the surgery. However Van Cleynenbreugel *et al*^[6] reported the donor lamellae thicknesses were ranged in 94-304 µm and there were no statistical correlation between donor lamellae pachymetry and visual outcome. In another study Shinton *et al* ^[16] concluded that there is no clear association between graft thickness and visual acuity. Pogorelov *et al* ^[17], Neff *et al* ^[18] and Busin and Albé^[4] resulted that thin graft groups had better visual acuity results. As seen, there is no clear evidence about the effect of graft thickness on visual acuity results in DSAEK surgery.

We used single pass technique in our DSAEK surgeries. We think that the thickness variability was resulted from the speed of the microkeratome. Despite the surgeries were done by same surgeon (Acar BT), variability of the microkeratome speed resulted in the variability of the graft. This was proved by a recent study. Vajpayee *et al*^[19] stated that thin donor lenticules for DSAEK can be achieved by slow, single pass technique and can be used for a successful DSAEK surgery. They could achieve donor lenticule thickness between 70-134 μ m at the last follow up of the patients. Moreover Busin *et al*^[20] and Hsu *et al*^[21] defined the double cut method to obtain ultrathin grafts.

In our study we found that thick graft group had more graft dislocation rates (31%) than medium (22%) and thin graft (13%) group which had caused more endothelial cell loss in thick graft group. In spite of successful attachment of the graft by rebubbling in thick and medium-thick graft groups, this caused more endothelial damage and worse ECD than thin graft group at 12mo postoperatively in contrast to the study of Cleynenbreugel ^[5]. In our study thin grafted group had less graft dislocation and also secondary procedures, which resulted in lesser endothelial loss. This seems to be another advantage of thin graft group in our study. In addition, thin graft group had better visual acuity results. We concluded that putting nearly same tissue instead of more tissue had caused better anatomical restoration and resulted in higher visual acuity results.

In conclusion, since thin lamellar endothelial grafts bring better visual acuities, lesser endothelial cell loss and lesser complication rates, it's very important to form thinner grafts. Thinner grafted DSAEK surgery is a standard technique for the treatment of endothelial diseases with faster visual rehabilitation, good anatomic and functional results.

This study has potential limitation. The sample size is relatively small. Further studies with larger sample size and longer follow-up are necessary for better comparison of surgical results.

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