Original Article

Preparation and Thickness Profile of Endothelial Keratoplasty Lenticules from Donated Whole Eyes with Previous Photorefractive Keratectomy

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Abstract

Purpose: To describe the preparation and thickness profiles of endothelial keratoplasty lenticules harvested from eyes with previous photorefractive keratectomy (PRK).
Methods: Donor whole eyes that underwent PRK were subjected to microkeratome-assisted dissection for Descemet stripping automated endothelial keratoplasty. Specular microscopy and Visante optical coherence tomography were performed on precut corneas. Endothelial cell indices and thickness profiles of endothelial keratoplasty lenticules were statistically analyzed. Postoperative reports for transplanted lenticules were recorded.
Results: Over a 6-month period, 2,929 whole eyes from 1,471 donors were screened for PRK. Twenty-five (0.85%) eyes from 14 donors were diagnosed with disciform haziness due to prior PRK and were used uneventfully for preparation of endothelial keratoplasty lenticules. Mean endothelial cell count was 3164.6 ± 311.0/mm^2 and mean central posterior lenticule thickness was 128 ± 34 μm. Posterior lenticules revealed an increase in thickness from the central to peripheral cornea (mean increase of 26.2 μm at pericentral and 90.4 μm at peripheral locations). Mean increase in thickness was statistically different between two peripheral locations (74.5 μm vs. 108.1 μm, $P = 0.047$). Postoperative reports of transplanted lenticules revealed no posterior flap detachment or loss of clarity at least three months after the surgery.
Conclusion: PRK donor whole eyes are potential sources for preparation of microkeratome-assisted thin endothelial keratoplasty lenticules with a high endothelial cell count. Although an asymmetric and significant increase in thickness was present at the peripheral cornea, neither attachment nor clarity of transplanted lenticules was affected by variations in thickness of precut corneas.

Keywords: Endothelial Keratoplasty; Eye Bank; Lenticule; Photorefractive Keratectomy

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INTRODUCTION

As a safe alternative to conventional penetrating keratoplasty, Descemet stripping automated endothelial

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keratoplasty (DSAEK) with rapid postoperative visual rehabilitation is the most popular transplantation technique for various types of endothelial disorders.[1-7] Preparation of microkeratome-assisted precut endothelial lenticules from either whole eyes[8] or excised corneoscleral discs[9-11] in eye banks has shortened the duration of surgery as well as anesthesia. Over the last decade in Iran, like the USA,[12] there has been an increasing rate of donor tissue use in endothelial keratoplasty techniques.[13]

Although there are no statistical data on the prevalence of corneal refractive surgeries in Iran, our impression is that donors with prior keratorefractive surgery are common in the eye donor population. According to the medical standards of the Central Eye Bank of Iran (CEBI), like those stated by the Eye Bank Association of America,[14] donor corneas with noninfectious anterior disorders can be used for the preparation of precut endothelial lenticules, provided that posterior stromal and endothelial layers are intact. Therefore, donor corneas with previous refractive surgery, preferably those with photorefractive keratectomy (PRK) and with very good to excellent endothelial rating, can be eligible for endothelial keratoplasty. Moshirfar et al[15] reported the first PRK donor for the preparation of precut tissue for DSAEK and stated that good post-microkeratome-cut tissue could be prepared from the potential PRK donor population. This study described the preparation of endothelial keratoplasty lenticules in a series of PRK donor whole eyes and their corresponding thickness profiles prepared at the CEBI.

METHODS

In a retrospective study, between October 2015 and March 2016, donor whole eyes that were screened for PRK at the CEBI were enrolled. The donors had nonreactive serology tests and a death to enucleation time of less than 24 h. Based on family interview, the donors had either no history or an unknown history of previous refractive surgery. Ethical approval, prior to commencement of the study, was obtained from the Institutional Review Board of the CEBI and the ethics committee of the Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Screening of Donated Whole Eyes for PRK

Donor whole eyes were first examined with slit lamp biomicroscopy for the presence of any corneal changes. Corneas with a peripheral scar due to prior laser *in situ* keratomileusis were excluded. The screening of donated whole eyes for prior PRK has been described previously.[16] In brief, after immersing whole eyes in 3% povidone iodine for 3 min and irrigation with 0.9% normal saline, the corneas were examined with gross inspection followed by repeat slit lamp biomicroscopy for the presence of a disciform, round-shaped, hazy area within the central 8 mm of the cornea, indicative of prior PRK. PRK corneas with very good to excellent endothelial rating, based on slit lamp examinations, were used for the preparation of endothelial keratoplasty lenticules.

Preparation of Endothelial Keratoplasty Lenticules

PRK whole eyes were cut for DSAEK by a trained eye bank technician using a manual microkeratome (Moria S. A 65073, Antony, France), as previously described.[10] Briefly, under sterile conditions and using an operating microscope (Topcon, OMS 90, Tokyo, Japan), corneal epithelium was removed and the eye was tightly wrapped in a sterile gauze. The cornea was marked from the limbus to the center and the limboscleral area was vacuumed. After intraocular pressure measurement with an Ocular Barraquer 65-90-mmHg Tonometer (Ocular Instruments, Bellevue, WA, USA), and with pressure maintained around 90 mmHg, central corneal thickness (CCT) was measured with an ultrasonic SP-100 Handy Pachymeter (USP) (Tomey GmbH, Erlangen, Germany). A 350- or 400-μm microkeratome head, chosen on the basis of values obtained with the USP, was passed over the cornea to create an anterior corneal cap. Then, the anterior edge of the lamellar dissection was undermined using a crescent knife, followed by relocation of the anterior corneal flap on the posterior stromal bed. After excision of the corneosclera and transfer of the tissue to Optisol GS (Bausch and Lomb, Irvine, CA, USA), the corneas were subjected to: i) specular microscopy for endothelial cell count, and ii) Visante optical coherence tomography (V-OCT) (Carl Zeiss Meditec, Inc., Dublin, CA, USA) for measurement of the central, paracentral, and peripheral thicknesses of the posterior endothelial keratoplasty lenticules (median interval between placement of the tissue in Optisol GS and performing V- OCT: 14 h, range 12-16 h). The measurement of the corneal thickness was performed at the most central, two paracentral (3.21 mm diameter) and two peripheral (6.29 mm diameter) locations. In case of corneal perforation during the microkeratome cut, V-OCT measurement was not performed and the cornea was excluded from the study.

Statistical Analysis

Values for endothelial cell indices and thickness of endothelial keratoplasty lenticules were presented as means and standard deviations. The generalized estimating equation was used to analyze the relationship between the two eyes of each donor. Multiple comparisons of the V-OCT thickness profile data of the posterior lenticules were adjusted with the Bonferroni test. All statistical analyses were performed with SPSS

Version 22.0 software (SPSS, Inc., Chicago, IL, USA), and *P* values less than 0.05 were considered statistically significant. Postoperative reports of all transplanted lenticules were also recorded.

RESULTS

Between October 2015 and March 2016, 2,929 donated whole eyes from 1,471 donors were screened for PRK; of these, 25 (0.85%) eyes from 14 donors had a disciform, round-shaped hazy area within the central 8 mm of the cornea indicative of prior PRK. The PRK donors had either no history or an unknown history of refractive surgery on family interview. Mean age of the PRK donors was 33.8 ± 8.4 years (range: 21–58) and 57.1% were male. In three PRK donors, only one eye from each donor was dissected. Endothelial keratoplasty lenticules were successfully prepared from the PRK whole eyes and no corneal perforation occurred after the microkeratome cut.

Donor characteristics and posterior lenticule preparation data as well as thickness profiles of PRK posterior lenticules measured by V-OCT are presented in Tables 1, 2 and Figure 1. Mean CCT obtained with USP was 653 ± 72 µm (range: 520–762) and the measured depth of cut by V-OCT (thickness of the anterior cap) ranged from 395 to 607 µm (mean: 472.9 ± 46.1 µm). Mean central posterior lenticule thickness obtained from V-OCT, after microkeratome cut and transfer of the cornea to Optisol GS, was 128 ± 34 µm (range: 56–182). The thickness profiles of the posterior lenticules [Table 2 and Figure 1] demonstrated an increase in thickness from the central to the peripheral cornea (mean increase of 26.2 µm at the paracentral and 90.4 µm at the peripheral locations). The mean increase of thickness was not statistically different between 2 paracentral locations (18.8 µm vs. 33.6 µm, $P = 0.102$) but the difference was significant between two peripheral positions (74.5 µm vs. 108.1 µm, $P = 0.047$).

Means endothelial cell count, percentage of polymegathism, percentage of hexagonality, and mean cell area in the precut PRK tissues were 3164.6 ± 311cells/mm^2, 39.4 ± 6.9%, 50.5 ± 11.3%, and 321.1 ± 32.8 µm^2, respectively. According to the postoperative reports, all endothelial keratoplasties were uneventful and no posterior flap detachment or loss of clarity occurred in the grafted lenticules at least three months after the surgery.

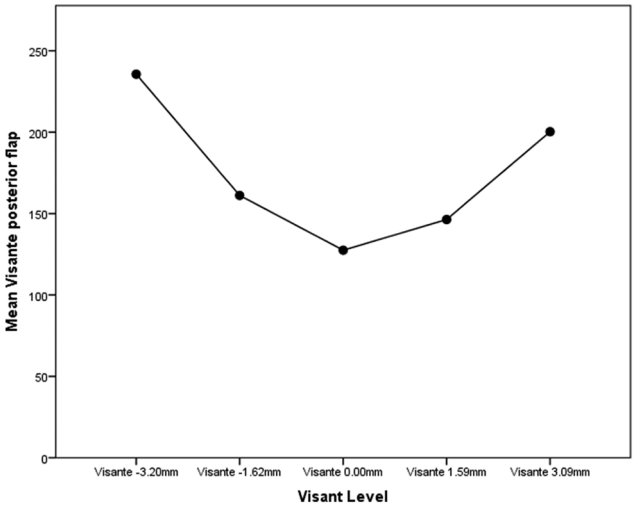

Figure 1. Mean thickness profiles of photorefractive keratectomy (PRK) endothelial keratoplasty lenticules in five locations on Visante optical coherence tomography (V-OCT). Note the increase in mean thickness from the central to the peripheral parts and the significantly different means of thickness between two peripheral locations.

Table 1: Donor Characteristics and Posterior Lenticule Preparation Data

Case no.	Age	Sex	Central corneal thickness (µm)*		Microkeratome head size		Central posterior lenticule thickness (µm)$		Cap thickness (µm)$	
			OD	OS	OD	OS	OD	OS	OD	OS
1	M	28	570	590	400	400	73	79	470	478
2	M	30	760	717	400	400	165	132	410	440
3	M	27	650	605	350	350	123	180	465	426
4	M	53	-	520	-	350	-	103	-	449
5	F	47	732	762	400	400	142	113	468	490
6	M	30	-	730	-	350	-	164	-	488
7	F	32	638	623	350	350	145	131	395	433
8	M	37	740	610	400	350	160	95	472	518
9	M	30	620	520	350	350	124	56	442	473
10	F	32	701	705	400	400	159	139	479	502
11	F	29	610	710	350	400	143	116	445	549
12	F	40	650	660	400	400	97	116	533	492
13	M	37	650	550	400	350	92	182	507	407
14	F	33	-	700	-	400	-	159	-	492

M, male; F, female; OD, right eye; OS, left eye. *Measured with ultrasound pachymetry; $Measured with Visante optical coherence tomography

Table 2. Thickness profile of PRK posterior lenticules measured by V-OCT in 5 locations

Case no.	Center		Pericentral 1 (1.59 mm)		Pericentral 2 (1.62 mm)		Peripheral 1 (3.09 mm)		Peripheral 2 (3.20 mm)	
	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
1	73	79	87	80	138	161	97	133	283	214
2	165	132	158	175	200	167	190	273	277	198
3	123	180	191	208	135	168	288	273	166	215
4	-	103	-	104	-	158	-	221	-	269
5	142	113	134	128	150	172	181	128	234	212
6	-	164	-	256	-	165	-	323	-	203
7	145	131	164	143	152	166	204	149	161	222
8	160	95	150	103	210	157	166	185	348	315
9	124	56	135	68	146	69	191	97	208	112
10	159	139	179	164	183	181	236	255	317	251
11	143	116	161	139	189	122	180	205	251	154
12	97	116	126	107	101	173	217	171	202	250
13	92	182	115	178	196	146	165	236	316	233
14	-	159	-	206	-	223	-	243	-	280

PRK, photorefractive keratectomy; V-OCT, Visante optical coherence tomography; OD, right eye; OS, left eye

DISCUSSION

Over a 6-month period, the CEBI identified 25 out of 2,929 donor whole eyes that were diagnosed as having prior PRK based on a published highly-sensitive and specific screening method.[16] Microkeratome-assisted thin endothelial keratoplasty lenticules with high endothelial rating could successfully be prepared from these donors. Although no published data on the prevalence of corneal refractive surgeries in Iran are available, given that PRK has comprised more of the market share of refractive surgery,[17] an increasing number of PRK donors among cornea donations to the CEBI is expected in future.

One of the main findings in the current study was an asymmetrical increase in thickness from the central to the peripheral parts of the endothelial keratoplasty lenticules that were prepared from PRK donor eyes. In contrast, in a recently published study,[18] this increase in thickness was symmetric in the lenticules that were prepared from non-PRK donated whole eyes. Given that the same procedure was used for the preparation of lenticules from both PRK and non-PRK eyes, surface ablation might have a significant effect on the microkeratome cut and the occurrence of an asymmetric cut at the periphery.

At the CEBI, the number of precut tissues used for DSAEK showed an increasing trend during the last decade,[13] from zero in 2007 to 1,224 in 2013,[13] and 1,733 in 2015 (unpublished data). To meet this increase in demand for precut endothelial keratoplasty lenticules, donor tissues with noninfectious anterior pathology that does not involve the posterior stroma and endothelium,[14] including donor corneas with previous PRK, have been successfully used for this purpose at the CEBI. Although the thickness profile of the PRK precut tissue for DSAEK demonstrated a meaningfully asymmetric increase of thickness towards the peripheral locations, it was not clinically significant and did not affect clarity or attachment of the graft. Variations in the thickness profile of a posterior lenticule when dissected manually from a freshly donated whole eye are expected to occur frequently. It would be interesting to analyze the effect of thickness profile of PRK endothelial keratoplasty lenticules on postoperative visual and refractive outcomes; however, these data are not available in the majority of the PRK donors.

The diagnosis of previous PRK surface ablation in donated corneas has been difficult when there is no history or an unknown history of refractive surgery on family interview.[15] However, with the introduction of a highly-sensitive and specific method for screening of whole eyes at the CEBI,[16] the identification of PRK donor corneas is no longer difficult. This screening method is safe, simple, and inexpensive, with a short learning curve for eye bank technicians who work with donor whole eyes.

The average CCT of PRK whole eyes in our series, before microkeratome cut and transfer to Optisol GS, was 653 μm, with a minimum thickness of 520 μm. In the only PRK cornea reported by Moshirfar et al, the CCT was 487 μm.[15] In their study, the donor cornea was mounted on an artificial anterior chamber, while the CCT measurements in our series were taken on whole eyes, which may account for the different measurements. In our series, a mean cutting depth of 473 μm and a mean residual bed thickness of 128 μm were obtained after passing a 350 or 400 μm microkeratome head. However, in Moshirfar's report,[15] the residual bed thickness after a 300-μm microkeratome pass was not measurable by optical pachymetry, and the depth of cut was not specified. They also did not use OCT to measure corneal thickness.

One of the concerns with using PRK donors may be the potential effects of intraoperative application of mitomycin

C (MMC) on corneal endothelial cells. It is unclear whether brief exposure to MMC during PRK exerts adverse effects on corneal endothelial cells.[19-23] In some studies, significant endothelial cell loss was observed following stromal application of 0.02% MMC.[19,20] however, in other series, short-term treatment with 0.02% MMC did not have any adverse effects on corneal endothelial cell populations.[21-23] In our series, the PRK corneas had very good to excellent endothelial rating and the ongoing possibility of adverse effects of MMC and the ongoing adverse effects of MMC on the corneal endothelium seem unlikely.

In addition to selection of PRK corneas with a high endothelial quality, another explanation for a very good endothelial rating after the microkeratome cut can be the unique technique used at the CEBI.[8] In this method, the anterior chamber of whole eyes supports the procedure, and risks from tissue manipulation and endothelial cell loss are lower.

Our study had some limitations. Since this study was retrospective and was mainly based on eye bank data, we had no access to the postoperative information including the amount of donor cornea flatness, hyperopic shift, V-OCT measurements, and the endothelial cell density of grafted lenticules.

In summary, because of the high success rate and popularity of PRK in Iran, eye donations with a history of prior PRK have been more common at the CEBI. Implementation of a highly-sensitive, specific, and simple method at the CEBI has enabled the identification of PRK donors and led to the use of their corneas for the preparation of endothelial keratoplasty lenticules. Investigation of the thickness profile of PRK precut tissues in our series revealed appropriate thinness at the central part and an asymmetric and significant increase in thickness at the peripheral parts of the lenticules. Nonetheless, such variations in the thickness profile of PRK precut lenticules did not have any influence on attachment or clarity of the grafts.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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