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/mm² 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 limbus-scleral 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 flap. 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.
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 ± 311 cells/mm², 39.4 ± 6.9%, 50.5 ± 11.3%, and 321.1 ± 32.8 µm², 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.](image)

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        | 28  | M   | 570    | 590    | 400    | 400    | 73     | 79     | 470    | 478    |
| 2        | 30  | M   | 760    | 717    | 400    | 400    | 165    | 132    | 410    | 440    |
| 3        | 27  | M   | 650    | 605    | 350    | 350    | 123    | 180    | 465    | 426    |
| 4        | 53  | M   | -      | 520    | -      | 350    | -      | 103    | -      | 449    |
| 5        | 47  | F   | 732    | 762    | 400    | 400    | 142    | 113    | 468    | 490    |
| 6        | 30  | M   | -      | 730    | -      | 350    | -      | 164    | -      | 488    |
| 7        | 32  | F   | 638    | 623    | 350    | 350    | 145    | 131    | 395    | 433    |
| 8        | 37  | M   | 740    | 610    | 400    | 350    | 160    | 95     | 472    | 518    |
| 9        | 30  | M   | 620    | 520    | 350    | 350    | 124    | 56     | 442    | 473    |
| 10       | 32  | F   | 701    | 705    | 400    | 400    | 159    | 139    | 479    | 502    |
| 11       | 29  | F   | 610    | 710    | 350    | 400    | 143    | 116    | 445    | 549    |
| 12       | 40  | F   | 650    | 660    | 400    | 400    | 97     | 116    | 533    | 492    |
| 13       | 37  | M   | 650    | 550    | 400    | 350    | 92     | 182    | 507    | 407    |
| 14       | 33  | F   | -      | 700    | -      | 400    | -      | 159    | -      | 492    |

* Measured with ultrasound pachymetry; $ Measured with Visante optical coherence tomography

M, male; F, female; 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. 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 refraction surgeries in Iran are available, given that PRK has comprised more of the market share of refractive surgery, 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, 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, from zero in 2007 to 1,224 in 2013 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, 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. However, with the introduction of a highly-sensitive and specific method for screening of whole eyes at the CEBI, 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. 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, 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.

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**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
C (MMC) on corneal endothelial cells. It is unclear whether brief exposure to MMC during PRK exerts adverse effects on corneal endothelial cells.\textsuperscript{[19-23]} In some studies, significant endothelial cell loss was observed following stromal application of 0.02% MMC,\textsuperscript{[19,23]} however, in other series, short-term treatment with 0.02% MMC did not have any adverse effects on corneal endothelial cell populations.\textsuperscript{[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.\textsuperscript{[18]} 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 prospective series.

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Nil.

**Conflicts of Interest**

There are no conflicts of interest.

**REFERENCES**

1. Lee WB, Jacobs DS, Musch DC, Kaufman SC, Reinhart WJ, Shtein RM. Descemet’s stripping endothelial keratoplasty: Safety and outcomes: A report by the American Academy of Ophthalmology. *Opthalmology* 2009;116:1818-1830.

2. Terry MA, Ousley PJ. Small-incision deep lamellar endothelial keratoplasty (DLEK): Six-month results in the first prospective clinical study. *Cornea* 2005a; 24:59-65.

3. Terry MA, Ousley PJ. Deep lamellar endothelial keratoplasty. Visual acuity, astigmatism, and endothelial survival in a large prospective series. *Opthalmology* 2005b; 112:1541-1548.

4. Ousley PJ, Terry MA. Stability of vision, topography, and endothelial cell density from 1 year to 2 years after deep lamellar endothelial keratoplasty surgery. *Opthalmology* 2005;112:50-57.

5. Terry MA, Ousley PJ. In pursuit of emmetropia: Spherical equivalent refraction results with deep lamellar endothelial keratoplasty (DLEK). *Cornea* 2003;22:619-626.

6. Price MO, Price FW Jr. Descemet’s stripping with endothelial keratoplasty. Comparative outcomes with microkeratome dissected and manually dissected donor tissue. *Opthalmology* 2006;113:1936-1942.

7. Gorovoy MS. Descemet-stripping automated endothelial keratoplasty. *Cornea* 2006;25:886-889.

8. Kanavi MR, Javadi MA, Javadi F, Chamani T. Preparation of pre-cut corneas from fresh donated whole globes for Descemet’s stripping automated keratoplasty: 3-year results at the Central Eye Bank of Iran. *Cell Tissue Bank* 2014;15:369-372.

9. Woodward MA, Titus M, Mavin K, Shtein RM. Corneal donor tissue preparation for endothelial keratoplasty. *J Vis Exp* 2012;64:e3847.

10. Terry MA. Endothelial keratoplasty: A comparison of complication rates and endothelial survival between precut tissue and surgeon-cut tissue by a single DSAEK surgeon. *Trans Am Ophthalmol Soc* 2009;107:184-193.

11. kitsmann AS, Goins KM, Reed C, Padnick-Silver L, Mascai MS, Sutphin JE. Eye bank survey of surgeons using precut donor tissue for descemet stripping automated endothelial keratoplasty. *Cornea* 2008;27:634-639.

12. Ple-Plakon PA, Shtein RM. Trends in corneal transplantation: Indications and techniques. *Curr Opin Ophthalmol* 2014;25:300-305.

13. Kanavi MR, Javadi MA, Motevasseli T, Chamani T, Rezaei Kanavi M, Kheiri B, et al. Trends in Indications and Techniques of Corneal Transplantation in Iran from 2006 to 2013; an 8-year Review. *J Ophthalmic Vis Res* 2016;11:146-152.

14. Eye Bank Association of America. Medical Standards. 2009;14-18.

15. Moshirfar M, Khalifa YM, Davis D, Fenzl CR, Espandar L, Chang JC, et al. Descemet stripping automated endothelial keratoplasty using donor corneas with previous laser in situ keratomileusis or photorefractive keratectomy: A case series and donor cap histopathology. *Cornea* 2012;31:533-537.

16. Kanavi MR, Javadi MA, Chamani T, Javadi A. Screening of donated whole globes for photorefractive keratectomy. *Cornea* 2011;30:1260-1263.

17. Kuo IC Trends in refractive surgery at an academic center: 2007-2009. *BMC Ophthalmol* 2011;11:11.

18. Kanavi MR, Nemati F, Chamani T, Kheiri B, Javadi MA. Measurements of donor endothelial keratoplasty lenticules prepared from fresh donated whole eyes by using ultrasound and optical coherence tomography. *Cell Tissue Bank* 2017;18:99-104.

19. Morales AJ, Zadok D, Moza-Retana A, Martinez-Gama E, Robledo NE, Chayet AS. Intraoperative mitomycin and corneal endothelium after photorefractive keratectomy: Nonrandomized controlled trial. *J Cataract Refract Surg* 2008;34:902-908.

20. Diakonis VF, Pallikaris A, Kymionis GD, Markomanolakis MM. Alterations in endothelial cell density after photorefractive keratectomy with adjuvant mitomycin. *Am J Ophthalmol* 2007;144:99-103.

21. Zhao LQ, Wei RL, Ma XY, Zhu H. Effect of intraoperative mitomycin-C on healthy corneal endothelium after laser-assisted subepithelial keratectomy. *J Cataract Refract Surg* 2008;34:1715-1719.

22. Sirak RK, Ryan DS, Edwards JD, Stutzman RD, Bower KS. The U.S. Army Surface Ablation Study: Comparison of PRK, MMC-PRK, and LASEK in Moderate to High Myopia. *J Refract Surg* 2014;30:256-264.