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SURGICAL TECHNIQUE

Combined use of a femtosecond laser and a microkeratome in obtaining thin grafts for Descemet stripping automated endothelial keratoplasty: an eye bank study

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Purpose: *To evaluate the use of a femtosecond laser combined with a microkeratome in the preparation of posterior corneal disks for Descemet stripping automated endothelial keratoplasty (DSAEK).*
Methods: *This experimental study involved ultrathin DSAEK tissue preparation of 22 donor corneas as unsuitable for transplantation. The first cut was performed with an Intralase® FS60 laser and the second cut with a Moria CBm 300-µm microkeratome. The thickness of the first cut was modified for each cornea to obtain a final graft thickness of less than 110 µm. Precut and postcut central pachymetry were performed with an ultrasonic pachymeter. Central endothelial cell density (ECD) was calculated before and 24 hours after tissue preparation.*
Results: *Final graft thickness was 105.0 ± 26.1 (SD) µm (range 65-117). The mean microkeratome head cut thickness was 324.5 ± 10.9 µm (range 310-345). Precut and postcut ECDs averaged 2250 ± 222 and 2093 ± 286 cells/mm², respectively, representing 6.9% of cell loss. No corneas were perforated.*
Conclusion: *Femtosecond FS60 lasers and Moria CBm 300-µm microkeratomes can be used sequentially to prepare consistently thin DSAEK grafts with no irregular cuts or cornea perforations.*

Keywords: *DSAEK, Eye bank, Femtosecond, Thickness*

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INTRODUCTION

Descemet stripping automated endothelial keratoplasty (DSAEK) has become the standard of care for Fuchs dystrophy and other causes of endothelial dysfunction (1). DSAEK consists of stripping a patient's diseased endothelium and replacing it with the healthy endothelium, Descemet membrane, and a layer of stroma prepared from a donor cornea with an automated microkeratome (2, 3).

An important limitation of DSAEK is that some eyes do not achieve good visual acuity despite a clear cornea and minimal residual astigmatism (4-6). This may be caused by interface irregularity, the presence of donor posterior

stroma, or a thick endothelial graft (7-9). The influence of endothelial graft thickness on visual acuity in DSAEK is controversial, with some authors reporting better results with thinner grafts and others finding no correlation (10-15). However, studies evaluating this subject have either focused on relatively thick grafts (160-170 µm) or on grafts prepared by manual dissection (11-15). When grafts having less than 130 µm are evaluated, a positive correlation between thickness and visual acuity is obtained (9, 10), suggesting that an effect may exist under a certain thickness. Obtaining thin grafts carries a higher risk of donor corneal perforation. Therefore, it is necessary to develop a technique to create thin posterior lamellar grafts without wasting donor corneas.

The present article presents a new technique that combines the sequential use of a femtosecond (FS) laser with a microkeratome to create posterior donor lenticules with a targeted thickness of less than 110 µm.

MATERIALS AND METHODS

This study was an institutional experimental laboratory investigation consisting of DSAEK tissue preparation on 22 human donor corneas. The corneas were unsuitable for transplantation due to positive donor serology results. Donor age and preservation to lamellar dissection time (storage time; see Tab. I) were recorded. Tissue was pre-

TABLE I - DONOR TISSUE DEMOGRAPHICS, DATA OBTAINED DURING EACH CUT, AND ENDOTHELIAL CELL DENSITY BEFORE AND 24 HOURS POSTCUT

Cornea no.	Age, y	Storage time, d	Initial CCT, µm	Programmed FS cut thickness, µm	Intermediate RSB, µm	Microkeratome mean cut depth, µm	Final RSB, µm	Precut ECD, cells/mm²	ECD 24 h postcut, cells/mm²
1	64	14	562	152	432	334	98	2320	2033
2	37	11	581	171	440	340	100	2222	1988
3	75	10	530	120	430	314	116	2342	2604
4	51	10	510	100	429	310	119	2062	1786
5	52	10	463					2045	
6	75	10	525	115	430	319	111	2227	1996
7	37	11	570	160	430	315	115	2083	1838
8	64	14	550	140	428	339	89	2597	2075
9	52	10	530	120	427	341	86	2506	1919
10	51	10	530	120	429	312	117	2481	1873
11	65	13	619	209	429	326	103	2070	1773
12	64	20	525	115	434	327	107	2141	2660
13	49	3	535	125	428	313	115	2227	2653
14	64	20	514	104	426	317	109	2506	2475
15	49	16	550	140	428	322	106	2096	2041
16	65	3	610	200	429	345	84	2053	2114
17	42	5	588	178	431	334	97	2217	2045
18	42	5	597	187	428	315	113	2193	2020
19	74	7	580	170	427	315	112	2183	2299
20	44	11	515	105	425	323	102	2882	1709
21	44	11	516	106	432	333	99	2028	1916
22	74	7	564	154	431	321	110	2028	2146

CCT = central corneal thickness; ECD = endothelial cell density; FS = femtosecond; RSB = residual stromal bed.

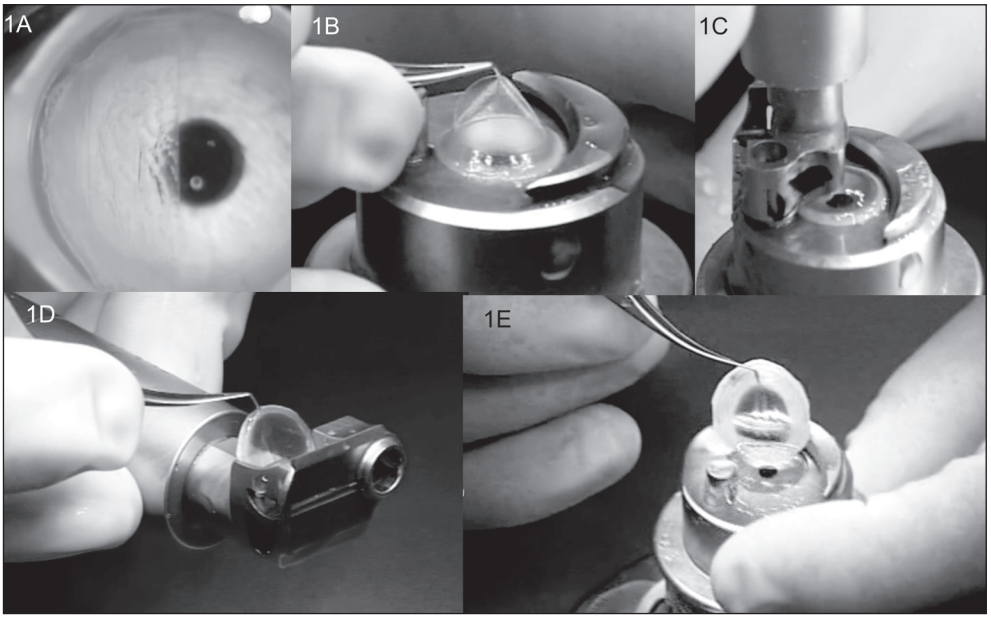

Fig. 1 - *Donor cornea is assembled in an artificial anterior chamber and a first cut is performed with the femtosecond laser **(1A)**. The thickness of this cut is chosen in order to leave approximately 410-430 µm of intermediate residual stromal bed. The tissue is easily removed **(1B)**. The second cut is performed with a 300-µm microkeratome head **(1C, 1D)** leaving a thin and smooth final residual stromal bed. The cornea is removed from the anterior chamber **(1E)**.*

served in Eusol-C® (Alchimia SRL, Padova, Italy) at a constant temperature of 4°C before use.

Each donor cornea had a 5-mm scleral rim and was mounted on an artificial anterior chamber (ALTK, Moria SA, Antony, France) filled with balanced salt solution (BSS®, Alcon Laboratories, Fort Worth, Texas, USA). The BSS® bottle was elevated to 220 cm and the tubing was clamped at 60 cm from the anterior chamber, to ensure a high and stable anterior chamber pressure.

In order to obtain the initial central corneal thickness (CCT), precut central pachymetry was performed with an ultrasonic pachymeter (Corneo Gage Plus® 50 MHz; Sonogage, Cleveland, Ohio, USA) after removal of the cornea epithelium. The first cut of the double-pass technique was performed with an Intralase® FS60 FS laser (Abbott Medical Optics, Santa Ana, California, USA), and the second cut with the Moria® CBm microkeratome 300 µm cutting head (Moria SA, Antony, France) (Fig. 1). The programmed FS cut thickness of the first cut was calculated as follows:

Programmed FS cut thickness = initial CCT – (theoretical microkeratome cut thickness + desired final graft thickness)

The theoretical microkeratome cut thickness was considered to be 300 µm and the desired final graft thickness was 110 µm.

By replacing these values, this formula can be simplified as follows:

Programmed FS cut thickness = initial CCT – 410 µm.

Care was taken when positioning the donor tissue on the anterior chamber so that there was a regular ring of sclera appearing around the cornea. The FS cut was centered, having as reference the hole inside the anterior chamber, visible on the screen of the Intralase® (Fig. 1A). No suction ring was used and docking was straightforward. The FS settings were full lamellar cut with a diameter of 9.5 mm, raster energy 1.5 µJ, and anterior side cut at 90° with 2.7 µJ. A new applanation cone was used for each cornea. After laser passage, the disc of tissue was removed and central pachymetry was evaluated to obtain intermediate residual stromal bed thickness (Tab. I). The second cut was performed immediately afterward with the 300 µm microkeratome head, keeping the manual rotation speed constant and with total duration of approximately 3-5 seconds. Central pachymetry was again repeated to obtain final residual stromal bed thickness. The tubing was unclamped with the BSS bottle at 150 cm to avoid turbulence inside the anterior chamber. Donor tissue was removed by gently pulling the scleral rim from the top of the anterior chamber and then immersed in the storage medium. Endothelial cell density was calculated before and 24 hours after the cuts, using a noncontact specular microscope (SP4000 noncontact specular microscope; Konan Medical Corp., Fair Lawn, New Jersey, USA). A technician marked the center of at least 50 endothelial cells and the computer software estimated the area and the cell density of the entire cornea.

RESULTS

Donor age was 56.1 ± 12.7 years (mean ± SD) and preservation to lamellar dissection time was 10.5 ± 4.6 days. Death to preservation time was less than 12 hours in all cases. Initial CCT after epithelial debridement was 548.4 ± 37.8 µm (range 463-619 µm; Tab. I). Cornea no. 5 (Tab. I) had an initial thickness of 463 µm, which would require a FS cut of 43 µm. This value is below the limit of the Intralase® depth range, which precluded cutting this cornea. The disc of tissue from the FS cut was easily removed in all cases.

Intermediate residual stromal bed was 429.7 ± 3.2 µm. The microkeratome mean cut depth was 324.5 ± 10.9 µm (range 310-345 µm; Tab. I). Final residual stromal bed was 105.1 ± 10.2 µm (range 84-119 µm). Average precut endothelial cell density was 2250 ± 222 cells/mm^2 and postcut was 2093 ± 286 cells/mm^2, representing 6.97% cell loss. No corneas were lost due to perforation, irregular cuts, or buttonholes.

DISCUSSION

Despite excellent postoperative results, best-corrected visual acuity after DSAEK is sometimes less than that obtained with penetrating keratoplasty (16, 17). Several studies report that better results might be achieved with a smoother interface and thinner grafts (2, 9, 10). Neff and colleagues (10) reported that grafts ≤131 µm provided a statistically significant improvement in best-corrected visual acuity compared with thicker grafts. Several attempts have been made to develop a technique to create very thin DSAEK grafts, without wasting donor tissue (18-21).

A FS laser, without a microkeratome, was previously used for this purpose. However, the resulting surface was not as smooth as from a microkeratome and concentric folds would form due to compression or irregularity of the posterior stroma (22, 23). In addition, interface scatter may later appear (21).

The method we describe here, the double-pass technique, combines the sequential use of a FS laser and a microkeratome. The first pass is performed with the laser to avoid the variability of the microkeratome, but for the second cut the latter is used to obtain a smooth stromal bed (24). Posterior donor lenticules with a mean thickness of 105 µm were obtained and no cornea was perforated. An important aspect of this technique is that the thinner cut is done first, leaving a strong and thick cornea for the microkeratome. Because the anterior stroma is the strongest (25), this approach avoids irregular cuts and buttonholes during the microkeratome cut.

The desired final graft thickness can be obtained by changing the parameters of the laser, which can be programmed to cut at a customized depth for each cornea, according to the initial pachymetry. This avoids using nomograms and allows having only one microkeratome head available, in this case a 300 µm, but other heads would probably work as well. It also has the advantage of using equipment already available in most cornea centers. The target thickness of 110 µm was chosen essentially for safety reasons. This way, at least 410 µm of corneal tissue are cut with a 300 µm microkeratome head, minimizing the risk of perforation. Since the microkeratome we use usually cuts more than 300 µm (26), we found that aiming for a final thickness of 110 µm would actually result in thinner grafts (than 110 µm) without perforating. Because the microkeratome cut depth depends on several factors, such as intrachamber pressure, tissue thickness, and manual rotation speed, these factors are kept constant (tubing clamped at 60 cm when bottle is raised at 220 cm, FS cut first, 3-5 seconds manual rotation speed) in order to reduce variability.

In terms of endothelial cell loss, the cut performed by the FS is superficial, similar to the flaps produced in laser-assisted in situ keratomileusis (LASIK). Therefore, this cut should not induce any more cell loss than a regular LASIK flap. The second cut is performed immediately after the first to avoid further manipulation of the donor cornea. In the present study, mean endothelial cell loss from precut to 24 hours postcut was 6.9%, which is higher than the 3%-4% reported by other authors (22, 26, 27). However, the time interval between cutting the cornea and counting the cells is likely to be important when evaluating cell death, with longer intervals (as was the case in this study) being associated with lower final cell densities (28).

In conclusion, the technique described in this study combines the sequential use of a FS laser and a microkeratome to obtain posterior lamellar grafts with a mean thickness of 105 µm. More research is needed to determine the visual outcomes of this approach and to clarify whether there is an ideal graft thickness both in terms of visual recovery and endothelial cell loss.

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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. Ophthalmology 2009;116:1818-30.
2. Price MO, Price FW Jr. Descemet's stripping with endothelial keratoplasty: comparative outcomes with microkeratome-dissected and manually dissected donor tissue. Ophthalmology 2006;113:1936-42.
3. Gorovoy MS. Descemet-stripping automated endothelial keratoplasty. Cornea 2006;25:886-9.
4. Melles GR. Posterior lamellar keratoplasty: DLEK to DSEK to DMEK. Cornea 2006;25:879-81.
5. Dapena I, Ham L, Melles GR. Endothelial keratoplasty: DSEK/DSAEK or DMEK—the thinner the better? Curr Opin Ophthalmol 2009;20:299-307.
6. Mearza AA, Qureshi MA, Rostron CK. Experience and 12-month results of Descemet-stripping endothelial keratoplasty (DSEK) with a small-incision technique. Cornea 2007;26:279-83.
7. Chen ES, Terry MA, Shamie N, Hoar KL, Friend DJ. Descemet-stripping automated endothelial keratoplasty: six-month results in a prospective study of 100 eyes. Cornea 2008;27:514-20.
8. Ham L, Dapena I, van der Wees J, Melles GR. Secondary DMEK for poor visual outcome after DSEK: donor posterior stroma may limit visual acuity in endothelial keratoplasty. Cornea 2010;29:1278-83.
9. Pogorelov P, Cursiefen C, Bachmann BO, Kruse FE. Changes in donor corneal lenticule thickness after Descemet's stripping automated endothelial keratoplasty (DSAEK) with organ-cultured corneas. Br J Ophthalmol 2009;93:825-9.
10. Neff KD, Biber JM, Holland EJ. Comparison of central corneal graft thickness to visual acuity outcomes in endothelial keratoplasty. Cornea 2011;30:388-91.
11. Van Cleynenbreugel H, Remeijer L, Hillenaar T. Descemet stripping automated endothelial keratoplasty: effect of intraoperative lenticule thickness on visual outcome and endothelial cell density. Cornea 2011;30:1195-200.
12. Seery LS, Nau CB, McLaren JW, Baratz KH, Patel SV. Graft thickness, graft folds, and aberrations after Descemet stripping endothelial keratoplasty for Fuchs dystrophy. Am J Ophthalmol 2011;152:910-6.
13. Ahmed KA, McLaren JW, Baratz KH, Maguire LJ, Kittleson KM, Patel SV. Host and graft thickness after Descemet stripping endothelial keratoplasty for Fuchs endothelial dystrophy. Am J Ophthalmol 2010;150:490-7.
14. Shinton AJ, Tsatsos M, Konstantopoulos A, et al. Impact of graft thickness on visual acuity after Descemet's stripping endothelial keratoplasty. Br J Ophthalmol 2012;96:246-9.
15. Villarrubia A, Palacín E, Aránguez C, Solana J, García-Alonso CR. [Functional results after endothelial queratoplasty: three years of experience.] Arch Soc Esp Oftalmol 2011;86:47-53.
16. Price FW Jr, Price MO. Descemet's stripping with endothelial keratoplasty in 200 eyes: early challenges and techniques to enhance donor adherence. J Cataract Refract Surg 2006;32:411-8.
17. Terry MA, Ousley PJ. Replacing the endothelium without corneal surface incisions or sutures: the first United States clinical series using the deep lamellar endothelial keratoplasty procedure. Ophthalmology 2003;110:755-64.
18. Busin M, Patel AK, Scorcia V, Ponzin D. Microkeratome-assisted preparation of ultrathin grafts for Descemet stripping automated endothelial keratoplasty. Invest Ophthalmol Vis Sci 2012;53:521-4.
19. Hsu M, Hereth WL, Moshirfar M. Double-pass microkeratome technique for ultra-thin graft preparation in Descemet's stripping automated endothelial keratoplasty. Clin Ophthalmol 2012;6:425-32.
20. Sikder S, Nordgren RN, Neravetla SR, Moshirfar M. Ultra-thin donor tissue preparation for endothelial keratoplasty with a double-pass microkeratome. Am J Ophthalmol 2011;152:202-8.
21. Hjortdal J, Nielsen E, Vestergaard A, Søndergaard A. Inverse cutting of posterior lamellar corneal grafts by a femtosecond laser. Open Ophthalmol J 2012;6:19-22.
22. Mootha VV, Heck E, Verity SM, et al. Comparative study of Descemet stripping automated endothelial keratoplasty

donor preparation by Moria CBm microkeratome, horizon microkeratome, and Intralase FS60. Cornea 2011;30:320-4.
23. Jones YJ, Goins KM, Sutphin JE, Mullins R, Skeie JM. Comparison of the femtosecond laser (IntraLase) versus manual microkeratome (Moria ALTK) in dissection of the donor in endothelial keratoplasty: initial study in eye bank eyes. Cornea 2008;27:88-93.
24. Rosa AM, Neto Murta J, Quadrado MJ, et al. Femtosecond laser versus mechanical microkeratomes for flap creation in laser in situ keratomileusis and effect of postoperative measurement interval on estimated femtosecond flap thickness. J Cataract Refract Surg 2009;35:833-8.
25. Hamilton DR, Johnson RD, Lee N, Bourla N. Differences in the corneal biomechanical effects of surface ablation compared with laser in situ keratomileusis using a microkeratome or femtosecond laser. J Cataract Refract Surg 2008;34: 2049-56.
26. Kelliher C, Engler C, Speck C, Ward D, Farazdaghi S, Jun AS. A comprehensive analysis of eye bank-prepared posterior lamellar corneal tissue for use in endothelial keratoplasty. Cornea 2009;28:966-70.
27. Chen ES, Terry MA, Shamie N, Hoar KL, Friend DJ. Precut tissue in Descemet's stripping automated endothelial keratoplasty donor characteristics and early postoperative complications. Ophthalmology 2008;115:497-502.
28. Rose L, Briceño CA, Stark WJ, Gloria DG, Jun AS. Assessment of eye bank-prepared posterior lamellar corneal tissue for endothelial keratoplasty. Ophthalmology 2008;115:279-86.