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Comparative Study of Descemet Stripping Automated Endothelial Keratoplasty Donor Preparation by Moria CBm Microkeratome, Horizon Microkeratome, and Intralase FS60

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Abstract

Purpose—To evaluate the quality of stromal bed and the safety on endothelium in preparation of donor tissue for Descemet stripping automated endothelial keratoplasty in a masked fashion using 2 mechanical microkeratomes and a femtosecond laser.

Methods—Deep anterior lamellar dissection was performed on 15 donor corneas. Central endothelial cell density was calculated using specular microscopy before and after the dissection. One cornea from each of 5 donor pairs was cut with the Moria ALTK system with the CBm microkeratome using the 300- μ m head and the mate cut with the Horizon disposable 300- μ m microkeratome. Five additional donor corneas were cut with the Intralase 60-kHz FS laser. The donor corneas were then bisected with half of the cornea used for Live/Dead assay to study central endothelial viability. The other halves were sent for scanning electron microscopy of the stromal bed. Qualitative surface roughness of the scanning electron microscopy images was graded by 2 masked observers, and quantitative surface roughness was assessed using roughness evaluation software.

Results—The Horizon group showed a smoother stromal bed compared with the Moria or Intralase groups by 2 masked observers. However, the Moria group had the smoothest quantitative score of all the groups when assessed by roughness evaluation software. There was no statistically significant difference among the 3 groups in the percentage change in the central endothelial cell density or percentage of viable central endothelium by Live/Dead assay after the dissection.

Conclusions—Both mechanical microkeratomes created smoother stromal bed dissections than the femtosecond laser. All systems provided good endothelial cell viability.

Keywords

Descemet stripping automated endothelial keratoplasty; posterior lamellar keratoplasty; endothelial cell density; microkeratome; femtosecond laser; roughness; surface

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Endothelial dysfunction remains the leading indication for corneal transplantation in the United States.¹ Visual rehabilitation after penetrating keratoplasty (PKP) may be limited by delayed wound healing, surgically induced astigmatism, suture-related complications, and anisometropia.^{2,3} In contrast, endothelial keratoplasty in the form of deep lamellar endothelial keratoplasty (DLEK) or Descemet stripping automated endothelial keratoplasty (DSAEK) allows selective replacement of diseased host endothelium, more rapid visual rehabilitation, minimal induced astigmatism, and a predictable change in corneal power postoperatively.⁴⁻⁶ DSAEK employs stripping of the diseased host endothelium and replacement with a thin layer of donor stromal tissue and endothelium harvested with an automated microkeratome.^{7,8} The manual lamellar dissections required to prepare the recipient cornea and donor tissue in DLEK surgery, which are technically difficult and time consuming for the surgeon, have been eliminated in DSAEK, helping this procedure become the procedure of choice for endothelial dysfunction.

Despite excellent postoperative results, the available data suggest that best-corrected visual acuity after DLEK or DSAEK surgeries is at times less than that of PKP patients.⁸⁻¹¹ Persistent subepithelial haze and interface haze have been suggested to affect the best-corrected visual acuity after DSAEK surgery.¹² The better visual outcomes after DSAEK in comparison to DLEK may be attributable to the smoother interface in DSAEK compared with the manually created DLEK interface.¹³

Despite several advantages of DSAEK over PKP, endothelial cell loss remains a concern with endothelial keratoplasty. Endothelial cell loss of 34% has been reported at the 6-month postoperative time period after DSAEK.¹⁴ Endothelial survival may be impacted by every step of the procedure including donor tissue preparation, folding of the donor disc, insertion site size and technique, intraoperative manipulation of the tissue to center and attach the donor disc, and the postoperative course.

Donor cornea preparation requires the surgeon or the eye bank to create a deep lamellar dissection to create a thin posterior stromal lenticule with viable endothelium. "Precut" donor tissue by the eye bank has been shown to have no increased risk of tissue-related complications and may increase surgeon's efficiency.¹⁵ In this masked study, we attempted to evaluate the quality of the stromal bed and the safety on the endothelium in the preparation of donor tissue for DSAEK using the Moria ALTK system with the mechanical CBm microkeratome, disposable Horizon mechanical microkeratome system, and Intralase FS60 femtosecond laser.

MATERIALS AND METHODS

Lamellar Resection

Deep lamellar cuts were performed on 15 donor corneas by an experienced corneal surgeon (S.M.V.). Donor age, death-to-preservation time, and preservation-to-lamellar dissection time were all recorded. Central endothelial density was calculated by specular microscopy before and after the cut. One cornea from each of 5 donor pairs was mounted on the Moria ALTK artificial chamber (Moria S.A., Antony, France), pressurized with balanced salt solution (Alcon Laboratories, Fort Worth, TX), and cut with the Moria CBm 300- μ m microkeratome head (Moria S.A.). The mate of the donor pair was cut by a disposable Horizon 300- μ m microkeratome after being mounted on the system's plastic artificial chamber with pressurized air (Refractive Technologies, Cleveland, OH). A new blade or a new disposable microkeratome was used for each donor cornea. Five additional corneas were cut with the Intralase FS60 femtosecond laser (Abbott Medical Optics, Santa Ana, CA) with the following settings: flap = 300 μ m, raster energy = 0.5 μ J or 0.6 μ J, spot sep/line

sep = 4 μ m/4 μ m after being mounted on the Moria artificial chamber with balanced salt solution infusion to gravity.

Endothelial Viability Assay

Donor corneas were bisected, with half used for Live/Dead assay (Invitrogen, Carlsbad, CA) to assess endothelial viability. Calcein AM and ethidium homodimer (EthD-1) were added to 1% phosphate-buffered saline in a 1:2 ratio. The sectioned corneas were incubated in the above mixture for 30–45 minutes at room temperature before imaging. Live/Dead images were obtained using Nikon TE300 inverted microscope (Nikon, Tokyo, Japan). Using MetaMorph 7.6 software (MDS, Ontario, Canada), maximum intensity projections of the live (green) and dead (red) stacks were overlaid (Fig. 1). For each corneal sample, images were obtained at 3 different central locations. All of the live cells and all of the dead cells were counted in each image to calculate the percentage of live cells. This percentage from the 3 images was averaged for each sample. Observers were masked to the technique used for the lamellar resection.

Electron Microscopy

Halves of the bisected donors were sent for scanning electron microscopy (SEM) of the stromal bed at $\times 14$, $\times 40$, and $\times 200$ magnifications. Specimens were fixed in 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer. After fixation, they were rinsed in buffer, postfixed in buffered 1% osmium tetroxide, and rinsed in water. They were then dehydrated in a graded ethanol series and transferred into hexamethyldisilazane (HMDS) with a graded series of ethanol and HMDS. After several changes in 100% HMDS, specimens were air dried overnight, mounted on aluminum stubs, and sputter coated with gold-palladium. Images were acquired in a Philips XL 30 ESEM (FEI, Hillsboro, OR) operating in high-vacuum mode with an accelerating voltage of 30 kV.

Stromal Bed Smoothness Grading

Qualitative surface roughness of the SEM images from the 2 microkeratome groups and the femtosecond group were graded using a roughness scale from 1 to 5 (1 = smoothest samples, 2 = next smoothest, 3 = median, 4 = rough, not worst, and 5 = roughest samples) by 2 masked observers using the methodology described by Sarayba et al.¹⁶ All SEM images ($\times 14$, $\times 40$, and $\times 200$ magnifications) of each sample were examined by the masked observers to grade the sample. Quantitative surface roughness (QnSR) of the $\times 200$ SEM images from all the groups was assessed in a masked fashion using the roughness evaluation module within Scanning Probe Image Processor software (SPIP v.3.3.5; Image Metrology, Lyngby, Denmark).

RESULTS

The donor age and death-to-preservation time were comparable in all 3 groups, but there was a delay in the lamellar dissection in the femtosecond batch of corneas (Table 1). There was no statistically significant difference in the baseline specular endothelial cell density, in the change of the central endothelial cell density after the lamellar dissection, or in the percentage of viable central endothelium after the lamellar dissection by Live/Dead assay among the 3 groups (Table 2).

The Horizon group showed a smoother stromal bed compared with the Moria or Intralase groups by masked observers with a statistically significant difference for qualitative surface roughness = 1.8, $P = 0.001$ (Table 3 and Fig. 2). The Moria group had the smoothest quantitative QnSR score of all the groups followed by Horizon and Intralase when assessed by roughness evaluation software (Fig. 3). Intralase stromal bed SEM images revealed

variable smoothness dependent upon femtosecond laser settings and surgical technique (Fig. 4).

DISCUSSION

The Horizon microkeratome stromal bed scored smoother than the Moria and Intralase groups by masked observers using $\times 14$, $\times 40$, and $\times 200$ SEM images. However, the SPIP software designed to study nanoengineered surfaces scored the Moria stromal bed with the lowest QnSR, which corresponds to a smoother surface. The software was used to grade only the highest resolution $\times 200$ images of the stromal bed. In contrast, the masked observers had access to a range of magnifications including $\times 14$ that serves as a useful overview image to examine the topography of the entire surface of the stromal bed. Based on our data, we feel that both the Moria and the Horizon give acceptable stromal bed smoothness required for DSAEK.

In this study, we were unable to consistently achieve a smooth deep stromal bed with the femtosecond laser. A study by Jones et al¹⁷ also found that the use of a manual microkeratome (Moria ALTK) resulted in a smoother stromal bed surface than the Intralase femtosecond laser. The flat applanation cone of the Intralase used to dock the laser to the corneal surface may compress the deep stromal lamellae, affecting the quality of deep stromal dissections. The longer preservation time of the donor tissue in our femtosecond subgroup may have had an impact on the corneal swelling and the quality of the lamellar dissection. The Intralase stromal bed was smoother with raster energy of $0.6 \mu\text{j}$ than with $0.5 \mu\text{j}$, use of a new applanation cone, attention to centration of the donor cornea with maintenance of an even meniscus 360 degrees around the donor tissue, and “light” docking to minimize compression of the donor. However, further refinements in settings and techniques such as using a double raster pass or higher energy may help improve the smoothness of the deep stromal bed dissections using a femtosecond laser. Based on this initial experience, a larger study with refined and consistent femtosecond treatment parameters would be useful to study the utility of this laser for DSAEK donor tissue preparation.

The Horizon microkeratome system is a relatively new instrumentation for DSAEK tissue preparation that uses a disposable linear microkeratome and plastic artificial chamber. The Horizon also differs from the Moria microkeratome in that it uses filtered air to pressurize the artificial chamber. In this study, there was no statistically significant difference among the 3 groups in the percentage change in the central endothelial cell density after the cut by specular microscopy or percentage of live central endothelial cells by Live/Dead assay. Although not statistically significant, we feel that the lower percentage of viable cells after the cut in Intralase group may be related to the longer preservation time of the donor tissue in this subgroup.

Vital staining with trypan blue and/or alizarin red stains only cells that have damaged cell membranes. Live/Dead assay simultaneously stains living endothelium, green (dependent upon functional intracellular esterases converting calcein AM to highly fluorescent calcein), and cells with damaged cell membranes, red. The Live/Dead assay seems to be a promising modern cell biology assay to assess the viability of the donor corneal endothelium after simulated surgical maneuvers.

The Moria unit requires sterilization before use and some assembly is required. The Horizon microkeratome is simple to use and disposable making it appealing for technicians to pre-cut tissue in an eye bank setting. A limitation of the Horizon microkeratome is the requirement to have a specially calibrated ultrasound pachymeter because air is used to pressurize the

donor cornea on the plastic artificial chamber, and standard ultrasound pachymeters are calibrated to measure corneal thickness with aqueous/fluid behind the endothelium. It is not possible to measure the residual stromal bed thickness after the lamellar cut with the specially calibrated pachymeter because of inaccuracy with measurements below 300 μm .

Further studies are required to assess the accuracy of the microkeratomes in achieving the intended stromal depth and compare the profiles of the resected anterior lamellar cap and resulting endothelial keratoplasty graft lenticule. The number of specimens evaluated in this study was limited. However, our study showed that both mechanical microkeratomes provided smoother stromal dissections than the femtosecond laser. We acknowledge that the smoothness achieved by the femtosecond laser may have been influenced by the longer preservation time of the donor corneas in that group. All systems seemed to provide good endothelial cell viability after the deep lamellar dissection.

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FIGURE 1.

Overlay image of maximum intensity projections of live (green) and dead (red with center mark) cells. Living cells have intracellular esterases that convert calcein AM to highly fluorescent calcein. Dead cells have damaged plasma membranes that allow ethidium homodimer-1 to enter and become fluorescent when bound to nucleic acids.

FIGURE 2. Representative SEM images ($\times 14$) of stromal bed of donor cornea cut with Horizon (A) compared with Moria microkeratome (B). Fine stromal ridges were occasionally seen with the Moria (arrow).

FIGURE 3. Representative SEM images ($\times 200$) of stromal bed cut with Horizon (A), Moria (B), and Intralase (C) used for quantitative assessment of smoothness with SPIP software.

FIGURE 4.

A, The femtosecond dissection resulted in a variable quality deep stromal bed with a smooth bed using raster energy = $0.6 \mu\text{J}$ and a new appplanation cone. B, Raster energy = $0.5 \mu\text{J}$ and a used appplanation cone resulted in a rough surface with a central depression (arrow) and peripheral pits (arrow-head) ($\times 14$). C, The femtosecond dissection occasionally resulted in significant pitting of the stromal bed ($\times 200$).

TABLE 1

Donor Tissue Demographics

Demographic	Group			<i>P</i> *
	Horizon	Moria	Intralase	
Donor age (yrs)	68.6	68.6	61.0	0.992
Death to preservation (h)	14.5	14.2	11.2	0.399
Preservation to DSAEK cut (h)	12.9	13.2	68.1	0.034

* Kruskal–Wallis test.

TABLE 2

Central Endothelial Cell Counts

Variable	Horizon	Moria	Intralase	<i>P</i> [*]
Precut central endothelial cell density (cells/mm ²)	2367 ± 889	2179 ± 795	2097 ± 677	0.860
Change in central endothelial cell density after tissue cut (%)	−0.32 ± 4.3	−2.1 ± 11.6	2.93 ± 5.4	0.600
Postcut viable cells (Live/Dead assay) (%)	91.4 ± 9.6	89.3 ± 9.7	82.8 ± 10.9	0.399

* One-way analysis of variance.

TABLE 3

Qualitative Surface Roughness (QISR) and Quantitative Surface Roughness (QnSR) Grading of SEM Images

Method	Horizon (Mean $\pm$ SD) (n = 5)	Moria (Mean $\pm$ SD) n = 5	Intralase (Mean $\pm$ SD) (n = 5)	P
QnSR (SPIP software)	2183.2 $\pm$ 301.7	1626.0 $\pm$ 208.1 *	2882.2 $\pm$ 206.3	0.003 *
QISR (masked observers) ‡	1.8	3.7	4.1	<0.001 ‡

* Moria QnSR score is statistically different from Intralase using Holm–Sidak method.

‡ Grading scheme of 15 with 1 being smoothest surface.

‡ Horizon smoother than others using Anova repeated measures.