ORIGINAL ARTICLE

Femtosecond and excimer laser-assisted endothelial keratoplasty (FELEK): A new technique of endothelial transplantation

La kératoplastie endothéliale assistée aux lasers femtoseconde et excimer (FELEK) : une nouvelle technique de greffe endothéliale

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Received 8 May 2013; accepted 5 July 2013
Available online 18 February 2014

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Introduction

Endothelial keratoplasty (EK) is becoming a popular alternative to penetrating keratoplasty (PK) for treatment of endothelial diseases such as pseudophakic bullous keratopathy and Fuchs’ dystrophy [1–3]. In the last few years, EK techniques have regularly improved, with Descemet-stripping automated endothelial keratoplasty (DMEK) [2], femtosecond laser-assisted Descemet-stripping endothelial keratoplasty (FLEK) [1,4,5], and most recently, Descemet membrane endothelial keratoplasty (DMEK) [6–8]. The advantages of EK compared to PK are the preservation of ocular structural integrity and minimal refractive changes with decreased risk of PK complications [2,9]. However, several studies showed that visual acuity results are still better with PK than with DSAEK [3,10,11], possibly due to an interface haze at the donor–recipient stromal interface, which might increase stray light [12–14].

Femtosecond laser for preparation of endothelial graft (FLEK) has been used for several years [4,5,15–18] and
provides more safety and reproducibility than with microkeratome, as previously observed in LASIK. Despite its advantages, FLEK yields lower visual acuity than conventional PK and probably DSAEK [5, 19]. Microkeratomies give a smoother interface than femtosecond laser [20, 21], and the quality and regularity of endothelial lamellar dissection might be a necessary condition to visual recovery after the grafting procedure [22].

In this study, we used scanning electron microscopy (SEM) to compare the smoothness and quality of the endothelial graft interface prepared by four different techniques, namely mechanical microkeratotomy, a single femtosecond laser lamellar cut, a double femtosecond laser lamellar cut, and combined femtosecond laser lamellar dissection with excimer laser surface photoablation. We further used the most satisfactory technique of endothelial graft preparation for the surgical procedure on three patients. Anatomical results were assessed using anterior segment spectral-domain optical coherence tomography (SD-OCT) and in vivo confocal microscopy (IVCM).

Materials and methods

Experimental preparation of endothelial grafts

Lamellar dissections were performed on four groups of two donor corneas each to investigate the four different techniques. For ethical reasons, we did not use viable donor corneas but expired donor corneas 1 month after death. Therefore, endothelial density could not be measured due to insufficient endothelial density.

The first group of corneas (group 1) was mounted on the Moria ALTK artificial chamber (Moria SA, Antony, France), pressurized with balanced salt solution (BSS) (Alcon Laboratories, Fort Worth, TX, USA) and cut with the Moria CBm 350-μm microkeratome head (Moria SA).

The corneas of group 2 were cut with the Intralase® Enabled Keratoplasty (IEK) module of the Intralase® FS150 femtosecond laser (Abbott Medical Optics, Santa Ana, CA, USA) for a single path full lamellar cut with the following settings: depth of flap = 400 μm, raster energy = 1.05 mJ, spot separation/line separation = 2 mm/2 mm, and diameter = 9.1 mm, after being mounted on the Moria artificial chamber pressurized with balanced salt solution (Alcon Laboratories).

The donor corneas of group 3 underwent two successive lamellar cuts with the Intralase® FS150 femtosecond laser (Abbott Medical Optics) punctuated by the lamellar dissection of the first lamellar cut. The parameters of the two lamellar cuts were, respectively: depth of cut = 300 μm and 100 μm, raster energy = 1.5 μJ and 0.5 μJ, spot separation/line separation = 4 mm/4 mm and 2 mm/2 mm, and diameter = 9.1 mm and 8.5 mm. The advantage of this double lamellar dissection was used of a lower energy for the second lamellar cut to obtain a more regular interface.

For the group 4 donor corneas, a single lamellar cut was performed with the Intralase® FS150 femtosecond laser with the following settings: lamellar depth = 400 μm, raster energy = 1.05 μJ, spot separation/line separation = 2 mm/2 mm and diameter = 9.1 mm (Fig. 1A). After manual removal of the corneal flap (Fig. 1B), the donor cornea still mounted on the Moria artificial chamber was transferred under an excimer laser, the Wavelight Allegretto platform (Alcon Laboratories), for laser photoablation. A photoablation therapeutic keratectomy procedure was performed with a photoablation depth of 30 μm, and a diameter of 8 mm for optical zone and 9.9 mm for transition zone (Fig. 1C). Then all the corneas were imaged in a scanning electron microscope to assess the quality of their stromal interface.

Scanning electron microscopy

The corneas were fixed by immersion in 0.1 M Cacodylate Buffer, pH 7.2, containing 1% paraformaldehyde, 2% glutaraldehyde and 2 mM CaCl₂, overnight at 4 °C. After a thorough washing in the same buffer (3 × 20 min) and postfixation in 2% aqueous OsO₄ for 1 h at 4 °C, the corneas were dehydrated at room temperature in sequential baths of ethanol (70%, 95%, 100%, 100% for 20 min each), ethanol/proplylene oxide (1:1:v/v) (10 min), propylene oxide (10 min), and hexamethyldisilazane (10 min) before being air dried. All samples were mounted on aluminum stubs and sputter-coated with gold-palladium. Images were acquired in a scanning electron microscope JEOL JSM-6510LV (JEOL Ltd., Tokyo, Japan) operating in high vacuum.

Endothelial keratoplasty on patients

The technique yielding the smoothest stromal interface (as assessed by SEM and described in the results section) was used to prepare the donor corneas for endothelial keratoplasty on patients. Three eyes of three patients were included in this interventional case series. The study was conducted at the Center for Clinical Investigations (CIC 503) of the Quinze-Vingts National Ophthalmology Hospital, with the approval of the Institutional Review Board of Saint-Antoine University Hospital (CPP—Île-de-France 5, number 10793). All subjects were informed of the aims of the study, and their informed consent was obtained. The mean age of the three female subjects was 76 years (range: 62–89 years). Three right eyes were operated. All preoperative visual acuities for distance vision were less than 20/400 in the operated eye. All patients had pseudophakic bullous keratoplasty, one with an anterior chamber intraocular lens (IOL) and two with a posterior chamber IOL. For the surgical technique, after preparation with the lamellar dissection technique chosen, the recipient cornea was trephined using an 8.5-mm Barron Vacuum Donor Cornea Punch (Katena products Inc., Denville, NJ, USA). The patients underwent the surgical procedure under a peribulbar anesthesia. A 20-gauge infusion was placed in a keratotomy in the inferonasal area of the cornea. A 4-mm corneal tunnel incision was made 1 mm from the limbus on the superonasal area. An 8.0-mm-diameter epithelial mark was made to outline the Descemet membrane stripping area and trypan blue dye (VisionBlue®, D.O.R.C. International, Zuidland, The Netherlands) was injected to stain the endothelium. Then the Descemet membrane was scored and stripped using a reverse Sinskey hook (Catalogue No. 50.1971B, D.O.R.C. International), so that an 8.0-mm-diameter “Descemetorhexis” was created and was removed from the eye. Another keratotomy was made inferotemporally. The anterior chamber IOL was removed if
present with McPherson forceps at this stage. The endothelial graft was loaded in the Endosaver® (Ocular Systems, Inc., Winston-Salem, NC, USA) injector (Fig. 1D), folded in it, and then inserted through the 4.0-mm incision. The 20-gauge infusion was removed and the graft slowly opened out over the iris while pushing it with the injector (Fig. 1E), endothelial side down, with an irrigation of BSS in the injector to maintain the anterior chamber. The donor lenticule was positioned using the BSS and Rycroft canula. Finally, an air bubble was injected under the endothelial graft to affix the donor tissue onto the recipient posterior stroma (Fig. 1F).

The corneal incision was sutured with one or two interrupted 10-0 nylon sutures. Postoperatively, the patient was asked to lie in a strict supine position for 24 h. All patients received one drop 0.1% dexamethasone sodium phosphate and tobramycin antibiotic ointment (TobraDex; Alcon Laboratories) was administered four times daily for 1 month, then three times daily for 1 month, and twice daily for 1 month.

The ophthalmologic examination included uncorrected distance visual acuity (UDVA) at 1 month and slit-lamp biomicroscopy analysis. All patients were then examined by SD-OCT on day 1 after the surgery to detect possible early graft detachment and at 1 month, and they underwent in vivo confocal microscopy at 1 month to assess the stromal cornea and interface of the endothelial graft.

## OCT examination and image analysis

An SD-OCT fitted with an anterior segment module (Spectralis® OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) was used. The OCT axial and lateral optical resolutions were 3.9 μm and 11 μm, respectively. All acquisitions were made using the high-resolution mode with an acquisition time of 19 ms per image. The instrument combines OCT technology with a confocal scanning laser ophthalmoscope (cSLO) to provide a live view of the eye so as to control the location of the OCT scan. Since OCT is a non-contact technique, it was performed before the ophthalmologic examination and oculus surface tests to avoid potential artifacts. For each eye, the central cornea was analyzed. A horizontal scan was used for imaging the central cornea to detect an early endothelial graft detachment.

The images were analyzed with a 600–800% zoom factor provided by the Heidelberg Eye Explorer software (Heidelberg Engineering GmbH) of the Spectralis® OCT. Endothelial graft thickness was measured after the first postoperative month using the cursors provided by the SD-OCT software.

## In vivo corneal confocal microscopy

All patients were examined with the Rostock Cornea Module of the Heidelberg Retina Tomograph (Heidelberg Engineering GmbH). The laser source used in the HRT II is a diode laser with a 670-nm wavelength. The acquired two-dimensional image is defined by 384 × 384 pixels covering an area of 400 × 400 μm with lateral digital resolution of 1 μm/pixel and digital depth resolution of 2 μm/pixel. Before microscopy evaluation, one drop of a topical anesthetic, oxybuprocaine 0.4% (oxybuprocaine, Laboratoires
Thea, Clermont-Ferrand, France) and one drop of a geltear substitute, carbomer 0.2% (Lacrinorm®, carbomer 980NF; Bausch and Lomb, Rochester, NY, USA) were instilled in the lower conjunctival fornix. Each eye was examined for less than 5 min. No ocular surface changes related to the IVCM evaluation were noted with the Heidelberg Retina Tomograph/Rostock Cornea Module. The objective of the microscope was an immersion lens (Olympus, Hamburg, Germany), magnification ×60, covered by a polymethylmethacrylate cap. The focal plane of analysis could be displaced manually over the entire cornea.

The examination was performed in the sagittal axis (anteroposterior axis) so that, as the operator proceeded, the corneal epithelium, subepithelial neural plexus, anterior stroma, posterior stroma, and endothelium were successively examined. For all eyes studied, several confocal microscopic images were taken of all corneal layers where the interface of the endothelial graft could be observed.

**Results**

**Quality of stromal interface in scanning electron microscopy**

SEM analysis of the four groups showed decreased smoothness of the stromal interface from groups 4 to 1, 3 and 2 (Figs. 2 and 3). In group 1, with the microkeratome, the interface was as smooth as in the central area of group 4, but regular rows of collagen fibrils were still visible (Figs. 2A and 3A). With a single lamellar cut with the Intralase® femtosecond laser (group 2), numerous marked lamellar irregularities were observed in the stromal bed, like wrenching strips (Figs. 2B and 3B). With a double layer pattern of lenticule cutting (group 3), the central corneal area corresponding to the second lamellar cut seemed to be slightly smoother than the peripheral area corresponding to the first lamellar cut (Figs. 2C and 3C). In group 4 with excimer photoablation, the interface appeared perfectly smooth (Figs. 2D and 3D). We obtained the best section quality with group 4, combining first a femtosecond laser lamellar cut 400 μm deep and then a 30-μm stromal excimer photoablation to smooth the collagen irregularities caused by the femtosecond laser and the lenticule removal.

We chose this new technique to prepare donor corneas in order to perform EK in three patients.

**Clinical results**

The three patients had a 1-month postoperative follow-up period. All corneas cleared after 1 month (Fig. 4), and we did not note any complications such as early graft detachment, high ocular pressure, pupillary block, infection, or allograft rejection. As we did not detect any graft detachment, no rebubbling was necessary. For one patient, the endothelial graft was decentered so we had to reposition the graft during the 1st week. At 1 month, UBVA increased to 20/40 for one

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**Figure 2.** Scanning electron microscopy (SEM) images of the stromal bed (×100) of the donor cornea cut with: the mechanical microkeratome (A), single femtosecond laser lamellar dissection (B), double femtosecond laser lamellar dissection (C), and the combination of femtosecond laser lamellar dissection with excimer laser surface photoablation (D). The smoothest stromal interface was observed on SEM with the combined used of femtosecond laser dissection and excimer photoablation followed by mechanical microkeratotomy, double femtosecond laser lamellar cut, and single femtosecond laser lamellar dissection.
patient but was still low (<20/200) for two patients including one aphakic patient and one with macular edema.

On the 1st postoperative day, SD-OCT confirmed the attachment of the endothelial graft. No interface was visible between the donor cornea and the recipient stromal bed the 1st postoperative day (Fig. 5A), but after 1 month, the interface became hyperreflective. Graft thickness at 1 month after surgery was 145 μm for the three grafts (Fig. 5B).

HRT in vivo microscopy showed subepithelial stromal haze (Fig. 6A) and kerocytes forming a honeycomb pattern (Fig. 6B). Stromal keratocyte activation also occurred with high reflectivity. Kerocytes were identified with visible cytoplasmic processes and bright nuclei inside an edematous area visible as stromal extracellular fluid cystic spaces.
In vivo confocal microscopy (IVCM) images (400 × 400 μm) of the cornea of patients after FELEK 1 month after the surgery. In the recipient stromal cornea, subepithelial haze (A) and keratocyte activation (with a honeycomb pattern and visible cytoplasmic processes) were observed (B). We noted multiple thin and small needle-like images inside host stromal haze with hyperreflective keratocytes (C). Small highly reflective particles were visible on the donor–recipient interface with haze (D) and on the endothelium (E).

Discussion

The current challenge in EK is to find the safest and most reproducible technique giving the best visual results to treat endothelial diseases. Use of femtosecond laser to prepare donor cornea offers this safety, accuracy, and comfort compared to microkeratome for DSAEK, as in LASIK for refractive surgery some years ago. However, visual results in FLEK seem to be lower than with a microkeratome, probably due to a possible interface haze with keratocyte activation and a more irregular interface with the endothelial graft [20,21]. Recently, a smoother interface was obtained with femtosecond laser when performing a double path procedure with lower energy delivered on the second path [23]. Our study compared the interface quality on SEM with four cutting techniques: the microkeratome, a single path with the femtosecond laser, and a double path with the femtosecond laser, and the combined use of the femtosecond laser and excimer photoablation, a procedure we suggest calling FELEK. The double lamellar femtosecond laser cut gave a better-quality stromal interface than the single lamellar cut and confirmed the results of Rousseau et al. [23]. Indeed, during the second path, a lower energy with a smaller separation of spots is delivered, which might create fewer strips of stroma induced by the laser. SEM found a smoother interface with microkeratome than with femtosecond laser lamellar cuts (in a single or double layer), as previously described in the literature because of the regular cut of its blade [20,21]. The most efficient technique in the present study was the use of femtosecond laser to create a deep lamellar dissection of the donor cornea. We obtained a thin endothelial graft with maximum safety and accuracy, with the femtosecond laser, which we smoothed with 30-μm excimer surface photoablation. The SEM images obtained showed a totally smooth surface with this technique because excimer laser regularized the stromal surface, as in refractive surgery. Combining the two lasers provided both precision for the dissection depth of the femtosecond laser and interface quality after excimer laser ablation. The low visual acuity of DSAEK could be attributed to the quality of the interface as well as the thickness of the endothelial graft. However, Shinton et al. found no correlation between central graft thickness and visual acuity [24], but Seery et al. showed that thicker grafts were associated with increased high-order aberrations (HOAs) [25]. In our study, the endothelial grafts were still rather thick, so we will adjust the parameters of femtosecond laser to obtain a deeper lamellar cut. Endothelial cell loss could not be assessed in the experimental part of the study because the grafts used for SEM were out of date for ethical reasons, but it is most likely that our procedure did not induce more endothelial cell loss than any other standard cutting procedure. Indeed in the study’s human subjects, the early clarity of grafts confirmed that no abnormal endothelial damage was induced by the double laser procedure, even though our series is too limited in time and number to provide definitive conclusions.

To our knowledge, the use of excimer laser to prepare the endothelial graft has never been published, and we applied this new technique on three patients who underwent EK. In our surgical procedure, we also used the Endosaver® injector to insert the graft into the anterior chamber. This injector was compatible with a small 4.0-mm corneal incision, and it was much easier to open out the graft than with the traditional Busin glide because of reduced manipulation of the graft and the less traumatic insertion into the anterior chamber. Recently, Gangwani et al. used another injector, the EndoGlide®, and showed less endothelial cell loss than with the standard Busin glide [26]. These new types of injector seem to be part of the material needed for EK but they require thin endothelial grafts for easier insertion.

Postoperative confocal imaging in these few patients allowed us to analyze the interface between the recipient cornea and the endothelial graft. In SD-OCT, this endothelial interface was almost invisible on day 1 but became hyperreflective at 1 month, probably due to an interface haze occurring there. The thickness measured was still high (145 μm), but in the future we will increase the depth of dissection to obtain a thinner graft. A haze occurring in the interface was observed on IVCM and corroborated with the hyperreflective interface seen on SD-OCT at 1 month but invisible on day 1. The interface haze might appear secondarily after 1 month. In IVCM, subepithelial haze, honeycomb-like keratocytes, and needle-shaped materials in the recipient corneal stroma such as the numerous hyperreflective particles found on the donor–recipient interface confirmed the previous IVCM results observed in DSAEK and non-DSAEK [27,28]. As we did not use a microkeratome...
to prepare the graft, hyperreflective particles at the donor–recipient interface cannot be metal particles from the microkeratome blade, which supports the hypothesis of degenerated keratocytes expressed by Chen et al. [29]. The visual outcomes in our small case series cannot be reported because of the short 1-month follow-up.

DSAEK and DMEK are classically accompanied by a postoperative hyperopic shift of approximately +1.50 diopters [30–33]. This hyperopic shift might be explained by the shape of the dissection where the center is thinner than the periphery of the endothelial graft [4,32]. Another advantage of this technique is that excimer photoablation can be programmed for a hyperopic treatment by photorefractive keratectomy to obtain an ablation profile with a thinner peripheral endothelial graft to reduce the hyperopic shift.

The new technique described in the current study, femtosecond and excimer lasers-assisted endothelial keratoplasty (FELEK) can be an alternative to the DMEK procedure, which provides the best visual results reported in the recent literature [7,34]. DMEK is a difficult manual technique and has a long learning curve, as the number of functional grafts increases with surgical experience [8], and has a higher rate of air rejections with a more uncomfortable postoperative period [34]. The FELEK will result in a faster learning curve thanks to the use of lasers, which automates the procedure and makes it reproducible. After these preliminary results, a larger cohort of patients operated with this technique should confirm better visual outcomes so that the endothelial outcomes can be studied more closely.

Disclosure of interest

The authors declare that they have no conflict of interest concerning this article.

Acknowledgements

We would like to thank Abdelkader Bensalem, Anne Carfantan, Laurent Perez, and Yves Holleville for their technical support.

Financial support: funded by Quinze-Vingts National Ophthalmology Hospital and Vision Institute, UMR5968.

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