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Excimer Laser Smoothing of Endothelial Keratoplasty Grafts

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Abstract

Purpose—To use excimer laser smoothing passes to reshape DSAEK endothelial grafts, and to evaluate the effect on the donor endothelium.

Methods—The stromal surface of microkeratome-cut DSAEK grafts was smoothed using excimer laser smoothing passes with masking fluid. Excimer laser hyperopic ablation was used to improve the uniformity of graft thickness within the optical zone. Fourier-domain optical coherence tomography (OCT) was used to measure endothelial graft pachymetry, plan ablations, and evaluate donor contour. Vital dye staining was performed to assess endothelial cell damage. Scanning electron microscopy (SEM) images of stromal surfaces were graded on a 5-point scale by masked observers to evaluate surface roughness.

Results—Four grafts underwent excimer laser smoothing. Vital dye staining showed no endothelial damage. Microkeratome-cut surfaces treated with laser smoothing (mean grade=2.04) were smoother than non-smoothed microkeratome-cut surfaces (mean grade=4.07, $p<0.01$), surfaces which underwent dry laser ablation (mean grade=3.63, $p<0.01$), and manually dissected interfaces (mean grade=4.75, $p<0.0001$). No difference was observed between stromal beds created by peeling Descemet's membrane (mean grade=1.64) compared to surfaces produced by microkeratome cutting followed by laser smoothing (mean grade=2.04, $p=0.14$). One graft underwent combined excimer smoothing and peripheral hyperopic ablation. The centre-periphery thickness difference was 15 μ m prior to ablation and 4 μ m afterward.

Conclusion—Laser smoothing passes can be used to improve the contour and smoothness of DSAEK grafts without damaging donor endothelial cells. Clinical trials are needed to determine whether reshaping donors using excimer laser can deliver improved visual outcomes following DSAEK.

Keywords

endothelial graft; DSAEK; excimer laser; smoothing; endothelium

Introduction

Descemet-stripping automated endothelial keratoplasty (DSAEK) has become the procedure of choice for endothelial disorders such as Fuch's endothelial dystrophy and pseudophakic bullous keratopathy. Several advantages associated with DSAEK have helped to make it a more popular procedure than traditional penetrating keratoplasty (PKP). In particular, reported visual recovery rates following DSAEK are more rapid, and because the integrity of the anterior corneal stroma is maintained, postoperative astigmatism is less, and the risk of wound dehiscence, which may occur following full thickness keratoplasty, is reduced. Further improvements in surgical success rates and visual outcomes have been achieved with the advent of standardized donor tissue preparation at eye banks using automated microkeratomes.^{1,2}

Despite these advances, the reported mean BCVA following successful DSAEK in eyes without other ocular comorbidities ranges from 20/40 to 20/30.³⁻⁶ Recently published studies have shown that the most common reason for regrafting following DSAEK is suboptimal visual outcomes, due to irregular donor graft thickness, leading to folds and wrinkles overlying the visual axis.⁷

We hypothesized that excimer laser smoothing passes could be applied to microkeratome cut donors in order to produce thinner donor buttons with improved contour and smoothness. The objectives of this study were firstly, to use excimer laser smoothing passes to reshape DSAEK endothelial grafts; and secondly, to examine the effects of this excimer laser treatment on the donor endothelium.

Materials and Methods

Eye bank corneas unsuitable for human transplantation were used in this study. The corneas were obtained from the Lions Eye Bank of Oregon, Portland, Oregon and were stored at 4°C in Optisol-GS (Optisol-GS, Bausch & Lomb, Irvine, CA) storage medium. All tissue was inspected by slit lamp and specular microscopy to ensure no significant endothelial trauma was present and that the stroma was free of pathology. Corneas were precut in order to create a free corneal cap with the Moria CB microkeratome (Moria, Doylestown, PA). Based on full thickness ultrasound pachymetry, a 250, 300 or 350µm head was chosen in order to achieve a target thickness of 150µm. A new blade was utilized for every cornea prepared. Post-resection, the anterior cap was replaced and tissue was inspected by slit lamp microscopy to ensure that no significant endothelial trauma was induced by the precutting procedure.

The microkeratome-cut DSAEK endothelial grafts were mounted on Barron artificial anterior chambers (Katena Products, Inc., Denville, NJ) which were filled with Optisol-GS through a side port. Saline bottles on an IV pole were used to maintain intraocular pressure (IOP). OCT and ultrasound pachymetry was used to measure the whole donor cornea and the endothelial graft thickness (Figure 1). The donor cap was removed using a forceps.

As controls, two corneas were prepared by Descemet's membrane stripping, which was performed by staining for one minute with trypan blue 0.06% (Visionblue, Dutch Ophthalmic USA, Kingston, NH), then peeling Descemet's off with a forceps.

One cornea was prepared as a manually dissected control. The Intralase FS 60 kHz femtosecond laser (Abbot Medical Optics, Inc., Santa Ana, CA) was used to create an 8.5mm diameter anterior side cut, with a 1mm diameter ring lamellar cut, aiming to leave a residual stromal bed of 100µm. White and blue Melles DALK spatulas (DALK Spatula

Dissector, Dutch Ophthalmic USA, Kingston, NH) were used to perform manual dissection starting from the deepest point on the ring lamellar cut identified on OCT.

Excimer Laser Smoothing

Excimer smoothing was performed using the VisX excimer laser (Abbot Medical Optics, Inc., Santa Ana, CA). Half the endothelial grafts were treated with five 10- μ m smoothing passes of an excimer PTK circle, at 6.5/0.5mm optical zone/transitional zone (oz/tz) diameters, and half were treated with eight 5 μ m smoothing passes with a 6.5/0.5mm oz/tz. Optisol-GS was used as a smoothing agent, and reapplied with a MeroCel sponge during programmed pauses between smoothing ablations, wiping the fluid in a different direction each time to minimize pooling.

For excimer laser smoothing passes, we defined the clinical ablation efficiency as follows:

$$\text{Ablation efficiency (\%)} = (\text{AD/TD}) * 100,$$

where AD = actual central corneal ablation depth in μ m,

and TD = target or programmed central corneal ablation depth in μ m.

Excimer Laser Hyperopic Ablation

Dry excimer laser peripheral hyperopic ablation of the microkeratome-cut surfaces was performed in order to improve the uniformity of the graft thickness within the optical zone. We used Munnerlyn's rule to translate the thickness difference between the central 2-mm diameter circle and the peripheral 5–6 mm diameter ring on the OCT pachymetry map, into a dioptric setting for peripheral excimer hyperopic ablation. Thus 1 dioptre of hyperopic ablation was performed for every 10 μ m of centre-periphery thickness difference measured by OCT. The hyperopic ablation was performed with a 6.0 mm optical zone and a 9.0mm treatment zone diameter.

Optical Coherence Tomography

A Fourier-domain OCT system operating at 830 nm wavelength (RTVue with corneal adaptor module by Optovue Inc., Fremont, CA) was used to obtain cross-sectional images. The system had a transverse scan width of 6 mm, an axial resolution of 5 μ m, and a speed of 26,000 axial scans per second. Pachymetry and line scans were performed on all donor corneas.

Vital Dye Staining and Quantitative Analysis of Endothelial Cell Loss

We followed the protocol for vital dye staining as published by Saad *et al.*⁸ The corneas were removed from the artificial anterior chambers and the endothelium was stained by adding trypan blue 0.25% dropwise to cover the endothelial surface. After 120 seconds, the stain was poured off, and the grafts were rinsed twice in balanced salt solution (BSS), drained to remove excess saline, and placed on a glass slide, with the endothelium facing up. The endothelial surface was covered with alizarin red S 0.2% dye for 90 seconds, which was poured off and the graft was again rinsed twice in BSS. The grafts were photographed under the operating microscope, and the percentage endothelial cell loss within the central 9mm diameter zone was quantified using Adobe Photoshop Version 8.0 software.⁸

Scanning Electron Microscopy

We removed the corneoscleral discs from the artificial anterior chambers and immediately immersed them in half-strength Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% calcium chloride and 0.1 M cacodylate buffer) and fixed at 4°C overnight. We rinsed the specimens in 0.1 M cacodylate buffer, and dehydrated them by

immersion in a graded series of ethyl alcohol solutions. They were treated with hexamethyldisilazane and air-dried. They were then mounted on aluminum stubs using colloidal silver liquid, and sputter coated with a thin film of gold/palladium. We viewed the specimens on a Scanning Electron Microscopy (SEM) imaging system (JEOL JSM-6390LV, JEOL Technics Ltd, Japan). Photographs at 100X magnification were taken at all 4 quadrants of each specimen.

Surface Roughness Grading

A subjective 5-point grading scale⁹ was used to compare the roughness on SEM of the microkeratome-cut stromal surfaces with and without smoothing, and the surface following dry excimer laser hyperopic ablation. As controls, SEM images of stromal bed surfaces following peeling of Descemet's membrane, and deep manual lamellar dissection using DALK spatulas, were included.

The SEM images were randomized and graded by 2 masked observers who were asked to rate the overall smoothness or roughness of the stromal bed. Photographs of each specimen were examined at 150x magnification. Photographs were labeled by number, and were presented in a random order to the masked observers. Each photograph was graded as follows: 1 = smoothest surface among all; 2 = next smoothest; 3 = intermediate group; 4 = rough, but not worst; 5 = roughest surface among all. The scores for individual specimens were averaged and the mean scores for the groups were compared using the Mann-Whitney and Kruskal-Wallis tests (Graphpad Prism).

Results

OCT images performed prior to removal of the donor anterior cap show the fresh microkeratome cuts which are visible as a line of increased reflectivity in the posterior corneal stroma (Figure 1). Four pre-cut corneas were treated with excimer smoothing passes. The SEM images of the stromal surface of donor endothelial grafts treated with excimer smoothing passes demonstrated improved smoothness of all four grafts. The transition from smoothed to non-smoothed areas was visible on both OCT and SEM images (Figures 2, 3).

There was a significant difference in roughness grading scores between the 5 groups ($p < 0.0001$, Figures 4 and 5). Microkeratome cuts with excimer laser smoothing (mean grade=2.04) reliably produced a smoother surface than microkeratome cuts alone (mean grade=4.07, $p < 0.001$), excimer ablation of dry microkeratome-cut surface (mean grade=3.63, $p < 0.01$), or manual dissection (mean grade=4.75, $p < 0.0001$). There was no difference in smoothness between the stromal bed surface following Descemet's membrane peeling (mean grade=1.64) and microkeratome cuts with excimer smoothing (mean grade=2.04, $p = 0.14$). We found no difference in smoothness scores between corneas where smoothing fluid was reapplied following every 5 μm compared to every 10 μm increment of smoothing ablation. Therefore for this analysis we combined these two groups into one.

The mean $\pm$ SD central donor endothelial graft thickness prior to smoothing was 139 ± 41 μm and mean central residual thickness following smoothing was 91 ± 20 μm on OCT pachymetry ($p < 0.05$). The mean $\pm$ SD clinical ablation efficiency of laser smoothing passes was 112 ± 42 percent (measured/nominal ablation depth).

The percentage endothelial cell loss within the central 9-mm diameter zone of the endothelium for the two corneas which underwent vital dye staining was 1.9% and 1.3% respectively (Figure 6).

For one endothelial graft, we combined dry excimer peripheral hyperopic ablation with central excimer smoothing passes using masking fluid, in order to improve the uniformity of graft thickness within the optical zone. Prior to laser treatment the mean peripheral thickness at the 5–6 mm diameter ring segment on the OCT pachymetry map was 121 μm , and the thickness within the central 2-mm diameter circle was 106 μm . Following laser treatment the peripheral thickness was 79 μm and the central thickness was 75 μm . Thus the centre-periphery thickness difference was reduced from 15 μm prior to ablation to 4 μm afterward.

Discussion

DSAEK postoperative visual outcomes are continuing to improve, with many surgeons now achieving postoperative visual acuities in the 20/30 range, and with gradual improvements in vision occurring up to 3 years postoperatively. Indeed, 20/20 postoperative acuities have been reported using lenticules harvested by a traditional microkeratome dissection. However, despite these excellent results, the visual acuity of a significant proportion of DSAEK patients remains in the 20/40 range. Letko et al have reported that the commonest indication for repeat endothelial keratoplasty (REK) is below expected visual outcomes, due chiefly to folds and wrinkling in the donor grafts.⁷ These folds are thought to occur due to gross variations in donor thickness produced by microkeratome cutting, or due to mismatch in the curvatures of donors and recipients. Letko et al also reported thicker donor grafts in patients who required REK. A thicker donor may be more prone to the formation of folds and wrinkles, because when it conforms against the posterior surface of a cornea with a different curvature, the degree of wrinkling may be more severe than with a thinner graft. This mismatch is most pronounced when the keratometric astigmatism of the donor and recipient are very different such as one being in the 30s and the other in the 50s.¹⁰ Eye banks do not screen donors for keratometric astigmatism, so there is no way of matching donor and recipient curvature. We postulate that by using excimer laser smoothing to produce thinner donors, it may be possible to reduce the formation of folds, and enable more rapid visual recovery and improved visual results following DSAEK.

In this study we demonstrated that excimer laser smoothing passes with masking fluid can be used to reliably reduced donor lenticule thickness, while also improving the stromal surface roughness of DSAEK endothelial grafts to a degree which is comparable to the recipient bed smoothness produced by stripping Descemet's membrane. While the method used in this study for "smoothing" the donor surface does make it smoother on SEM, it does not decrease the gross irregularities which occur with microkeratome cuts, where donors tend to be thicker on one side than the other. We used Fourier-domain OCT to guide excimer reshaping of the donor endothelial grafts, following initial microkeratome cutting at the eye bank. Theoretically, more precise reshaping of microkeratome-cut donors could be done using a high resolution OCT linked to an excimer laser, however this technology is not yet available. However we have shown that peripheral hyperopic ablations can be used to reduce centre-periphery thickness differences, and potentially toric ablations might also be used to reshape donors.

A hyperopic refractive shift^{6,11,12} of approximately +1.0 D has been well described following DSAEK, due to the meniscus shape the graft adopts on the posterior corneal lamella, which shortens the posterior radius of curvature. In this study we demonstrate that OCT-guided excimer peripheral hyperopic ablations could theoretically be used to reduce thickness differences between the graft centre and periphery, thereby diminishing this meniscus effect and potentially improving refractive outcomes. However even a plano-powered lenticule, by nature of its thickness, may have the effect of reducing the posterior corneal curvature, still causing a hyperopic shift in refraction, although possibly less significantly. Dry peripheral hyperopic ablations also create a rough surface at the graft

periphery in contrast to the central smoothed area, which may help encourage graft adhesion.

Due to the fixed beam width of the VisX laser, only the central 6.5mm of the graft was smoothed. Smoothing the central 6.5 mm zone of the donor lenticule leaves only a 1 mm wide rim of “rough” stromal surface, assuming an 8.5 mm graft. This could potentially adversely impact donor adhesion. Alternatively, this zone of roughness at the periphery might serve as an area of temporary mechanical adhesion until endothelial pump function resumes, comparable to the technique described by Terry *et al.* of scraping the periphery of the recipient bed surface in order to promote graft adhesion in DSAEK.¹³ Further studies will be necessary to quantify the effect of laser smoothing passes on DSAEK donor adhesion.

An increasingly popular technique which could improve endothelial graft surface smoothness and visual recovery is Descemet’s membrane endothelial keratoplasty (DMEK).¹⁴ The peeled Descemet’s membrane surface is very smooth, as we also demonstrated here. However, in early studies DMEK has been reported to have a relatively high rate (up to 10%) of graft damage during preparation,¹⁴ due to the technical difficulty of handling the very thin tissue, which tends to roll up like a carpet.^{2,14} However multiple centers are now finding the rate of damaged or lost donor during DMEK preparation to be around 1% or less as techniques and learning curves have improved. The other disadvantage of DMEK is that the reported postoperative dehiscence rate is also relatively high,¹⁴ possibly due to the very smooth interface.¹³ In contrast, laser smoothing retains a thin layer of stroma that might make the graft easier to manipulate. The rough stromal surface at the periphery might also promote better adhesion than the Descemet’s surface. Further studies will be needed to determine if our new technique can provide better results than DMEK.

A major limitation of this study is the small number of corneas examined. We hope our results will be replicated by others. Also, most likely due to variations in corneal hydration,¹⁵ the measured ablation depth on pre-ablation and post-ablation OCT scans varied widely from the nominal laser setting, during ablations performed over smoothing fluid. However, this should be clinically acceptable because of the relatively shallow and planar nature of the smoothing ablation. Finally, a clinical study is necessary to test whether this excimer laser smoothing provides significant improvements in visual outcomes after DSAEK surgery, without causing any increase in graft dehiscence or failure.

A key finding of this study is that significant endothelial damage does not appear to occur with excimer smoothing passes, even on donor endothelial grafts less than 150µm thick. Indeed, in the central 9mm zone of the endothelium used for grafting, where the laser energy was applied, endothelial cell loss was negligible compared to losses of up to 30% which occur during insertion of DSAEK grafts¹⁶. Clinical and histopathologic studies have indicated that in the long-term, adhesion of the donor after DSAEK is primarily achieved through good endothelial pump function.^{6,17–20} Microkeratome donor preparation has been proven to cause no change in donor endothelial cell density.¹⁷ If excimer smoothing ablations at 100µm distance are also non-detrimental to the endothelium, this greatly broadens the scope for using excimer lasers not only to enhance DSAEK graft and optical surface quality, but also has positive implications for anterior lamellar keratoplasty techniques which utilize deep laser ablation. Until now surgeons performing deep excimer laser anterior lamellar keratoplasty have retained stromal beds 200µm thick,¹⁸ because of concerns regarding endothelial cell damage, although 2 cases of successful excimer treatments 100µm from Descemet’s membrane have been reported.^{19,20} Further studies are required in order to confirm that excimer smoothing passes are not harmful to donor graft endothelium.

Finally, if this technique was to be used by either eye banks or by surgeons, some increase in costs and tissue preparation time are to be anticipated. An excimer laser would be required either in the eye bank or in the operating room, and we estimate that the smoothing process would add approximately 20 minutes to the tissue preparation time. A precedent already exists for using lasers in eyebanking, because many eyebanks in the USA use femtosecond lasers off-label to cut donor corneas for intralase-enabled penetrating keratoplasty (IEK). So with the correct technician training and protocols it should be possible for eyebanks to perform endothelial graft reshaping and smoothing as an off-label application of the excimer laser.

In conclusion, excimer laser smoothing passes can be used to reshape DSAEK endothelial grafts without damaging donor endothelial cells. OCT-guided hyperopic ablation could be combined with central smoothing to make graft thickness more uniform. Clinical studies are required to evaluate whether a laser-reshaped donor can further improve visual outcomes following DSAEK surgery.

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Figure 1.
OCT images of microkeratome-cut DSAEK donor corneas.
A. Shows the interface between the anterior stroma and the DSAEK donor endothelial graft.
B. Shows the peripheral edge of the microkeratome cut. Due to the microkeratome cut pattern the graft becomes thicker towards the periphery.

Figure 2.

OCT images illustrating the effect of excimer smoothing on DSAEK endothelial grafts.

A. Shows the transition from the non-smoothed area on the left to the smoothed area on the right.

B. A donor endothelial graft before smoothing. Microscopic surface roughness is evident. Endothelial graft thickness is 94 μm.

C. The same donor endothelial graft following excimer smoothing passes, thickness is now 68 μm.

Figure 3. Transition from smoothed to non-smoothed areas visible on (A) OCT (arrow) and (B) SEM imaging.

Figure 4.

A-F. Scanning electron microscopy images illustrating the quality of stromal surfaces following different preparation methods. . Magnification: (A) 20x, (B) to (F), 150x.

(A) SEM image showing a donor endothelial graft where half the central 6mm zone has been smoothed;

(B) Illustrates the smooth surface produced by microkeratome cutting followed by excimer smoothing of the stromal surface of the endothelial graft;

(C) Illustrates the surface produced by excimer ablation of the dry microkeratome-cut surface;

(D) Illustrates the extremely smooth surface produced by peeling Descemet's membrane;

(E) Illustrates the surface produced by microkeratome-cutting, surface irregularities produced by chatter of the microkeratome blade are visible;

(F) Shows the rough surface with broken collagen fibrils produced by manual dissection.

Results of Surface Roughness Grading of Scanning Electron Microscopy Images

Figure 5.

Graph comparing the stromal bed smoothness grade of scanning electron microscopy images. DP = Descemet's peeling; LS= laser with smoothing fluid on microkeratome-cut surface; LD= laser ablation of dry microkeratome-cut surface; MK = microkeratome-cut surface; MN = manual dissection.

Figure 6. Photographs illustrating the results of vital dye staining of 2 endothelial grafts following excimer smoothing. Endothelial cell losses (quantified using Adobe Photoshop software) were less than 2% within the central 9mm diameter zone in both specimens.