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Deep Laser-Assisted Lamellar Anterior Keratoplasty with Microkeratome-Cut Grafts

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Abstract

Background—The goals of this laboratory study were to evaluate the interface quality in laser-assisted lamellar anterior keratoplasty (LALAK) with microkeratome-cut grafts, and to achieve good graft–host apposition.

Methods—Simulated LALAK surgeries were performed on six pairs of eye bank corneoscleral discs. Anterior lamellar grafts were pre-cut with microkeratomers. Deep femtosecond (FS) laser cuts were performed on host corneas followed by excimer laser smoothing. Different parameters of FS laser cuts and excimer laser smoothing were tested. OCT was used to measure corneal pachymetry and evaluate graft–host apposition. The interface quality was quantified in a masked fashion using a 5-point scale based on scanning electron microscopy images.

Results—Deep FS laser cuts at 226–380 μm resulted in visible ridges on the host bed. Excimer laser smoothing with central ablation depth of 29 μm and saline as a smoothing agent did not adequately reduce ridges (score = 4.0). Deeper excimer laser ablation of 58 μm and Optisol-GS as a smoothing agent smoothed ridges to an acceptable level (score = 2.1). Same sizing of the graft and host cut diameters with an approximately 50 μm deeper host side-cut relative to the central graft thickness provided the best graft–host fit.

Conclusions—Deep excimer laser ablation with a viscous smoothing agent was needed to remove ridges after deep FS lamellar cuts. The host side cut should be deep enough to accommodate thicker graft peripheral thickness compared to the center. This LALAK design provides smooth lamellar interfaces, moderately thick grafts, and good graft–host fits.

Keywords

anterior lamellar keratoplasty; femtosecond laser; excimer laser

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Introduction

Anterior lamellar keratoplasty is a selective transplantation procedure in which the anterior layers of cornea of variable depth are replaced by donor tissues. Compared to penetrating keratoplasty (PKP), anterior lamellar keratoplasty retains the host endothelium, which reduces the risk of allograft rejection and graft failure. Moreover, retaining ocular structural integrity theoretically reduces intraoperative and postoperative vision-threatening problems: including suprachroidal hemorrhage, endophthalmitis, traumatic globe rupture, and secondary glaucoma. Since manual lamellar dissection of the stroma and baring Descemet's membrane in deep anterior lamellar keratoplasty (DALK) require skilled hands and a steep learning curve,^{1–3} several techniques have attempted to standardize anterior lamellar keratoplasty using either microkeratome, femtosecond (FS) laser or excimer laser.^{4–13}

Previously, we reported a preliminary laboratory study of a laser-assisted lamellar anterior keratoplasty (LALAK) technique in which a FS laser was used to produce the side cut on a full-thickness stromal graft, and an excimer laser was used to prepared the host bed.⁴ The diameter of the broad-beam excimer laser limited the transplant size. Also, the graft was much thicker than the host ablation depth. In another laboratory study, we explored the use of the FS laser to make both the host cut and the graft lamellar cut. It was found that a lamellar cut depth of 31% stromal thickness or shallower produced an acceptable interface smoothness, while deeper cuts produced significant interface ridges.⁵ Such thin grafts may not be adequate for deep scars or keratoplasty in keratoconus. In this laboratory study, we remedy the shortcomings of our previous schemes by developing a new LALAK procedure that is able to achieve thick grafts, deep host cuts, smooth interfaces, and large diameters. We explored different settings for the microkeratome, corneal punch, FS laser, and excimer laser that could achieve all these goals.

Methods

Preparation of eye-bank eyes

Thirteen eye-bank corneoscleral discs were obtained from Lions VisionGift (Portland, Oregon, USA). They had no history of refractive surgery or central corneal opacity but were unsuitable for transplantation. Six of them were cut using microkeratome (Moria, France) after wiping off the epithelium at the eye-bank (Graft 1 to Graft 6) (Table 1). In each microkeratome cut, a new blade was used. Several sizes of microkeratome heads (200–300 μm) were used for the donor cuts. The central graft thickness, measured by Fourier-domain optical coherence tomography (OCT) (RTVue, Optovue, Inc.), was provided by the eye bank based on the difference in residual stromal thickness before and after the microkeratome cuts. The other seven intact corneoscleral discs were used as hosts in the simulated LALAK surgery (Host 1 to Host 7, suturing was not performed in Host 7).

The central and peripheral thickness of another eight microkeratome-cut corneas, using a 300 μm head, were measured by OCT at the eye bank. Each cornea was placed in a view chamber filled with Optisol-GS (Bausch & Lomb, Inc.) and mounted on a custom holder to be scanned by OCT. The anterior lamellar graft thickness was compared at the center and the 8 mm diameter.

Host femtosecond laser cut design

The host cornea was mounted on a Barron artificial anterior chamber (Katena Products, Inc.). The epithelium was removed by wiping with a dry polyvinyl alcohol sponge (Merocel, Medtronic Inc., Mystic, CT). The FS laser (Intralase iFS 150 kHz, Abbott Medical Optics, Inc) was used to make anterior side cuts at a 125 degree angle in order to produce recessed side pockets (energy 2.4 μ J; spot and line separations 4 μ m and 4 μ m) and full lamellar cuts (energy 0.7 μ J; spot and line separations 6 μ m and 7 μ m) (Figure 1). We tested different depth and diameter settings of FS side cuts and full lamellar cuts (Table 2). The target anterior side cut depth was deeper than the central graft thickness by 15 μ m (Host 1), 46 μ m (Host 2), 4 μ m (Host 3), and 50 μ m (Host 4 to Host 7). Side cut diameters were either 8.0 mm or 8.2 mm. For Host 1, the target full lamellar cut depth was to match central graft thickness with a 15 μ m reserve for excimer laser smoothing ($389+15=404\mu$ m). For Host 2 to Host 7, the full lamellar cut depth was set to leave at least 200 μ m residual stroma with a 50 μ m reserve for excimer laser smoothing (e.g. Host 2, $556-250=306\mu$ m).

Excimer laser smoothing passes

After FS laser cuts, excimer laser smoothing was performed using myopic ablation of the WaveLight Allegretto excimer laser (Alcon Inc). The smoothing agent was applied with a Merocel sponge every two seconds during the ablation. The fluid was wiped in multiple directions each time to minimize pooling. Different smoothing agents and excimer laser ablation depths were tested. A balanced salt solution with -1.25D myopic ablation (8.0mm OZ, 29 μ m central ablation depth) was used only for Host 1, and Optisol-GS with -2.50D myopic ablation (8.0mm OZ, 58 μ m central ablation depth) was used for Host 2 to Host 7. Digital photographs were taken through the operating microscope before and after excimer laser smoothing.

Suturing technique

The caps of microkeratome-cut corneas were punched with either 8.0 mm or 8.25 mm Barron cornea punches (Katena Products, Inc.) (Table 1). Then the grafts (Graft 1 to Graft 6) were sutured onto the hosts (Host 1 to Host 6). Eight cardinal interrupted sutures combined with a 16-bite single running were placed using 10-0 nylon sutures. Suturing was not performed for Host 7.

Optical coherence tomography

A Fourier-domain OCT system (RTVue) was used to obtain high-resolution cross-sectional images. The system had transverse scan widths of 9.0 mm, axial resolution of 5 μ m, and operated at 26,000 axial scans per second. Pachymetry scans were performed on hosts after four procedural steps: the removal of the host epithelium, the FS laser cut, the excimer laser smoothing, and the suturing. The actual FS laser lamellar cut depth was measured just after the FS laser cut. The host stromal bed quality was assessed on OCT images before and after excimer smoothing. After suturing, the graft-host fitting was evaluated by OCT.

Surface quality grading using scanning electron microscopy

After simulated LALAK surgery, the sutures were removed. The graft and host tissues were immersed in Karnovsky fixative (2% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% calcium chloride and 0.1M cacodylate buffer) and fixed overnight at 4 degrees. After fixation, the specimens were rinsed in an osmium tetroxide 1.0% solution at room temperature for two hours and then dehydrated by immersion in a graded series of ethyl alcohol solutions. After treatment with hexamethyldisilazane and air drying, the specimens were mounted on aluminum stubs using colloidal silver liquid then sputter coated with a thin film of gold-palladium. The specimens were viewed on a scanning electron microscope (SEM) imaging system (Jeol JSM-6390LV, Jeol Ltd.). The quality of the stromal surfaces was quantified using a subjective 5-point integer scale (1=best quality; 5=worst quality) using the same baseline scores as our previous study.⁵ The surface quality metrics included ridge and roughness indices. The ridge grading was based on 23X SEM images that reflected the macroscopic surface quality. The roughness grading was based on 100X SEM images at quadrants around the center that reflected the microscopic surface quality. The SEM images were presented in random orders to two masked observers. The scores were then averaged to minimize inter-observer and intra-location differences.

Results

Simulated LALAK procedures were performed using six grafts and seven hosts. We obtained high resolution cross-sectional OCT images of the graft-host fitting after simulated LALAK (Figure 2A–2F). In Pair 1 and Pair 3, there was anterior and posterior bulging in the suture zone. The graft fit into the host side pocket with less bulging in Pair 2, Pair 4, Pair 5, and Pair 6.

The actual FS laser lamellar cut depths measured by OCT images were between 226 and 380, which were almost the same as the laser settings in all hosts (Table 2). After excimer laser smoothing, the OCT-measured residual host stromal thickness were 111 μm (Pair 1), 235 μm (Pair 2), 200 μm (Pair 3), 234 μm (Pair 4), 224 μm (Pair 5), and 208 μm (Pair 6), which were all similar to the target (Table 2).

Just after the FS laser cuts were made, noticeable concentric ridges were apparent through the operating microscope on all host bed surfaces (Figure 3A, 3B). After excimer laser smoothing, there were still residual ridges in Host 1 (Figure 3C), whereas ridges were significantly decreased in Host 2 to Host 7 (Figure 3D). Significant reduction of ridges could also be observed on OCT images (Figure 4A, 4B).

Table 3 shows the surface quality scores based on SEM images. The mean difference between two masked observers was 0.46 ± 0.52 for ridge scores and 0.42 ± 0.50 for roughness scores. In microkeratome-cut anterior lamellar grafts, ridge scores were between 1.0 and 2.5 (Figure 5A and 5B). The worst ridge score, 4.0, was noted in Host 1 (Figure 5C), whereas relatively good ridge scores, equal to or smaller than 3.5, were evident in Host 2 to Host 6, with an average score of 2.1 (Figure 5D).

Based on the additional eight microkeratome-cut corneas using the 300 μm heads, the mean central graft thickness was $296 \pm 18 \mu\text{m}$, and the mean peripheral graft thickness was $320 \pm 33 \mu\text{m}$. The mean difference between the peripheral and central graft thickness was $24 \pm 26 \mu\text{m}$.

Discussion

A good fit at the graft-host junction with a smooth anterior surface after keratoplasty is of paramount importance for retaining ocular integrity and healthy ocular surfaces. In this laboratory study, we evaluated the graft-host fit using Fourier-domain OCT system after simulated LALAK. We tested host anterior side cuts that were 4 μm , 15 μm , and 46–50 μm deeper than central graft thickness. OCT images showed the graft fit into the deep host side pocket with less bulging (side cut 46–50 μm deeper than central graft thickness). Additionally, when the 300 μm microkeratome head was used, the peripheral graft thickness was measured to be $24 \pm 26 \mu\text{m}$ greater than the central graft thickness. Because the host side cut must be equal to or deeper than the peripheral graft thickness to prevent anterior bulging, our results indicate that a host side cut depth about 50 μm deeper than central graft thickness might be optimal.

In this study, the diameter of the graft also influenced the graft-host fitting. An oversized graft created by an 8.25 mm punch lead to peripheral bulging in Pair 1. A better fitting of Pair 2 suggested that an 8.0 mm diameter graft might be appropriate when the host side cut was 8.0 mm in diameter. Similarly, grafts created by 8.25 mm punches and hosts created by 8.2 mm diameter FS laser side cuts provided a good fit (Pair 4–6). Thus same sizing seems appropriate when matching a mechanically trephined graft with a FS laser cut bed.

The graft-host interface quality is important for visual performance after lamellar keratoplasty. Although the FS laser has many feasible characteristics for corneal surgery, one drawback is that deep lamellar cuts (approximately deeper than 200 μm) produce irregular surfaces.^{5, 14, 15} Previous studies showed that deep FS laser lamellar cuts in endothelial grafts for Descemet's stripping automated endothelial keratoplasty (DSAEK) lead to less smooth interfaces¹⁵ and worse postoperative distance corrected visual acuity (by four Snellen lines)¹⁶ compared to microkeratome-cut grafts.

In the current study, many concentric ridges in the host bed were noted by gross inspection after the deep FS laser cuts were made. To resolve this problem, we used an excimer laser smoothing technique.¹⁷ After applying a 58 μm excimer laser ablation with Optisol-GS used as a smoothing agent, the ridges were significantly reduced (average score = 2.1). This was apparent on digital photos, OCT, and SEM images. On the other hand, applying a 29 μm excimer laser ablation with the balanced salt solution proved to be inadequate and ridge score remained high (4.0). The balanced salt solution seemed to be unsuitable for smoothing, probably because of its lower viscosity compared to the Optisol-GS which contained 1% dextran. The roughness scores of microkeratome-cut grafts were variable (from 1.38 to 4.00) even though all grafts were cut with new blades. The ridge scores of the microkeratome-cut grafts were consistently good (less than 2.5).

Many techniques have been proposed to perform anterior lamellar keratoplasty on keratoconic eyes.^{6–13} Busin et al. reported results from microkeratome-assisted lamellar keratoplasty, using 200 μm heads for recipient lamellar cuts and 300 μm heads for donor cuts. The incidence of postoperative irregular astigmatism was 18%, leading them to suspect that ‘keratoconus memory’ of the residual recipient stroma could influence the postoperative corneal topography and limit the postoperative vision.^{11, 12} Tan et al. reported a single case of 2-stage microkeratome-assisted lamellar keratoplasty, using a 180 μm head for the recipient cut, and a 350 μm head for the donor cut. This resulted in 20/25 vision.¹³ Spadea reported the excimer laser-assisted lamellar keratoplasty with 200 μm residual beds. Their final visual results were similar to those achieved with PKP.⁹ Mosca et al. reported anterior lamellar keratoplasty using a 60-kHz FS laser (Intralase FS60, Abbott Medical Optics, Inc.) for both the graft and host cuts in twenty-one patients, thirteen of who had keratoconus.⁷ They created grafts with an average thickness of 353 μm and host beds with an average thickness of 185 μm . This resulted in a 0.45 decimal uncorrected visual acuity and a 0.63 decimal best spectacle-corrected visual acuity at 12 months. Lu et al. used a 500-kHz FS laser (VisuMax, Carl Zeiss Meditec, AG) to the graft and host cuts in nine patients with keratoconus and post-LASIK keratectasia.⁶ They created grafts with an average thickness of 399 μm and host beds with an average thickness of 84 μm , resulting in a mean 0.25 decimal uncorrected visual acuity and 0.49 decimal best spectacle-corrected visual acuity at 16 months. Based on these studies, it seems that thinner host stromal beds and thicker grafts may have less “keratoconus memory”, resulting in a better visual outcome.

However, thinner host stromal beds have the risk of endothelial damage by excimer laser. In our previous experiment of excimer laser smoothing on 150 μm host beds, no significant endothelial damages were noted.¹⁷ Also, excimer laser treatments in human corneas having as deep as 100 μm bed thickness without endothelial damage have been reported.^{18, 19} As a result, it is reasonable to believe that a 200 μm residual stromal bed can be void of endothelial damages by excimer smoothing.

We propose a clinical strategy implementing LALAK for keratoconus. First, the host corneal thickness is measured. According to the host thickness information, the eye bank creates a microkeratome-precut graft with a proper sized microkeratome head. Based on the central graft thickness, the FS side cut for the host is set to be about 50 μm deeper. The host full-lamellar cut depth is set to leave at least a 200 μm residual stroma plus a 50 μm reserve for excimer laser smoothing. Lastly, 50 μm of excimer laser ablation is performed using the Optisol-GS as the smoothing agent.

The main limitation of this study is the small number of corneas examined. In addition, the proposed LALAK technique requires the availability of both the excimer and FS lasers, which are expensive. Although the excimer laser needs to be located in the operating room, the FS laser does not need to be on site. Another limitation is that the stromal interface might negatively influence visual restoration in LALAK compared to Descemet-baring DALK. In previous reports, which compared visual outcomes between pre-descemet DALK with a residual stromal bed and descemet DALK without a stromal interface, faster visual recovery was found from the descemet DALK. However, similar final visual acuity was achieved in both.^{20, 21} In terms of endothelial keratoplasty, visual recovery in

DSAEK is generally slower than DMEK, and the visual outcome in DSAEK (0.39 ± 0.1 logMAR) is worse than DMEK (0.25 ± 0.1 logMAR).²² As a result, because of the stromal interface, the visual restoration in LALAK might be slower and worse than that of the Descemet-baring DALK. This is similar to the difference between DSAEK and DMEK. Clinical trials are needed to determine if equivalent visual outcomes could be produced between LALAK and DALK.

In conclusion, in this new LALAK design, the use of the FS laser makes the surgical procedure more predictable than DALK. In this procedure, we achieved three goals: 1) smooth lamellar interfaces, 2) moderately thick grafts, 3) and a good graft-host fit. We discovered that using deep excimer laser ablation with a viscous smoothing agent is needed to remove ridges on the host bed created by the deep FS lamellar cuts. Microkeratomes were used to produce moderately thick grafts with acceptable stromal interfaces. To achieve a good graft-host fit, the host side cut should be deep enough to accommodate thicker graft peripheral thickness compared to the center. In addition, it is recommended to set the host anterior side cut diameter the same as the graft punch diameter.

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Figure 1.

Host cut design for femtosecond laser cutting. The anterior side cuts at a 125 degree angle produced recessed side pockets. The side-cut diameter was either 8.0 mm or 8.2 mm, depending on the graft diameter. The side-cut and full lamellar-cut depths were different in each case.

Figure 2.

OCT images after suturing in Pair 1 – Pair 6 (A–F). The central scans and side scans were montaged. We made measurements of the graft and host thickness at the center. **A:** In Pair 1, graft and host thickness were 358 μm and 111 μm , respectively. At the suture zone, there was anterior bulging and anterior surface mismatch (arrows), and posterior bulging (arrow heads). **B:** In Pair 2, graft and host thickness were 328 μm and 235 μm , respectively. The graft fit into the host side pocket with less bulging at the suture zone. **C:** In Pair 3, graft and host thickness were 252 μm and 200 μm , respectively. At the suture zone, there was anterior bulging and anterior surface mismatch (arrows), and posterior bulging (arrow heads). **D:** In Pair 4, graft and host thickness were 295 μm and 234 μm , respectively. The graft fit into the host side pocket with less bulging at the suture zone. **E:** In Pair 5, graft and host thickness were 348 μm and 224 μm , respectively. The graft fit into the host side pocket with less bulging at the suture zone. **F:** In Pair 6, graft and host thickness were 174 μm and 208 μm , respectively. The graft fits into the host side pocket with less bulging at the suture zone.

Figure 3.

Photographs before and after excimer smoothing. **A:** Before excimer smoothing, many concentric ridges are noted on bed surface in Host 1. **B:** Before excimer smoothing, many concentric ridges are noted on bed surface in Host 2. **C:** After 29 μm excimer laser smoothing with a balance salt solution as a smoothing agent, concentric ridges decreased but still persisted in Host 1. **D:** After a 58 μm excimer laser smoothing with the Optisol-GS as a smoothing agent, ridges at the central area decreased significantly in Host 2.

Figure 4. OCT images before and after excimer smoothing. **A:** Significant ridge-like interface irregularities were noted (arrows) just after femtosecond laser cuts before cap lifting in Host 3. **B:** After excimer smoothing and cap replacement, the interface became almost smooth (arrows).

Figure 5.

SEM images at 23X magnification in interface after simulated surgery. In each image, the ridge score is indicated at the upper zone as a white colored value, and the roughness scores are indicated at the 4 quadrants as 4 black colored values. **A:** A microkeratome-cut anterior lamellar graft with the least ridge (ridge score 1.0). **B:** A microkeratome-cut anterior lamellar graft with ridge score 2.5. **C:** The worst ridge score, 4.0, with many concentric ridges (arrows) in Host 1. **D:** A host bed surface with the least ridge (ridge score 1.0) in Host 7.

Table1

Graft information

	Microkeratome head (μm)	Central stromal thickness (μm)	Punch diameter (mm)
Graft 1	300	389	8.25
Graft 2	300	384	8.00
Graft 3	300	343	8.00
Graft 4	250	417	8.25
Graft 5	300	343	8.25
Graft 6	200	242	8.25

Table 2

Host information and laser settings

	Central stromal thickness (μm)	Minimal stromal thickness (μm)	FS laser side cut diameter setting (mm)	FS laser side cut depth setting (μm)	FS laser lamellar cut depth setting (μm)	Actual FS laser lamellar cut (μm)	Excimer laser ablation depth setting (μm)	Target residual stromal thickness (μm)
Host 1	533	506	8.0	404	374 [*]	380	29 (B [†])	144 [§]
Host 2	564	556	8.0	430	306 ^{**}	310	58 (O [‡])	229 [§]
Host 3	518	508	8.0	347	258 ^{**}	261	58 (O [‡])	231 [§]
Host 4	588	572	8.2	467	322 ^{**}	352	58 (O [‡])	237 [§]
Host 5	478	466	8.2	393	216 ^{**}	236	58 (O [‡])	233 [§]
Host 6	509	478	8.2	292	228 ^{**}	233	58 (O [‡])	252 [§]
Host 7	468	463	8.2	393	213 ^{**}	226	58 (O [‡])	226 [§]

^{*} Match graft thickness – 15μm reserve for smoothing (–1.25D, 8.0OZ, 29μm central ablation)^{**} At least 200μm residual stroma – 50μm reserve for smoothing (–2.50D, 8.0OZ, 58μm central ablation)[†] Masking agent: Balanced salt solution (B), Optisol-GS (O)[§] Calculated by: Central stromal thickness - FS laser lamellar cut depth setting – 0.5 × Excimer laser ablation depth setting (assuming the excimer laser ablation efficiency is 50% when smoothing agent is used to mask the stromal bed)

Table 3

Surface quality grading based on scanning electron microscopy images

	Ridge score	Roughness score
Graft 1	1.5	1.75
Graft 2	1.5	2.38
Graft 3	2.0	3.50
Graft 4	1.0	3.38
Graft 5	1.0	2.38
Graft 6	2.5	4.00
Host 1	4.0	1.50
Host 2	2.0	2.25
Host 3	2.5	3.00
Host 4	1.0	1.75
Host 5	3.5	4.00
Host 6	2.5	3.75
Host 7	1.0	1.38