Dual Laser-Assisted Lamellar Anterior Keratoplasty with Microkeratome-Cut Graft

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Abstract

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

**Methods:** Simulated LALAK surgeries were performed on 6 pairs of eye bank corneoscleral discs. Anterior lamellar graft was precut with 200-300 µm microkeratome heads and trephined with Barron punch, then sutured onto the deep femtosecond (FS) laser cut host bed. The host bed was smoothed with excimer laser. Different parameters for FS laser cut and excimer laser smoothing were tested. Fourier domain optical coherence tomography was used to measure corneal pachymetry and evaluate graft-host apposition. The graft and host surface quality was evaluated in a masked fashion using a 5-point subjective scoring method based on scanning electron microscopy images.

**Results:** Deep FS laser cut at depths of 226-380 µm resulted in grossly visible ridges on host bed. Excimer laser smoothing with central ablation depth of 29 µm and saline as smoothing agent did not adequately smooth the ridges (ridge score=4.0). Deeper excimer laser ablation of 58 µm with Optisol-GS as smoothing agent reduced ridges to an acceptable level (average ridge score=2.1). Oversizing of the microkeratome-cut graft diameter by 0.25 mm relative to FS-laser-cut host resulted in excessive bulging at the graft-host junction after suture. Same sizing of the graft and host cut diameters with approximately 50 µm deeper host side cut relative to the central graft thickness provided the best fit.

**Conclusions:** To achieve good graft-host fit in LALAK, host side cut should be deep enough to accommodate thicker graft peripheral thickness compared to center. It is recommended to set the host anterior side cut diameter the same as the graft trephine diameter. Deep excimer laser ablation with a viscous smoothing agent was needed to remove ridges produced by deep FS lamellar cut. This LALAK design provides for smooth lamellar interface, moderately thick graft, and good graft-host fit. Laser cuts make the procedure more predictable than deep anterior lamellar keratoplasty, but clinical trials are needed to determine if similar visual outcome could be produced.

**Keywords:** Anterior lamellar keratoplasty; Femtosecond laser; Excimer laser

Introduction

Anterior lamellar keratoplasty is a selective transplantation in which the anterior layers of cornea of variable depth are replaced by donor tissue. Comparing to penetrating keratoplasty (PKP), anterior lamellar keratoplasty retains host endothelium which reduces risks 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 stroma and baring Descemet membrane in deep anterior lamellar keratoplasty (DALK) needs skilled hands with steep learning curve, [1-3] several technique 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 dual laser-assisted lamellar anterior keratoplasty (LALAK) technique in which a FS laser was used to produce side cut on a full thickness stromal graft and an excimer laser was used to prepared the host bed.4 However, this diameter of the broad-beam excimer laser limited the transplant diameter, and the graft was much thicker than the recipient ablation depth. In another laboratory study, we explored the use of FS laser to make both host and graft lamellar cut, and found that lamellar cut depths of 31% stromal thickness or shallower produced acceptable interface smoothness. But deeper cuts produced significant interface ridges [5]. Such thin graft may not be adequate for keratoplasty in keratoconus or deep scars. In the current study, we remedy the shortcomings of our previous schemes by developing a new LALAK procedure that is able to achieve thick graft, deep host cuts, smooth interface, and large diameter. The laboratory study explored different settings for 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) with epithelium off at the eye-bank (Graft 1 to Graft 6) (Table 1).

In each microkeratome cut, a new blade was used to cut cleanly. Several kinds of microkeratome head (200-300 µm depth) were used for donor cut, and the information of central graft thickness measured by Fourier-domain optical coherence tomography (OCT) system (RTVue, Optovue, Inc) was provided by the eye bank based on the difference in residual stromal thickness before and after microkeratome cut. The other 7 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 8 microkeratome-cut corneas with 300 µm head was measured by OCT at the eye bank. The corneas were placed in view chamber filled with Optisol-GS (Bausch & Lomb, Inc) and mounted on a custom holder to be scanned on OCT. The anterior lamellar graft thickness at the center and the 8 mm diameter were compared.

Host femtosecond (FS) laser cut design

The host cornea was mounted on a Barron artificial anterior chamber (Katena Products, Inc). Then, the epithelium was removed by wiping with a dry polyvinyl alcohol sponge (Merocel, Medtronic Inc., Mystic, CT). An FS laser (Intralase iFS 150 kHz, Abott Medical Optics, Inc) was used to make anterior side cuts at 125-degree side cut angle to produce recessed side pockets (energy 2.4 µJ; spot and line separations 4 µm and 4µm) and full lamellar cuts (energy 0.7 µJ; spot and line separations 6 µm and 7µm) (Figure 1). We tested different depth and diameter settings of FS side cut and full lamellar cut (Table 2).

For Host 2 to Host 7, full lamellar cut target depth was set to leave at least 200 µm residual stroma with 50 µm reserve for excimer laser smoothing (e.g. Host 2, 556-250=306).

Table 1: Graft information.

| Microkeratome head (µm) | Central stromal thickness (µm) | Minimal stromal thickness (µm) | FS side cut depth setting (µm) | FS laser side cut depth setting (µm) | FS laser lamellar cut depth setting (µm) | Actual FS laser ablation depth (µm) | FS laser ablation depth setting (µm) | Target residual stromal thickness (µm) |
|------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------------|----------------------------------------|------------------------------------|--------------------------------------|-----------------------------------|
| Host 1                 | 533                          | 506                           | 8.00                          | 404                                 | 374*                                   | 380                                | 29 (B†)                              | 144§                              |
| Host 2                 | 564                          | 556                           | 8.00                          | 430                                 | 306**                                  | 310                                | 58 (O†)                              | 229§                              |
| Host 3                 | 518                          | 508                           | 8.00                          | 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§                              |

Table 2: Host information and laser settings.

Anterior side cut target depth was deeper than central graft thickness in 15 µm (Host 1), 46 µm (Host 2), 4 µm (Host 3), and 50 µm (Host 4 to Host 7). Side cut diameter was either 8.0 mm or 8.2 mm. Full lamellar cut target depth was to match central graft thickness with 15µm reserve for excimer laser smoothing for Host 1 (389+15=404). For Host 2 to Host 7, full lamellar cut target depth was set to leave at least 200 µm residual stroma with 50 µm reserve for excimer laser smoothing (e.g. Host 2, 556-250=306).

Excimer laser smoothing passes

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

**Figure 1:** Host cut design for femtosecond laser cutting. The anterior side cuts at 125 degrees 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 depth and full lamellar-cut depth were different in each case.

**Suturing technique**

The caps of microkeratome-cut corneas were punched with either 8.0 mm or 8.25 mm diameter 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), respectively. Eight cardinal interrupted combined with 16-bite single running were placed using 10-0 nylon sutures (Figure 2). Digital photographs were taken through the operating microscope after graft was sutured. Only for Host 7, suturing was not performed.

**Figure 2:** Photograph of Pair 2 after simulated dual laser-assisted lamellar anterior keratoplasty. A 16-bite single running suture with 8 interrupted sutures were noted.

**Optical coherence tomography**

A Fourier-domain OCT system (RTVue) was used to obtain high-resolution cross-sectional images. The system had a transverse scan width of 9.0 mm, an axial resolution of 5 µm, and a speed of 26,000 axial scans per second. Pachymetry scans were performed on hosts after removal of the host epithelium, after FS laser cut, after excimer laser smoothing, and after suturing. The actual FS laser lamellar cut depth was measured just after FS laser cut. The host stromal bed interface irregularity was assessed on OCT images before and after excimer smoothing. After suturing, the graft-host fitting were evaluated according to the corneal shape at the suture zone on 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 at 4 degree overnight. After fixation, the specimens were rinsed in osmium tetroxide 1.0% solution at room temperature for 2 hours and then dehydrated by immersion in a graded series of ethyl alcohol solutions. After treatment with hexamethyldisilazane and air drying, they were mounted on aluminum stubs using colloidal silver liquid and sputter coated with a thin film of gold-palladium. The specimens were viewed on a SEM imaging system (Jeol JSM-6390LV, Jeol Ltd.). The quality of the stromal surfaces was evaluated using a subjective 5-point integer scale based on previous baseline score (1=best quality; 5=worst quality).

**Results**

Simulated LALAK were performed using 6 grafts and 7 hosts. We obtained high resolution cross-sectional OCT images of graft-host fitting after simulated LALAK (Figures 3A-3F). In Pair 1 and Pair 3, there was much bulging anteriorly and posteriorly in the suture zone. The graft fit into the host side pocket with the less bulging in Pair 2, Pair 4, Pair 5, and Pair 6.

The actual FS laser lamellar cut depth measured on OCT images were between 226 and 380, which were almost 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).
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 were much 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 were much 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 fit into the host side pocket with less bulging at the suture zone.

Just after FS laser cuts, noticeable concentric ridges were apparent on all host bed surface through operating microscope (Figures 4A and 4B). After excimer laser smoothing, there were still residual ridges in Host 1 (Figure 4C), whereas ridges were significantly decreased in Host 2 to Host 7 (Figure 4D). Significant reduction of ridges could also be observed on OCT images (Figures 5A and 5B).

![Figure 3: OCT images after suturing in Pair 1 - Pair 6 (A-F).](image1)

### 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            |

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

![Figure 6: 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 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.](Image)

Based on the other 8 microkeratome-cut corneas by 300 µm head, the mean central graft thickness was 296 ± 18 µm, and mean peripheral graft thickness was 320 ± 33 µm. The mean difference between the peripheral and central graft thickness was 24 ± 26 µm.

**Discussion**

Good fit at the graft-host junction and a smooth anterior surface after keratoplasty is of paramount importance for retaining ocular integrity and healthy ocular surface. In this laboratory study, we evaluated graft-host fit using Fourier-domain OCT system after simulated LALAK. We tested different depth of host anterior side cut with 125 degree angle 4 µm, 15 µm, and 46-50 µm deeper than central graft thickness. Among all, the graft fit into the deep host side pocket (side cut 46-50 µm deeper than central graft thickness) with less bulging on OCT images. Additionally, when 300 µm microkeratome head was used, the peripheral graft thickness was measured to be 24 ± 26 µm thicker than central graft thickness. Because the host side cut must be equal or deeper than the peripheral graft thickness to prevent anterior bulging of the graft, our results indicate that a host side cut depth that is 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. Oversized graft created by 8.25 mm diameter punch lead to much peripheral bulging in Pair 1. Better fitting of Pair 2 suggests that 8.0 mm diameter might be appropriate for graft punching when host side cut with FS laser is 8.0 mm in diameter. Similarly, grafts created by 8.25 mm punch and hosts created by 8.2 mm diameter FS laser side cut provided good fit (Pair 4-6). Thus same sizing seems appropriate when matching a mechanically trephined graft with a FS laser cut bed.

Graft-host interface quality is important for visual performance after lamellar keratoplasty. Although FS laser have many feasible characteristics for corneal surgery, a drawback is that deep lamellar cuts (approximately deeper than 200 µm) produce irregular surface [5,14,15]. Previous experience with deep FS laser lamellar cut in endothelial graft preparation for Descemet stripping automated endothelial keratoplasty (DSEAEEK) showed worse interface smoothness [15] and worse postoperative distance corrected visual acuity (by 4 Snellen lines)16 compared to microkeratome-cut grafts. In the current study, many concentric ridges in the host bed were notable by gross inspection after the deep FS laser cuts. To resolve this problem, we used excimer smoothing technique [17]. After 58 µm excimer laser ablation with Optisol-GS as smoothing agent, the ridges were significantly reduced (average score=2.1), which was apparent on digital photos, OCT, and SEM images. On the other hand, 29 µm excimer laser ablation with balanced salt solution was inadequate and ridge score was high (4.0). Balanced salt solution seems to be unsuitable for smoothing agent probably because of its lower viscosity compared to Optisol-GS which contains 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. On the other hand, the ridge scores of 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 el al. reported microkeratome-assisted lamellar keratoplasty, using 200 µm head for recipient lamellar cut and 300 µm head for donor cut. The incidence of postoperative irregular astigmatism was 18%, and they expected ‘keratoconus memory’ of residual recipient stroma could influence postoperative corneal topography and limit postoperative vision [11,12]. Tan et al. reported single case of 2-stage microkeratome-assisted lamellar keratoplasty, using 180 µm head for recipient cut, and 350 µm head for donor cut, resulting in 20/25 vision [13]. Spadea reported excimer laser-assisted lamellar keratoplasty with 200 µm residual bed, and final visual results was similar to those achieved with PKP [9]. Mosca et al. reported anterior lamellar keratoplasty using 60-kHz FS laser (Intralase FS60, Abbott Medical Optics, Inc) for both graft and host cuts in 21 patients including 13 patients with keratoconus [7]. They created the graft with mean 352.86 µm thickness and host bed with mean 185.14µm residual thickness, resulted in mean 0.45 decimal uncorrected visual acuity and 0.63 decimal best spectacle-corrected visual acuity at 12 months. Lu et al. used 500-kHz FS laser (VisuMax, Carl Zeiss Meditec, AG) to both graft and host cuts in 9 patients with keratoconus and post-LASIK keratectasia.6 They created graft with mean 399 µm thickness and host bed with mean 84.4 µm residual thickness, resulted in 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 seem to have the benefit of reduced “keratoconus memory” which leads to better visual outcome.

On the other hand, a thinner host stromal bed has risks of endothelium damage by excimer laser. In our previous experiment of
Excimer smoothing for 150 µm bed, no significant endothelial damages were noted [17]. Also, excimer laser treatments in humans as deep as 100 µm bed thickness without endothelial damage have been reported [18,19]. Therefore, at the 200 µm residual stromal bed, the endothelium would not be damaged by excimer smoothing.

We propose the clinical strategy of LALAK for keratoconus as below. First, the host corneal thickness is measured by OCT. According to the host thickness information, eye bank creates microkeratome-prefect donor with proper-sized microkeratome head. Then based on central graft thickness, the FS side cut for the host was set to be about 50 µm deeper. The host full-lamellar cut depth was set to leave at least 200 µm residual stroma plus 50 µm reserve for excimer laser smoothing. At last, 50 µm for excimer laser ablation is performed with Optisol-GS as smoothing agent.

The main limitation of this study is the small number of corneas examined. In addition, LALAK technique requires the availability of both excimer and FS lasers, which are expensive. Excimer laser need to be at operating room, but FS laser do not need to be onsite. Since the cost of using a FS laser and an excimer laser is expensive compared to manual PK or DALK, the cost could prohibit to spread the procedure widely. Another limitation is that stromal interface might influence visual restoration negatively in LALAK compared to descemet-baring DALK. In previous reports which compared visual outcome between predesecmetric DALK with residual stromal bed and descemetic DALK without stromal interface, faster visual recovery was found from descemetic DALK, and similar final visual acuity was achieved in 2 procedures [20,21]. In terms of endothelial keratoplasty, visual recovery in DSAEK is generally slower than DMEK, and visual outcome in DSAEK (0.39 ± 0.1 logMAR) is worse than DMEK (0.25 ± 0.1 logMAR) [22]. Therefore, because of the stromal interface, the visual restoration in LALAK might be slower and worse than that of descemet-baring DALK, similar to the difference between DSAEK and DMEK. Clinical trials are needed to determine if equivalent visual outcome could be produced between LALAK and DALK.

In conclusion, in this new LALAK design, the use of FS laser makes it more predictable than DALK. In this procedure, we achieved: 1) smooth lamellar interface, 2) moderately thick graft, and 3) good graft-host fit. Deep excimer laser ablation with a viscous smoothing agent was needed to remove ridges on the host bed produced by deep FS lamellar cut. Microkeratome was used to produce moderately thick graft with an acceptable stromal interface. At last, to achieve good graft--host fit, host side cut should be deep enough to accommodate thicker graft peripheral thickness compared to center. In addition, it is recommended to set the host anterior side cut diameter the same as the graft trephine diameter.

Proprietary Interests
Maolong Tang, Yan Li, and David Huang have significant financial interests in Optovue, Inc., a company that may have a commercial interest in the results of this research and technology. This potential individual conflict of interest has been reviewed and managed by the Oregon Health & Science University.

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