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Interface quality of different corneal lamellar–cut depths for femtosecond laser–assisted lamellar anterior keratoplasty

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Abstract

PURPOSE—To evaluate interface quality of different corneal lamellar–cut depths with the femtosecond laser and determine a feasible range of depth for femtosecond laser–assisted lamellar anterior keratoplasty.

SETTING—Casey Eye Institute, Portland, Oregon, USA.

DESIGN—Experimental study.

METHODS—Full lamellar cuts were made on 20 deepithelialized human cadaver corneas using the femtosecond laser. The cut depth was 17% to 21% (100 μ m), 31%, 35%, 38% to 40%, and 45% to 48% of the central stromal thickness. Scanning electron microscopy images of cap and bed surfaces were subjectively graded for ridge and roughness using a scale of 1 to 5 (1 = best). The graft–host match was evaluated by photography and optical coherence tomography in a simulated procedure.

RESULTS—The ridge score was correlated with the cut depth ($P = .0078$, $R = 0.58$) and better correlated with the percentage cut depth ($P = .00024$, $R = 0.73$). The shallowest cuts had the least ridges (score 1.25). The 31% cut depth produced significantly less ridges (score 2.15) than deeper cuts. The roughness score ranged from 2.19 to 3.08 for various depths. A simulated procedure using a 100 μ m host cut and a 177 μ m (31%) graft had a smooth interface and flush anterior junction using an inverted side-cut design.

CONCLUSIONS—The femtosecond laser produced more ridges in deeper lamellar cuts. A depth setting of 31% stromal thickness might produce adequate surface quality for femtosecond laser–

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assisted lamellar anterior keratoplasty. The inverted side-cut design produced good edge apposition even when the graft was thicker than the host lamellar-cut depth.

The femtosecond laser is widely used to perform laser in situ keratomileusis (LASIK)¹ and penetrating keratoplasty (PKP).² However, it is not as commonly used for anterior or posterior lamellar keratoplasty because deep lamellar cuts produce irregular surfaces.³ The resulting poor interface quality negatively affects contrast sensitivity and visual performance.⁴ Given this limitation, we speculated that by limiting the depth of the femtosecond laser lamellar cut, a sufficiently smooth graft–host interface could be obtained. Within the depth limit, femtosecond laser–assisted anterior keratoplasty could be useful for the treatment of keratoconus and some anterior stromal scars. We called such a procedure femtosecond laser–assisted lamellar anterior keratoplasty.

As a first step of developing the femtosecond laser–assisted lamellar anterior keratoplasty technique, we performed laboratory experiments to determine the smoothness of the laser-cut interface as a function of depth so that the feasible range of cut depth could be decided. We used scanning electron microscopy (SEM) to evaluate the corneal interface quality after femtosecond laser cuts over a range of depths to determine the depth limit that produced sufficiently smooth graft–host interfaces. Using maximum graft thickness within this limit, a simulated femtosecond laser–assisted lamellar anterior keratoplasty procedure designed to augment a keratoconic eye was tested in the laboratory.

MATERIALS AND METHODS

Preparation of Eye-Bank Eyes

Eye-bank corneoscleral disks with no history of refractive surgery or central corneal opacity but unsuitable for transplantation were used in this study. They were obtained from 2 eye banks (Lions Visiongift, Portland, Oregon, USA; Sightlife, Seattle, Washington, USA). The corneas were preserved at 4°C in tissue storage medium (Optisol-GS, Bausch & Lomb).

Femtosecond laser cuts were performed at Casey Eye Institute, Portland, Oregon, USA, and at Sightlife using the same laser setting. To perform the laser cuts, the corneas were mounted on Barron artificial anterior chambers (Katena Products, Inc.) filled with the tissue storage medium through a side port. The corneal epithelium was removed using a polyvinyl alcohol sponge (Merocel). Central stromal thickness was measured by a Fourier-domain optical coherence tomography (OCT) system (RTVue, Optovue, Inc.) operating at 830 nm wavelength for lamellar cut-depth calculation. Ultrasound (US) pachymetry (Corneo-Gage Plus, Sonogage, Inc.) was used on samples cut by Sightlife because OCT was not available. Saline bottles on an intravenous pole were used to maintain intraocular pressure at 65 mm Hg during applanation. Once the proper applanation area was obtained, the fluid line was clamped off and the applanation cone was lowered further until adequate pressure was obtained for the laser cut.

After femtosecond laser cutting (Intralase FS 60 kHz and iFS 150 kHz, Abbott Medical Optics, Inc.), the cap was lifted from the stromal bed using a Seibel LASIK flap lifter. Digital photographs were taken through the operating microscope oculars, and OCT

scanning of the interface was performed. The stromal bed (corneoscleral disk) was dismantled from the artificial anterior chambers. Both cap and bed were immediately immersed in Karnovsky fixative (2.0% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% calcium chloride and 0.1 M cacodylate buffer) and fixed at 4°C overnight in preparation for further SEM processing.

Femtosecond Laser–Assisted Lamellar Anterior Keratoplasty Technique for Keratoconus

Graft and Recipient Design—The femtosecond laser was used to make side cuts (energy 2.4 μ J; spot and line separations 4 μ m and 4 μ m) and full lamellar cuts at different depths (energy 0.7 μ J; spot and line separations 6 μ m and 7 μ m). The donor side-cut angle was 135 degrees to produce a beveled edge, and the recipient side-cut angle was 150 degrees to produce recessed side pockets (Figure 1). The lengths of the donor and recipient side cuts were equal so they would match precisely. The recipient full lamellar-cut depth was set at 100 μ m. The donor lamellar graft was intended to be as thick as possible without having excessive interface ridges or roughness. The donor-cut depth was set as a percentage of the central stromal thickness after the epithelium had been wiped off. A range of donor lamellar-cut depths was investigated, and the optimum depth was then determined for a simulated femtosecond laser–assisted lamellar anterior keratoplasty procedure performed in the laboratory.

Wound Closure Technique for Simulated Femtosecond Laser–Assisted Lamellar Anterior Keratoplasty—The graft was sutured onto the host cornea in a simulated femtosecond laser–assisted lamellar anterior keratoplasty procedure. Four cardinal 10-0 nylon sutures were placed; then, the graft rim was tucked into the host side pocket using a Sinskey hook. The graft was then sutured with 24-bite running sutures. The cardinal sutures were then removed. Digital photographs were taken through the operating microscope before and after the graft was sutured.

Optical Coherence Tomography

A Fourier-domain OCT system was used to obtain high-resolution cross-sectional images. The system had a transverse scan width of 6.0 mm, an axial resolution of 5 μ m, and a speed of 26,000 axial scans per second. After removal of the epithelium, pachymetry scans were performed. The central stromal thickness was used as the denominator in calculating the percentage depth of the laser cut for donor corneas. Pachymetry scans were again taken after the femtosecond laser cut was made. High-resolution line scans were taken after suturing was performed in the simulated femtosecond laser–assisted lamellar anterior keratoplasty procedure.

Scanning Electron Microscopy

After fixation (see above), the corneal cap and bed 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.).

Surface-Quality Grading

The quality of cap and stromal bed surfaces was evaluated based on SEM images. A subjective 5-point integer scale⁵ (1 = best quality among all; 5 = worst quality among all) was used. The surface quality metrics included 2 indices; that is, ridge and roughness. The ridge grading was based on $\times 27$ magnification SEM images that reflected the macroscopic surface quality (Figure 2, A). The roughness grading was based on $\times 100$ magnification SEM images at 4 locations that reflected the microscopic surface quality (Figure 2, B and C). The SEM images were presented in random order to 2 masked observers. The scores for the cap and bed surfaces were averaged to obtain 1 interface score for each laser cut. The ridge and roughness scores were correlated with the femtosecond laser-cut depth and percentage cut depth. The Mann-Whitney (nonparametric) test was used to compare the means of different groups.

RESULTS

Twenty samples were included in the data analysis (Table 1). The mean age of the donor corneas was $60.2 \text{ years} \pm 12.4 \text{ (SD)}$, and there were 12 male eyes (60%) and 8 female eyes (40%). The mean postmortem time (time of death to time of experiment) was 5.0 ± 1.5 days. The lamellar-cut depths ranged from 100 to 272 μm as measured from the anterior surface of Bowman layer. This was equivalent to 17% to 48% of the stromal depth, calculated using the depth setting for lamellar cut as the numerator and the central stromal thickness after epithelial removal as the denominator.

Photographs and OCT images provided assessment of stromal bed quality immediately after laser cutting and graft lifting. Quantitative grading was not attempted because only gross ridges could be clearly appreciated in these images. In photographs (Figure 3) and OCT images (Figure 4), the setting of 100 μm and 31% depth showed smooth surfaces with no appreciable ridges, while ridges of various severities were observed in deeper cuts.

Formal masked grading was performed based on SEM images, which provided the most detail on cut-surface quality (Figure 5). The mean difference between the 2 masked observers was 0.39 ± 0.81 for ridge scores and 0.28 ± 0.71 for roughness scores. There were no significant correlations between postmortem time and ridge or roughness scores.

The ridge scores were significantly correlated with the cut depth ($P = .0078$, $R = 0.58$) and even better correlated with the percentage cut depth ($P = .00024$, $R = 0.73$). For further statistical analysis, the specimens were divided into the following 5 groups based on percentage depth: 17% to 21% (100 μm , $n = 3$), 31% ($n = 5$), 35% ($n = 5$), 38% to 40% ($n = 4$), and 45% to 48% ($n = 3$) of total stromal thickness. When the ridge scores were analyzed by group (Figure 6), the 17% to 21% group had the fewest ridges, the 31% depth group had an intermediate ridge score, and the deeper groups (35% to 48%) had the most ridges. The ridge score was statistically significantly lower at 31% depth than for deeper cuts ($P < .05$).

The roughness score did not vary systematically with cut depth (Figure 7); there was no monotonic trend. The roughness score ranged from 2.19 to 3.08 for various depths.

Most femtosecond laser cuts were performed at Casey Eye Institute. Six cuts (31% depth and 35% depth, n = 3 each) were performed at the Sightlife Eye Bank. There was no significant difference between ridge or roughness scores between the cuts performed at the 2 sites using separate femtosecond laser systems of the same model and settings. The 2 31% depth cuts at Casey had ridge scores of 1.7 and 2.5. The 3 31% depth cuts at Sightlife had ridge scores of 2.8, 1.7, and 2.0.

The 31% stromal thickness appeared to produce adequate surface quality for femtosecond laser–assisted lamellar anterior keratoplasty. Therefore a simulated femtosecond laser–assisted lamellar anterior keratoplasty was performed using eye-bank corneas, with the anterior lamellar graft cut at 31% depth (177 μm thick) and the recipient bed cut at 100 μm depth using the dovetail design (Figure 1). After suturing, the graft appeared to fit well on the bed (Figure 8, A). The OCT cross-section showed a smooth lamellar interface and a good peripheral graft–host match with a smooth anterior junction and a slight posterior peripheral bulge (Figure 8, B).

DISCUSSION

Femtosecond lasers are used in PKP because they can create more complex interlocking donor and host wound edges, thereby enhancing wound stability and strength.^{6,7} The femtosecond laser is also desirable for both anterior and posterior lamellar keratoplasty because of its excellent control in cut depth. Shallow femtosecond laser cuts for LASIK flaps have improved with increasing laser pulse repetition rate,^{5,8} which allows use of lower pulse energy⁹ and narrower spot separation. The laser is now commonly used for LASIK and consistent with excellent visual outcomes.^{10,11}

However, applications that require deeper lamellar cuts, including anterior and posterior lamellar keratoplasties, have been limited by the interface quality of the laser lamellar cut. It is commonly understood that deeper laser cuts have a more irregular stromal bed surface. Several previous studies^{3,5,12,13} have assessed stromal bed surface quality after femtosecond laser lamellar cuts. These studies evaluated factors such as surgery protocol,³ laser frequency,⁵ laser manufacturer,¹² and laser energy setting.¹³ Soong et al.³ found that femtosecond laser lamellar cuts deeper than 180 μm could produce ridges on the interface. A smooth interface for lamellar keratoplasty is very important for obtaining good visual outcomes, as evidenced by the advantage of big-bubble dissection over manual dissection in deep anterior lamellar keratoplasty.¹⁴ A smooth lamellar interface has also been found to be important for postoperative quality of vision in endothelial keratoplasties, as evidenced by the stepwise improvement in corrected distance visual acuity (CDVA) when surgeons moved from manual dissection¹⁵ to Descemet-stripping, automated endothelial keratoplasty, which produces smoother microkeratome cuts,¹⁶ and finally to Descemet membrane endothelial keratoplasty, which yields the smoothest interface.¹⁷

To improve the interface quality of deep femtosecond laser cuts, different laser settings have been evaluated. The general consensus is that lower pulse energy^{13,18} and raster pattern¹⁹ produces smoother interfaces for femtosecond laser cuts from 200 to 500 μm in depth. Lombardo et al.¹⁸ advocate pulse energy of 0.50 μJ over higher pulse energies of 0.65 to

1.00 μJ on a 150 kHz femtosecond laser system. Ziebarth et al.¹³ suggest 180 nJ pulse energy is better than 340 nJ using a 500 kHz laser system. Sarayba et al.¹⁹ found that the raster pattern produced smoother interface than the spiral pattern on a 15 kHz laser system. However, even with optimization of laser parameters, deeper lamellar cuts still create ridges that could interfere with visual outcomes.^{3,20} In published studies,^{21,22} anterior lamellar keratoplasty with a femtosecond laser lamellar cut produced only fair CDVA that averaged between 20/32 and 20/38 at a 12-month follow-up. The problem of laser lamellar–cut quality can be circumvented using the laser to make only the side cuts and then using the big-bubble technique^{23–25} or the excimer laser²⁰ to prepare the lamellar bed. However, this negates the benefit of using the femtosecond laser for safer and more predictable lamellar dissection. The approach taken in our study was to accept the problem with ridge formation in deeper femtosecond laser lamellar cuts and to systematically find the deepest cut that could be made without excessive ridges. Accepting this depth limit, we could then devise a femtosecond laser–assisted lamellar anterior keratoplasty procedure that could have a useful clinical indication.

As expected, we found that deeper depth cuts produced significantly more ridges. A revealing finding was that the severity of interface ridges was more closely correlated with the percentage cut depth than the number of micrometers in the cut depth. The anterior corneal lamellae have more bridging fibers between them that increase shear strength, and these are relatively lacking in the posterior stroma.^{26,27} We hypothesize that the lack of interlamellar fibers causes the femtosecond laser cavitation bubbles to preferentially create cleavage between lamellar planes rather than across lamellae in the posterior stroma. When the laser dissection eventually jumps across lamellae, a ridge is then formed. The lack of shear strength in the posterior stroma also creates visible concentric posterior striae when the cornea is applanated by the applanation plate of the femtosecond laser.³ However, the applanation effect does not entirely explain the ridges because they still appear in deep lamellar cuts even when a curved applanation lens that conforms better to corneal curvature is used.²⁸ Our results indicate that by limiting the lamellar cut to 31% depth or shallower, the degree of ridge irregularity could be acceptable with ridge scores between 1 and 3. Whether this will provide good visual outcome will have to be confirmed in a clinical trial.

We did not find a systematic correlation between cut depth and microscopic roughness. Although the 100 μm cut depth was slightly smoother than deeper cuts, the difference was not statistically significant. Because the 100 μm cut depth is similar to a common LASIK setting, which produces excellent visual outcomes,²⁹ it is likely that the microscopic smoothness of deeper lamellar cuts is also compatible with good visual outcomes. However, the uniformity in microscopic roughness between different cut depths could also be attributed to the small sample size. In addition, artifacts resulting from SEM tissue preparation could lead to errors in the roughness score. Even though the SEM preparation technique used in this study was similar to that used in established studies,^{30,31} the dehydration and metal coating of corneas for SEM imaging could introduce shrinkage and microscopic roughness, which might affect the accuracy in qualifying microscopic surface features.^{32,33}

Given the limitations imposed by a 31% thickness anterior lamellar graft, an attractive application is to augment the keratoconic cornea to improve the corneal shape and topography. In this application, it is not necessary to remove much of the host stroma, assuming that it is clear. Therefore we chose a 100 μm host cut so that minimum ridges would be produced and the host cornea would be minimally weakened. This shallow cut would slightly flatten the cone where there is epithelial thinning over the apex.^{34,35} Therefore, a femtosecond laser lamellar cut through the intact epithelium would remove more tissue from the apex than from the surrounding cornea. On the donor side, it is preferable to make the femtosecond laser lamellar cut after removing the epithelium, which can become irregular as a result of exposure in the eye-bank harvesting process. Cut at 31% depth, the lamellar graft would be approximately 160 μm in stromal thickness given the average stromal thickness. To accommodate the thicker graft of 160 μm on a shallower lamellar bed of 50 μm depth, assuming 50 μm epithelial thickness, we made the host side cut deeper than the lamellar cut. The graft edge was beveled and the recipient side cut was angled to form a side pocket to receive the graft bevel. Both side-cut angles were “inverted” in Intralase terminology. In LASIK, the inverted side cut improves the strength of the healed wound.³⁶ The recipient side-cut angle was shallower than the donor. This minimizes the host side-cut depth to lessen the weakening of the host cornea. The lengths of the donor and host side cuts were calculated to match. A simulation of this femtosecond laser–assisted lamellar anterior keratoplasty design was performed in the laboratory using eye-bank corneas. Optical coherence tomography imaging of the peripheral graft–host junction showed good apposition with a smoother anterior junction. There was a slight posterior peripheral bulge that would have had no visual consequence because it was outside the optical zone.

The design of the inverted side-cut femtosecond laser–assisted lamellar anterior keratoplasty procedure for keratoconus is similar to that of epikeratoplasty, with a thinner graft and better junctional fit using the femtosecond laser. Epikeratoplasty was successful in flattening keratoconic corneas, but the visual outcome was not as good as PKP, probably because of striae in the host cornea resulting from a graft–host mismatch.³⁷ Whether femtosecond laser–assisted lamellar anterior keratoplasty can overcome this limitation will have to be determined in a clinical trial.

In addition to the interface quality and wound apposition, the performance of femtosecond laser–assisted lamellar anterior keratoplasty for keratoconus treatment might also be affected by potential uneven host stromal thickness profile after a femtosecond laser lamellar cut. The reason is that the femtosecond laser dissects the host cornea at a constant depth from the anterior corneal surface. Therefore, in ectatic disorders such as keratoconus where the anterior surface is irregular, the contour of the resulting stromal bed might be as uneven as the initial surface.

One drawback of this study was that US pachymeter was used on 6 corneas to measure total stromal thickness when OCT was not available. We performed a previous study of the correlation between Fourier-domain OCT and US pachymeter³⁸ and found US pachymetry was 3.7% greater than OCT pachymetry. For lamellar cuts of 31% of total stromal thickness, it translated to 1.1% variation in percentage cut depth (approximately 6 μm). We considered

this discrepancy acceptable because of its magnitude was similar to the variation in femtosecond laser–created LASIK flaps.³⁹

In conclusion, interface ridges after femtosecond laser lamellar cuts were more closely correlated with the percentage cut depth than the absolute cut depth. A depth setting of 31% stromal thickness could provide a graft with adequate surface quality. Simulated femtosecond laser–assisted lamellar anterior keratoplasty showed good graft–host apposition. An ongoing clinical trial will determine whether femtosecond laser–assisted lamellar anterior keratoplasty is useful in keratoconus treatment.

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WHAT WAS KNOWN

- The irregularity of the cornea lamellar surface caused by deep femtosecond laser cuts limits the application of the femtosecond laser in anterior and posterior lamellar keratoplasty.
- Previous studies tried to overcome the limitation by optimizing parameters, such as energy pulse and energy pattern. However, the correlation between cut depth and surface quality has not been studied.

WHAT THIS PAPER ADDS

- More ridges were produced in deeper lamellar cuts in femtosecond laser–assisted lamellar anterior keratoplasty designed to augment a keratoconic eye.
- A depth setting of 31% stromal thickness may produce adequate surface quality for femtosecond laser–assisted lamellar anterior keratoplasty.

Figure 1.

The graft and host cut profiles for a dovetail-shaped femtosecond laser-assisted lamellar anterior keratoplasty (D = graft lamellar cut depth [mm], Fs = femtosecond).

Figure 2.

Scanning electron microscope evaluation of cut surfaces. *A*: Magnified images were used to assess the severity of ridges (*arrows*) (original magnification $\times 27$). *B*: Four rectangular areas in the center were selected for higher magnification imaging (original magnification $\times 100$). *C*: Images magnified were used to grade microscopic roughness (original magnification $\times 100$).

Figure 3.

The stromal bed after femtosecond laser cuts. With cut depths of 100 μm (A) and 31% of the total stromal thickness (178 μm) (B), no ridges were apparent. C: With a cut depth of 45% of the total stromal thickness (259 μm), significant ridges were apparent.

Figure 4.

The OCT images of the cornea after femtosecond laser cuts but before cap lifting. At a cut depth of 100 μm (A) or 31% of the total stromal thickness (178 μm) (B), the interface was smooth. C: At a cut depth of 45% of total stromal thickness (259 μm), significant ridge-like irregularities were present in the interface profile.

Figure 5.

The SEM images used to anchor the 5-point grading scale. *A*: Stromal bed surface cut with femtosecond laser at 100 μm showed minimum ridges. *B*: Stromal bed surface of a deep cut had the most ridges and was used to define grade 5 ridges. *C*: Stromal bed surface cut at 100 μm had a very smooth surface and was used to define the grade 1 roughness score. *D*: Stromal bed surface (original magnification $\times 100$) of a deep cut showed the most roughness and was used to define the grade 5 roughness.

Figure 6.

Mean stromal bed ridge grading after femtosecond laser cut. Error bars denote standard errors of the means. Percentage depth was obtained by dividing the laser setting for lamellar cut depth by the central stromal thickness.

Figure 7.

Mean stromal bed roughness grading after femtosecond laser cut. Error bars denote standard errors of the means.

Figure 8.

Simulated femtosecond laser-assisted lamellar anterior keratoplasty. *A*: Smooth graft–host junction with a 24-bite running suture. *B*: The OCT image shows good apposition of the dovetail graft edge into the host side cut. The anterior wound junction was smooth except for mild suture-induced bunching in the host tissue. There was a slight posterior bulge under the peripheral wound junction. The lamellar interface was smooth.

Table 1

Donor cornea Information and femtosecond laser lamellar cut depth.

No	Donor Age (Y)	Postmortem Time (D)	Central stromal Thickness (μm) [*]	Femtosecond Laser (kHz)	Cut Depth (μm)	Cut Depth (%)
1	72	8	588	60	100	17
2	80	6	476	60	100	21
3	55	3	535	150	100	19
4	55	7	645	60	200	31
5	65	5	655	60	203	31
6 [†]	43	5	571	150	177	31
7 [†]	79	5	574	150	178	31
8 [†]	67	3	611	150	189	31
9 [†]	53	4	517	150	181	35
10 [†]	52	3	623	150	218	35
11 [†]	56	5	611	150	214	35
12	47	5	486	60	170	35
13	56	4	460	60	161	35
14	44	4	534	60	203	38
15	84	4	680	150	272	40
16	44	6	510	150	204	40
17	71	8	570	60	228	40
18	55	5	438	150	197	45
19	66	5	576	60	259	45
20	60	4	521	150	250	48

^{*} Central stromal thickness measured by optical coherence tomography after epithelial removal (n=14).

[†] Corneal stromal thickness measured by ultrasound pachymeter (n = 6).