Published in final edited form as:

Cornea. 2015 November ; 34(11): 1499–1503. doi:10.1097/ICO.0000000000000614.

Laboratory Evaluation of Femtosecond Laser Lamellar Cuts in Gamma-Irradiated Corneas

Chenxing Zhang, MD^{1,2}, Liang Liu, MD^{1,3}, Maolong Tang, PhD¹, Yan Li, PhD¹, Winston Chamberlain, MD¹, and David Huang, MD, PhD¹

¹Center for Ophthalmic Optics & Lasers (www.COOLLab.net), Casey Eye Institute and Department of Ophthalmology; Oregon Health & Science University; Portland, OR, USA

²Southwest Eye Hospital, Third Military Medical University, Chongqing, China

³Department of Ophthalmology, Peking Union Medical College Hospital, Beijing, China

Abstract

PURPOSE—To evaluate the stromal interface quality after femtosecond laser full lamellar cuts in Gamma-irradiated corneas (VisionGraft Sterile Cornea) and determine the limits of cut depth using VisionGraft as donor corneas for laser-assisted lamellar anterior keratoplasty.

METHODS—Fourteen VisionGraft corneas underwent full lamellar cuts using femtosecond laser. The percent cut depth was 17-21% (100μm, n=2), 31-35% (n=3), 38-40% (n=3), 45-48% (n=3) and 50% (n=3) of total stromal thickness (not including epithelium). The cap and stromal bed surfaces were imaged with scanning electron microscope (SEM). The quality of cut surfaces was graded by 2 masked observers based on two indices: ridge and roughness. The ridge grading indicated macroscopic irregularity. The roughness grading indicated microscopic irregularity. The grading was on a subjective integer scale of 1-5 (1=best, 5=worst), which was used previously in a published study of cut quality in fresh corneas.

RESULTS—The ridge grading ranged from 1.50 for the shallowest cut to 2.25 for the deepest cut and was weakly ($r=0.279$) but significantly ($P=0.037$) correlated with percent cut depth. The roughness grading ranged from 2.63 to 2.56 and bore no trend with percent cut depth ($r=0.006$, $P=0.968$).

CONCLUSION—Compared to previously published results in fresh corneas, where ridge grading exceeded 3 for cuts deeper than 31%, the cut quality was better for VisionGraft. Even at depths up to 48% of total stromal thickness, ridge grading was not worse than shallow cuts. Thus Gamma irradiated corneas could provide smoother interface than fresh eye bank cornea for laser-assisted lamellar anterior keratoplasty.

Correspondence to: David Huang, MD, PhD, 3375 SW Terwilliger Blvd., Portland, OR, USA 97239-4197, davidhuang@alum.mit.edu, (503) 494-5131.

Authors Chenxing Zhang and Liang Liu are co-first authors who contributed equally to this manuscript.

Proprietary Interests: David Huang has a significant financial interest in Carl Zeiss Meditec, Inc. 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. These potential conflicts of interest have been reviewed and managed by OHSU. The authors have no financial interest in VisionGraft, Tissue Bank International, iFS femtosecond laser, or AMO.

Keywords

Femtosecond laser; scanning electron microscope; VisionGraft; anterior lamellar keratoplasty

INTRODUCTION

The femtosecond laser is widely used to perform penetrating keratoplasty.¹ However, the irregular stromal interface created by deep lamellar cuts limit the application of femtosecond laser in lamellar keratoplasty² because it negatively affects contrast sensitivity and visual performance.³ To improve the interface quality of deep femtosecond laser lamellar cuts, several studies have tried to optimize the laser settings.⁴⁻⁶ However, optimizing the laser settings alone was not enough to produce sufficiently smooth stromal interface.^{2, 7} Our previous study⁸ suggested that, for fresh cadaver corneas, the interface quality after femtosecond laser cuts was strongly correlated with percent cut depth. Acceptable interface quality could be achieved if the cut depth was less than 31% of total stromal thickness, which was usually less than 200µm. This depth may not be adequate for procedures such as deep anterior keratoplasty.

To improve the depth limit of femtosecond laser lamellar cuts, we experimented with Gamma-irradiated cornea (VisionGraft sterile cornea; Tissue Banks International, Baltimore, MD). We hypothesized because of the increased interlamellar cohesion after Gamma-irradiation might result in less ridges on the interface than those on fresh corneas.⁸ In addition, VisionGraft sterile cornea is considered suitable for procedures that do not require viable corneal endothelium such as anterior lamellar keratoplasty.⁹

There has been no study demonstrating the feasibility of using VisionGraft corneas in deep anterior keratoplasty. Therefore, as a first step to establish feasibility, we evaluated in this study the effect of femtosecond laser lamellar cut depth on the stromal interface quality of VisionGraft corneas in a series of laboratory experiments. This information was used to determine the depth limit that could produce acceptable interface quality on VisionGraft corneas in femtosecond laser-assisted lamellar anterior keratoplasty.

MATERIALS AND METHODS

Preparation of VisionGraft Sterile Corneas

VisionGraft sterile corneas with complete scleral rims were used in this study. Femtosecond laser cuts were performed at Casey Eye Institute (Portland, OR, USA). The VisionGraft corneas were mounted on Barron artificial anterior chambers (Katena Products, Inc., Denville, NJ, USA) filled with albumin through a side port. Central stromal thickness was measured by an 830 nm wavelength Fourier-domain OCT system (RTVue, Optovue, Inc., Fremont, CA, USA). Saline bottles on an intravenous pole were used to maintain intraocular pressure at 65 mmHg during applanation. Once the proper applanation area was obtained, the fluid line was clamped off and the applanation cone lowered further until adequate pressure was obtained for the laser cut. After femtosecond laser cut (Intralase iFS 150 kHz, Abbot Medical Optics, Inc., Santa Ana, CA, USA), the cap was then lifted from the stromal

bed using a Seibel LASIK flap lifter. Digital photographs were taken through the operating microscope oculars. The stromal bed (corneo-scleral disc) was dismantled from the artificial anterior chambers. Both cap and bed were immediately immersed in Karnovsky fixative (2% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% calcium chloride and 0.1 M cacodylate buffer) and fixed at 4°C overnight in preparation for SEM processing.

Femtosecond Laser Settings

The femtosecond laser was used to make side cuts (energy 2.4 μJ , spot and line separations 4 and 4 μm) and full lamellar cuts at different depths (energy 0.7 μJ , spot and line separations 6 and 7 μm). The side-cut angle was 135° to produce a beveled edge (Fig. 1). The lamellar cut depth was set as a percentage of the central stromal thickness on VisionGraft. The specimens were divided into groups based on percent cut depth, rather than the micron depth, because the former has stronger correlation with interface quality according to our previous study on fresh corneas.⁸

Optical Coherence Tomography

A Fourier-domain OCT system was used to obtain cross-sectional images. The system had a transverse scan width of 6 mm, an axial resolution of 5 μm , and a speed of 26,000 axial scans per second. Before femtosecond laser cuts, pachymetry scans were performed. The central stromal thickness was used as the denominator in calculating the percent depth of the laser cut. Pachymetry scans were performed again after the femtosecond laser cut.

Scanning Electron Microscopy

After fixation, the corneal cap and bed specimens were rinsed in 1% osmium tetroxide 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 Technics Ltd., Peabody, MA, USA).

Interface Quality Grading

The SEM images of both cap and stromal bed surfaces were graded for ridges and roughness. A subjective 5-point integer scale (1=best, 5=worst) was used to evaluate the surface quality. The ridge grading was based on 27× SEM images that reflected the macroscopic surface quality (Fig. 2A). The roughness grading was based on 100× SEM images at four locations that reflected the microscopic surface quality (Fig. 2B, 2C). The set of SEM images used to anchor the 5-point grading scale (Fig. 3A-3D) was the same as the one used in the previous study on fresh cadaver corneas⁸ to make the gradings comparable. The SEM images were presented in random order to two masked observers who were familiar with electron microscopy. The scores for the cap and bed surfaces were averaged to obtain one interface score for each sample. Spearman test was used to determine correlation between cut depths and ridge/roughness scores. The Mann–Whitney U test was used to compare the difference of each group. The Wilcoxon signed-rank test was utilized to compare the differences of ridge/roughness scores between cap and stromal bed.

RESULTS

Fourteen VisionGraft sterile corneas were included in the study. The lamellar cut depths ranged from 100-302 μ m or 17-50% of total stromal thickness (Table 1). The specimens are divided into 5 groups based on percent depth: 17-21% (100 μ m, n=2), 31-35% (n=3), 38-40% (n=3), 45-48% (n=3) and 50% (n=3).

Photographs and OCT images provided assessment of stromal bed quality immediately after laser cutting and graft lifting. However, quantitative grading was not attempted because only gross ridges could be appreciated in these images. In photographs (Fig. 4B, 4D) and OCT images (Fig. 4A, 4C), the setting of 100 μ m provided the smooth interface without any perceivable ridges. Meanwhile, the depth setting at 50% produced slightly more ridges.

Masked grading was performed based on SEM images. The ridge scores were weakly correlated with the percent cut depth, ($r=0.279$, $P=0.037$). The 17-21% group had the fewest ridges (Fig. 5A). However, the ridge scores had no significant ($p>0.05$) differences between all the various depth settings. The average ridge scores of cut depth less than 48% of total stromal thickness was all within the range of 1 ~ 2.

The roughness scores were not correlated with the percent cut depth ($r=0.006$, $P=0.968$). There was no significant difference ($p>0.05$) between various depth settings (Fig. 5B).

The average ridge score for the bed surfaces (2.4 ± 0.6) was higher than that for the cap interfaces (1.4 ± 0.6 , $P = 0.001$). The average roughness score was also higher for the bed and (3.0 ± 0.8) than that for the cap interfaces (2.4 ± 0.5 , $P = 0.025$).

DISCUSSION

Femtosecond laser has been used extensively in penetrating keratoplasty for its precise creation of complex donor and host wound edges configurations.^{10, 11} It is also desirable for both anterior and posterior lamellar keratoplasty because of its precise edge configuration, accurate depth cut control and good predictability. However, the application of femtosecond laser in lamellar keratoplasty has been limited by the irregularity interface caused by deep femtosecond laser lamellar cuts. The most common irregularity was the concentric macroscopic striae on the cut surface.^{2, 12} A smooth interface is important to obtain good visual outcome, as evidenced by the advantage of big bubble dissection over manual dissection in deep anterior lamellar keratoplasty.¹³ To improve interface quality, different research groups have optimized laser settings by using lower pulse energy,⁵ tighter spacing and raster pattern⁶, or by choosing a high-frequency laser.⁴ These approaches had only limited success. Deeper lamellar cuts could still create ridges that may interfere with visual outcome.^{2, 7} Currently, the compromised approach was using femtosecond laser to make side cuts, and then using big-bubble technique,¹⁴⁻¹⁶ or the excimer laser⁷ to prepare the lamellar bed. Our previous laboratory observation on fresh cadaver corneas indicated that the interface ridge strongly correlated with cut depth. The deepest lamellar cut which could produce acceptable interface quality was about 31% of total stromal thickness.⁸ This usually translated to less than 200 μ m cut depth, which was not enough for procedures such as deep anterior lamellar keratoplasty. We hypothesized that smooth deeper cuts could be obtained

in Gamma-irradiated corneas like VisionGraft because of the increased interlamellar cohesion after Gamma-irradiation and preservation in high concentration protein solution. Therefore, in this study, we performed femtosecond laser lamellar cuts over a range of depth on VisionGraft corneas in order to determine the depth limit which could create interfaces without excessive ridges. A greater depth limit could potentially expand the application of femtosecond laser in anterior lamellar keratoplasty.

Gamma irradiation is a validated technique for sterilizing many donor tissue against bacteria, fungal tissues and viruses.¹⁷ The benefits for the usage of Gamma irradiation on corneal tissue include longer preservation life more than 1 year, lower risk of infectious disease transmission⁹, and lower costs¹⁸, which could be helpful in tackling the shortage of eye bank corneas in developing countries. Previous studies have shown that VisionGraft sterile corneas have good biocompatibility for corneal procedure and could be considered to be substitute for fresh donor corneas in lamellar keratoplasty.^{9, 19}

In this study, as the first step to establish the feasibility of using VisionGraft corneas in deep anterior keratoplasty, we evaluated the stromal interface quality after femtosecond laser lamellar cut at different depth. We used the same set of anchoring images for grading as the previous study on fresh cadaver corneas⁸ to make the gradings comparable between the two studies. For shallow cuts less than 31% of total stromal thickness, the ridge gradings were similar between VisionGraft corneas (ridge scores ranged 1.50 to 1.67) and fresh corneas (ridge scores ranged 1.25 to 2.15)⁸. However, for cuts deeper than 31%, VisionGraft corneas clearly have less ridges (ridge scores ranged 1.92 to 2.25) than fresh corneas (ridge scores ranged 3.30 to 3.83)⁸. It has been suggested that in fresh corneas, the anterior corneal lamellae have more bridging fibers between them that increase shear strength, and these are relatively lacking in the posterior stroma,^{20, 21} the less compactly arranged posterior stromal fiber wrinkled by external pressure such as the action of applanation lens might possibly be one of reasons for ridges on fresh cadaver corneas. We speculated the stronger interlamellar cohesion of VisionGraft corneas could decrease the stromal fiber wrinkling induced by applanation and lead to less ridges on the interface compared with those on fresh corneas.⁸

Meanwhile, we did not find any systematic correlation between cut depth and microscopic roughness. No significant difference has been found between all depth settings. This finding is similar to that for fresh cadaver corneas⁸. It is likely that the microscopic smoothness of deeper lamellar cuts is also compatible with good visual outcome. The bed surface seemed to always have more ridges and roughnesses than the interface of its cap after femtosecond laser lamellar cut. We speculate that the ridges are produced by the laser cut jumping across corneal lamellar planes. This may produce lamellar edges that retracts on the bed due to limbal anchoring and the relatively sparse interlamellar crossing fibers in the posterior stroma. The retracted edges exaggerates the ridges and microscopic roughness under SEM. The cap is not anchored to the limbus (not under radial tension) and the anterior stroma has denser interlamellar crossing fibers. Therefore the ridges are less apparent in SEM of the cap. However, if the lamellar steps are too obvious in the bed they must exist in the cap as well, and could very well affect the graft interface.

In conclusion, smooth stromal interfaces could be obtained after cut depth up to 48% of total stromal thickness using VisionGraft sterile corneas, which is much deeper than that using fresh cadaver cornea.⁸ This and the additional benefits long shelf-life of VisionGraft may potentially expand the application of laser-assisted lamellar anterior keratoplasty to wider range of cornea diseases including keratoconus and corneal opacities.

Acknowledgments

This study was supported by NIH grant R01 EY018184, a grant from Optovue, Inc., a grant from Research to Prevent Blindness and Chinese NSFC grant 81100688.

REFERENCES

1. Baradaran-Rafii A, Eslani M. Femtosecond laser-assisted corneal transplantation. *Br J Ophthalmol*. 2013; 97(6):675–6. [PubMed: 23137665]
2. Soong HK, Mian S, Abbasi O, Juhasz T. Femtosecond laser-assisted posterior lamellar keratoplasty: initial studies of surgical technique in eye bank eyes. *Ophthalmology*. 2005; 112(1):44–9. [PubMed: 15629819]
3. Reinhart WJ, Musch DC, Jacobs DS, et al. Deep anterior lamellar keratoplasty as an alternative to penetrating keratoplasty a report by the american academy of ophthalmology. *Ophthalmology*. 2011; 118(1):209–18. [PubMed: 21199711]
4. Ziebarth NM, Dias J, Hurmeric V, et al. Quality of corneal lamellar cuts quantified using atomic force microscopy. *J Cataract Refract Surg*. 2013; 39(1):110–7. [PubMed: 23141078]
5. Lombardo M, De Santo MP, Lombardo G, et al. Surface quality of femtosecond dissected posterior human corneal stroma investigated with atomic force microscopy. *Cornea*. 2012; 31(12):1369–75. [PubMed: 22262224]
6. Sarayba MA, Maguen E, Salz J, et al. Femtosecond laser keratome creation of partial thickness donor corneal buttons for lamellar keratoplasty. *J Refract Surg*. 2007; 23(1):58–65. [PubMed: 17269245]
7. Cleary C, Song JC, Tang M, et al. Dual laser-assisted lamellar anterior keratoplasty with top hat graft: a laboratory study. *Cornea*. 2012; 31(7):791–7. [PubMed: 22378114]
8. Zhang CBM, Tang M, Li Y, Huang D. Laboratory Evaluation of Interface Quality of Different Corneal Lamellar Cut Depth for Femtosecond Laser-Assisted Lamellar Anterior Keratoplasty. *J Cataract Refract Surg*. 2015 In press.
9. Daoud YJ, Smith R, Smith T, et al. The intraoperative impression and postoperative outcomes of gamma-irradiated corneas in corneal and glaucoma patch surgery. *Cornea*. 2011; 30(12):1387–91. [PubMed: 21993467]
10. Farid M, Kim M, Steinert RF. Results of penetrating keratoplasty performed with a femtosecond laser zigzag incision initial report. *Ophthalmology*. 2007; 114(12):2208–12. [PubMed: 18054639]
11. Bahar I, Kaiserman I, Lange AP, et al. Femtosecond laser versus manual dissection for top hat penetrating keratoplasty. *Br J Ophthalmol*. 2009; 93(1):73–8. [PubMed: 18927225]
12. Vetter JM, Holtz C, Vossmerbaeumer U, Pfeiffer N. Irregularity of the posterior corneal surface during applanation using a curved femtosecond laser interface and microkeratome cutting head. *J Refract Surg*. 2012; 28(3):209–14. [PubMed: 22373034]
13. Borderie VM, Sandali O, Bullet J, et al. Long-term results of deep anterior lamellar versus penetrating keratoplasty. *Ophthalmology*. 2012; 119(2):249–55. [PubMed: 22054997]
14. Buzzonetti L, Petrocelli G, Valente P. Femtosecond laser and big-bubble deep anterior lamellar keratoplasty: a new chance. *J Ophthalmol*. 2012; 2012:264590. [PubMed: 22496960]
15. Farid M, Steinert RF. Deep anterior lamellar keratoplasty performed with the femtosecond laser zigzag incision for the treatment of stromal corneal pathology and ectatic disease. *J Cataract Refract Surg*. 2009; 35(5):809–13. [PubMed: 19393878]

16. Price FW Jr, Price MO, Grandin JC, Kwon R. Deep anterior lamellar keratoplasty with femtosecond-laser zigzag incisions. *J Cataract Refract Surg.* 2009; 35(5):804–8. [PubMed: 19393877]
17. Bright, RWSJ.; Gambill, VM. Sterilization of human bone by irradiation. Little, Brown and Co.; Boston, MA: 1983.
18. Akpek EK, Aldave AJ, Aquavella JV. The use of precut, gamma-irradiated corneal lenticules in Boston type 1 keratoprosthesis implantation. *Am J Ophthalmol.* 2012; 154(3):495–8. e1. [PubMed: 22633353]
19. Utine CA, Tzu JH, Akpek EK. Lamellar keratoplasty using gamma-irradiated corneal lenticules. *Am J Ophthalmol.* 2011; 151(1):170–4. e1. [PubMed: 21145036]
20. Aptel F, Olivier N, Deniset-Besseau A, et al. Multimodal nonlinear imaging of the human cornea. *Invest Ophthalmol Vis Sci.* 2010; 51(5):2459–65. [PubMed: 20071677]
21. Bergmanson JP, Horne J, Doughty MJ, et al. Assessment of the number of lamellae in the central region of the normal human corneal stroma at the resolution of the transmission electron microscope. *Eye Contact Lens.* 2005; 31(6):281–7. [PubMed: 16284509]

Figure 1.

The profile for an inverted side cut-shaped femtosecond laser lamellar cut. D, graft lamellar cut depth in μm ; .Fs, femtosecond.

Figure 2.

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

Figure 3.

Set of SEM images used to anchor the 5-point grading scale. This set of SEM images was the same as the one used in the previous study on fresh cadaver corneas (Zhang C, Bald M, Tang M, Li Y, Huang D. Laboratory Evaluation of Interface Quality of Different Corneal Lamellar Cut Depth for Femtosecond Laser-Assisted Lamellar Anterior Keratoplasty. J Cataract Refract Surg 2015: In press) to make the gradings comparable. (A) A stromal bed surface cut with femtosecond laser at 100 μ m showed minimum ridges. (B) A stromal bed surface of a deep cut had the most ridges and was used to define grade 5 ridges. (C) A stromal bed surface cut at 100 μ m had a very smooth surface and was used to define the grade 1 roughness score. (D) A stromal bed surface (100x) of a deep cut showed the most roughness and was used to define the grade 5 roughness.

Figure 4.

OCT images (A, C) of the cornea after femtosecond laser cuts but before cap lifting. Photographs (B, D) of the stromal bed after femtosecond laser cuts and cap lifting. The lamellar cut depth was 100 μm for A and B. The cut depth was 50% of total stromal thickness (302 μm) for C and D. In all images, no obvious interface irregularities were present.

Figure 5.

Ridge grade (A) and roughness grade (B) at different depths after femtosecond laser lamellar cut on Gamma-irradiated corneas. 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.

Table1

Central corneal thickness and femtosecond laser lamellar cut depth for each specimen.

Specimen	Central stromal thickness (μm)	Lamellar cut depth (μm)	Percent depth *
1	574	100	17%
2	595	100	17%
3	592	184	31%
4	629	195	31%
5	574	201	35%
6	558	212	38%
7	635	254	40%
8	540	216	40%
9	633	285	45%
10	614	276	45%
11	578	277	48%
12	604	302	50%
13	520	260	50%
14	588	294	50%

* Percent depth is ratio between laser lamellar cut depth and central stromal thickness.