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Surface Quality of Porcine Corneal Lenticules after Femtosecond Lenticule Extraction

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Key Words
Lenticule extraction · Porcine corneal lenticules · Femtosecond laser

Abstract
Purpose: To determine the surface characteristics of porcine corneal lenticules after Femtosecond Lenticule Extraction. ***Methods:*** The Carl Zeiss Meditec AG VisuMax® femtosecond laser system was used to create refractive corneal lenticules on 10 freshly isolated porcine eyes. The surface regularity on the corneal lenticules recovered was evaluated by assessing scanning electron microscopy images using an established scoring system. ***Results:*** All specimens yielded comparable score results of 5–7 points (SD = 0.59) per lenticule (score range minimum 4 to maximum 11 points). Surface irregularities were caused by tissue bridges, cavitation bubbles or scratches. ***Conclusion:*** The Femtosecond Lenticule Extraction procedure is capable of creating corneal lenticules of predictable surface quality. However, future studies should focus on the optimization of laser parameters as well as surgical technique to improve the regularity of the corneal stromal bed.

Introduction

The rapid development of new laser in situ keratomileusis (LASIK) flap creation techniques requires continuous quality management of the operating mode, precision and safety of these procedures. In addition to flap cut complications, attention should be paid to the laser cutting quality because it has been assumed that smoother optical interfaces are associated with better vision quality.

Complications associated with repeated refractive surgical treatments and cutting techniques have major economic relevance [1]. New technologies such as femtosecond laser systems seem to offer a promising alternative to mechanical microkeratomes. Initial clinical comparisons of femtosecond laser versus microkeratome cuts give evidence for the superiority of the laser technique [2–7].

Femtosecond lasers can create 3-dimensional cuts [8] permitting the surgeon to design different shapes within the cornea. As an example, the relatively new procedure to correct myopia has been termed Femtosecond Lenticule Extraction [9]. In this procedure, the flap and the refractive lenticule are created in a 1-step procedure using

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Table 1. Criteria for evaluating cut surface characteristics

	Criterion and magnification	Appearance	Score
A	Surface relief, ×10	No visible roughness	2
		Roughness visible	1
B	Regularity of surface structure, ×50	Almost completely maintained	3
		Partially maintained	2
		Not maintained	1
C	Portion of surface irregular, ×50	<10% of cut surface	3
		10–20% of cut surface	2
		>25% of cut surface	1
D	Position of the irregular area, ×50	No irregularities	3
		Peripheral only	2
		All over	1

a femtosecond laser. Early peer-reviewed publications [9–11] as well as initial clinical reports [12] stimulated interest in this new technology because it allowed a refractive change without the use of an excimer laser.

The aim of the study was to investigate the surface quality of porcine corneal lenticules which were extracted during the Femtosecond Lenticule Extraction procedure. A scoring system established by Wilhelm et al. [13] was used to analyze the regularity of the cutting surface.

Materials and Methods

Freshly isolated porcine eyes (n = 10) were used in this study. They were kept in a wet chamber at +4°C and used within 5 h of animal death. Prior to surgery, the eyes were held at room temperature for 2 h to allow deswelling of the corneal stroma.

Laser System and Surgical Procedure

The Carl Zeiss Meditec AG VisuMax® femtosecond laser system was used for the Femtosecond Lenticule Extraction [9–12]. Both a flap and a lenticule of intrastromal corneal tissue were sequentially cut after which the lenticule was manually removed and the flap repositioned.

The laser produced ultrashort pulses of light at a repetition rate of 200 kHz. The energy level was set at 185 nJ, focused at a precise depth in the corneal tissue. The laser energy settings were the same for all eyes.

A plasma state develops with optical breakdown, and small gas bubbles are formed from the vaporization of tissue. A dense layer of bubbles is created in a spiral fashion with a spot distance of 3 × 3 μm resulting in the cleaving of tissue planes. Thus, a cohesive cut surface is generated.

Four subsequent femtosecond incisions were performed to create the corneal lenticule: (1) the posterior surface of the refractive lenticule; (2) the lenticule border; (3) the anterior surface of the refractive lenticule which was extended centripetally as a flap, and (4) a side cut to make the flap opening. The flap was opened and reflected surgically using an iris spatula (G-16193, Geuder, Heidelberg, Germany). The refractive lenticule was subsequently grasped with forceps and extracted.

The diameter of the flap was set at 8.0 mm and that of the lenticule at 7.5 mm. Intended flap thickness was 160 μm. A 120-degree side cut angle was chosen for both, the flap and the lenticule. Lenticule thickness varied from 54 to 142 μm (average 93 μm). However, the front of the lenticules, which lies right beneath the 160-μm flap, was examined.

Tissue Preparation and Scanning Electron Microscopy

All specimens were coprocessed at the same time. For scanning electron microscopy (SEM), lenticules were smoothly placed on a filter paper, to prevent curling of the tissue, and immersed in 3% glutaraldehyde, 100 mM HEPES (pH 7.4), 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 25 mM NaN_3 at room temperature for 2 h, and then at 4°C overnight. The samples were treated with 2% tannic acid for 1 h, 2% osmium tetroxide for 2 h and with 2% uranyl acetate for 1 h with washing steps in between. The samples were dehydrated in a graded series of aqueous ethanol solution (20–100%) and then critical-point dried by amylacetate and CO_2. Finally, the samples were mounted on aluminum stubs, sputtered with gold and examined in a DSM 940A (Zeiss, Oberkochen, Germany). The description of the morphology of the lenticules as well as the evaluation of their surface quality were carried out on the basis of the electron micrographs.

Surface Regularity Index

For evaluation of the surface morphology of the corneal lenticules, an established scoring system [14, 15] was applied (table 1). The transformation of qualitative to quantitative information was carried out on the basis of 4 selective criteria. In addition to the surface relief and the regularity of the surface structure, the score evaluated the extent of surface irregularities as well as the position of the irregular area.

Characteristic features of the cut morphology were first described, and the scoring system was then applied. A maximum of 11 points could be assigned to each lenticule resulting in a possible number of 110 points for all specimens. All criteria are characterized in table 1. Irregularities were scored at 10-fold and 50-fold magnification. For the evaluation of the entire lenticule surface (criterion A) the 10-fold magnification was used. All other criteria were analyzed at 50-fold magnification.

As the criteria are based on subjective measures, two observers graded the findings in a blinded fashion. Wilhelm et al. [13] did not describe in detail what they meant by rough or irregular. Therefore, we studied their specimens to be able to assess ours in the same manner.

Results

The quality of the cut surface was mainly affected by surface irregularities. Three types could be differentiated. First, a serrated surface could be due to tissue bridges. Second, ring-like slots of different size, sometimes con-

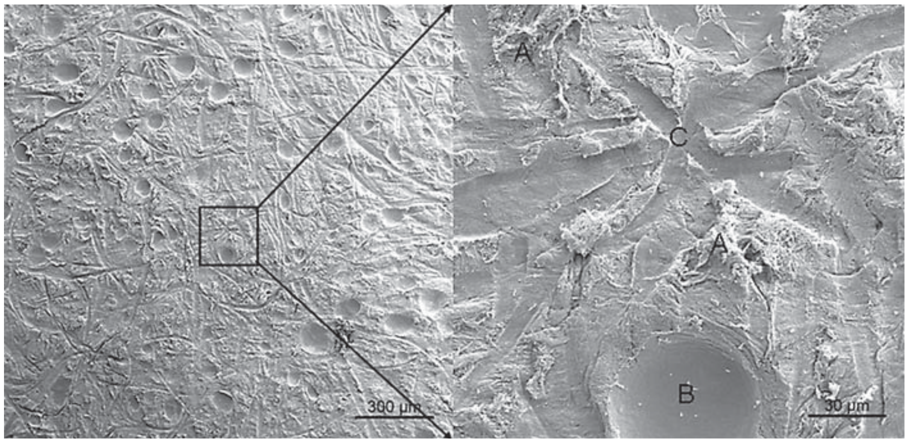

Fig. 1. Demonstration of typical irregularities at a magnification of ×50 (**a**) and at one of ×500 (**b**). A = Tissue bridges; B = cavitation marks; C = groove.

Table 2. Score results for 10 porcine corneal lenticules with average (avg.), standard deviation (SD) and sum (Σ)

Specimen	Surface relief (max. 2)	Regularity of surface (max. 3)	Portion of surface irregular (max. 3)	Position of the irregular area (max. 3)	Score
1	2	2	1	1	6
2	1	2	1	1	5
3	1	2	1	1	5
4	1	3	2	1	7
5	1	2	1	1	5
6	1	2	2	1	6
7	1	2	1	1	5
8	1	2	1	1	5
9	1	2	2	1	6
10	1	3	1	1	6
Avg.	1.1	2.2	1.3	1.0	5.6
SD	0.32	0.42	0.48	0.00	0.59
Σ	11	22	13	10	56

fluent, were found in the stromal bed. Those were related to cavitation bubbles. Third, in some specimens, grooves run criss-cross over the cutting surface. Specimens did not necessarily contain all 3 entities. A representative sample of these irregularities is shown in figure 1.

A summary of the score results is given in table 2. Examination of the lenticules at lower magnification revealed surface roughness in 90% of the samples (criterion A; SD ±0.32). The VisuMax achieved 11 out of the 20 possible points for criterion A. If areas of roughness appeared on the lenticule surface, they always affected the entire surface area. An isolated localization of surface irregularities was not found in any of the evaluated specimens (criterion D; SD ±0).

Despite the presence of irregularities, 80% of the corneal lenticules showed homogeneous areas at least in part (criterion B; SD ±0.42). A total of 22 out of possible 30 points were obtained for criterion B. In 7 out of 10 samples, irregularities affected 10–20% of the lenticule surface. In 30% of the specimens, more than a quarter of the total area was inhomogeneous (criterion C; SD ±0.48).

In total, the 10 lenticules achieved a surface irregularity index of 56 out of 110 potential points. Beyond that, score results were well comparable between the specimens varying from 5 to 7 points per lenticule (SD = 0.59; score range 4–11 points). The regularity index showed only minor deviation among the individual specimens. Figure 2 shows 3 typical scanning electron micrographs for score results of 5, 6 and 7 points, respectively.

Discussion

LASIK is one of the common methods in corneal refractive surgery. The variety of flap creation devices for this procedure is increasing. Numerous studies have compared the mechanical microkeratomes with the developing femtosecond laser technology [2, 4, 5]. Femtosecond lasers offer an attractive alternative because of their variable range of application. Furthermore, there is evidence for higher predictability, better biomechanical flap characteristics and, for this reason, better safety [16].

The Femtosecond Lenticule Extraction is a classical example of the promising modalities that the femtosecond laser technology offers [9–12]. This new procedure is an all-femtolaser technique which no longer requires an excimer laser. The flap creation as well as the refractive correction are performed by the same laser. From a prac-

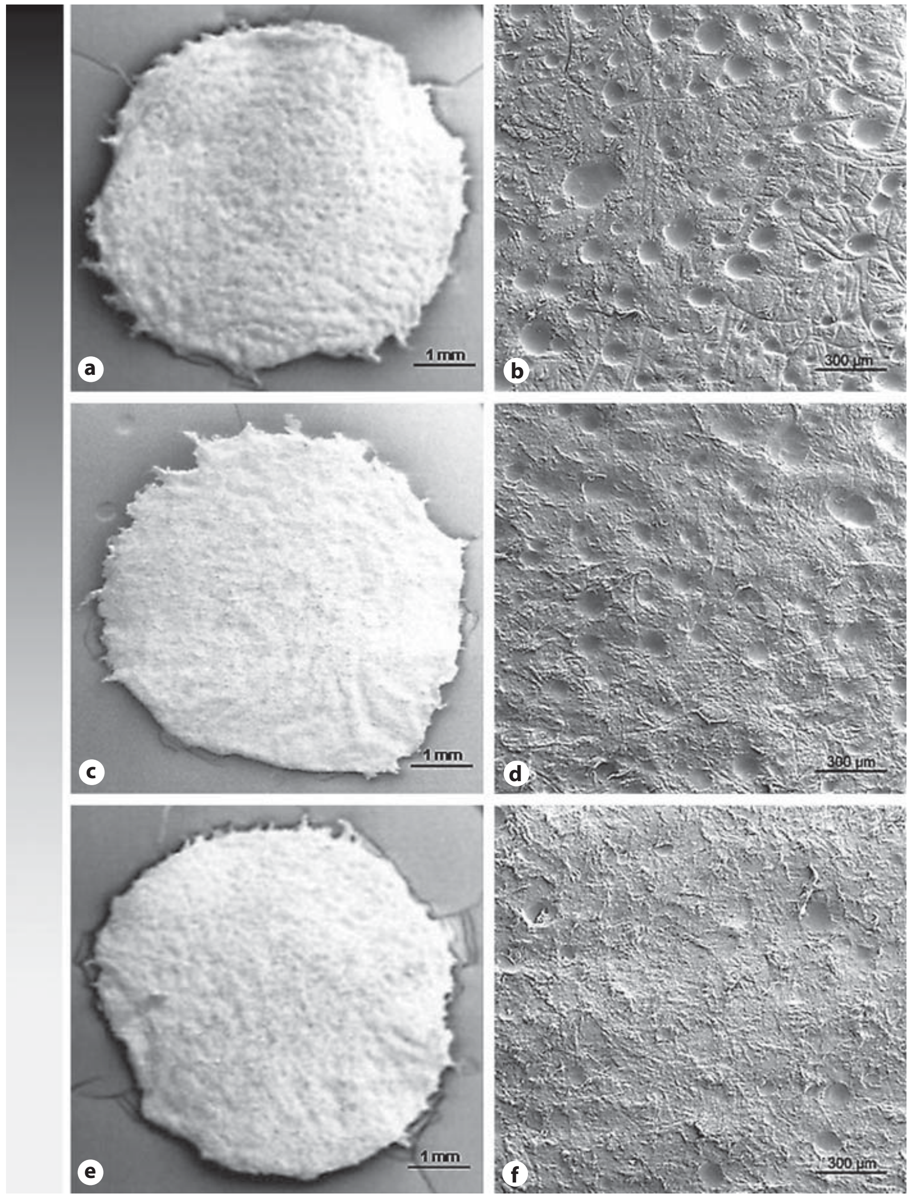

Fig. 2. Representative SEM images of samples with ascending quality. **a**, **c**, **e** Overview (×10). **b**, **d**, **f** Center of lenticule (×50). With examples of the lowest score result (5 points; **a**, **b**), a score result of 6 (**c**, **d**) and the highest score result of 7 points (**e**, **f**).

tical point of view, the use of one laser unit decreased the effort, length and costs of the procedure.

The mechanism of action of the femtosecond laser is based on nonlinear absorption of light and consecutive disruption of the corneal tissue. A plasma state develops with optical breakdown, and small gas bubbles are formed from the vaporization of tissue. A series of bubbles are created resulting in cleaving of tissue planes. Tissue bridges remain when the bubble layer is not continuous [8].

These different morphological features could impact visual and refractive outcomes. For this reason, the surface quality of the refractive corneal lenticules on freshly isolated porcine eyes has been examined in the present paper. Evaluation of the cutting surface was conducted on scanning electron micrographs using an established scoring system [13].

The extracted lenticules showed comparable surface regularity score results. The score only varied between 5 and 7 points among the 10 evaluated corneal lenticules. The theoretical score range was 4–11 points indicating that the lenticules showed only minor deviation among the individual specimens. However, the cut surface of the refractive lenticules appeared slightly rough, indicating average surface quality as analyzed by the score.

Slightly higher surface regularity score results between 5 and 9 points were reached for microkeratome flap cuts [13].

One reason for the surface irregularities seemed to be tissue bridges embedded in areas of cavitation bubbles. This phenomenon could be described as residual fibers between flap and lenticule interface after bubble formation had been completed. The more tissue bridges remain after the laser application, the more surgical manipulation is needed, possibly affecting the surface quality of the interface. Binder [16] described the necessity for manual dissection of residual tissue adhesions when the Intralase femtosecond laser was used for flap creation. Remaining collagen fibers along the cutting surface demanding additional surgical manipulation have also been demonstrated [17].

This emphasizes the importance of gentle surgical dissection. Some potential optical side effects might be induced by surgical manipulation.

Secondly, evaluation of surface morphology revealed some grooves that run criss-cross over the lenticule surface. This phenomenon has not been described yet in previous reports. One could assume that those might be related to surgical manipulation. In cases where extraction of the lenticule was easily achieved, only sporadic stromal tissue bridges remained in the microscopy specimens. On the other hand, artificial grooves were seen in cases of tighter adhesions where surgical dissection was more difficult. The structure of the surface seemed to be considerably affected in those cases. However, the grooves did not influence the surface regularity index score results as much as tissue bridges and cavitation marks. This is due to the fact that grooves only affected parts of the specimens, whereas tissue bridges and cavitation bubbles were present on the entire surface. One could therefore discuss to include the grooves as special criterion of the score.

Third, laser scanning electron micrographs of porcine refractive lenticules showed several crater-like cavities along the cutting surface. These were attributed to gas bubbles and the craters became bigger as single bubbles merged. Kermani and Oberheide [18] also described bubble formations in micromorphological examinations. The correlation between magnitude of bubble formation and energy settings of the femtosecond laser systems requires further investigation. A comparison of commercially available femtosecond lasers in refractive surgery demonstrates that there is less bubble formation in devices with high pulse frequency and low pulse energy [8]. The smaller the bubbles, the more precise and homogeneous is the cut [8]. On the other hand, an advantage of the bubble creation seems to be the easier flap opening, because the bubble layer helps to locate the incision [8]. Moreover, a reduction in spot size makes it easier to elevate the flap [16]. Thus, further studies are needed to systematically assess parameter variation – especially spot size and pulse energy.

Artifacts due to the preparation of the lenticules for SEM should also be taken into account. For SEM, specimens were gently placed on a filter paper using cellulose sponges. Although precautions were taken, such as wetting of the sponges, scratches on the lenticule surface could possibly be generated. Later alterations were not to be expected since specimens were immediately placed into fixative. In previous studies applying the same technique [13–15], the authors did not describe any influence of tissue preparation for SEM on the surface morphology.

The occurrence of tissue irregularities like grooves and tissue tears increased the area of the wound bed. This may result in an augmented wound healing reaction. Other studies have shown that the application of the laser itself presented a trigger for postoperative inflammation. Several studies associated keratocyte cell death to femtosecond laser flap creation, especially when higher energy levels were applied [19, 20]. Histopathological examination of rabbit corneas after femto-LASIK showed a markedly greater tendency toward early postoperative inflammation, including more stromal cell death, greater stromal cell proliferation and greater monocyte influx [19, 20]. The accelerated inflammatory reaction was associated with more central diffuse lamellar keratitis and prominent scar formation at the flap edge. Laser pulse frequency as well as energy level affected the inflammatory reaction. Lower frequency of the femtosecond laser and higher energy level increased the inflammatory response (15 kHz with 2.5 μJ vs. 30 kHz with 0.9 μJ and 60 kHz with 1.1 μJ) [19].

This inflammatory reaction might in part be responsible for side effects unique to the femtosecond laser, such as the transient light sensitivity syndrome in which patients have a delayed-onset photophobia without loss in visual acuity. To date, the exact pathophysiological mechanism remains unclear, though an intensified wound healing reaction in the stromal interface has been discussed as a possible cause. It was postulated that higher laser energies activate keratocytes, which are increased in the wound bed in cases of transient light sensitivity syndrome, and cause delayed recovery. The incidence of transient light sensitivity syndrome decreased significantly after the use of lower energies or higher pulse frequencies in femto-LASIK [21, 22].

Mechanical microkeratomes have been used to create LASIK flaps for over 2 decades. In comparison, femtosecond laser technology for lamellar keratotomy for LASIK is in its early stages compared with classical LASIK, and improvement of cutting properties of the femtosecond lasers should make their advantages over conventional techniques clearer in the future. With the Femtosecond Lenticule Extraction procedure, one is able to create corneal lenticules of predictable surface quality. The score results are comparable to microkeratome cuts although slightly lower. This is partly due to different morphological features such as tissue bridges and grooves, which were not seen after mechanical flap preparation. The surface regularity of tissue cut by the femtosecond laser seems to be further influenced by the laser settings and the surgical dissection. Further experimental and clinical studies are needed to closely investigate this context. Parameters should be looked at with regard to visual outcome and complication rate. To better compare different settings, an objective software-based analysis mode for the surface morphology would be desirable.

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Disclosure Statement

None of the authors have a financial or proprietary interest in any material or method mentioned.

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