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Abstract. Femtosecond near-infrared lasers are widely used for a number of ophthalmic procedures, with flap cutting in the laser-assisted *in situ* keratomileusis (LASIK) surgery being the most frequent one. At the same time, lasers of this type, equipped with harmonic generators, have been shown to deliver enough ultraviolet (UV) power for the second stage of the LASIK procedure, the stromal ablation. However, the speed of the ablation reported so far was well below the currently accepted standards. Our purpose was to perform high-speed photorefractive keratectomy (PRK) with femtosecond UV pulses in rabbits and to evaluate its predictability, reproducibility and healing response. The laser source delivered femtosecond 206 nm pulses with a repetition rate of 50 kHz and an average power of 400 mW. Transepithelial PRK was performed using two different ablation protocols, to a total depth of 110 and 150 μm. The surface temperature was monitored during ablation; haze dynamics and histological samples were evaluated to assess outcomes of the PRK procedure. For comparison, analogous excimer ablation was performed. Increase of the ablation speed up to 1.6 s/diopter for a 6 mm optical zone using femtosecond UV pulses did not significantly impact the healing process. [DOI: 10.1117/1.JBO.20.5.051037]

Keywords: femtosecond laser; laser-assisted *in situ* keratomileusis; photorefractive keratectomy; corneal ablation; transepithelial ablation.

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1 Introduction

Since the first appearance of near infrared (NIR) femtosecond lasers in refractive surgery as a tool for flap creation,[1] the number of applications for these lasers in ophthalmic surgery has grown significantly. It is now possible to complete at least myopic correction using only the femtosecond laser,[2,3] although this procedure still has not made pulsed ultraviolet (UV) lasers obsolete. The excimer laser has been the laser source of choice for photorefractive keratectomy (PRK) since the introduction of the procedure;[4,5] later on, it also took a choice position in laser-assisted *in situ* keratomileusis (LASIK)-type surgeries. Excimer laser-based systems evolved from high energy, low repetition rate lasers that covered a large area of the cornea in a single shot into ~1000 Hz repetition rate flying spot machines with sophisticated scanning patterns and fast eye tracking systems.[6–9] Most practical disadvantages of the excimer lasers (intense maintenance required, toxic gases used as gain medium, relatively low stability of output, poor beam quality) have been successfully overcome or at least became manageable by the use of sophisticated engineering solutions and application protocols.[10,11] The clinical outcomes of UV systems for corneal ablation based upon nanosecond solid-state lasers with harmonic generators[12,13] have been demonstrated to be equivalent to those of excimer lasers.[14] However, the inherent advantages of solid-state lasers like better shot-to-shot stability did not prove to be decisive in gaining a considerable market share. This situation could change if solid-state technology would allow for significant integration of the equipment used for both stages of the LASIK treatment, thus reducing the cost of the systems.[15,16] In this regard, the use of high power femtosecond lasers capable of producing substantial UV power via harmonic generation appears a straightforward solution.

In our previous work, we have shown that UV pulses produced as the fifth harmonic of a femtosecond Ytterbium doped laser enable highly accurate and predictable ablation on model materials, as well as on the corneas of enucleated porcine eyes.[17] The ablation threshold fluence with femtosecond pulses was similar to that reported for excimer lasers, which indicated that absorption was dominated by linear single photon process.[17] Our experiments, in which rabbit corneas were treated *in vivo* with femtosecond UV pulses, did not show any adverse effects that could be induced specifically by high peak intensity, and the healing responses were similar to those after excimer ablation.[18] To be able to compare the results of ablation due to the two lasers, we adjusted the average power of our femtosecond UV source to that of the excimer laser we had at our disposal. As a result, the ablation was relatively slow, at 3.7 s/diopter (s/D), which is well below the industry standards currently accepted. A faster ablation speed is considered an advantage due to several factors. First, it has been proven that corneal hydration affects the excimer laser ablation rate.[19] Longer exposure of the ablated surface can enhance the dehydration of

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the corneal stroma, resulting in less predictable refractive outcomes. A shorter duration of the procedure is more comfortable for patients and surgeons, and the risk of undesired eye movements is smaller.[6] Sufficient ablation speed is also a requirement for the transepithelial photorefractive keratectomy ablation (TransPRK), an advanced "no-touch" surface ablation technique.[20–22]

The aim of the present study was to perform high-speed corneal ablation on rabbit corneas using high average power (400 mW) femtosecond UV pulses at a 50 kHz repetition rate to investigate the predictability and reproducibility of ablation of both the epithelium and stroma and to evaluate the healing responses. The transepithelial version of PRK was chosen for several reasons. First, variations in the eye treatment prior to ablation, such as mechanical stress, dehydration, and the shape of the area from which the epithelium was removed, can be avoided. Second, the chances of contamination in the cornea and remaining epithelium could be significantly reduced. Third, this type of procedure requires a large amount of tissue to be removed, thus extending the UV exposure time and facilitating the detection of potential problems (e.g., surface roughness, tissue damage due to heating).

2 Materials and Methods

2.1 *Femtosecond Laser System*

To increase the ablation rate of the corneal stroma, we have modified the laser system that was previously described in detail.[17,18] First, the laser was switched to the standard pulse duration mode (280 ± 10 fs pulses instead of 200 ± 10 fs in the short pulse mode), which is more power efficient. This alteration enabled us to increase the average power of the fundamental laser radiation (1030 nm) up to 5 W at a 50 kHz pulse repetition rate, which in turn resulted in an average UV power up to 400 mW. Second, higher-speed galvanometer scanners (hurrySCAN II7, Scanlab AG) were installed to reduce the time intervals when the laser is switched off during acceleration. Third, the UV pulse generator was equipped with a beam shaper to achieve a near second order super-Gaussian beam profile at the corneal surface (Fig. 1). The beam diameter at the $1/e^2$ intensity level was estimated to be 115 ± 5 μm, which is in good agreement with the 113 ± 10 μm obtained using the knife-edge method.[23] Assuming the super-Gaussian intensity distribution, the peak fluence with these laser settings was 135 ± 5 mJ/cm^2, which is close to the value we used in our previous experiments on rabbits.[18]

We also modified the scanning pattern to maintain low local heating and to achieve a smooth ablation surface at a higher pulse repetition rate. The tissue was removed by ablating circular layers, each of which consisted of five sublayers. The laser beam was scanned in a raster pattern with the distance between adjacent raster lines in a sublayer of 275 μm and the distance between adjacent spots in the raster line of 55 μm. Each of the following sublayers was displaced by 55 μm in the direction perpendicular to the raster line. The layers were rotated with respect to each other (Fig. 2). This arrangement ensured efficient cooling due to heat migration into the depth of the cornea and in the direction perpendicular to the raster line while minimizing the number of lost laser pulses. The myopic ablation of stroma was realized as a stack of ablation layers with varying diameter. In order to minimize the heating of the central part of the cornea, the ablation layer with the smallest diameter was followed by one with the largest diameter, and so on, until the process was completed by two layers of approximately equal diameter.

2.2 *Animals and Surgery*

Approval for this study was obtained from the Lithuanian State Food and Veterinary Service (number 0213, issued 2011.02.12). Thirty-three pigmented rabbits aged 3 to 6 months and weighing 1.3 to 3.2 kg were included in the study. Anesthesia was achieved by an intramuscular injection of ketamine hydrochloride (Bioketan, Vetoquinol) (35 mg/kg) and xylazine hydrochloride (Xylazine 2%, Alfasan) (5 mg/kg). After the application of a sterile drape, one drop of topical anesthetic proxymetacaine hydrochloride 5 mg/ml solution (Alcaine, Alcon) was applied, and the eye was immobilized using a vacuum suction ring. Before ablation, another drop of topical anesthetic was administered. At the end of the surgery, the corneas were flushed with a balanced salt solution (BSS) (Distra–Sol, OphconD.Roesee.K.) and dried with a sterile sponge. Dexamethasone/chloramphenicol ointment (OftanDexa-Chlora, Santen) was applied, and a sterile bandage was placed for several hours. During the postoperative period,

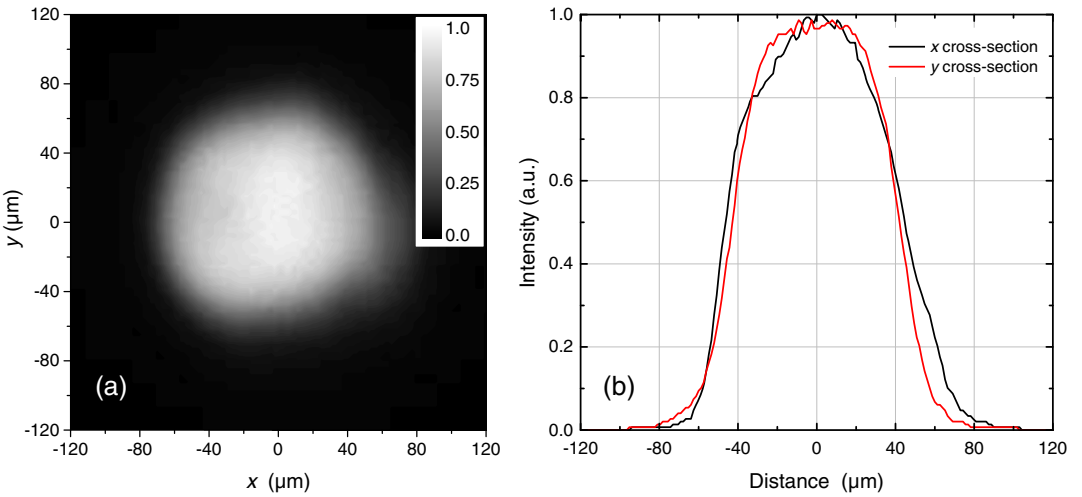

Fig. 1 (a) Image of the UV beam at the ablation plane and (b) intensity distributions along the *x* and *y* axes. The CCD camera recorded the fluorescence of a sapphire plate positioned at the ablation plane.

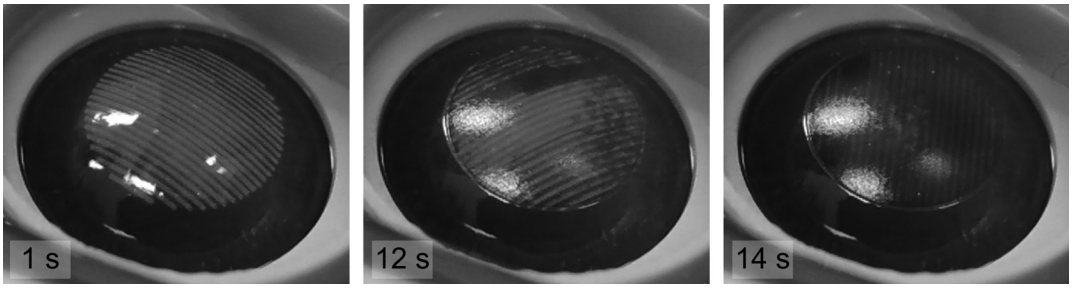

Fig. 2 The nonstop transepithelial femtosecond ablation procedure. Figure shows the progress of the disappearance of the blue fluorescence as the ablation progresses from the epithelium to the stroma (Video 1 QuickTime, 11.7 MB) [URI: http://dx.doi.org/10.1117/1.JBO.20.5.051037.1].

dexamethasone/chloramphenicol drops (OftanDexa-Chlora, Santen) were applied four times per day for 5 days.

The study involved calibration and three treatment modalities: nonstop TransPRK with femtosecond UV pulses, ablation with excimer pulses, and the modified TransPRK with femtosecond UV pulses.

2.2.1 *Calibration*

To determine the amount of removed tissue per layer as well as the number of layers required to completely remove the corneal epithelium, myopic TransPRK of 190 scanning layers was performed in two eyes of two rabbits. We were not able to accurately measure the ablation speed of the corneal epithelium and corneal stroma separately because we had no ability to measure the thickness of the epithelia of the individual corneas before ablation. However, we were able to determine the exact moment of epithelial debridement by clearly observing the disappearance of the blue fluorescence[24,25] as the ablation procedure progressed (Fig. 2). The epithelium of all eyes was completely removed after 64 scanning layers. The published values of rabbit epithelium thickness range from 32.2 to 47.7 μm,[26–31] implying that the rate of the epithelial ablation in our study was ~0.6 μm/scanning layer.

2.2.2 *Nonstop TransPRK with femtosecond UV pulses*

Monolateral nonstop TransPRK at ~110 μm in depth for an optical zone of 6 mm with a 0.6-mm transition zone was performed in 20 rabbits. We estimated that this would include 60 to 80 μm of the stromal tissue and that the myopic ablation of 70 μm central depth within the 6 mm optical zone corresponded to a ~5.0 D refraction change. The intended ablation profile is shown in Fig. 3.

The central corneal thickness (CCT) was measured using an ultrasound pachymeter (Pocket II, Quantel Medical SA) on a dry surface before and after ablation. An infrared thermal camera (ThermaCAM S65, FLIR Systems, Inc.) was used for the monitoring of the surface heating. The temperature increase presented in graphs corresponds to the hottest spot, which was in the center of the ablated area.

2.2.3 *Ablation with excimer pulses*

Monolateral transepithelial ablation was performed using a commercial excimer system (Technolas 217z100, Technolas Perfect Vision GmbH) in 10 rabbits. An eyelid speculum was inserted, and surgery was performed without a suction ring using an eye-tracking system. Laser parameters included a repetition rate of 50 Hz, fluence of 120 ± 5 mJ/cm^2, pulse duration of 18 ns, and beam diameter of 2 mm. The epithelium was removed in a 6.6 mm zone using the phototherapeutic keratectomy (PTK) mode, followed by myopic ablation in the planoscan mode of 70 μm of the stroma in a 6-mm optical zone. This mode was chosen to keep the ablation profile close to the one applied in TransPRK with femtosecond UV pulses (Fig. 3), i.e., to remove the epithelium without reshaping the cornea and to perform myopic ablation only in the stroma. The PTK depth was set to 55 μm, which exceeded the expected epithelial thickness. The intensity of the blue fluorescence decreased abruptly during the last pass of the PTK stage, which we assumed to be an indication of complete epithelial removal.

According to the manufacturer's protocol, the procedure was frequently paused. During PTK, the ablation was paused seven times for ~2 s; the adjustment of laser parameters for myopic ablation after PTK took ~3 min; and during stromal ablation, the process was paused three times for ~2 s. Pachymetry was performed before ablation, after the removal of the epithelium, and after the ablation of the stroma. Measurements after stromal ablation were possible only after moistening the probe tip with BSS.

2.2.4 *Modified TransPRK with femtosecond UV pulses*

The modified procedure was introduced to make better comparisons between the femtosecond and excimer systems, as the excimer ablation depth resulted in a value of 151.4 ± 19.7 μm instead of the expected 110 μm (see Results for details). We modified the femtosecond procedure to match this final depth (150 μm) by setting deeper spherical ablation of the stroma and included pauses to reproduce the duration of the excimer ablation. Extension of the epithelial ablation stage was not used as there was strong evidence that the extra depth in the excimer ablation resulted from the excessive removal of the stroma. The process of the epithelial removal was well

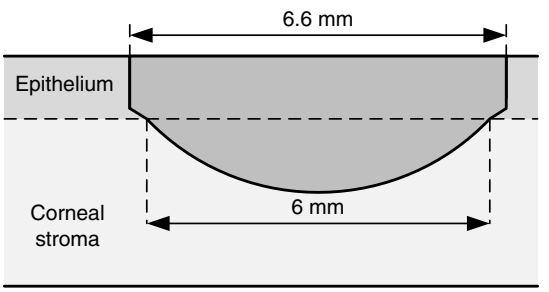

Fig. 3 The attempted ablation profile of the TransPRK with femtosecond UV pulses. Eighty percent of the epithelium was ablated without the transition zone, forming a cylinder with abrupt edges. The transition zone was initiated in the deep epithelium and led to the 6 mm optical zone in the stroma, where the myopic ablation was performed.

defined by the blue fluorescence of the epithelial tissue. Besides, pachymetry measurements after the re-epithelialization at 1 week indicated that in excimer cases, the stromal, not the epithelial ablation was deeper than in nonstop femtosecond UV cases (Table 1). In addition, the increased depth using the epithelial ablation pattern would have produced sharper edges in the transition zone, which could have affected the reepithelialization process and the final haze score.[32] Five rabbits previously treated by excimer ablation underwent the modified TransPRK with femtosecond UV pulses for previously untreated eyes at 2.5 months after excimer surgery. Pachymetry measurements were taken at the same stages as in the excimer ablation procedure and no moistening of the probe tip was required.

2.3 *Evaluation of the Residual Smoothness*

Both eyes of one animal underwent transepithelial excimer ablation, as described above. For comparison, the modified TransPRK with femtosecond UV pulses was performed on the previously untreated eyes of three rabbits after the completion of the follow-up period of 6 months. The animals were humanely killed immediately after the procedure, and their eyes were enucleated for histological examination.

2.4 *Follow-up and Corneal Haze Grading*

Two eyes after nonstop TransPRK with femtosecond UV pulses were excluded from the follow-up due to postoperative keratitis; however, their intraoperative data (CCT and ablation rates) were included. Ten rabbits were followed-up for four weeks, and eight were followed-up for up to 6 months. Five eyes after the excimer ablation were followed-up for four weeks, and five for two months. The follow-up period after the modified femtosecond UV ablation was four weeks.

Postoperative slit-lamp biomicroscopy examinations, photography and pachymetry were performed without general anesthesia weekly for four weeks and monthly thereafter. The level of haze in the corneas was graded according to the Fantes scale,[33] which scores the highest degree of haze at grade 4. Corneal haze score data were analyzed using the statistical nonparametric Mann–Whitney test ($p = 0.05$), as has been previously reported.[18]

2.5 *Corneal Collection and Histology*

To evaluate the smoothness, five corneas (two after the excimer and three after the modified TransPRK with femtosecond UV pulses) were obtained immediately after treatment. Five eyes after femtosecond UV 110 μm, five after excimer 150 μm and four after femtosecond UV 150 μm ablation were obtained at four weeks after treatment, as previous studies have shown that the haze in rabbit corneas peaks at 1 month after both excimer PRK[34] and femtosecond UV ablation.[18] Light microscopy of the specimens stained with hematoxylin and eosin was performed as previously described.[18]

3 Results

3.1 *Ablation Depth and CCT Changes*

The results of pachymetric CCT measurements and calculated thickness changes are presented in Table 1.

To evaluate the reproducibility of the epithelial debridement, 11 video-records of the nonstop femtosecond UV ablation process were analyzed. We found that the mean variation of the

Table 1 Ultrasound pachymetry measurements of the central corneal thickness changes during different ablation modes.

	Nonstop TransPRK with femtosecond UV pulses, 110 μm	Excimer ablation, 150 μm	Modified TransPRK with femtosecond UV pulses, 150 μm
		Mean central corneal thickness, μm	
Before ablation (D1)	347.0 ± 30.5	370.7 ± 26.0	359.2 ± 20.1
After removal of epithelium (D2)	—	282.6 ± 18.6	293.4 ± 12.9
After myopic stromal ablation (D3)	237.7 ± 26.4	219.3 ± 17.9	210.8 ± 9.4
One week after ablation (D4)	269.6 ± 23.5	249.9 ± 18.8	239.6 ± 13.4
		Thickness of removed corneal tissue, μm	
Epithelial removal stage (until change in fluorescence) (D1-D2)	—	93.4 ± 12.0	65.8 ± 11.6
Stromal ablation stage (D2-D3)	—	62.4 ± 9.8	82.6 ± 8.0
		Total thickness of removed corneal tissue, μm	
Immediately after ablation (D1-D3)	109.3 ± 10.5	151.4 ± 19.7	148.4 ± 15.9
One week after ablation (D1-D4)	75.8 ± 23.3	120.8 ± 17.4	119.6 ± 28.3
		Estimated thickness of epithelium after regrowth, μm	
One week after ablation (D4-D3)	34.9 ± 20.5	30.6 ± 19.5	28.8 ± 13.9

epithelial thickness within the ablation zone of 6 mm in diameter for each individual rabbit was ~12% of the maximal thickness (4 to 6 μm). No pronounced difference in the debridement time between the center and periphery of the ablated area was evident, and areas with maximal epithelial thickness were distributed randomly. Thickness measurements after epithelial debridement were not possible in the nonstop procedure, however, pachymetry immediately after stromal ablation revealed high reproducibility (see Table 1). The attempted total ablation depth of 110 ± 15 μm was achieved in 90% of the eyes treated with high-speed nonstop femtosecond UV ablation (Fig. 4). In five corneas treated with the modified femtosecond UV ablation, the average achieved ablation depth (148.4 ± 15.9 μm) also closely matched the intended ablation depth (150 μm).

When removing the epithelium during the excimer procedure, the stromal surface was exposed after completing PTK ablation with a depth setting of 55 μm. Although it was more difficult to visually observe the disappearance of the blue fluorescence during this process due to the illumination of the operative field, it was easily detectable on the video. The depth of the PTK in the center of the ablation zone was unexpectedly high, 93.4 ± 12 μm instead of the expected 55 μm. The total ablation depth was thus 151.4 ± 19.7 μm, significantly greater than planned (110 μm). There are a number of factors that could lead to a rather large mismatch between attempted and measured corneal ablation depths in the case of excimer ablation. The accuracy of the pachymetry measurements could have been affected by the change in the hydration of the cornea, since some force had to be applied to establish the acoustic contact between the probe and the ablated surface of the stroma. The actual thickness of the stroma could have been reduced due to dehydration because of long treatment time and high absorption of water at the excimer wavelength, and the ablation rate of stroma also could have been affected. Yet another possible source of discrepancy is the fact that the diameter of the treatment zone was only approximately three times the beam diameter, which makes it difficult to achieve a perfectly uniform ablation.

The resultant stromal ablation depth measured after the re-epithelialization in both variations of the ablation procedure with femtosecond UV pulses was found to be as expected; in the eyes ablated with the excimer pulses, this value was 120.8 ± 17.4 μm instead of the intended 70 μm. At this point, pachymetry measurements should not have been affected by differences in hydration of the stroma, and other possible measurement errors should be similar for all ablation cases. Therefore, we assume that more than the planned stromal tissue was ablated during excimer treatment, and most likely during the PTK stage.

We should also note that significant deviation of the measured ablation depth from the attempted one, which strongly depended on the ablation time, was observed when TransPRK was performed in humans.[35]

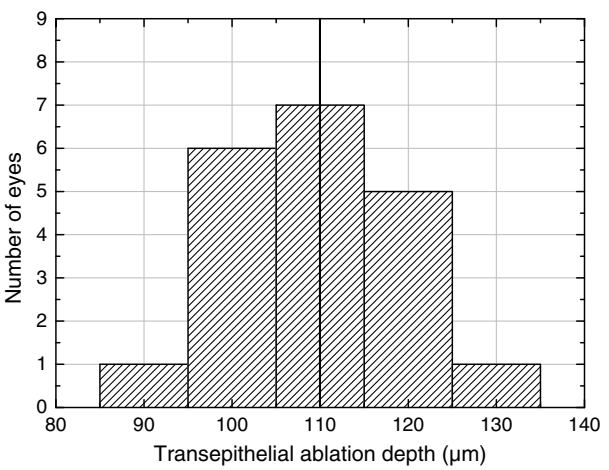

Fig. 4 Statistical distribution of the ablation depth of the nonstop TransPRK with femtosecond UV pulses.

3.2 *Ablation Speed*

The CCT data after femtosecond ablation procedures were analyzed, and the ablation rate of the corneal tissue was evaluated. Assuming that the ablation speed of the corneal epithelium and stroma was the same, it could be estimated that ~280 pL of corneal tissue per 1 mJ have been removed using the high-speed femtosecond UV laser system. As a result, a refractive change of 1-D in the 6 mm optical zone could be created in ~1.6 s. Alternatively, evaluation of the refraction change was made by measuring the change of the CCT[36] after re-epithelialization and resulted in speed values of ~1.45 s/D for the nonstop procedure and ~1.62 s/D for the modified procedure. In the latter case, the speed was calculated by taking into account only the actual ablation time, i.e., with pauses excluded. The durations of the stages of the ablation are presented in Table 2.

3.3 *Temperature Rise*

The graphs of the temperature increase for different kinds of treatments are presented in Fig. 5. During the nonstop femtosecond TransPRK, the increase in the maximum corneal surface temperature peaked at the end of the procedure and was <7°C (Fig. 5). The temporal dependence of the surface temperature was directly related to the laser beam scanning algorithm. Ablation of the epithelium was performed within the area of 6.6 mm in diameter and lasted ~15 s. After that, the ablation zone was reduced to 6 mm, and myopic ablation started (Fig. 2, see video from the second 15). During the myopic ablation, the average power density was higher in the center of the ablation zone in contrast to the epithelial ablation, where the laser power was distributed uniformly over the ablated area. No pauses for cooling were introduced, and stroma was already preheated due to the heat transfer from the epithelium. These factors caused an additional temperature increase during the stromal ablation.

The red line in Fig. 5 (color online) represents the dynamics of the corneal surface temperature during the myopic ablation with excimer laser pulses (epithelial ablation is not included). The recorded increase in the corneal surface temperature was less than 4.5°C during the entire excimer ablation procedure. The lowest corneal surface temperature was registered during the modified femtosecond TransPRK. Due to the pauses, the overall increase in temperature did not exceed 3°C, whereas the maximum temperature increase during the epithelial removal was only 1.5°C (Fig. 5, blue line, color online).

3.4 *Postoperative Complications*

Two cases of keratitis were observed after the nonstop TransPRK with femtosecond UV pulses. In one animal, an epithelial defect with corneal infiltration and hemorrhage under the nictitating membrane was noted on the seventh day after

Table 2 Durations of the ablation stages.

	Nonstop TransPRK with femtosecond UV pulses, 110 μm	Excimer ablation, 150 μm	Modified TransPRK with femtosecond UV pulses, 150 μm
Epithelial ablation (including pauses)	15 s (15 s)	55 s (~69 s)	15 s (29 s)
Pause between epithelial and stromal ablations	—	~180 s	180 s
Stromal ablation (including pauses)	8 s (8 s)	30 s (~36 s)	14 s (20 s)
Total procedure duration	23 s	~285 s	229 s

surgery. In another case, the healing process was uncomplicated for three weeks, but at week 4, a ring infiltrate in the ablation zone developed. While excimer surgery took place in an operating room, procedures with femtosecond UV pulses were performed in a laboratory room where aseptic conditions could not be completely ensured. Another source of infection could have been a nondisposable suction ring, which was used to immobilize the eyes during the femtosecond UV ablation.

3.5 *Subepithelial Haze Measurements*

Figure 6 shows the development of haze after different treatments. No statistically significant difference was found in the data on the corneal haze score after nonstop TransPRK with femtosecond UV pulses and excimer laser ablation during the follow-up period of 1 month. The least intense haze was observed after the modified femtosecond UV treatment. This value was even lower than after the shallower ablation with femtosecond UV pulses.

Comparing the degree of haze in the contralateral eyes of the same animals treated by excimer and the modified TransPRK, we found that the latter treatment induced less haze. Statistical analysis showed a significant difference in the corneal haze at 1 week ($p = 0.0135$) and at 1 month ($p = 0.0415$) after the modified TransPRK with femtosecond UV pulses compared with haze after the other procedures; however, there were only five rabbits in this group.

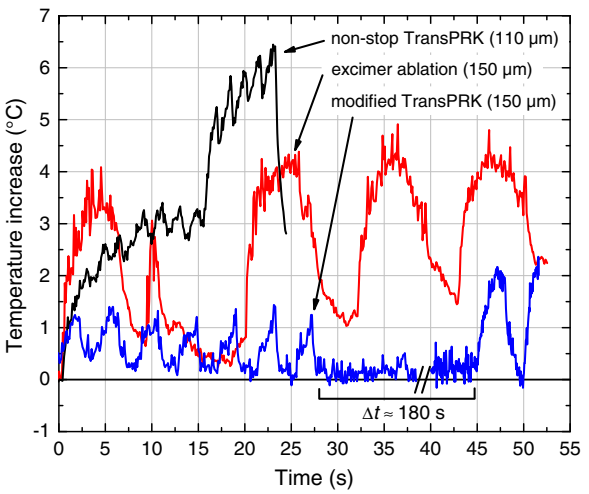

Fig. 5 Corneal surface temperature changes during the ablation process at the center of the treatment zone. Δt indicates the pause between the epithelial and stromal ablations.

3.6 *Histological Examination*

In samples taken immediately after ablation, relatively smooth ablated surfaces were observed. Light microscopy revealed no noticeable differences in the surface smoothness after excimer (150 μm) or femtosecond UV ablation (150 μm).

At 1 month, all corneas after the femtosecond UV ablation of 110 μm exhibited normal epithelial thickness, with minor variations in smoothing out the stroma (Fig. 7). In three eyes, possible edema of solitary epitheliocytes was noted. In most of the corneas treated by excimer or the modified femtosecond UV ablation, reduced epithelial thickness was observed. Light microscopy of the specimens with corneal haze demonstrated similar subepithelial fibrous layers in the eyes after different treatments. The deeper stroma and inner layers revealed no visible changes.

4 Discussion

After upgrading the laser system, the myopic corneal ablation speed in rabbit corneas increased by nearly a factor of four compared with the laser setup used in our previous experiments.[18] The main reason for this increased speed was the increased repetition rate of the laser (50 kHz against 20 kHz) and thus of the average power of the UV radiation. Further, fewer pulses were lost due to the faster scanners, and the ablation was more efficient due to the beam intensity distribution with relatively less power in subthreshold wings.

Quite inevitably, higher average power applied led to a temperature increase at the surface of the cornea, which is assumed

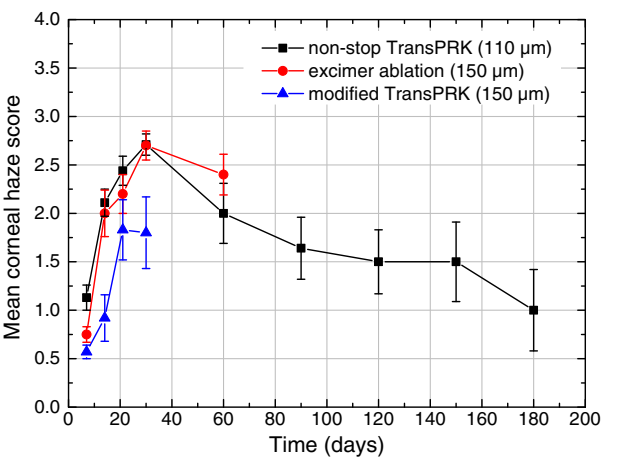

Fig. 6 Mean corneal central subepithelial haze score dynamics after different treatments.

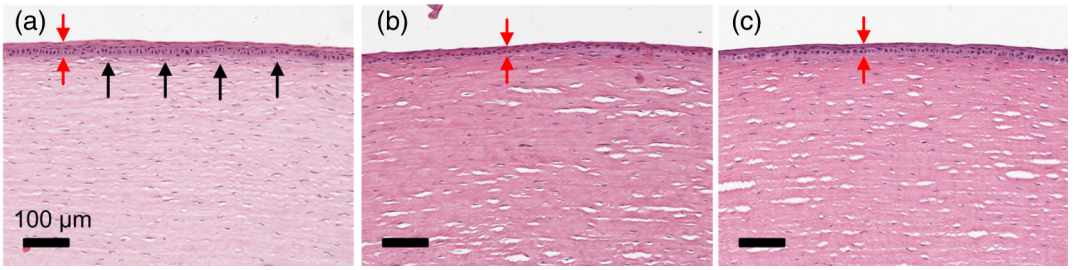

Fig. 7 One month after (a) the nonstop TransPRK with high-speed femtosecond UV pulses (110 μm), (b) excimer pulses (150 μm), and (c) modified ablation with femtosecond UV pulses (150 μm). The epithelium is thinner in (b) and (c) (red arrowheads, color online). Black arrowheads indicate subepithelial fibrosis (light microscopy).

to be a risk factor for the development of postoperative haze. In our case, increased power seems not to have influenced the healing process significantly. The healing process and its outcomes in terms of haze and histopathology evaluations were similar to those described in our previous study, in which we compared the outcomes at 1 month after femtosecond UV (180 ± 5 mW, 20 kHz) or excimer ablation.[18] This similarity arises despite the fact that in the nonstop TransPRK procedure, the ablation of the stroma began immediately after epithelial debridement, when the temperature was already elevated. Although the temperature did not exceed the accepted safe level of 7°C,[37] the rabbits after TransPRK treatment with the excimer laser developed less haze if the values were weighed against the thickness of the removed cornea. It is interesting that the lowest degree of haze developed in the corneas that were treated with the deeper femtosecond UV ablation with pauses. One of the possible causes could be the reduced temperature, which was even lower than in the excimer case. Although the correlation between the corneal temperature during the surgery and the amount of haze has been observed, the sample size (five rabbits) precludes making definite conclusions. The different groups and ages of the rabbits treated by the excimer and modified femtosecond procedures are among the factors that could have impacted the outcomes. Therefore, the thorough testing of the hypothesis that haze is caused by the thermal stress induced by the ablation remains a subject of a separate study.

It has been shown by Mrochen et al. that both linear and circular scanning with spot overlap led to high temperature increase, which could be significantly reduced by optimizing the scanning pattern.[38] A computer algorithm called "intelligent thermal effect control" has been developed to avoid thermal overload without increasing the treatment duration.[39,40] The algorithm assumes the capability of the scanning system of moving the laser beam to any location between the subsequent laser shots. With a repetition rate of tens of kilohertz as in our case, the available scanners are too slow to control every pulse, which means only the distances between adjacent spots in the raster lines can be optimized. Shraiki and Arba-Mosquera[37,41] have proposed a model for the evaluation of thermal load based on the local frequency, i.e., the repetition rate of laser pulses delivered on the same location. Our evaluation shows that during the nonstop femtosecond ablation of the epithelium the local frequency was 13 Hz, whereas during the stromal ablation stage, it reached up to 31 Hz. These numbers are smaller than those for typical excimer applications,[37] however, the temperature rise may be influenced by the spot size. It is quite obvious that the temperature increase would be significantly less if standard PRK or LASIK procedures, rather than transepithelial techniques, were used for the same depth of stromal ablation. The need for temperature control may arise only if large refractive errors are to be corrected.

In the present study, TransPRK with the epithelium ablated first was chosen to observe the healing process after the "worst" treatment conditions in terms of the surface heating.

We see several possibilities of reducing the peak temperature when a high repetition rate laser is to be used for TransPRK. The simplest of these is the introduction of brief pauses at the final stage of corneal shaping, which would cause only a minor increase in the overall procedure time. Another possibility is to blend the shaping of the cornea with the epithelial removal to avoid prolonged scanning of the laser beam over a small area, or to use reverse TransPRK when the myopic ablation is performed first while the cornea is still at normal temperature.[20,22] Some additional temperature reduction may be achieved by increasing the fluence further above the threshold. In our case, the entire ablation was performed with fluence below saturation, which is referred to as a high fidelity regime.[42] In addition to the optimization of the laser and scanning parameters, additional means, such as cooling the cornea prior to ablation by chilled BSS,[43] may also be applied.

For the TransPRK type of surgery, it is important to remove the epithelium in a predictable manner; otherwise, extra tissue may be removed from the cornea unnecessarily. Our findings show that with femtosecond UV pulses, the process is highly reproducible. The variable thickness of the epithelium over the debridement area was found to span only a few micrometers, not significantly affecting the residual thickness of the cornea. It is worth noting that the excitation of the fluorescence after femtosecond UV pulses at 206 nm provides a very good contrast between the epithelium and the cornea and that fast scanning allows for easy surface monitoring. This feature potentially can be used to provide real-time feedback for the detection of complete epithelial removal. The necessary detector would be a regular color CCD camera.

It is worth noting that the stromal surface after femtosecond UV ablation seemed to be less dried out than after excimer ablation. This difference was confirmed by the fact that in eyes treated with excimer ablation, it was not possible to perform pachymetry after stromal ablation without moistening the tip of the sensor. By contrast, this measurement was possible on eyes ablated with femtosecond pulses, even if pauses were introduced to prolong the procedure time. The difference could be related to the different wavelengths used. Dair et al. estimated that the absorption coefficient in BSS for the longer 213 nm

wavelength was significantly less than for the 193 nm.[44] We speculate that while the longer 206 nm wavelength used in our study was less absorbed by BSS than the 193 nm, the evaporation of the fluids from the surface was decreased. As BSS strongly absorbs the 193 nm light, ablation depth and residual surface smoothness is affected by the presence of fluid on the surface during ablation.[44] For that reason, ablation with excimer pulses is usually performed after eliminating the excessive fluid. On the other hand, if the process of ablation is long, the cornea may significantly dry out, and overcorrection could occur.[19,44,45] Therefore, the lower influence of high-speed femtosecond UV ablation on corneal hydration is an advantage, as it lowers the requirements for the application of surface fluids and allows for less strict environmental parameters.

It must be noted that femtosecond UV pulses deliver much higher intensity than excimer pulses of equal fluence, which, in principle, may lead to undesirable modification of the biological tissue. Therefore, cytotoxicity and genotoxicity of 206-nm femtosecond pulses have to be assessed. As for the wavelength difference, previous studies that compared the unscheduled DNA synthesis induced by excimer (193 nm) and solid-state UV (213 nm) laser irradiation found no significant differences between the two.[46] However, the effect of the shorter pulse duration of our laser remains a subject of a separate study.

The achieved speed of ~1.6 s/D in a 6-mm optical zone is comparable to the fastest reported ablation speed in human corneas. For example, the treatment time of the new WaveLight Concept System1000 laser (Concept System 1000; WaveLight GmbH) is ~1.2 s/D of myopia within a 6.5 mm optical zone.[6] However, this system is not available on the market. The speed of the WaveLight EX500 excimer laser, which is a part of the WaveLight Refractive Suite (WaveLight GmbH), is ~1.4 s/D.[10] With the SchwindAmaris 1050RS system (SchwindAmaris 1050RS, Schwind eye-tech-solutions GmbH & Co. KG), one diopter is corrected in 1.3 s.[11]

The size of the spot used in our experiments (~115 μm) appears to be excessively small for achieving even the best possible spatial resolution required for custom ablation.[47,48] Reduced spot size, however, may be advantageous because the small-scale surface irregularities created by such a spot are more easily smoothed out by reepithelialization.[49]

In conclusion, our experiments achieved an ablation speed of ~1.6 s/myopic diopter in the 6 mm optical zone without significant impact on the healing outcomes in rabbits. The feasibility of TransPRK is an additional advantage of ablation with femtosecond UV pulses. Other advantages include inaudible ablation, high spatial resolution, better stability, and low maintenance.

Although modern sophisticated nanosecond systems enable highly precise surface refractive treatments, they are designed for UV treatment only. A single solid-state laser source for combined UV and NIR applications, including all-femtosecond LASIK, femtosecond corneal and cataract surgery, would be a convenient, multipurpose device for anterior segment surgeons.

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References

1. I. Ratkay-Traub et al., "Ultra-short pulse (femtosecond) laser surgery: initial use in LASIK flap creation," *Ophthalmol. Clin. North. Am.* **14**(2), 347–355 (2001).
2. W. Sekundo et al., "First efficacy and safety study of femtosecond lenticule extraction for the correction of myopia: six-month results," *J. Cataract Refract. Surg.* **34**(9), 1513–1520 (2008).
3. R. Shah, S. Shah, and S. Sengupta, "Results of small incision lenticule extraction: all-in-one femtosecond laser refractive surgery," *J. Cataract Refract. Surg.* **37**(1), 127–137 (2011).
4. S. L. Trokel, R. Srinivasan, and B. Braren, "Excimer laser surgery of the cornea," *Am. J. Ophthalmol.* **96**(6), 710–715 (1983).
5. T. Seiler, G. Kahle, and M. Kriegerowski, "Excimer laser (193 nm) myopic keratomileusis in sighted and blind human eyes," *Refract. Corneal Surg.* **6**(3), 165–173 (1989).
6. R. Khoramnia et al., "Safety, efficacy, predictability and stability of laser in situ keratomileusis (LASIK) with a 1000-Hz scanning spot excimer laser," *Acta Ophthalmol.* **90**(6), 508–513 (2012).
7. M. Tomita et al., "Safety, efficacy, and predictability of laser in situ keratomileusis to correct myopia or myopic astigmatism with a 750 Hz scanning-spot laser system," *J. Cataract Refract. Surg.* **40**(2), 251–258 (2014).
8. M. Bohac et al., "Comparison of wavelight allegretto eye-Q and schwind amaris 750 S excimer laser in treatment of high astigmatism," *Graefes. Arch. Clin. Exp. Ophthalmol.* **252**(10), 1679–1686 (2014).
9. S. Arba-Mosquera and T. Klinner, "Improving the ablation efficiency of excimer laser systems with higher repetition rates through enhanced debris removal and optimized spot pattern," *J. Cataract Refract. Surg.* **40**(3), 477–484 (2014).
10. http://www.alconsurgical.com (accessed January 4, 2015).
11. http://www.eye-tech-solutions.com (accessed January 4, 2015).
12. I. Anderson et al., "Treatment of irregular astigmatism with a 213 nm solid-state, diode-pumped neodymium: YAG ablative laser," *J. Cataract Refract. Surg.* **30**(10), 2145–2151 (2004).
13. A. M. Roszkowska et al., "Experimental and clinical investigation of efficiency and ablation profiles of new solid-state deep-ultraviolet laser for vision correction," *J. Cataract Refract. Surg.* **30**(12), 2536–2542 (2004).
14. A. M. Roszkowska et al., "One-year clinical results of photorefractive keratectomy with a solid-state laser for refractive surgery," *J. Refract. Surg.* **22**(6), 611–613 (2006).
15. O. Kittelmann, G. Korn, and M. Lenzner, "Device and procedure for refractive laser surgery," U.S. Patent No. 7,721,743 (2010).
16. O. Kittelmann, G. Korn, and M. Lenzner, "Device and procedure for refractive laser surgery," U.S. Patent No. 8,313,479 (2012).
17. M. Vengris et al., "Corneal shaping and ablation of transparent media by femtosecond pulses in deep ultraviolet range," *J. Cataract Refract. Surg.* **36**(9), 1579–1587 (2010).
18. E. Danieliene et al., "Corneal stromal ablation with femtosecond ultraviolet pulses in rabbits," *J. Cataract Refract. Surg.* **39**(2), 258–267 (2013).
19. P. J. Dougherty, K. L. Wellish, and R. K. Maloney, "Excimer laser ablation rate and corneal hydration," *Am. J. Ophthalmol.* **118**(2), 169–176 (1994).
20. I. M. Aslanides et al., "Comparison of single-step reverse transepithelial all-surface laser ablation (ASLA) to alcohol-assisted photorefractive keratectomy," *Clin. Ophthalmol.* **6**, 973–980 (2012).
21. A. Stojanovic et al., "One-step transepithelial topography-guided ablation in the treatment of myopic astigmatism," *PLoS One* **8**(6), e66618 (2013).
22. I. M. Aslanides et al., "Single-step transepithelial ASLA (SCHWIND) with mitomycin-C for the correction of high myopia: long term follow-up," *Clin. Ophthalmol.* **2015**(9), 33–41 (2014).
23. D. K. Cohen, B. Little, and F. S. Luecke, "Techniques for measuring 1-μm diam Gaussian beams," *Appl. Opt.* **23**(4), 637–640 (1984).

24. S. Tuft et al., "Characterization of the fluorescence spectra produced by excimer laser irradiation of the cornea," *Invest. Ophthalmol. Vis. Sci.* **31**(8), 1512–1518 (1990).
25. S. Korkmaz et al., "A clinical and confocal microscopic comparison of transepithelial PRK and LASEK for myopia," *J. Ophthalmol.* **2014**, 784185 (2014).
26. N. S. Tsiklis et al., "Photorefractive keratectomy using solid state laser 213 nm and excimer laser 193 nm: a randomized, contralateral, comparative, experimental study," *Invest. Ophthalmol. Vis. Sci.* **49**(4), 1415–1420 (2008).
27. H. F. Li et al., "Epithelial and corneal thickness measurements by in vivo confocal microscopy through focusing (CMTF)," *Curr. Eye Res.* **16**(3), 214–221 (1997).
28. J. V. Jester et al., "Area and depth of surfactant-induced corneal injury correlates with cell death," *Invest. Ophthalmol. Vis. Sci.* **39**(6), 922–936 (1998).
29. B. Masters, "Three-dimensional confocal microscopy of the living in situ rabbit cornea," *Opt. Express* **3**(9), 351–355 (1998).
30. B. J. Reiser et al., "In vitro measurement of rabbit corneal epithelial thickness using ultrahigh resolution optical coherence tomography," *Vet. Ophthalmol.* **8**(2), 85–88 (2005).
31. W. M. Petroll et al., "Quantitative 3-dimensional corneal imaging in vivo using a modified HRT-RCM confocal microscope," *Cornea* **32**(4), e36–e43 (2013).
32. S. Serrao and M. Lombardo, "Corneal epithelial healing after photorefractive keratectomy: analytical study," *J. Cataract Refract. Surg.* **31**(5), 930–937 (2005).
33. F. E. Fantes et al., "Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys," *Arch. Ophthalmol.* **108**(5), 665–675 (1990).
34. R. R. Mohan et al., "Apoptosis, necrosis, proliferation, and myofibroblast generation in the stroma following LASIK and PRK," *Exp. Eye Res.* **76**(1), 71–87 (2003).
35. S. Adib-Moghaddam et al., "On-line pachymetry outcome of ablation in aberration free mode trans PRK," *Eur. J. Ophthalmol.* **24**(4), 483–489 (2014).
36. M. H. Niemz, "Lasers in ophthalmology," Chapter 4.1 in *Laser-Tissue Interactions: Fundamentals and Applications*, pp. 170–172, Springer, Berlin Heidelberg New York (2007).
37. M. Shraiki and S. Arba-Mosquera, "Simulation of the impact of refractive surgery ablative laser pulses with a flying-spot laser beam on intrasurgery corneal temperature," *Invest. Ophthalmol. Vis. Sci.* **52**(6), 3713–3722 (2011).
38. M. Mrochen et al., "Effect of time sequences in scanning algorithms on the surface temperature during corneal laser surgery with high-repetition-rate excimer laser," *J. Cataract Refract. Surg.* **35**(4), 738–746 (2009).
39. U. Brunsmann et al., "Minimisation of the thermal load of the ablation in high-speed laser corneal refractive surgery: the 'intelligent thermal effect control' of the AMARIS platform," *J. Mod. Opt.* **57**(6), 466–479 (2010).
40. D. de Ortueta et al., "In vivo measurements of thermal load during ablation in high-speed laser corneal refractive surgery," *J. Refract. Surg.* **28**(1), 53–58 (2012).
41. S. Arba-Mosquera and M. Shraiki, "Analysis of the PMMA and cornea temperature rise during excimer laser ablation," *J. Mod. Opt.* **57**(5), 400–407 (2010).
42. S. Arba-Mosquera and T. Hollerbach, "Ablation resolution in laser corneal refractive surgery: the dual fluence concept of the AMARIS platform," *Adv. Opt. Technol.* **2010**, 538541 (2010).
43. Y. Kitazawa et al., "Cooling effect on excimer laser photorefractive keratectomy," *J. Cataract Refract. Surg.* **25**(10), 1349–1355 (1999).
44. G. T. Dair et al., "Absorption of 193-and 213-nm laser wavelengths in sodium chloride solution and balanced salt solution," *Arch. Ophthalmol.* **119**(4), 533–537 (2001).
45. W. S. Kim and J. M. Jo, "Corneal hydration affects ablation during laser in situ keratomileusis surgery," *Cornea* **20**(4), 394–397 (2001).
46. P. P. Van Saarloos and J. Rodger, "Histological changes and unscheduled DNA synthesis in the rabbit cornea following 213-nm, 193-nm, and 266-nm irradiation," *J. Refract. Surg.* **23**(5), 477–481 (2007).
47. D. Huang and M. Arif, "Spot size and quality of scanning laser correction of higher-order wavefront aberrations," *J. Cataract Refract. Surg.* **28**(3), 407–416 (2002).
48. A. Guirao, D. R. Williams, and S. M. MacRae, "Effect of beam size on the expected benefit of customized laser refractive surgery," *J. Refract. Surg.* **19**(1), 15–23 (2003).
49. M. Bueeler and M. Mrochen, "Simulation of eye-tracker latency, spot size, and ablation pulse depth on the correction of higher order wavefront aberrations with scanning spot laser systems," *J. Refract. Surg.* **21**(1), 28–36 (2004).

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