VILNIUS UNIVERSITY

Eglė Danielienė

CORNEAL ABLATION WITH FEMTOSECOND ULTRAVIOLET PULSES

Summary of Doctoral Dissertation

Biomedical sciences, medicine (06 B)

Vilnius, 2015

Extramural doctoral degree.

Scientific consultants:

prof. dr. Janina Tutkuvienė (Vilnius University, Biomedical Sciences, Medicine – 06B).

prof. dr. Osvaldas Rukšėnas (Vilnius University, Biomedical Sciences, Biophysics – 02B).

The Doctoral Dissertation will be defended at the Medical Research Council of Vilnius University:

Chairman – prof. dr. Eugenijus Lesinskas (Vilnius University, Biomedical Sciences, Medicine – 06B).

Members:

prof. dr. Ingrida Janulevičienė (Lithuanian University of Health Sciences, Biomedical Sciences, Medicine – 06B);

prof. dr. Nomeda Rima Valevičienė (Vilnius University, Biomedical Sciences, Medicine – 06B);

prof. habil. dr. Ričardas Rotomskis (Vilnius University, Biomedical Sciences, Biophysics – 02B);

dr. Giedrius Kalesnykas (University of Tampere, Finland, Biomedical Sciences, Medicine – 06B).

The dissertation will be defended at the open session of the Medical Research Council of Vilnius University at 3:00 p.m. on 20th of May, 2015 in the Great Hall of the Medical Faculty of Vilnius University.

Address: M. K. Čiurlionio 21, Vilnius 03101, Lithuania.

The summary of the doctoral dissertation was mailed out on the 19th of April, 2015.

The dissertation is available in the Library of Vilnius University and online at *www.vu.lt/lt/naujienos/ivykiu-kalendorius*.

VILNIAUS UNIVERSITETAS

Eglė Danielienė

RAGENOS ABLIACIJA FEMTOSEKUNDINIAIS ULTRAVIOLETINIAIS IMPULSAIS

Daktaro disertacijos santrauka

Biomedicinos mokslai, medicina (06 B)

Vilnius, 2015

Disertacija ginama eksternu.

Moksliniai konsultantai:

prof. dr. Janina Tutkuvienė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06B).

prof. dr. Osvaldas Rukšėnas (Vilniaus universitetas, biomedicinos mokslai, biofizika – 02B).

Disertacija ginama Vilniaus universiteto Medicinos mokslo krypties taryboje:

Pirmininkas – prof. dr. Eugenijus Lesinskas (Vilniaus universitetas, biomedicinos mokslai, medicina – 06B).

Nariai:

prof. dr. Ingrida Janulevičienė (Lietuvos sveikatos mokslų universitetas, biomedicinos mokslai, medicina – 06B);

prof. dr. Nomeda Rima Valevičienė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06B);

prof. habil. dr. Ričardas Rotomskis (Vilniaus universitetas, biomedicinos mokslai, biofizika – 02B);

dr. Giedrius Kalesnykas (Tamperės universitetas, Suomija, biomedicinos mokslai, medicina – 06B).

Disertacija bus ginama viešame Medicinos mokslo krypties tarybos posėdyje 2015 m. gegužės mėn. 20 d. 15 val. Medicinos fakulteto Didžiojoje auditorijoje.

Adresas: M. K. Čiurlionio g. 21, Vilnius 03101, Lietuva.

Disertacijos santrauka išsiuntinėta 2015 m. balandžio mėn. 19 d.

Disertaciją galima peržiūrėti Vilniaus universiteto bibliotekoje ir VU interneto svetainėje adresu: *www.vu.lt/lt/naujienos/ivykiu-kalendorius*.

CONTENTS

ABBREVIATIONS

BSS	—	balanced salt solution
CCD	—	charge coupled device
CCT	—	central corneal thickness
D	—	diopter
Fs	—	femtosecond (10^{-15} s)
Hz	—	hertz
LASIK	—	laser-assisted in situ keratomileusis
MMC	—	mitomycin C
mW	—	milliwatt ((10^{-3} W)
nm	—	nanometer (10^{-9} m)
PRK	—	photorefractive keratectomy
PTK	—	phototherapeutic keratectomy
ReLEx	—	refractive lenticule extraction
SD	—	standard deviation
SEM	—	standard error of the mean
TransPRK	—	transepithelial photorefractive keratectomy
UV	—	ultraviolet
μm	—	micrometer (10^{-6} m)

1. INTRODUCTION

The aim of the corneal refractive surgery is to correct refractive errors predominantly in healthy corneas, this is why it is especially important to ensure safe, predictable and long-lasting results. Laser-assisted in situ keratomileusis (LASIK) was introduced in 1990 (Pallikaris, Papatzanaki et al. 1990) and currently is the most popular refractive procedure (Duffey and Leaming 2005; Reinstein, Archer et al. 2012).

The most important modification of LASIK was the introduction of a femtosecond laser for flap creation instead of a mechanical microkeratome in 2001 (Ratkay-Traub, Juhasz et al. 2001). Studies have demonstrated advantages of the femtosecond laser over microkeratome, including improved safety and accuracy (Binder 2004; von Jagow and Kohnen 2009; Murakami and Manche 2011), faster visual recovery, and better correction outcomes (Tanna, Schallhorn et al. 2009). Besides flap creation, the number of applications for the femtosecond lasers has grown significantly. Tissue resection in lamellar and penetrating keratoplasty (Farid, Kim et al. 2007; Por, Cheng et al. 2008), femtosecond-assisted astigmatic keratotomy (Nubile, Carpineto et al. 2009), corneal tunnel incisions for the insertion of intrastromal ring segments (Pinero, Alio et al. 2009), and the relatively recently added lens fragmentation (Nagy, Takacs et al. 2009) and capsulorhexis (Nagy, Takacs et al. 2009; Friedman, Palanker et al. 2011; Tackman, Kuri et al. 2011) in cataract surgery are among these procedures.

New techniques for correcting myopia, which employ near infrared femtosecond laser only, have been developed as well. In refractive lenticule extraction (ReLEx), a flap and a lenticule of intrastromal corneal tissue are cut simultaneously using a femtosecond laser, followed by manual removal of the lenticule (Sekundo, Kunert et al. 2011; Shah, Shah et al. 2011). While these minimally invasive methods appear to be safe and promising, they are not yet indicated for hyperopic corrections and retreatments. This limitation makes them additional, rather than superseding, techniques to the currently used ultraviolet (UV) laser pulses.

Modern femto-LASIK is performed using two laser sources: a near infrared (wavelength ~1000 nm) femtosecond solid-state or fiber laser for flap creation and a nanosecond UV laser (~200 nm) for stromal ablation. Argon fluoride excimer laser (193 nm) is the standard source of choice for UV ablation. 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 (Khoramnia, Salgado et al. 2012; Arba-Mosquera and Klinner 2014; Bohac, Biscevic et al. 2014; Tomita, Watabe et al. 2014). 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. UV systems for corneal ablation based upon nanosecond solid-state lasers with harmonic generators is another choice (Anderson, Sanders et al. 2004; Roszkowska, Korn et al. 2004). The clinical outcomes using solid-state systems have been demonstrated to be equivalent to those of excimer lasers (Roszkowska, Korn et al. 2004). However, inherent advantages of solid-state lasers like better shot-to-shot stability did not prove to be decisive in gaining a considerable market share. The situation could change if both stages of the LASIK treatment were performed using a single system driven by one solid-state laser.

The rapidly growing number of applications for ultrashort pulses in micromachining has fueled the rapid progress in the development of high-power, reliable, compact and versatile femtosecond laser systems (Raciukaitis, Grishin et al. 2006). Owing to their high-frequency conversion efficiency with ultrashort pulses, these lasers are capable of sub-watt average power at a wavelength near 200 nm. An example of such a laser is the “Pharos” femtosecond system (Light Conversion Ltd., Vilnius, Lithuania) (www.lightcon.com). Its high repetition rate of up to 1 MHz potentially allows for high-speed intrastromal flap cutting. In addition, it delivers multi-watt power at 5-50 kHz repetition rates that are optimal for the generation of UV pulses. It is important to note that switching between the two regimes can be performed in seconds without manual intervention. A prototype of this laser, with a fifth harmonic generator, was previously tested for ablation efficiency on poly(methyl methacrylate), gelatin, and freshly enucleated porcine eyes (Vengris, Gabryte et al. 2010). Ablation was smooth and predictable and showed high lateral resolution; both myopic and hyperopic ablations were possible. Miclea et al. (Miclea, Skrzypczak et al. 2011) performed flap cuttings and removal of intrastromal lenticules using the "Pharos" femtosecond system in *ex vivo* pig eyes. However, it was not clear whether the living tissue could tolerate such a high peak intensity with a short pulse duration, and what the effects of the strongly reduced laser spot size could be.

1.1. Aim and objectives of the study

The aim was to optimize and characterize a novel method of corneal stromal ablation based on high repetition rate femtosecond UV pulses at a wavelength of 206 nm using *in vivo* model.

Objectives:

1. To determine the range of parameters of the femtosecond UV pulses acceptable for corneal ablation of *in vivo* rabbit eyes.
2. To compare the process of myopic ablation using femtosecond UV and nanosecond UV excimer pulses: to evaluate temperature dynamics, surface dehydration and stromal surface smoothness after ablation.
3. To evaluate the characteristics of high-speed myopic ablation with femtosecond UV pulses in transepithelial photorefractive keratectomy (TransPRK) mode.
4. To compare the healing response between rabbit eyes treated with femtosecond UV and nanosecond UV excimer pulses, by evaluating the development and grades of haze as well as histological changes.

1.2. Positions to defend

1. Corneal ablation with femtosecond UV pulses is effective and predictable thus femtosecond solid-state lasers could be used instead of nanosecond systems in refractive surgery.
2. Ablation with femtosecond UV pulses is comparable to the ablation by nanosecond excimer UV pulses in terms of reproducibility, stromal surface temperature increase and smoothness of the ablated stroma.
3. Despite significant differences in peak intensity and pulse repetition rate, corneal wound-healing response and outcomes are comparable to those after ablation with nanosecond UV excimer pulses.

1.3. Scientific novelty

At present corneal ablation in refractive surgery is performed using only nanosecond UV pulses, generated by excimer or solid-state lasers. To our knowledge, in this study, for the first time, femtosecond UV pulses of high repetition rate and small spot diameter were applied for corneal ablation *in vivo*. Femtosecond UV pulses were applied to perform photorefractive keratectomy (PRK), myopic high-speed transepithelial PRK (TransPRK), and to mark the deepithelialization zone.

The quality of ablation and healing outcomes were evaluated for different repetition rates (20 and 50 kHz), power levels (180, 200 and 400 mW) and spot sizes (130 and 280 µm). When applying maximal power (400 mW), the ablation speed comparable to the fastest reported ablation speeds in human corneas using only high-fidelity regime was achieved.

The healing process after ablation with femtosecond UV pulses was monitored and the healing outcomes were evaluated after ablation of different depth (30 and 130 µm), ablation with Mitomycin C (MMC) and TransPRK (110 and 150 µm).

It was shown for the first time that the healing outcomes after ablation with femtosecond UV pulses were comparable to those after ablation with nanosecond UV excimer pulses.

1.4. Practical significance of the study

Ablation with femtosecond UV pulses can replace ablation with nanosecond pulses, which means that one solid-state femtosecond laser system can be used instead of two devices in the LASIK surgery. Such a system would have the following advantages in comparison with current laser systems:

- Smaller spots and high repetition rates support finer ablation profiles for custom treatment.
- Longer wavelength (206 nm) is less absorbed by BSS than the 193 nm excimer laser radiation, the hydration level of the stroma has less influence on the ablation rate. This also relaxes the requirements for the environment control.
- The UV laser beam instead of mechanical marker for the deepithelialization zone is more precise and sterile.
- With a high repetition rate and low energy of individual pulses, shock waves are essentially eliminated, thus, ablation is inaudible and less stressfull.
- Both stages of LASIK can be completed without moving the patient and surgeon between two different devices; therefore, speed, safety and comfort of the procedure would be significantly improved.
- Installation and maintenance costs of the integrated system would be significantly lower, and less space in the operating room would be required.
- There would be no need for the surgeon to learn the new technique, because the new device would be used to perform standard LASIK stages. Surface ablation procedures, including TransPRK, would be also feasible.
- We believe that the same device could be used for the whole range of procedures, currently performed with femtosecond laser, including ReLEx, cataract surgery, keratoplasty, etc.

2. MATERIALS AND METHODS

Approval for the study was obtained from the Lithuanian State Food and Veterinary Service (numbers 0180, 0213). Experiments were performed in the research laboratory of "Light Conversion" Ltd., and Laser Research Center of Vilnius University in 2009–2013. Excimer surgery was performed in the eye clinic "Akių Lazerinės Chirurgijos Centras". Histological analysis was performed in the National Center of Pathology.

In this study we have performed three series of experiments:

Series 1. Development of the protocol for surgical procedure as well as for the evaluation of healing outcomes of the corneal ablation with the femtosecond UV pulses in rabbits' eyes.

The aim was to adapt the "Pharos" femtosecond laser system for the treatment of rabbits' eyes, to develop an immobilization device for the animals during surgery, to prepare a protocol for surgical procedures and postoperative treatment, to estimate available methods for the evaluation of the healing outcomes, and to perform pilot surgeries using an excimer laser.

Series 2. Evaluation of the procedure and healing outcomes of PRK with femtosecond UV pulses and comparison with PRK using excimer pulses.

Experiments were performed to determine the effectiveness and optimum parameters of femtosecond UV pulses in ablating the corneal stroma and to compare the healing response between eyes treated with femtosecond UV pulses and those treated with standard excimer PRK. The average power of the femtosecond UV pulses was adjusted to the excimer laser we had at our disposition. Pilot experiments were conducted to evaluate the influence of the surface roughness, mitomycin C (MMC) and stromal hydration on the ablation process and outcomes.

Series 3. High-speed non-stop myopic transepithelial ablation.

In this series, the average power of the femtosecond UV pulses and thus the ablation speed was increased, and the predictability and reproducibility of the ablation of both the epithelium and stroma, as well as healing responses after the high-speed TransPRK were evaluated. The transepithelial version of

PRK was chosen in order not only to reduce the contamination in the cornea, but also to avoid variations in eye treatment prior to the ablation (mechanical stress, dehydration, different shapes of deepithelialization areas) and to detect the potential problems (tissue damage due to heating, surface roughness). For comparison, analogous treatment with excimer pulses was performed.

2.1. Laser systems

Corneal stromal ablation was performed using the fifth harmonic of a solid-state UV femtosecond system based on the "Pharos-SP" laser. The maximum repetition rate was 200 kHz, and the laser could produce 6 W of average power at repetition rates greater than 70 kHz. In the first and second series, the laser was operated at repetition rates of 5 kHz, 20 kHz and 25 kHz. Myopic spherical ablation was performed by removing circular corneal layers of progressively increasing radii. Each layer was removed by passing the surface 4 times avoiding overlapping of ablation spots.

The ablation process was monitored using a visible-range CCD camera (CMLN-13S2M, Point Grey Research, Inc.) under conventional or slit-like illumination with a low-power green laser. An infrared thermal camera (ThermaCAM S65, FLIR Systems, Inc.) was used for the observation of surface heating. Excimer ablation was performed with a commercial excimer system (Technolas 217z100 system, Technolas Perfect Vision GmbH). The main characteristics of both laser systems used in the second series of the experiments are presented in Table 1.

In order to increase the ablation rate of the corneal stroma, in the third series the laser system was modified. First, the average power of the fundamental laser radiation (1030 nm) was increased up to 5 W at 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 in order to reduce the time intervals when the laser is switched off during acceleration. And third, the UV pulse generator was equipped with a beam shaper to achieve near second order super-Gaussian beam profile at the corneal surface. The peak fluence with these laser settings was 135 ± 5 mJ/cm^2. The scanning pattern was also modified for maintaining low local heating and achieving smooth ablation surface at higher pulse repetition rate.

Table 1. Parameters of UV radiation sources for corneal stromal ablation. Excimer parameters obtained from www.fda.gov

	Femtosecond solid-state laser		Excimer laser
Repetition rate	5 kHz	20 kHz	50 Hz
Wavelength	206 nm		193 nm
Pulse duration	200-300 fs		18 ns
Beam profile at corneal surface	Gaussian		Flat top
Beam diameter $1/e^2$	280 ± 2 μm	130 ± 2 μm	2 mm
Mean power after focusing optics	200 ± 5 mW	180 ± 5 mW	235 ± 5 mW*
Peak fluence	0.13 ±0.01 J/cm^2	0.13 ± 0.01 J/cm^2	0.12 J/cm^2
Myopic ablation duration (depth ~30μm, optical zone diameter 5 mm)		11 s	10 s

Means ± standard deviation (SD)

* Measured using optical power meter (Nova II with thermal power sensor 10A, Ophir Optronics, Ltd.)

2.2. Animals and surgery

In the first series, 16 rabbits were treated, the second series included 32 animals, and the third – 33 rabbits.

Procedures performed in the first and second series are presented in tables 2 and 3. For PRK, the central 6.5 mm corneal area was circumscribed by femtosecond UV laser incision and mechanical debridement of the corneal epithelium was carried out. Myopic spherical laser ablation was performed, using femtosecond UV pulses in a 5 mm optical zone with a 0.5 mm transition zone. Conventional excimer PRK was performed without a suction ring using an eye-tracking system. A corneal marker was used, instead of a UV laser, to circumscribe the incision. Central corneal thickness (CCT) was measured using an ultrasound pachymeter (Pocket II, Quantel Medical SA).

To evaluate the predictability of the process and to determine the amount of removed tissue per layer, ablation of a constant area was performed until perforation of the cornea (calibration of the ablation rate).

For slit-like incisions, a laser beam was repeatedly scanned along a straight line. To maintain a similar heat deposition rate, as in the case of 5 mm area ablation, a pause of 0.5 s between the scans was introduced.

Table 2. Procedures performed in the first series of experiments

Procedure	**Number of procedures**
Myopic ablation with femtosecond UV pulses	24
Ablation with hydration	1
Hypermetropic ablation with femtosecond UV pulses	1
Mechanical removal of the epithelium with suction only	1
Myopic ablation with nanosecond UV excimer pulses	4
Calibration of ablation rate	5
Total number of procedures*	**36**

* In three eyes, two procedures with the interval of five months were performed - after the myopic ablation the same eyes were used for calibration. Ablation with hydration was performed in the eye which underwent myopic ablation five months earlier.

Table 3. Procedures performed in the second series of experiments (BSS – balanced salt solution, MMC – mitomycin C)

Procedure	**Number of rabbits**
Ablation rate calibration (both eyes)	3
Ablation with different spot sizes (280 μm and 130 μm) in contralateral eyes	4
Slit-like incision, contralateral eye control	2
Deep vs. shallow ablation in contralateral eyes	7
Femtosecond UV vs. nanosecond UVexcimer pulses in contralateral eyes	9
Surface irregularity*	1
Myopic ablation with hydration*	2
Ablation with MMC vs. BSS*	3
Mechanical removal of the epithelium, contralateral eye control	1
Total number of rabbits	**32**

* see Results section

The third series involved calibration (4 eyes) and three treatment modalities: non-stop TransPRK with femtosecond UV pulses, ablation with excimer pulses, and the modified TransPRK with femtosecond UV pulses:

- Monolateral non-stop TransPRK of ~110 μm in depth for an optical zone of 6 mm with a 0.6 mm transition zone was performed in twenty rabbits. As the published values of rabbit epithelium thickness range form 32.2 to 47.7 μm (Li, Petroll et al. 1997; Jester, Li et al. 1998; Masters 1998; Reiser, Ignacio et al. 2005; Tsiklis, Kymionis et al. 2008; Petroll, Weaver et al. 2013), we estimated, that the 110 μm ablation would include 60-80 μm of the stromal tissue, while myopic ablation of 70 μm central depth within the 6 mm optical zone corresponded to a ~5.0 D refraction change. CCT was measured on a dry surface before and after the ablation.
- For comparison, monolateral transepithelial ablation with excimer pulses was performed in 10 rabbits. The epithelium was removed in 6.6 mm zone using the phototherapeutic keratectomy (PTK) mode, followed by the myopic ablation of 70 μm of the stroma in 6 mm optical zone. This mode was chosen in order to keep the ablation profile close to the one applied in TransPRK with femtosecond UV pulses, 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. 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 performed with pauses. Pachymetry measurements after stromal ablation were possible only after moistening of the probe tip with BSS.
- The modified TransPRK with femtosecond UV pulses was introduced to make better comparisons between the femtosecond and excimer systems, as the excimer ablation depth resulted in 151.4 ± 19.7 μm instead of the expected 110 μm. We modified the femtosecond procedure to match this final depth (150 μm) by setting deeper spherical ablation 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. Five rabbits, previously treated by excimer ablation, underwent the modified TransPRK with femtosecond UV pulses on the previously untreated eyes at 2.5 months after excimer surgery.

- For the evaluation of the surface roughness two eyes of one rabbit after the excimer ablation and three eyes after the modified TransPRK were enucleated immediately after the procedures.

Postoperative slit-lamp biomicroscopy examinations, photography and pachymetry were performed at 1-3 days and then weekly until one month and monthly thereafter up to 6 months. The level of haze in the corneas was graded according to the Fantes (Fantes, Hanna et al. 1990) scale, which scores the highest degree of haze at grade 4.

For the histological examinations, the tissue specimens were stained with hematoxylin and eosin and examined using light microscopy. Transmission electron microscopy was performed on two eyes after the femtosecond UV treatment in the second series.

2.3. Statistical analysis

Statistical analysis of the corneal haze score was performed using the STATISTICA software package, version 8.0 (StatSoft Inc.). The non-parametric Mann-Whitney test (Kwam and Vidakovic, 2007) was used for the reliable comparison of two representative groups with different treatments. A significance level of $p<0.05$ was chosen to determine whether the haze of one group was different from the other. Graphs present mean corneal haze score with standard error of the mean (SEM).

3. RESULTS

3.1. Results of the first and second series of the experiments

Calibration. It was estimated, that 1.25 ± 0.04 μm of corneal tissue per layer was removed. For myopic correction, the achieved ablation speed was 1 D per 3.7 s at a 20 kHz repetition rate within a 5 mm optical zone.

Spot size. We compared the surface quality and haze development up to six months of corneas ablated with different spot sizes (280 μm and 130 μm) and at different pulse repetition rates (5 kHz and 20 kHz). Having observed no detectable differences in terms of post-ablation smoothness and healing response we performed the subsequent experiments using a spot size of 130 μm and a repetition rate of 20 kHz, at which higher average power of the UV radiation could be produced.

Slit-like incisions. To check for possible heat-induced damage at the edges of the processed area, slit-like femtosecond UV ablations were performed on two eyes. Light microscopy of histological sections revealed smooth edges of V-shaped ablation with no evidence of stromal melting (Figure 1).

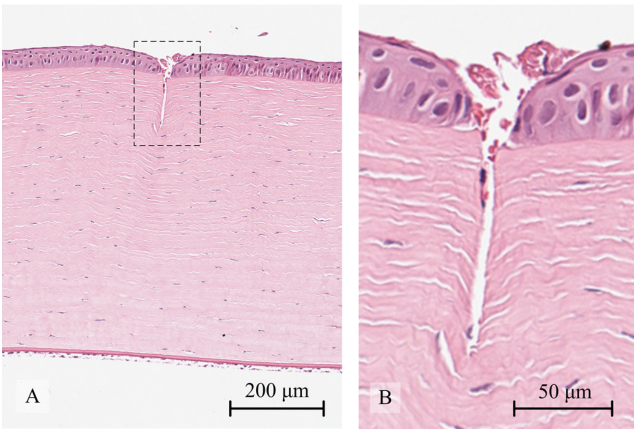

Figure 1. A slit-like incision performed with femtosecond UV laser pulses. Smooth edges, no evidence of thermal damage. Epithelial debris is visible in the interface (light microscopy)

Temperature rise. During femtosecond ablation (30 µm), the maximum measured temperature change was approximately 2 °C at 3 s after the beginning of the procedure (red line in Figure 2). At the beginning of the procedure, only a small area in the center of the cornea was being ablated because of the myopia-correction algorithm of beam scanning. Therefore, heat accumulated in this small area.

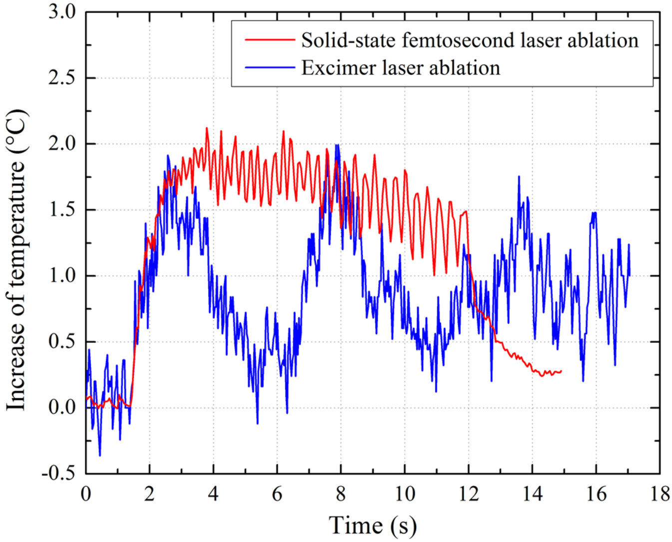

Figure 2. Surface temperature dynamics during the ablation (30 µm) process (20 kHz, 180 mW). According to the manufacturer's protocol, excimer laser was switched off every 5 s for a period of 2.5 s. The measurements were taken at the hottest spot, which was in the center of the ablation

Subepithelial haze measurements and histological examination. Central subepithelial corneal haze was observed to appear beginning at one week after surgery, to peak at approximately 1-2 months and to decrease gradually afterwards over the observation period of six months.

Deep vs. shallow femtosecond ablation. In seven rabbits, deep femtosecond UV ablation (130 µm, ~13.0 D) in one eye and shallow ablation (30 µm, ~2.5 D) in the contralateral eye were performed. In histological samples taken immediately after ablation, relatively smooth ablated surfaces were observed (Figure 3). The deeper ablation produced a greater amount of haze over the entire follow-up of six months.

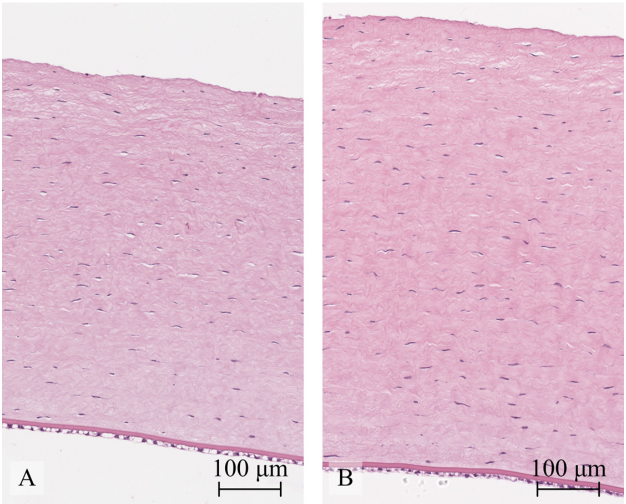

Figure 3. Corneal surface immediately after (A) deep (130 µm) and (B) shallow (30 µm) ablation with femtosecond UV pulses. No damage to deeper stroma is observed (light microscopy)

Femtosecond vs. excimer ablation. Nine rabbits received femtosecond UV ablation (30 µm) in one eye and conventional excimer PRK (30 µm) in the contralateral eye.

On average, the eyes treated by femtosecond UV pulses exhibited slightly less haze at one month than those treated by excimer ablation (1.5 ± 1.1 and 2 ± 1.1, respectively), but the difference was statistically insignificant ($p = 0.05$) (Figure 4). Light microscopy specimens revealed a normal epithelium at one month after the ablation in most specimens, except for several corneas with thinner spots; one specimen exhibited mild epithelial hyperplasia after femtosecond UV ablation. The specimens with corneal haze demonstrated subepithelial fibrous layers, the thickness and structure of which were similar in eyes treated by femtosecond UV pulses vs. excimer ablation.

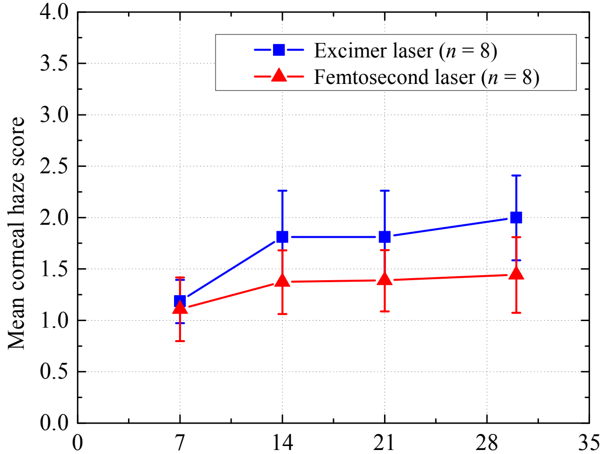

Figure 4. Mean corneal haze (±SEM) score dynamics after ablation performed with femtosecond and excimer lasers, blue and red lines, respectively (n = number of eyes)

Additional experiments. In order to evaluate the healing process under the influence of some factors, we performed the following experiments with femtosecond UV pulses:

- *Surface irregularities.* The left eye underwent PRK of 30 µm, and in the right eye irregular surface was formed: after the standard ablation of 20 µm, the process was carried on forming "pits" of 20 µm depth. Haze at six months was similar in both eyes (0.5). Histological specimens showed minimal epithelial thickness irregularities in the left eye and minimal subepitheliai fibrosis in the right eye.
- *Stromal hydration.* In two rabbits ablation of 130 µm after the mechanical debridement ot the epithelium was performed. In right eyes hydration was applied immediately after the ablation (BSS was instilled for 6 minutes) and in left eyes ablation of 5 µm was peformed, BSS was instilled for 7 minutes, and ablation of 125 µm followed. Normal healing was observed in one eye only. In one case, after prolonged reepithelialization (more than 10 days) grade 4 gaze developed at one month. Two corneas healed with uneven surfaces

and subepithelial edema in histological specimens at 6 months. In two eyes ablation in hydrated corneas until perforation was performed and the change of the corneal thickness was recorded. Ablation of the hydrated cornea was faster, however, the ablation rate of the stromal tissue was lower since the increase of speed was less than the increase in thickness.

- *Ablation with MMC.* In three animals deep ablation (130 µm) with application of 0,02% MMC for 2 minutes and irrigation with 30 mL BSS was performed in one eye, while contralateral eyes received BSS treatment instead. As expected, haze was less pronounced in the eyes treated with MMC.

3.2. Results of the third series of the experiments

Calibration. We were able to determine the exact moment of epithelial debridement by clearly observing the disappearance of the blue fluorescence (Tuft, al-Dhahir et al.1990; Korkmaz, Bilgihan et al 2014) as the ablation procedure progressed (Figure 5). The rate of the epithelial ablation in our study was approximately 0.6 µm per scanning layer.

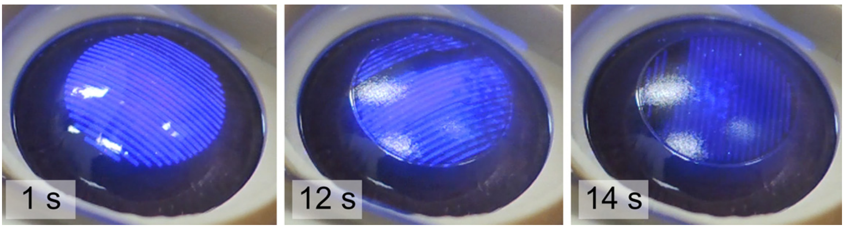

Figure 5. The non-stop transepithelial femtosecond UV ablation procedure. Figure shows the progress of the disappearance of the blue fluorescence as the ablation progresses from the epithelium to the stroma

Ablation depth and central corneal thickness changes. The results of pachymetric CCT measurements and calculated thickness changes are presented in Table 4.

Table 4. Ultrasound pachymetry measurements of the central corneal thickness changes during different ablation modes

	Non-stop TransPRK with femtosecond UV pulses, 110 μm	**Excimer ablation, 150 μm**	**Modified TransPRK with femtosecond UV pulses, 150 μm**
	Mean central corneal thickness, μm ± SD		
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± SD		
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± SD		
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 regeneration, μm± SD		
One week after ablation (D4-D3)	34.9 ± 20.5	30.6 ± 19.5	28.8 ± 13.9

We have found that the mean variation of the epithelial thickness within the ablation zone of 6 mm in diameter for each individual rabbit was ~12% of the maximal thickness (4-6 μm). Pachymetry immediately after stromal ablation revealed high reproducibility (Table 4) and in all cases was possible without moistening.

The attempted total ablation depth of 110 ± 15 μm was achieved in 90% of eyes treated with high-speed non-stop femtosecond UV ablation. 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).

Ablation speed. It was estimated that, with the high-speed femtosecond UV laser system, ~280 pL of corneal tissue per 1 mJ could be removed. 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 (Niemz 2007) after reepithelialization, and resulted in speed values of ~1.45 s/D for the non-stop procedure, and ~1.62 s/D for the modified procedure.

Temperature rise. The temperature increases across the different treatments are presented in Figure 6. The temporal dependence of the surface temperature was directly related to the laser beam scanning algorithm. The lowest corneal surface temperature was registered during the modified femtosecond TransPRK.

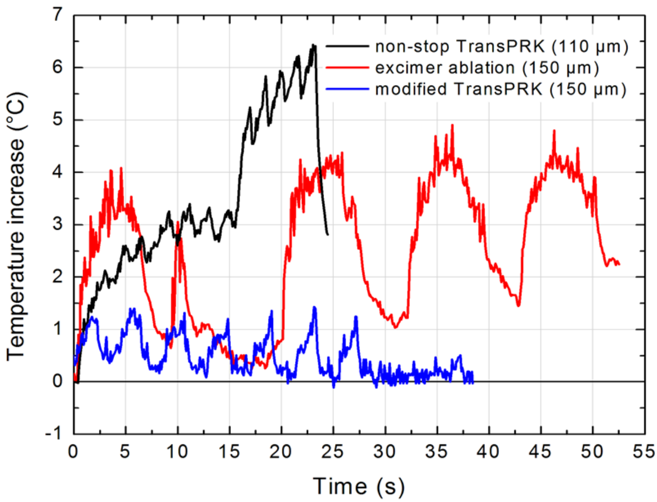

Figure 6. Corneal surface temperature changes during the high-speed transepithelial ablation process

Subepithelial haze measurements. Figure 7 shows the development of haze after different transepithelial treatments. No statistically significant difference was found in the data on the corneal haze score after non-stop TransPRK with femtosecond UV pulses and excimer laser ablation during the follow-up period of one month. The least intense haze was observed after the modified femtosecond UV treatment.

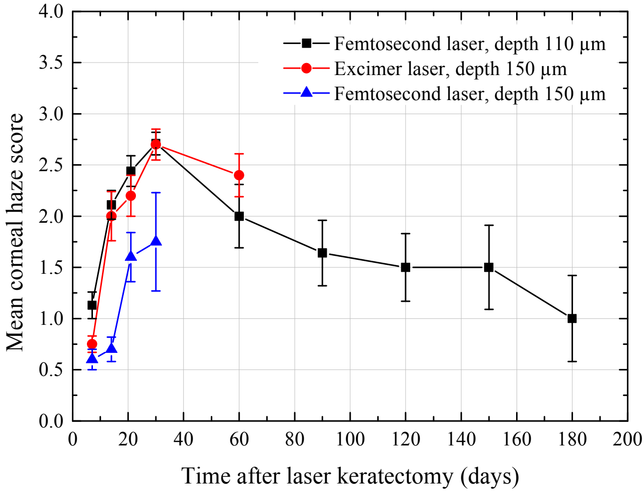

Figure 7. Mean corneal central subepithelial haze (±SEM) score dynamics after different transepithelial treatments

Histological examination. In samples taken immediately after ablation, relatively smooth ablated surfaces were observed. At one month, all corneas after a femtosecond UV ablation of 110 µm exhibited normal epithelial thickness, with minor variations in smoothing out the stroma (Figure 8). 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.

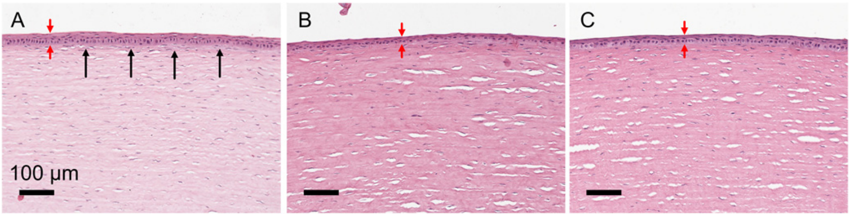

Fig 8. One month after (A) the non-stop TransPRK with high-speed femtosecond UV pulses (110 µm), (B) excimer pulses (150 µm), and (C) modified ablation with femtosecond UV pulses (150 µm). The epithelium is thinner in (B) and (C) (red arrowheads). Black arrowheads indicate subepithelial fibrosis (light microscopy)

4. DISCUSSION

4.1. Predictability and reproducibility of the ablation. Stromal surface quality after the procedure

Our findings show that, with femtosecond UV pulses, the ablation is accurate and highly reproducible. 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. 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.

Light microscopy of the histological samples revealed similar surface roughness after excimer and femtosecond UV treatments. The size of the spots used in our experiments (~115 μm and ~130 μm) appears to be excessively small for achieving even the best possible spatial resolution required for custom ablation (Huang and Arif 2002; Guirao, Williams et al. 2003). 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 (Bueeler and Mrochen 2005). However, the application of small-diameter beams in the clinical practice requires an adequate eye-tracking system or new methods of the beam scanning and eye-movement compensation, which may be developed.

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. In contrast, this measurement was possible on eyes ablated with femtosecond pulses, even if pauses were introduced to prolong the procedure time. We speculate that, while the longer 206 nm wavelength used in our study was less absorbed by BSS than the 193 nm (Dair, Ashman et al. 2001), the evaporation of the fluids from the surface was decreased. 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.

4.2. Temperature increase

In micromachining, ablation by femtosecond pulses is referred to as cold ablation (Steen and Mazumder, 2010). Ultrashort pulses have an obvious advantage in cases of highly transparent or reflecting materials in which multiphoton absorption plays a key role. During PRK after mechanical removal of the epithelium, we observed similar heating of the corneas using either UV (femtosecond or excimer) laser, which might indicate that in cases of high absorption and relatively low fluence, there might be only minor differences between femtosecond and nanosecond pulses in this regard. In these cases, the beam of the femtosecond laser had a near Gaussian profile, the laser operated at the repetition rate of 20 kHz, and the average power of the UV radiation was 200 mW.

For the high-speed non-stop TransPRK the higher average power (400 mW) was applied which caused the increase of the surface temperature up to 6,5°. The ablation was more efficient due to the super-Gaussian beam intensity distribution with relatively less power in subthreshold wings, but the temperature rise during this procedure was the highest.

We see several possibilities to reduce the peak temperature along with the use of a high repetition rate laser. The simplest of these is the introduction of brief pauses at the final stage of corneal shaping, which would cause only minor increases in the overall procedure time. The modified TransPRK with pauses, which was introduced in our study to reproduce the excimer ablation, appeared to cause the lowest temperature rise. 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 (Aslanides, Padroni et al. 2012; Aslanides, Georgoudis et al. 2015). 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 (Arba-Mosquera and Hollerbach, 2010). In addition to the optimization of the laser and scanning parameters, additional means, such as cooling the cornea prior to ablation by chilled BSS (Kitazawa, Maekawa et al. 1999), may also be applied.

In our 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. It is quite obvious that the temperature increases would be significantly less if standard PRK or LASIK procedures, rather than transepithelial techniques, were used for the same depth of corneal ablation. The need for temperature control may arise only if large refractive errors are to be corrected.

4.3. Ablation speed

In the first and second series of the experiments, we maintained the peak fluence and average power of the UV beam close to those of the excimer system we had at our disposal. As a result, the ablation was relatively slow, at 3.7 seconds per diopter (s/D), which is well below the industry standards currently accepted.

Faster ablation speed is considered an advantage due to several factors. It has been proven that corneal hydration affects the excimer laser ablation rate (Dougherty, Wellish et al. 1994) and, consequently, refractive outcomes. A shorter duration of the procedure is more comfortable for patients and surgeons, and the risk of undesired eye movements is smaller (Khoramnia, Salgado et al 2012). Manufacturers of excimer systems compete in technological advances enabling high-speed ablation. The treatment time of the fastest systems is 1.2-1.4 s/D (Khoramnia, Salgado et al. 2012; www.alconsurgical.com; www.eye-tech-solutions.com). In our study, after upgrading the femtosecond laser system, the myopic corneal ablation speed in rabbit corneas increased by nearly a factor of four. The achieved speed of ~1.6 s/D in a 6 mm optical zone for myopic ablation is comparable to the fastest reported ablation speed in human corneas.

4.4. Healing response

Our experiments have shown that, despite large differences in peak intensity, spot size and repetition rate, healing of the rabbit corneas after femtosecond UV stromal ablation was very similar to that after excimer ablation. In general, the course of healing followed very closely that of a rabbit model described in published studies (Marshall, Trokel et al. 1988; Gaster, Binder et al. 1989; Tuft, Zabel et al. 1989; Hanna, Poliquen et al. 1989; McCally, Connolly et al. 2006; Netto, Mohan et al. 2006).

There is great variability among rabbits in the corneal healing response after refractive surgery in both eyes of the same animal (McCally, Connolly et al. 2006). In our case, the scatter of the haze density between individual rabbits far exceeded the differences between the eyes of the same animal ablated with different laser sources.

Healing in the rabbit model is considered to be similar to that in the human clinical situation, although the subepithelial haze is denser and occurs more frequently and rapidly in rabbits (McCally, Connolly et al. 2006). When comparing subepithelial haze intensity after the ablation of the average power of 200 mW and depth of 30 μm, we found no significant differences in haze formation between eyes treated by femtosecond UV pulses and eyes treated by excimer PRK.

In order to achieve higher ablation speed, the higher average power (400 mW) was applied. Quite inevitably, during the non-stop procedure it led to a temperature increase at the surface of the cornea, which was assumed 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. Although the temperature did not exceed a safe level of 7°C (Shraiki and Arba-Mosquera 2011), the rabbits after TransPRK treatment with the excimer laser developed less haze if the values were weighed against the thickness of removed cornea. Certain factors, such as different groups of rabbits or conditions in the operating room, could have affected this result; however, the influence of the rise in temperature cannot be excluded. It is interesting that the lowest degree of haze developed in the corneas that were treated with the deeper (150 μm) transepithelial femtosecond UV ablation with pauses. One of the possible causes could be the reduced temperature, which was even lower than in the excimer cases, however, the sample size (5 rabbits) precludes making definite conclusions.

It must be noted that femtosecond UV pulses deliver much higher intensity (about 10000 times) 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 (Van Saarloos and Rodger 2007). However, the effect of the shorter pulse duration and higher intensity of our laser remains a subject of a separate study.

4.5. Possible applications of the femtosecond laser with harmonic generator

Our experiments on rabbits show the possibility of the clinical application of the UV femtosecond laser for corneal ablation. The outcomes of the surgeries performed are comparable with the results obtained in rabbits using nanosecond excimer lasers as UV sources. Although modern sophisticated nanosecond systems enable highly precise surface refractive treatments, they are designed for UV treatment only. The ability to switch femtosecond laser harmonics seamlessly allows for fast changeover from UV to infrared radiation, implying that a wide range of ophthalmic procedures could be performed using a single solid-state laser device. We believe that the same laser could also be used for advanced intrastromal procedures that require only infrared femtosecond pulses as well as for femtosecond laser-assisted cataract surgery.

5. CONCLUSIONS

1. Corneal ablation with femtosecond UV pulses in rabbits was efficiently performed according to the programmed profile, the surface was smooth and the depth reproducible.
2. The tempeature rise of the cornea during femtosecond ablation can be contained within safe limits even with relatively high average power. When applying the same time pattern as in the reference excimer procedure, the surface temperature is lower, and stroma less dehidrated in the case of femtosecond pulses.
3. With the increased average power (400 mW) of the femtosecond UV pulses, the speed of high fidelity myopic ablation of ~1,6 s /D in 6 mm optical zone was achieved, which is close to the performance of modern excimer laser based systems.
4. Despite huge difference in peak intensity, the healing process and subepithelial haze formation was similar between eyes ablated with femtosecond UV and excimer lasers. There were no significant differences in histological specimens as well.
5. Experiments show that a femtosecond solid-state laser is capable of replacing nanosecond excimer laser in the corneal refractive surgery. The possibility to perform both LASIK stages using a single solid-state device is especially appealing.

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7. PUBLICATIONS AND CONFERENCE PRESENTATIONS

Publications

1. **Danieliene E.**, Gabryte E., Danielius R., Vengris M., Vaiceliunaite A., Morkunas V., Ruksenas O. Corneal stromal ablation with femtosecond ultraviolet pulses in rabbits. J. Cataract Refract. Surg. 39**,** 258-267 (2013).
2. **Danieliene E.**, E Gabryte E., Vengris M., Ruksenas O., Gutauskas A., Morkunas V., Danielius R. High-speed photorefractive keratectomy with femtosecond ultraviolet pulses. J. Biomed. Opt. 20(5), 051037 (2015). doi:10.1117/1.JBO.20.5.051037
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Conference presentations

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8. AUTHOR'S PROFESSIONAL AND SCIENTIFIC BIOGRAPHY

Eglė Danielienė is an ophthalmologist in a group private practice in Vilnius, and a resident manager at the Faculty of Medicine at the Ear, nose, throat and eye diseases clinic. She graduated from the Medical Faculty of Vilnius in 1983 cum laude. Starting as a medical student, she was involved in research on laser retinal therapies. The scientific period was interrupted by a career as a clinical ophthalmologist until recent years. E. Danielienė is a member of the European Strabismology Association and an international member of the American Academy of Ophthalmology. She is an elected member of the board of the Lithuanian Eye Doctors' Association and a frequent lecturer at the meetings of the Lithuanian ophthalmologists. She has attended more than 60 national and international courses and conferences. The most important postgraduate training abroad took place at the Oslo Ulleval hospital (Norway), the New York Eye and Ear Infirmary (USA) and Berlin Charite Hospital (Germany). At present E.Danieliene's areas of interest are corneal laser refractive surgery, pediatric refractive errors and strabismus.

9. SUMMARY IN LITHUANIAN

Santrumpos

D	— dioptrija
DNR	— dezoksiribonukleorūgštis
FRK	— fotorefrakcinė keratektomija
fs	— femtosekundė (10^{-15} s)
Hz	— hercas
IR	— infraraudonoji (spinduliuotė)
J	— džaulis
Yb:KGW	— iterbiu legiruotas kalio gadolinio volframatas
LASIK	— lazerinė *in situ* keratomilezė
MHz	— megahercas (10^{6} Hz)
MMC	— mitomicinas C
nm	— nanometras (10^{-9} m)
RCS	— ragenos centrinės dalies storis
ReLEx	— refrakcinė lentikulo ekstrakcija
SBT	— subalansuotas druskų tirpalas
TransFRK	— transepitelinė fotorefrakcinė keratektomija
UV	— ultravioletinė (spinduliuotė)
µm	— mikrometras (10^{-6} m)
W	— vatas

Įvadas

Dažniausia refrakcinė operacija yra lazerinė *in situ* keratomilezė (LASIK) (Duffey and Leaming 2005; Reinstein, Archer et al. 2012). Procedūra, kai ragenos lopelis atidalijamas femtosekundiniu lazeriu, veikiančiu artimajame infraraudonajame (IR) diapazone, o stroma abliuojama nanosekundiniu (dažniausiai eksimeriniu) ultravioletiniu (UV) lazeriu, vadinama femto-LASIK.

Lazerine sistema „Pharos“, sukurta UAB „Šviesos konversija“ (Lietuva), dirbant iki ~1 MHz pasikartojimo dažniu, kai bangos ilgis 1030 nanometrų (nm), galima gauti pakankamos energijos impulsus ragenos intrastrominei fotodisrupcijai, o sumažinus pasikartojimo dažnį iki 5–50 kilohercų (kHz) ir naudojant harmonikų generatorių, galima generuoti santykinai didelės energijos ~200 nm bangos ilgio UV impulsus, tinkamus fotoabliacijai. Tai leidžia vieną lazerinę sistemą panaudoti abiem LASIK etapams, t. y. visą operaciją atlikti vienu femtosekundiniu prietaisu, užuot naudojus du.

Miclea ir kt. ((Miclea, Skrzypczak et al. 2011) sėkmingai panaudojo lazerinį prietaisą „Pharos" ragenos stromos lopeliams atpjauti ir intrastrominiams iki 30 μm storio lentikulams formuoti *ex vivo* kiaulių akyse. Antrasis LASIK etapas, t. y. stromos abliacija, lazerio „Pharos" prototipu su penktosios harmonikos generatoriumi buvo atliktas polimetilmetakrilate, želatinoje ir šviežiai enukleuotose kiaulės akyse (Vengris, Gabryte et al. 2010). Šiose medžiagose, formuojant miopinius ir hipermetropinius lęšius, abliacija buvo greita, tiksli ir prognozuojama. Gyvųjų audinių abliacijai iki šiol femtosekundiniai UV impulsai nebuvo naudojami, todėl buvo neaišku, kaip šie audiniai reaguotų į labai trumpus nepaprastai didelio intensyvumo ir mažo dėmės dydžio impulsus.

Darbo tikslas

Optimizuoti ir charakterizuoti inovatyvų ragenų abliacijos kietojo kūno lazerio didelio pasikartojimo dažnio femtosekundiniais UV (bangos ilgis 206 nm) impulsais metodą *in vivo*.

Darbo uždaviniai

1. Nustatyti femtosekundinių UV impulsų parametrus, tinkamus triušių ragenų abliacijai *in vivo.*
2. Palyginti miopinės abliacijos femtosekundiniais UV ir nanosekundiniais UV eksimerinio lazerio impulsais (bangos ilgis 193 nm) ypatybes: paviršiaus temperatūros kitimą ir paviršiaus audinių džiūvimą, stromos paviršiaus lygumą po procedūros.
3. Ištirti greitos abliacijos femtosekundiniais UV impulsais ypatumus, atliekant miopinę transepitelinę fotorefrakcinę keratektomiją (Trans-FRK).

4. Palyginti ragenos gijimo procesą ir jo baigtis po abliacijos femtosekundiniais UV impulsais ir nanosekundiniais UV eksimerinio lazerio impulsais, vertinant subepitelinės miglos vystymąsi ir laipsnį, histologinius pakitimus.

Ginamieji teiginiai

1. Ragenos abliacija femtosekundiniais UV impulsais yra efektyvi ir atkartojama, todėl femtosekundiniai kietojo kūno lazeriai refrakcinėje chirurgijoje galėtų pakeisti šiuo metu naudojamus nanosekundinius.
2. Abliacijos femtosekundiniais UV impulsais kokybė, palyginti su analogiška procedūra nanosekundiniais UV eksimeriniais impulsais, nėra blogesnė vertinant abliacijos atkartojamumą, stromos paviršiaus džiūvimą, paviršiaus kaitimą ir lygumą po procedūros.
3. Nors esama didelių impulsų intensyvumo ir pasikartojimo dažnio skirtumų, gijimas ir baigtys po abliacijos femtosekundiniais UV impulsais ir nanosekundiniais UV eksimerinio lazerio impulsais yra panašūs.

Mokslinio darbo naujumas

Šiame darbe pirmą kartą, mūsų žiniomis, *in vivo* ragenos abliacijai panaudoti femtosekundiniai didelio pasikartojimo dažnio ir mažo dėmės dydžio UV impulsai. Femtosekundiniais UV impulsais *in vivo* buvo atliktos FRK ir miopinė TransFRK. Atliekant FRK, mechaniškai šalintino epitelio skersmuo buvo apibrėžiamas lazeriu. Padidinus UV spinduliuotės galią iki 400 mW ir pakeitus abliacijos profilį iš Gauso į supergausinį, buvo pasiektas abliacijos greitis, atitinkantis šiuolaikinių eksimerinių sistemų greitį, abliuojant tik tikslios abliacijos režimu. Pirmą kartą buvo palyginta ir nustatyta, kad abliuojant femtosekundiniais UV impulsais gijimo baigtys nėra blogesnės, nei abliuojant nanosekundiniais UV eksimerinio lazerio impulsais.

Praktinė darbo reikšmė

Femtosekundiniais UV impulsais galima atlikti ragenos abliaciją, todėl viena lazerine sistema būtų galima atlikti abu LASIK etapus. Klinikinėje praktikoje įdiegta ši procedūra turėtų ne tik kietojo kūno lazeriui būdingų pranašumų, palyginti su dujiniu, tačiau ir dar kelis pranašumus prieš dabar naudojamas eksimerines sistemas, kurių svarbiausi yra šie:

- Dėl mažesnio dėmės dydžio didelio pasikartojimo dažnio impulsais galėtų būti formuojami ypač tikslūs abliacijos profiliai.
- 206 nm bangos ilgio UV impulsus mažiau sugeria vanduo ir subalansuotas druskų tirpalas (SBT), todėl abliacija turėtų mažiau skirtis skirtingo hidracijos lygio ragenose. Ne tokia svarbi tampa aplinkos drėgmė.
- Vietoj mechaninio žymeklio deepitelizacijos plotą FRK operacijoms būtų galima apibrėžti lazeriu.
- Operacija yra beveik negirdima.
- Integravimas padidintų operacijų greitį ir patogumą. Universali sistema užimtų mažiau vietos operacinėje, leistų sumažinti instaliacijos ir priežiūros išlaidas.
- Manome, kad tą patį daugiafunkcį prietaisą būtų galima panaudoti ir kitoms femtosekundiniu lazeriu atliekamoms procedūroms, pavyzdžiui, refrakcinei lentikulo ekstrakcijai (ReLEx), femtosekundinei kataraktos chirurgijai ir pan.

Tyrimo medžiaga ir metodai

Buvo gauti Valstybinės maisto ir veterinarijos tarnybos leidimai Nr. 0180, Nr. 0213 eksperimentams su triušiais. Atlikome tris eksperimentų serijas su 81 tiriamuoju gyvūnu:

- pirmoji buvo skirta triušio ragenos abliacijos femtosekundiniais UV impulsais operacijos ir baigčių vertinimo protokolui parengti (16 triušių),
- antroji – FRK femtosekundiniais UV impulsais įvertinti ir palyginti su FRK nanosekundiniais UV eksimerinio lazerio impulsais (32 triušiai),
- trečioji – greitai transepitelinei abliacijai optimizuotais didesnės galios femtosekundiniais UV impulsais įvertinti ir palyginti su transepiteline abliacija nanosekundiniais UV eksimerinio lazerio impulsais (33 triušiai).

Pirmosios ir antrosios serijų procedūros parodytos pirmoje ir antroje lentelėse.

1 lentelė. Pirmosios eksperimentų serijos procedūros. Operuotos abi akys

Procedūra	**Procedūrų skaičius**
Miopinė abliacija femtosekundiniais UV impulsais	24*
Abliacija su hidracija	1
Hipermetropinė transepitelinė abliacija femtosekundiniais UV impulsais	1
Mechaninis epitelio pašalinimas be abliacijos	1
Miopinė abliacija nanosekundiniais UV eksimerinio lazerio impulsais	4
Abliacijos našumo kalibracija	5
Iš viso	**36**

* Trijose akyse buvo atlikta miopinė abliacija femtosekundiniais UV impulsais, o po 5 mėnesių tose pačiose akyse atlikta abliacijos našumo kalibracija. Vienoje akyje praėjus 5 mėnesiams po femtosekundinės abliacijos buvo atlikti hidracijos eksperimentai.

2 lentelė. Antrosios serijos procedūros (MMC – mitomicinas C, SBT – subalansuotas druskų tirpalas)

Procedūra	**Triušių skaičius**
Kalibracija (abi akys)	3
Mažos (130 μm) ir didelės (280 μm) dėmės kontralateralinėse akyse	4
Plyšio pavidalo abliacija, kita akis kontrolinė	2
Gili (130 μm) ir negili (30 μm) abliacija kontralateralinėse akyse	7
Femtosekundiniai UV ir nanosekundiniai UV eksimeriniai impulsai kontralateralinėse akyse (30 μm)	9
Paviršiaus lygumas	1
Miopinė abliacija su hidracija	2
Abliacija su MMC, kita akis su SBT (130 μm)	3
Kontrolinis (vienoje akyje mechaniškai pašalintas epitelis)	1
Iš viso	**32**

Trečiojoje serijoje atlikta abliacijos našumo kalibracija (4 akyse) ir trijų tipų ragenos abliacijos: nepertraukiama TransFRK femtosekundiniais UV impulsais (110 μm) (20 akių), transepitelinė abliacija nanosekundiniais UV eksimeriniais impulsais (10 akių) ir modifikuota TransFRK femtosekundiniais UV impulsais (150 μm) su pauzėmis (5 akys). Penkios akys (2 po eksimerinės ir 3 po modifikuotos TransFRK) paaukotos paviršiaus lygumo histologiniams tyrimams.

Abliacijos eiga buvo stebima naudojant vaizdo kamerą (CMLN-13S2M, Point Grey Research, Inc.), pritaikius įprastinį arba plyšio pavidalo apšvietimą mažos galios žaliu lazeriu. Ragenos paviršiaus temperatūros pokyčiams registruoti buvo naudojama infraraudonųjų spindulių kamera (Thermacam S65, FLIR Systems, Inc.).

Gyvūnai buvo stebimi iki 6 mėnesių. Buvo atliekama pachimetrija (Pocket II, Quantel Medical SA), ragenos buvo fotografuojamos, subepitelinės miglos laipsnis buvo vertinamas pagal Fanteso ir kt. skalę. Histologiniams tyrimams audiniai buvo dažomi hematoksilinu ir eozinu, preparatai buvo tiriami šviesos mikroskopu.

Subepitelinės miglos statistinė analizė buvo atlikta, naudojant „Statistica“ programinę įrangą (versija 8.0, Statsoft, Inc.). Skirtingai abliuotų imčių palyginimui taikytas neparametrinis Mann–Whitney testas. P reikšmė $<0{,}05$ buvo pasirinkta siekiant nustatyti, ar šiose grupėse subepitelinė migla skyrėsi.

Lazerinė sistema. Ragenos abliacija buvo atliekama naudojant kietojo kūno lazerio „Pharos-SP“ Yb:KGW pagrindu sukurtą ir trumpiems femtosekundiniams UV impulsams optimizuotą sistemą. Antrosios serijos eksperimentuose buvo pasirinktas energijos tankis ($0{,}13\pm0{,}01$ J/cm^2), atitinkantis eksimerinio lazerio energijos tankį (0,12 J/cm^2). Impulsų pasikartojimo dažnis buvo 5 ir 20 kHz, impulso trukmė 200-300 fs, dėmės skersmuo 130 ir 280 μm. Trečiosios serijos eksperimentuose, siekiant pagreitinti abliacijos procesą, vidutinė UV spinduliuotės galia nuo 200 mW buvo padidinta iki 400 mW, vietoje Gauso buvo suformuotas supergausinis impulso profilis ir buvo pakeistas skenavimo algoritmas. Naudoto pluoštelio skersmuo buvo

115 μm.

Eksimerinio lazerio „Technolas 217z100“ (Technolas Perfect Vision GmbH) impulsų pasikartojimo dažnis buvo 50 Hz, impulso trukmė 18 ns, dėmės skersmuo 2 mm.

Rezultatai

Atlikome skirtingų dėmių dydžių (130 μm ir 280 μm) FRK, skirtingų gylių (130 μm ir 30 μm) FRK, FRK su MMC, dviejų tipų TransFRK (nepertraukiamą 110 μm ir modifikuotą150 μm su pauzėmis). Palyginimui, eksimeriniu lazeriu atlikome FRK (30 μm) ir TransFRK (150 μm).

Abliacijos efektyvumas ir atkartojamumas. Paviršiaus lygumas. Abliacijos femtosekundiniais UV impulsais našumas buvo stabilus, t.y. pašalintų audinių kiekis per laiko vienetą nesikeitė ir ragenos storis laike kito pagal tiesinę funkciją. Atkartojami buvo visi mūsų taikyti femtosekundinės abliacijos variantai, įskaitant transepitelinį. Iš pachimetrijos matavimų, atliktų po stromos abliacijos, matyti, kad nepertraukiamos TransFRK femtosekundiniais impulsais abliacijos gylis 90% atvejų buvo 110±15 μm, t. y. atitiko planuotą.

Stromos lygumą po abliacijos vertinome šviesos mikroskopijos preparatuose. Iškart po abliacijos (FRK ir TransFRK) paruoštuose histologiniuose preparatuose skirtingais impulsais (femtosekundiniais ir nanosekundiniais eksimeriniais) paveiktų ragenų stromos paviršius atrodė panašaus lygumo. Nebuvo matyti skirtumo tarp paviršiaus lygumo po gilios ir negilios abliacijų.

Paviršiaus temperatūros kitimas. Atlikę plyšio pavidalo įpjovas, histologiniuose preparatuose nudegimo požymių nenustatėme.

Kai femtosekundinio lazerio pluoštelis buvo beveik gausinio profilio, didelė impulso energijos dalis kaupėsi pluoštelio kraštuose, ir ši ikislenkstinė energija nebuvo panaudojama abliacijai, o tik kaitino. Pakeitus pluoštelio profilį į supergausinį, daugiau energijos teko abliacijai, ji tapo efektyvesnė, o ragenos kaitimas sumažėjo.

Ragenos labiausiai kaito (iki 7 °C) nepertraukiamos TransFRK femtosekundiniais UV impulsais metu. Mūsų naudotu eksimeriniu lazeriu abliacija pagal gamintojų nurodymus buvo atliekama su pauzėmis, todėl maksimalus temperatūros pakilimas buvo žemesnis (~4,5 °C). Atlikdami modifikuotą TransFRK femtosekundiniais UV impulsais su pauzėmis nustatėme, kad temperatūra pakilo mažiau (~3 °C), nei abliuojant eksimeriniu lazeriu Akivaizdu, kad temperatūra pakiltų mažiau atliekant ne TransFRK, o to paties gylio standartinę FRK ar LASIK. Be to, temperatūros pakilimas gali būti reikšmingas tik koreguojant didesnes refrakcijos ydas, kai abliacija užtrunka ilgiau.

Audinių hidracija ir džiūvimas. Ragenos labiau džiūvo abliuojant eksimeriniu lazeriu. Po abliacijos eksimeriniu lazeriu stromos paviršius buvo sausesnis nei po TransFRK femtosekundiniais UV impulsais, įskaitant ir gerokai ilgesnę modifikuotą procedūrą. Po abliacijos femtosekundiniais UV impulsais pachimetriją atlikti buvo paprasta, o po abliacijos nanosekundiniais UV eksimeriniais impulsais dėl sauso paviršiaus ragenos storį pachimetru buvo įmanoma pamatuoti tik sudrėkinus daviklio viršūnę. Mūsų nuomone, džiūvimo skirtumus labiausiai lemia skirtingi lazerių bangų ilgiai. Femtosekundinių UV impulsų bangos ilgio (206 nm) spinduliuotę dėl ilgesnio bangos ilgio SBT turėtų sugerti mažiau nei eksimerinio lazerio, todėl aplinkos ir paviršiaus drėgmė turėtų mažiau veikti abliacijos našumą.

Abliacijos greitis. Viena trumparegystės dioptrija, kai impulsų vidutinė galia buvo 180-200 mW ir optinė zona 5 mm, buvo pašalinama per 3,7 s. Padidinus impulsų vidutinę galią iki 400 mW, viena dioptrija 6 mm optinėje zonoje buvo pašalinama per ~1,6 s.

Gijimo baigtys. Po gilios (130 μm) abliacijos dviejų skersmenų (130 ir 280 μm) dėmėmis nenustatę skirtumų tarp paviršių lygumo, gijimo eigos ir baigčių abiem būdais operuotose akyse, tolesniems eksperimentams pasirinkome 130 μm dydžio dėmes su 20 kHz pasikartojimo dažniu.

Po FRK gijimas buvo panašus kontralateralinėse akyse, operuotose skirtingais lazeriais (femtosekundiniu ir eksimeriniu). Nors šiose akyse buvo atlikta negili abliacija (30 μm), tačiau visose išsivystė subepitelinė migla. Ji buvo kiek mažiau intensyvi femtosekundiniais UV impulsais abliuotose ragenose (vidutinė subepitelinė migla atitinkamai 1,5±1,1 ir 2,0±1,1), bet skirtumas buvo statistiškai nepatikimas (p=0,05). Ragenose su ryškesne subepiteline migla susidarė subepitelinės fibrozės sluoksniai, kurių storis ir struktūra buvo panašūs akyse, operuotose skirtingais lazeriais.

Vertinant transepitelinių abliacijų baigtis, silpniausia subepitelinė migla buvo stebėta po modifikuotos TransFRK femtosekundiniais impulsais. Ji buvo net silpnesnė nei po seklesnės nepertraukiamos TransFRK abliacijos femtosekundiniais impulsais. Lyginant subepitelinės miglos laipsnį to paties triušio kontralateralinėse akyse, jis praėjus savaitei ir praėjus mėnesiui buvo mažesnis po modifikuotos TransFRK femtosekundiniais impulsais nei po transepitelinės abliacijos eksimeriniu lazeriu. Skirtumas statistiškai reikšmingas (atitinkamai p = 0,0135 ir p = 0,0415), tačiau reikia atsižvelgti į tai, kad šioje grupėje buvo tik 5 triušiai. Nerasta statistiškai patikimo subepitelinės miglos laipsnio skirtumo tarp akių po nepertraukiamos TransFRK femtosekundiniais UV impulsais ir transepitelinės abliacijos eksimeriniu lazeriu (p>0,05). Reikia pažymėti, kad didesni gijimo skirtumai buvo stebėti tarp individualių triušių negu tarp to paties triušio akių.

Prieš naudojant femtosekundinius UV impulsus klinikinėje praktikoje, būtina ištirti jų mutageninį poveikį. Dezoksiribonukleorūgštis (DNR) stipriausiai absorbuoja 240–270 nm bangos ilgio UV spindulius, taigi mūsų naudotas gerokai trumpesnis bangos ilgis šia prasme nepavojingas, tačiau femtosekundinių UV impulsų intensyvumas yra apie 10 000 kartų didesnis už eksimerinio lazerio, todėl reikėtų atlikti neplaninės DNR sintezės tyrimus.

Išvados

1. Triušio ragenų abliacija femtosekundiniais UV impulsais vyko pagal suprogramuotą profilį efektyviai ir tolygiai, gylis buvo atkartojamas, stromos paviršius buvo lygus.

2. Temperatūra net abliacijos didelės galios impulsais metu neviršijo saugaus lygio. Abliuojant femtosekundiniais UV impulsais su pauzėmis, paviršius kaito mažiau, nei abliuojant nanosekundiniais UV eksimerinio lazerio impulsais. Po abliacijos femtosekundiniais UV impulsais stromos

paviršius buvo mažiau išdžiūvęs.

3. Atliekant miopinę transepitelinę abliaciją, kai femtosekundinių UV impulsų galia buvo 400 mW ir impulsų pasikartojmo dažnis 50 kHz, abliacijos greitis 6 mm optinėje zonoje pasiekė ~1,6 s/D dirbant tik tikslios abliacijos režimu. Šis greitis yra artimas modernių eksimerinių sistemų greičiui.
4. Nepaisant didelių spinduliavimo intensyvumo skirtumų, ragenų gijimas bei subepitelinės miglos vystymasis vyko panašiai kaip po abliacijos eksimeriniu lazeriu. Histologiniuose preparatuose esminių skirtumų nebuvo pastebėta.
5. Mūsų eksperimentai leidžia teigti, kad femtosekundinis kietojo kūno lazeris galėtų pakeisti nanosekundinį dujinį eksimerinį lazerį ragenos lazerinėje refrakcinėje chirurgijoje. Tai būtų ypač naudinga LASIK procedūroje, kur abu operacijos etapus būtų galima atlikti viena lazerine sistema.