Graefe's Arch Clin Exp Ophthalmol
(2003) 241:511–517

LABORATORY INVESTIGATION

DOI 10.1007/s00417-003-0683-z

Alexander Heisterkamp
Thanongsak Mamom
Omid Kermani
Wolfgang Drommer
Herbert Welling
Wolfgang Ertmer
Holger Lubatschowski

Intrastromal refractive surgery with ultrashort laser pulses: in vivo study on the rabbit eye

Received: 23 April 2002
Revised: 18 March 2003
Accepted: 20 March 2003
Published online: 14 May 2003

A. Heisterkamp (✉) · H. Welling
W. Ertmer · H. Lubatschowski
Laser Zentrum Hannover e.V.,
Hollerithallee 8, 30419 Hannover,
Germany
e-mail: ah@lzh.de
Tel.: +49-511-2788228
Fax: +49-511-2788100

T. Mamom · W. Drommer
Institut für Pathologie,
Tierärztliche Hochschule Hannover,
Hannover, Germany

O. Kermani
Laserforum Köln e.V.,
Cologne, Germany

Abstract *Background:* Femtosecond (fs) laser pulses may offer new possibilities in the field of refractive surgery, especially when using the laser as a microkeratome. By induction of nonlinear absorption processes the laser can be used to perform intrastromal cuts. The conventional microkeratome, associated with numerous potential side effects, can possibly be replaced. Furthermore, refractive lenticules can be prepared within the stroma and removed in a single-step operation. *Methods:* In 10 rabbits, cuts were made to create both a lamellar flap and an intrastromal refractive lenticule. The flap was lifted, the lenticule was extracted and, finally, the flap was repositioned (intrastromal laser keratomileusis, ILK). The corneal samples were collected up to 120 days after treatment and processed for histopathological analysis. *Results:* All flaps could be opened and prepared lenticules could be extracted in one piece by the surgeon. The treated corneas developed a mild wound healing reaction, comparable to that known from excimer laser in situ keratomileusis (LASIK) studies. The wound healing was restricted to the flap–stroma interface, most pronounced at the periphery of the flaps. *Conclusions* The use of the fs-laser offers new possibilities in preparation of corneal flaps, possibly providing advantages over conventional microkeratomes. Furthermore, the fs-laser has the potential to create intrastromal refractive lenticules for complete refractive procedures (ILK).

Introduction

Laser in situ keratomileusis (LASIK) has become a safe and well-established procedure for vision correction in the past years. However, possible risks and complications relating to the use of the mechanical microkeratome remain [3]. Furthermore, the procedure suffers from relatively low predictability of the thickness of the created flaps [2, 15]. With respect to the recent developments of wavefront sensing and customised ablation, a more defined and predictable flap thickness is highly desirable. Furthermore, precision of excimer laser photoablation depends highly on both environmental circumstances and tissue consistency, especially the water content of the treated cornea.

A possible alternative to the mechanical knife might be the use of ultrashort laser pulses. Due to the very high intensity of these pulses, it is possible to achieve a cutting effect underneath the surface and inside the bulk of transparent materials by focusing the radiation into the sample [12, 14]. The high field intensities at the focus lead to generation and acceleration of free electrons and, thus, to an optical breakdown [13]. In refractive corneal surgery, this so-called photodisruptive process can be used to achieve a cutting effect inside the corneal stroma.

Photodisruptive processes are well established in ophthalmology. Nanosecond (ns) pulses of a Nd:YAG laser are used for the treatment of secondary cataract (laser capsulotomy) [1]. If femtosecond (fs) pulses are applied, however, the mechanical and thermal side effects are reduced by several orders of magnitude. This is due mainly to the intensity-dependent threshold of approximately 10_{11} W/cm^3 for creating a plasma. Thus, a cutting effect

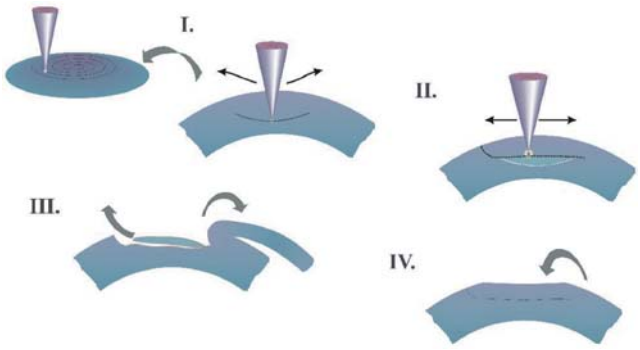

Fig. 1 Procedure of the fs-LASIK. First, the lenticule is created by scanning the laser spot in a spiral pattern (*I*). Subsequently, a second cut is performed, which generates the flap (*II*). Afterwards, the flap is opened and the lenticule extracted (*III*). Finally, the flap is closed (*IV*)

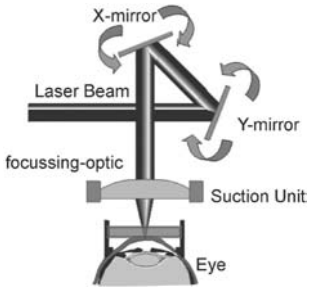

Fig. 2 Surgery set-up: the eye is fixed by a suction unit, and the laser pulses are guided by a computer-controlled scanner towards the treated eye

can be reached at energies in the range of only 1 μJ or less, when focusing the ultrashort laser pulses to spot sizes of several micrometers in diameter.

In media with high aqueous saturation, such as the cornea, a vapour-filled bubble develops (and oscillates) at the breakdown region. This is because of the explosive heating of the media. Due to photodissociation of involved water, the bubble contains hydrogen [6, 11]. The bubble remains within the stroma for several seconds and can interfere with subsequent laser pulses. Additionally, a shock wave is generated at the focal region. In corneal tissue possible mechanical damage due to these phenomena might relate to adjacent tissue such as the epithelium and the endothelium. These effects scale with the energy of the laser pulse and are, therefore, significantly reduced when fs-laser pulses are applied.

A dependency of the cutting precision on the duration of the applied pulses can be found at pulse widths also below 1 picosecond (ps), as published by the authors [4]. The mechanical side effects are significantly stronger when the laser pulse energy is only slightly increased. Therefore, the cutting precision of longer pulses in the picosecond range is highly irregular and of poor quality if compared to laser cuts performed by laser pulses with durations of only 150 fs. Only such ultrashort laser pulses, enabling highest cutting precision and smoothness, make the fs-laser a potential tool for refractive corneal surgery. Possibly, by preparation of two intrastromal lamellar cuts, both the lamellar flap and a refractive lenticule could be prepared in a single-step operation fully replacing the microkeratome and the excimer laser [7, 9].

In a first scan, a deep intrastromal cut is set in order to define the posterior shape of the refractive lenticule that later is removed (see I and II in Fig. 1). Thereafter, a second scan performs a surface-parallel cut, defining the anterior shape of the lenticule (II). This second cut also allows the opening of the corneal flap and the extraction of the lenticule (III). As a result, a change of the cornea's curvature and the refractive power of the eye is achieved (IV). The shape of the lenticule has to correspond to the desired change in refractive power. The creation of the intrastromal lenticule calls for a very high cutting precision. In this paper we present preliminary in-vivo rabbit eye studies to evaluate the potential of ultrashort fs pulses in refractive corneal surgery for intrastromal laser keratomileusis (ILK) (Fig. 1).

Experimental methods

Laser system

The laser system used in these studies consisted of a titanium–sapphire amplifier, seeded by an erbium-fibre laser oscillator, which was developed at the Laser Zentrum Hannover. The pulses from the Er^{3+} oscillator, at a repetition rate of 16 MHz, had a central wavelength of 775 nm and approximately 2 mW output power. Subsequently, these pulses were amplified by means of chirped pulse amplification (CPA) in a titanium–sapphire regenerative amplifier pumped by a frequency-doubled Nd:YLF laser, (Thales-Laser, formerly B.M. Industries). The system allowed variable repetition rates of up to 3 kHz at output power of 1 W. The minimum achievable pulse duration of the system was approximately 130 fs.

During all experiments, the pulse duration was controlled by a self-constructed single-shot autocorrelator. The energy was adjusted by a variable attenuator or with the help of a half-wave plate in front of the compressor system, resulting in the attenuation of the uncompressed pulses at the grating of the compressor set-up.

Surgery set-up

In order to perform intrastromal cuts, a computer-controlled two-axis scanner system (Model XY15M2-S, GSI Lumonics), was used to guide the amplified pulses over a focusing unit towards the treated eye as shown in Fig. 2. The focusing unit consisted of a special scan optic (Sill-Optics) with a focal length of 75 mm, leading to a theoretical spot size of approximately 5 μm.

To keep the treated eye in a fixed position, a suction unit was integrated into the focusing system. A suction mask contained a glass plate, which flattened the cornea over the working field of 6 mm in diameter. At the edges of the glass plate suction of 200 mmHg was applied to keep the eye in position. The whole suction ring was mounted on a microtranslation stage, which allowed the variation of the Z-position along the beam axis by moving the treated eye within a submicrometer resolution. Combined with the two scanning mirrors, a three-dimensional translation of the laser focus over the working field of 6 mm in diameter was possible.

Intrastromal cuts

The in-vivo experiments were conducted on 10 adult New Zealand White rabbits. Although rabbit eyes show anatomic differences from the human eye, e.g. the lack of Bowman's layer, the differences do not play an important role in the corneal wound healing process at the central cornea after LASIK operation. The anterior part of the cornea, including corneal epithelium, basement membrane (Bowman's layer in humans) and anterior stroma, remains intact during the performed procedure.

One eye of each animal was subjected to the surgery while the other served as untreated control. In all animals, corneal flaps of 3 mm and lenticules of 2.8 mm with a central thickness of 60 µm were created.

To study wound healing and long-term effects, the postoperative follow-up was chosen to be 3, 7, 14, 28, 60 (two animals), 90 (two animals), 110 and 120 days. During the surgery, the animals were kept under general anaesthesia using 10% ketamine and 2% xylazine hydrochloride. Additionally, a topical anaesthesia (oxybuprocaine hydrochloride 0.4%) was given at the cornea before fixation of the eye by the suction unit, using preservative-free eye-drops. Directly after treatment, antibiotic eye-drops (tobramycin) were applied and continued for 5 times daily on the first 3 days. To prevent the animals from opening the flap, they were restrained by means of special collars during the first week after surgery. Corneal transparency was observed with a portable slit-lamp biomicroscope during follow-up. The animals were killed and the eyes were enucleated and fixed in 5% cacodylate-buffered glutaraldehyde. Plastic embedding technique (GMA/MMA: glycol methacrylate/methyl methacrylate) was applied and serial sections of 1.5 µm thickness were prepared and stained with toluidine blue and haematoxylin–eosin (H&E) for histopathological evaluation.

The animals used in these studies were treated according to the "Principles of laboratory animal care" and the stipulations of the German Law on the Protection of Animals.

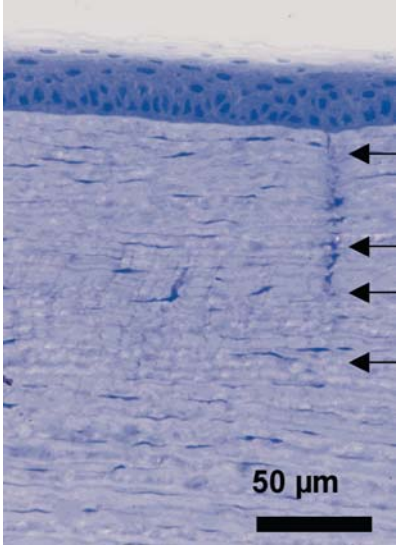

Fig. 3 Histological section of a rabbit eye, 7 days after operation. The vertical cut of the flap edge is indicated by *arrows*. [5]

Results

Preparing corneal flaps

In previous studies, corneal flaps were created with a right angle between the horizontal cutting plane and the vertical cut towards the epithelium. Figure 3 shows a histological section of a flap with 90° angle 7 days after treatment; the vertical cut is marked by arrows [5].

As a consequence of the steep angle between these cuts, the opening of the flap by the surgeon was difficult. Therefore, the angle applied in this study was changed to a value of 60°, allowing easy opening of the flap followed by lenticule extraction as described in the following section. No significant wound healing reaction inside the corneal stroma could be found. In Fig. 4a and Fig. 4b the edge of a flap created at an angle of 60° and the flap hinge can be seen 60 days after surgery. As shown in these histological images, in eight of the animals no proliferation of epithelial cells was visible. However, in two animals, killed at 7 and 14 days after operation, a proliferation of

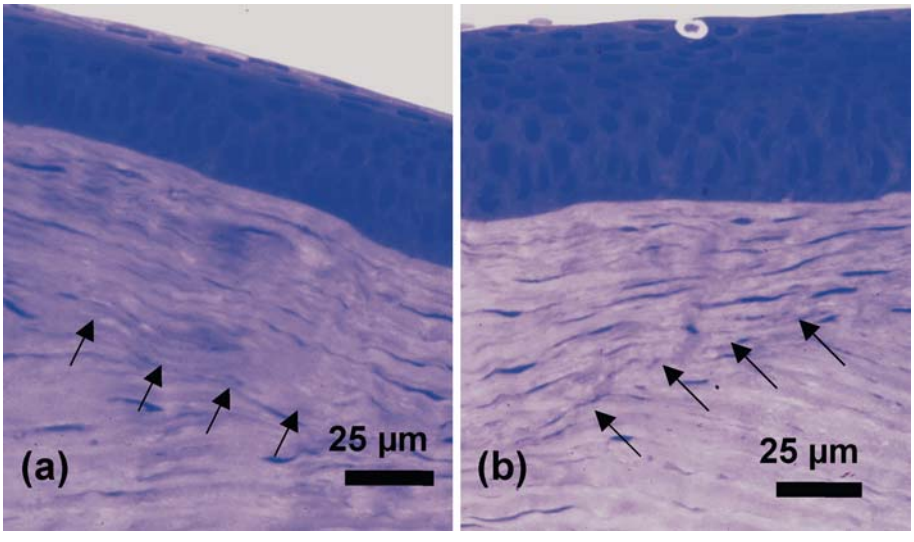

Fig. 4 **a** Hinge of the flap 60 days after operation. **b** In the same eye at the edge of the flap, the angle of 60° can be clearly seen. The flap interface is indicated by *arrows*

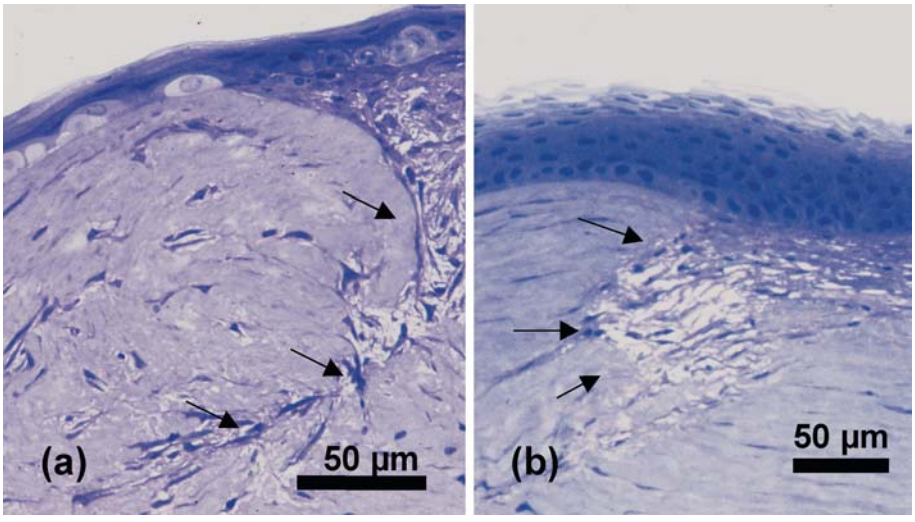

Fig. 5a, b Edge of the flap in rabbit eyes with proliferation of epithelial cells: **a** 7 days after operation and **b** 14 days after operation, a slight oedema is seen (*arrows*)

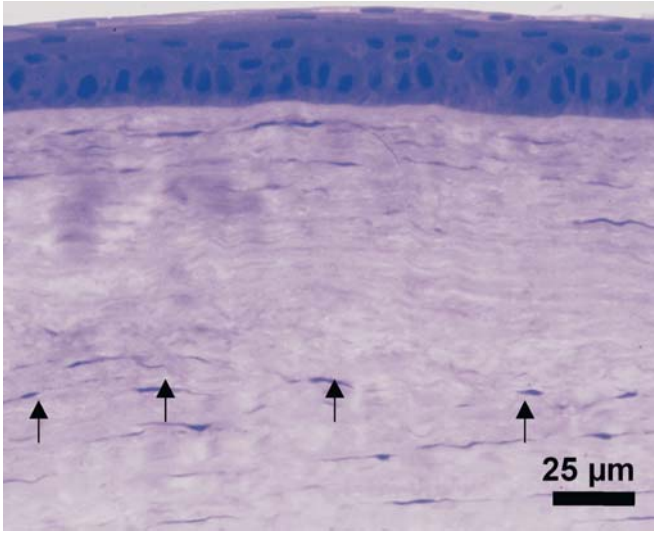

Fig. 6 Central part of a histological section of a rabbit eye 60 days after operation. A lenticule 60 µm thick was extracted. The cutting line is indicated by *arrows*

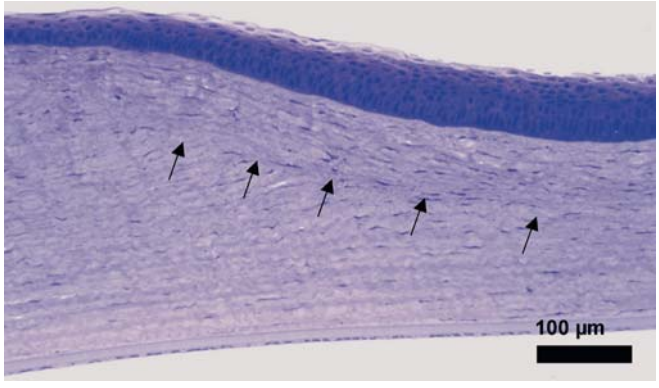

Fig. 7 Histological section of a rabbit eye 28 days after operation. The edge of the treated area can be seen. The curvature of the eye is changed due to the extraction of the lenticule

epithelial cells was noticed, probably due to imperfect replacement of the corneal flap; see Fig. 5a and Fig. 5b.

Intrastromal lenticules

In all treated animals, the lenticules could be extracted in one piece. After opening of the flap, the lenticules adhered to the bottom of the remaining stroma and could be extracted by gentle traction. The flap could be repositioned and within several minutes after surgery the cornea was almost transparent. Mild corneal opacity, most probable due to oedema, was observed at the central cornea and disappeared within 24–48 h after surgery.

Central epithelium remained intact. The edges of the cut were still visible on slit-lamp observation, even 120 days after the treatment. Figure 6 shows a histological picture of the central part of a rabbit cornea 60 days after laser treatment. The cornea shows only mild wound healing reaction, although a lenticule of 60 µm thickness was extracted. The surface of the cornea is smooth. Only some activated keratocytes mark the connecting zone between the flap and stromal bed at a depth of approximately 180 µm from the original corneal surface (see arrows in Fig. 6). No wound healing reaction in terms of excessive collagen production could be noticed at the central cornea. The anterior and posterior stroma (the layers in front and behind the focal region) revealed no significant wound healing-associated changes. Descemet's membrane and endothelium remain intact.

Due to the small working field of the suction ring used for rabbit eyes, the change in curvature induced by the extraction of the 60 µm lenticule is high, as can be seen in Fig. 7: A corneal histological image from the rabbit 28 days after treatment is shown. The edge of the created

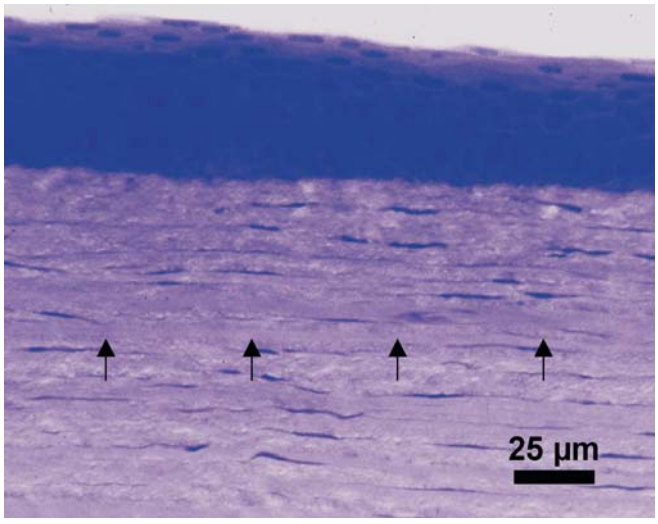

Fig. 8 Histological section of a rabbit eye 120 days after operation. The cutting line is indicated by *arrows*

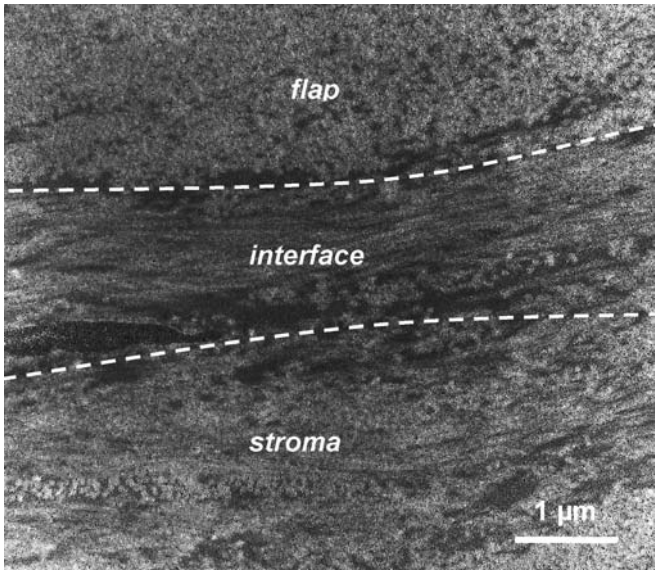

Fig. 9 Transmission electron microscopy image of the transition zone in a treated eye between flap and stroma. The image shows the central part of the eye shown in Fig. 8

flap shows no wound healing reaction. The flap can be identified by the changed orientation of the corneal stroma (arrows). The steep change in curvature, however, leads to pronounced hyperplasia of the epithelial layer. Figure 8 shows a histological section of a rabbit cornea 120 days after operation. Figure 9 shows a transmission electron microscopy image of the same sample, displaying the flap–stroma interface in high magnification. Fine unorganised collagen fibrils, still present 120 days after operation, can be seen in an area approximately 1 µm thick.

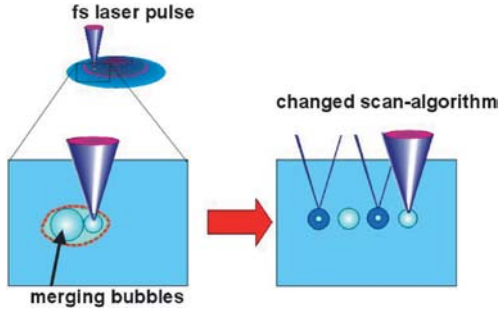

Fig. 10 Gas bubbles of successive laser pulses merge inside the stroma. By the use of appropriate scan algorithms these problem can be suppressed

Discussion

The results of previous ex-vivo experiments showed that intrastromal cuts could be performed with very high accuracy when using laser pulse durations of some hundreds of femtoseconds and pulse energies of around 1 µJ [4]. Other working groups have already proofed the high precision of fs pulses in comparison with ps or ns pulses [8, 14]. Laser pulses close to 1 ps in duration (930 fs) do induce noticeable vacuolation and irregular cuts [5]. By using shorter pulse durations (130 fs) higher precision in tissue processing is achieved, allowing both the processing of smooth flaps and the extraction of intrastromal lenticules inside the living animal [4].

In comparison to the microkeratome cuts, the cutting surface generated by the fs-laser (150 fs pulse duration) is of equal or higher quality [10]. The wound healing reaction after treatment was found to be very similar to that known from excimer LASIK studies [16]. No visible thermal or mechanical damage of the corneal tissue could be noticed by light microscopy. Side effects of the fs-photodisruption are negligible with respect to mechanical or thermal damage to the surrounding tissue. Nevertheless, the in-vivo impact of several nonlinear effects, such as self-focusing or UV-light production, is still to be evaluated [6].

In the first two eyes, due to interaction of the bubbles created by each laser pulse, tissue bridges remained a problem when the flap was to be opened. The opening, nevertheless, was feasible, but was performed with some surgical stress. Figure 10 visualises the process: If two successive laser pulses are placed too close to each other, the produced bubbles merge, leading to large intrastromal bubbles. These bubbles possibly deflect following laser pulses, resulting in remaining tissue bridges. To overcome this problem, special care has to be taken with regard to the scanning pattern of the laser pulses inside the cornea. Figure 10 shows an example: In a first spiral pattern, the pulses are placed at relatively large distances from one another. Afterwards, in a second scan, the puls-

es are placed in the spaces between the previous set of pulses. At that time, the gas of the bubbles created during the first scan has partly dissolved in the liquid of the surrounding tissue.

Due to these phenomena, a strong dependence on the energy and spot size of the laser was found in all experiments. Therefore, a minimum energy in the range of 1 μJ and small spot sizes below 5 μm diameter were used. Additionally, fixation of the treated eye and control of the focal plane inside the cornea represent a further limiting factor and, thus, a key issue in the achievable precision.

As proofed by ex-vivo and in-vivo experiments, refractive surgery by means of the fs-laser seems feasible, as already shown by other working groups [8]. Compared to a conventional microkeratome, the fs-laser may offer advantages with regard to potential complication of the flap-making process. Certainly higher flexibility and predictability in the cutting geometry is possible.

As a full refractive procedure, ILK with ultrashort laser pulses has to be performed with micrometer precision if changes in refractive power of one or below one dioptre are desired. Such high precision has already been demonstrated in ex-vivo studies by the authors. The precision in living specimens is under investigation. The lenticules, as produced in this study, were well defined inside the stroma and easy to extract. The change of refractive power that was achieved in the treated eyes could not be evaluated because of the small size of the optical zones imposed by the limited working field of the suction unit. The construction of new suction devices is under way. However, the treatment of larger optical zones triggers another problem, i.e. the surgical time.

By scanning working fields of up to 10 mm in diameter, the surgery may take about 20 min with the 150 fs-laser pulse system at 3 kHz, as used in this study. Certainly surgical time is significantly shorter when longer pulse durations are applied, but then the quality of the cuts is below clinically acceptable thresholds. However, laser systems providing repetition-rates of up to 300 kHz at pulse energies of several microjoules have recently become available. Surgical times in the range of 20 s even with ultrashort pulses seem possible in the near future. In order to achieve the desirable large treatment zones of up to 10 mm diameter and short application times below 60 s with resolution so high that refractive changes in the range of 0.1 dioptre can be achieved, intensive effort in the development on the laser hardware is necessary.

In summary, ILK with ultrashort fs-laser pulses (120–150 fs) has been shown to be possible in the animal model. The procedure offers tissue processing of very high precision, in the micrometer range, with negligible side effects. The wound healing reaction is gentle and corresponds to the experiences with LASIK.

Close to clinical application is the creation of corneal flaps with ultrashort laser pulses, providing the advantage of high flexibility and predictability of the desired flap geometry. However, fs-ILK as propagated in this paper certainly needs further development of the laser hardware and the delivering optics.

Acknowledgements This work was supported by the German Ministry of Education and Research (BMBF), project-nr. 13N7793, and the German Science Foundation, project-nr. Lu498/1.

References

1. Aron-Rosa D, Aron J, Griesemann J, Thyzel R (1980) Use of the neodymium:YAG laser to open the posterior capsule after lens implant surgery. J Am Intra-Ocular Implant Soc 6:352–354
2. Behrens A, Seitz B, Langenbucher A, Kus MM, Rummelt C, Küchle M (1999) Evaluation of corneal flap dimensions and cut quality using a manually guided microkeratome. J Refract Surg 15:118–123
3. Gimbel HV, Iskander NG, Peters T, Penno EA (2000) Prevention and management of microkeratome-related laser in situ keratomileusis complications. J Refract Surg 16 [Suppl]:226–229
4. Heisterkamp A, Ripken T, Lubatschowski H, Drommer W, Lütkefels E, Mamom T, Welling H, Ertmer W (2001) Optimisation of the parameter for intrastromal refractive surgery with ultrashort laser pulses. Proc SPIE 4245:246–255
5. Heisterkamp A, Mamom T, Kermani O, Drommer W, Welling H, Ertmer W, Lubatschowski H (2002) Intrastromal refractive surgery with ultrashort laser pulses in living animals. Proc SPIE 4611:136–143
6. Heisterkamp A, Ripken T, Mamom T, Drommer W, Welling H, Ertmer W, Lubatschowski H (2002) Nonlinear side effects of fs pulses inside corneal tissue during photodisruption. Appl Phys B 74:1–7
7. Juhasz T, Loesel FH, Horvath C, Kurtz RM, Mourou G (1998) Femtosecond laser for ultra-accurate surgery: application to corneal surgery. Ultrafast Phenomena IX:687–691
8. Kurtz RM, Horvath C, Liu H, Krueger RR, Juhasz T (1998) Lamellar refractive surgery with scanned intrastromal picosecond and femtosecond laser pulses in animal eyes. J Refract Surg 14:541–548
9. Lubatschowski H, Maatz G, Heisterkamp A, Hetzel U, Drommer W, Welling H, Ertmer W (2000) Application of ultrashort laser pulses for intrastromal refractive surgery. Graefes Arch Clin Exp Ophthalmol 238:33–39
10. Lubatschowski H, Heisterkamp A, Drommer W, Kermani O, Welling H, Ertmer W (2001) Application of ultrashort laser pulses for refractive surgery of the eye. LaserOpto 6:40–46

11. Maatz G, Heisterkamp A, Lubatschowski H, Barcikowski S, Fallnich C, Welling H, Ertmer W (2000) chemical and physical side effects at application of ultrashort laser pulses for intrastromal refractive surgery. J Opt A Pure Appl Opt 2:59–64
12. Niemz MH, Klancnik EG, Bille JF (1991) Plasma-mediated ablation of corneal tissue at 1053 nm using a Nd:YLF oscilllator/regenerative amplifier laser. Lasers Surgery Med 11:426–31
13. Noack J, Vogel A (1999) Laser-induced plasma formation in water at nanosecond to femtosecond time scales: calculation of thresholds, absorption coefficients, and energy density. IEEE J Quant Elec 35 (8):1156–1167
14. Stern D, Schoenlein R, Puliafito C, Dobi E, Birngruber R, Fujimoto J (1989) Corneal ablation by nanosecond, picosecond, and femtosecond lasers at 532 nm and 625 nm. Arch Ophthalmol 107
15. Vongthongsri A, Srivannaboon S, Horatanaruang O, Nariptaphan P (2000) Laser in situ keratomileusis corneal flap creation with the Nidek MK-2000 and the Carriazo Barraquer microkeratomes. J Refract Surg 16:272–275
16. Wachtlin J, Langenbeck K, Schründer S, Zhang EP, Hoffmann F (1999) Immunohistology of corneal wound healing after photorefractive keratectomy and laser in situ keratomileusis. J Refract Surg 15:451–458