

An in vitro study of femtosecond laser photodisruption in rabbit sclera

Fagang JIANG (✉)¹, Xiaobo YANG¹, Nengli DAI², Peixiang LU², Hua LONG², Ling CUI¹

¹ Department of Ophthalmology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

² State Key Laboratory of Laser Technology, Huazhong University of Science and Technology, Wuhan 430074, China

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Abstract To explore the possibility of photodisruption in rabbit sclera by femtosecond (fs) laser and seek appropriate incision techniques and relevant parameters, a fs laser (800 nm/50 fs) with different pulse energies was applied to irradiate rabbit sclera in vitro. By moving a computer-controlled three-axis translation stage to which the sample was attached, the laser achieved three types of incisions: transscleral channel, snake pattern and linear cut. The irradiated samples were observed by light microscopy and scanning electron microscopy (SEM). In comparison with fs laser, Nd:YAG was used as control. The experimental results show that through an objective lens with numerical aperture (NA) of 0.2, the fs laser with power intensity larger than 955 TW/cm² and pulse energy ranging from 37.5–125 μJ, cutting depths from 30–70 μm may be achieved after linearly scanning in sclera at a translation speed of 0.1 mm/s. However, it failed to make any photodisruption when the power intensity was below 796 TW/cm² or the pulse energy was less than 31.25 μJ under the same condition. Compared with the Nd:YAG laser, the inner wall of the channel was smoother and the damage to the surrounding tissues was slight with the fs laser. The high precision of intrascleral photodisruption and minimal damage to surrounding tissues by a fs laser display its potential application in the treatment of glaucoma.

Keywords laser biology and medicine, femtosecond laser, photodisruption, sclera

1 Introduction

Previous attempts at laser sclerostomy using nanosecond or picosecond pulses of Nd:YAG, Er:YAG and excimer lasers were ineffective because of the strong laser-induced thermal damage and heavy mechanical trauma due to large pulse energy or long exposure time. The severe collateral damage stimulates fibrosis and scarring, which can subsequently lead to the closure of fistula [1]. Recent progress now allows a laser system to provide ultra-short laser pulses with femtosecond (fs) duration. Owing to its non-contact processing, extremely high intensity and ultra-short pulse duration, the fs laser has the remarkable characteristic of biological tissue ablation with minimal collateral damage [2–4]. At present, fs laser is beginning to be used to create corneal flap for laser in situ keratomileusis (LASIK) in clinics. However, there is still limited research on laser-sclera interaction for glaucoma treatment [5,6].

This experiment aims to explore the probability of photodisruption in rabbit sclera with a fs laser, seek appropriate incision techniques and relevant parameters and predict its potential application in the treatment of glaucoma.

2 Materials and methods

2.1 Experimental setup and materials

The experimental setup is shown in Fig. 1. Fs pulses were generated by a regeneratively amplified Ti:Sapphire laser system, emitting at a wavelength of 800 nm with pulse duration of 50 fs and repetition rate of 1 kHz. The pulse energy could be adjusted from 0 to 2 mJ by rotating a polarization plate. The beam was then focused by a 0.2 NA aspheric lens to the sclera sample sandwiched between microscope slides (each 1 mm thick). The size of the focal spot on the surface was 10 μm.

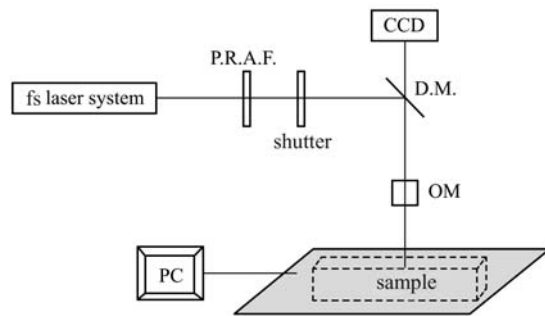


Fig. 1 Fs laser processing system with a mechanical shutter and CCD camera

The scleral sample between two microscope slides was mounted on a computer controlled three-axis translation stage [7], as shown in Fig. 2. The process of laser ablation was observed by a CCD camera [8]. Samples after laser exposure were fixed in 2% glutaraldehyde and prepared for SEM.

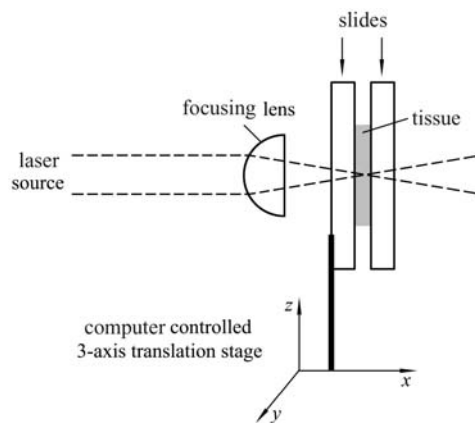


Fig. 2 Three-axis translation stage and sample arrangement

Ocular tissues were obtained from rabbits sacrificed by an intravenous injection of excessive 20% urethane solution. Full thickness scleral pieces (approximately $5\text{ mm} \times 5\text{ mm}$ and 0.5 mm thick) were cut from the limbal region. Samples were preserved in physiological saline solution to keep them hydrated [9].

2.2 Measurement of transmission spectrum of hydrated sclera

The transmission of hydrated sclera with wavelength in the visible and near infrared spectrum was measured by a spectrophotometer (HITACH U-3310).

2.3 Linear cut with pulse energy smaller than $31.25\text{ }\mu\text{J}$

A fs laser beam ($800\text{ nm}/50\text{ fs}/1\text{ kHz}$) with pulse energies ranging from $6.25\text{--}31.25\text{ }\mu\text{J}$ and maximum power intensity

of $796\text{ TW}/\text{cm}^2$ was applied to form linear cuts on the inner surface of the sclera. The laser beam was focused $100\text{ }\mu\text{m}$ below the surface. Eleven linear cuts were attempted with various pulse energies. Each piece of treated tissue was hit only once by the laser. The translation speed of laser spot was $0.1\text{ mm}/\text{s}$.

2.4 Linear cut with pulse energy larger than $37.5\text{ }\mu\text{J}$

In another procedure, pulse energy was increased and the power intensity exceeded $955\text{ TW}/\text{cm}^2$. The laser beam was focused $500\text{ }\mu\text{m}$ below the surface. Eleven linear cuts were attempted in all. The cutting pulse energy of the first line was $125\text{ }\mu\text{J}/\text{pulse}$, decreasing by $12.5\text{ }\mu\text{J}$ after each cut until it dropped to $37.5\text{ }\mu\text{J}/\text{pulse}$ for the eighth line. The first eight lines were made by single-shot laser exposure. The pulse energy was then fixed at $75\text{ }\mu\text{J}/\text{pulse}$ to ablate the last three lines with scanning frequencies of 2, 3 and 4 times, respectively. The translation speed of laser spot was also $0.1\text{ mm}/\text{s}$.

2.5 Transscleral channel creation

A fs laser with $200\text{ }\mu\text{J}$ pulse energy was used to create a transscleral channel from the inner surface of sclera [10]. The laser beam was focused at $200\text{ }\mu\text{m}$ beneath the surface. The total exposure time was 4 ms .

2.6 Snake pattern creation

A fs laser with $300\text{ }\mu\text{J}$ pulse energy was applied to create a snake pattern on the inner surface of sclera. The laser beam was focused at $200\text{ }\mu\text{m}$ beneath the surface. The translation speed of laser spot was $50\text{ }\mu\text{m}/\text{s}$.

2.7 Comparison of fs laser and Nd:YAG laser for laser sclerostomy

Compared with the Nd:YAG laser ($10\text{ mJ}/\text{pulse}$), the fs laser ($450\text{ }\mu\text{J}/\text{pulse}$) was used to create full thickness fistulae in hydrated scleral sample in vitro. Tissues after laser exposure were then fixed in 4% buffered formaldehyde and processed for paraffin embedding. Serial $5\text{ }\mu\text{m}$ sections were cut and stained with hematoxylin and eosin (H&E). The structure of the two types of incisions was examined by light microscopy.

3 Results

3.1 Transmission spectrum of hydrated sclera in vitro

As shown in Fig. 3, although the transmission of hydrated sclera increases with increasing wavelength in the visible and near infrared spectrum, the transmission

stays constant at a very low level. Hydrated sclera scatters and absorbs effectively for 800 nm light since the transmission is only about 0.68% at the wavelength.

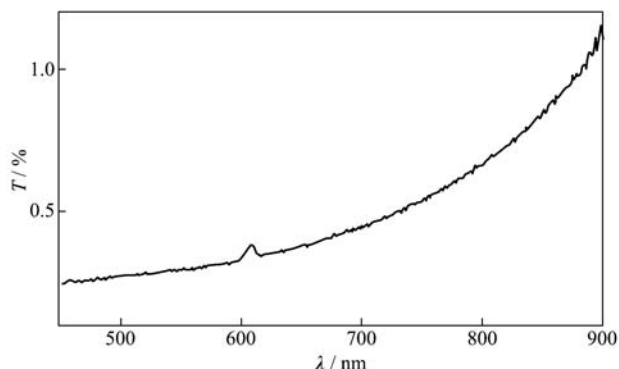


Fig. 3 Transmission spectrum of hydrated sclera in vitro at range of visible and near infrared light

3.2 Linear cut creation

A cross section of the sclera sample irradiated by pulse energies ranging from 6.25 to 31.25 μJ was observed by scanning electron microscopy (SEM). It was shown that the inner surface of sclera irradiated by laser beam was intact without any visible morphological change. No photodisruption effect occurred when the laser power intensity was below 796 TW/cm². It was essential to increase the pulse energy above the threshold to have such an effect.

Figure 4 shows the photodisruption effect in hydrated sclera after raising the pulse energy. The depths of incisions from left to right were 70, 65, 55, 50 and 45 μm, respectively.

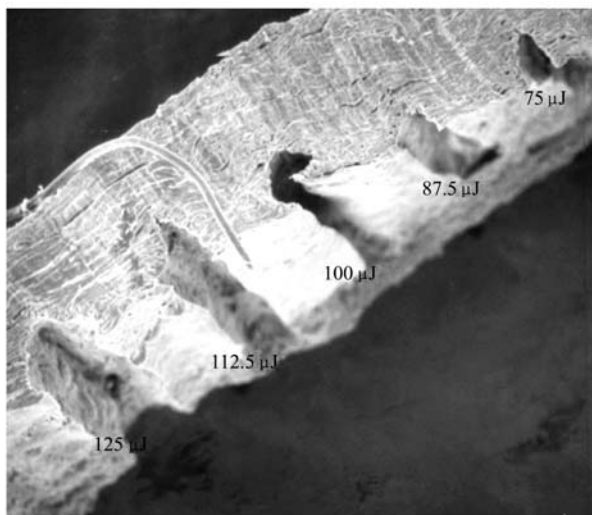


Fig. 4 Cross section of hydrated sclera ablated by fs laser with pulse energies of 75, 87.5, 100, 112.5 and 125 μJ, respectively (SEM image; × 121)

In Fig. 5, the depths of incisions were 30, 45, 65 and 130 μm from left to right. The corresponding pulse energies and laser scanning frequencies are also shown. The depths of incisions increased with increasing scanning frequencies, and a full thickness channel was created after a fourth scanning of the laser beam with fixed pulse energy of 75 μJ.

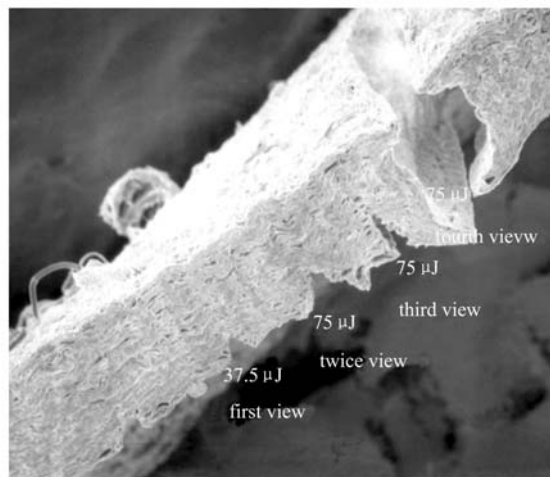


Fig. 5 Another part of cross section in sclera ablated by fs laser with pulse energies of 37.5 and 75 μJ at varying laser scanning frequencies (SEM image; × 100)

As shown in Fig. 6, there is an approximately linear correlation between single pulse energy and the depth of incision when pulse energy ranges from 37.5 to 125 μJ, with the other laser parameters kept constant.

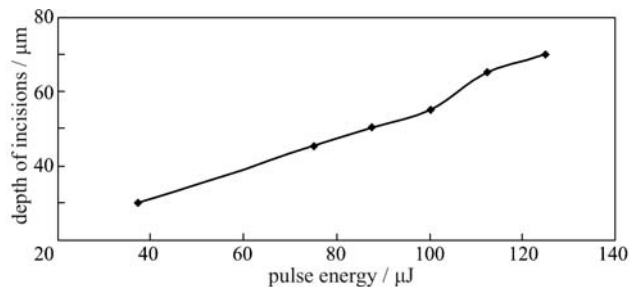


Fig. 6 Effects of pulse energy on depth of incision

The incisions shown in Figs. 4 and 5 show two significant features. First, the edges of channels are smooth and regular. Second, no collateral damage is presented in the surrounding tissues adjacent to the photodisrupted volume.

3.3 Transscleral channel creation

Figure 7 shows the laser created transscleral channel characterized by a regularly tubular shape and a smooth

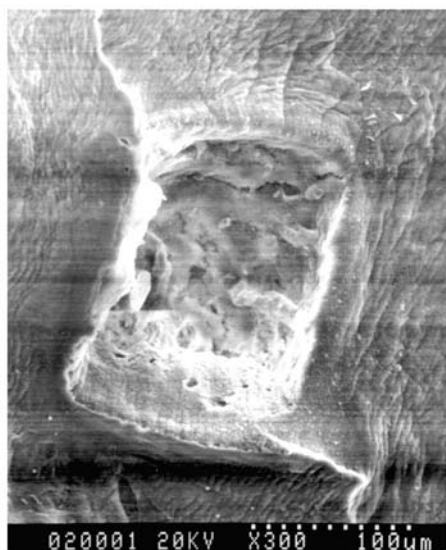
edge. It is noted that the channel was unobstructed with a small amount of debris at the opening and within the channel, leaving the surrounding tissues unaltered.



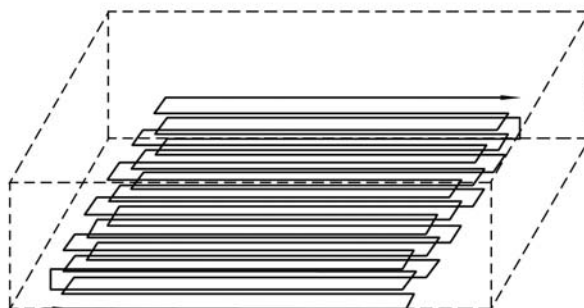
Fig. 7 Fs laser created channel in hydrated sclera in vitro (SEM image; $\times 1000$)

3.4 Snake pattern creation

A $200\ \mu\text{m} \times 200\ \mu\text{m}$ square snake pattern was successfully created as shown in Fig. 8(a), using the pattern schematized in Fig. 8(b). This incision pattern could be used to perform a transscleral procedure analogous to deep sclerostomy, where a block of inner surface sclera is removed. By changing the pulse energy or the translation speed of the laser spot, a desired pattern with a predictable thickness can be created.



(a)



(b)

Fig. 8 (a) Fs laser created snake pattern in hydrated sclera in vitro (SEM image; $\times 300$); (b) scanning pattern of laser focus

3.5 Comparison of fs laser and Nd:YAG laser for laser sclerostomy

Figures 9 and 10 show the histology of laser sclerostomy lesions using fs laser and Nd:YAG laser, respectively. A full thickness fistula could be created by 220–250 pulses of an Nd:YAG laser.

As observed with light microscopy, the edges of fistula created by fs laser were sharp with mild damage to the surrounding tissues, which maintained the normally tight structure of collagen fibrils (black arrow). In contrast, tissues surrounding the fistula created by Nd:YAG laser appeared loose and disordered, indicating serious laser-induced damage (white arrow).

4 Discussion

4.1 Mechanism of fs laser ablation of biological tissues

The ablation of biological tissues by ultra-short pulse laser depends on the nonlinear energy absorption process known as photodisruption. The photodisruption effect only occurs when the power intensity is larger than the threshold level. Below this threshold level, the laser pulse will pass through the material without causing any damage [7]. At a high optical intensity, the target will be ionized by multi-photon absorption, which is required to initiate an electron avalanche. This avalanche leads to optical breakdown, and the resulting plasma mediates the ablation process [11]. When photodisruption occurs near the threshold energy level,

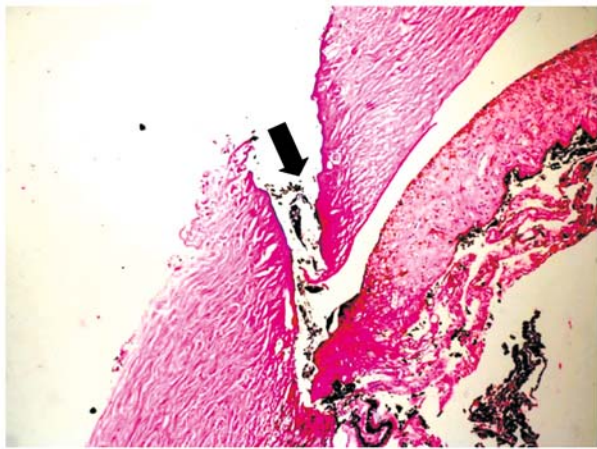


Fig. 9 Sclera fistula created by fs laser (H&E; $\times 100$)

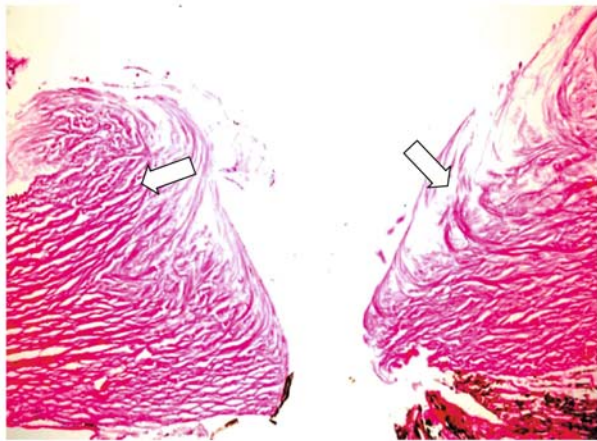


Fig. 10 Sclera fistula created by Nd:YAG laser (H&E; $\times 200$)

most of the absorbed energy is used to create the plasma and only a small amount of the absorbed energy is dissipated by other mechanisms such as shock wave formation, bubble oscillation, and thermal diffusion. Fs pulses, therefore, allow precise subsurface disruption with almost no collateral damage [7].

Since the threshold intensity is necessary for the optical breakdown, a decrease in pulse energy can only be achieved by shortening the laser pulse duration. In this experiment, by focusing the fs laser beam to a spot size of $10\ \mu\text{m}$, a high power intensity of $100\ \text{TW}/\text{cm}^2$ was achieved at pulse energies of decades of microjoules. As a consequence, the secondary thermal and mechanical effects should be reduced dramatically with fs-photodisruption. Targeted high precision photodisruption, which has been demonstrated in this paper, predicts minimally invasive surgery inside cells [12], tissues [13,14] or even a whole organ of the human body such as the eye [15,16] and tooth [17,18].

4.2 Influence of light transmission of hydrated sclera on photodisruption effect

In contrast with transparent cornea, sclera is which mainly composed of water and collagen fibrils, appears white under a physiological condition and has a low light transmission in the visible and near infrared spectrum. Previous studies attempted to increase the transmission of sclera through a dehydrating agent [7] or increasing the wavelength of the laser beam [9]. High precision subsurface incisions without damaging the superficial tissues were successfully made in hydrated sclera in vitro by a fs laser at a long wavelength of $1700\ \text{nm}$ [9].

In this experiment, since the laser beam was unable to focus effectively beneath the surface, a strong power intensity significantly larger than the threshold was required for subsurface photodisruption. As a result, the overlying tissues had been broken down before subsurface photodisruption occurred.

4.3 Prospect of application of fs laser sclerostomy in treatment of glaucoma

In this article, it is demonstrated that the fs laser can create various types of intrascleral incisions easily at different depths in vitro, which may be useful for draining aqueous and lowering intraocular pressure (IOP) for the treatment of glaucoma.

As a result of ultra-short pulse duration, a fs laser can reach the threshold intensity even at a pulse energy of a microjoule level, which is two or three orders of magnitude smaller than that of a nanosecond or picosecond pulsed laser. Furthermore, the edge of channel is smoother and damage to the surrounding tissues is slighter using the fs laser than using a longer pulse laser [19,20]. The advantages of sclerostomy with fs laser might contribute to less scar formation and long-term patency of fistula after operation. Highly precise photodisruption in sclera with ultra-short laser pulses offers a large potential for minimally invasive treatment of glaucoma.

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