

Eye and Glaucoma Research

Ultraviolet Laser Ablation of Trabecular Meshwork and Limbus in Rats

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Abstract

Background/Aims: The purpose of this study was to determine the ability of intraocularly delivered 213 nm UV laser pulses to ablate the trabecular meshwork and to monitor the healing process in an *in vivo* rat model. The long-term aim is to develop a new form of glaucoma surgery to cut a permanent drainage channel through the trabecular meshwork.

Methods: The rat was anaesthetized and its head securely mounted in a stereotaxic device. The eye was stabilized by suturing to an eye ring held in the stereotaxic frame. The fiberoptic delivery probe entered the eye through a freshly ablated channel through the cornea and passed across the anterior chamber under gonioscopic observation, to touch the trabecular meshwork on the nasal side. Laser lesions were then induced using either 100 pulses at a fluence of 0.4 J.cm⁻² or 50 pulses at 0.6 J.cm⁻². The rat eye was enucleated either immediately at the end of the laser treatment, or after a two week recovery period, and processed for histology.

Results: Both laser fluence levels were able to ablate the trabecular meshwork tissue and the adjoining limbus tissue with a clean border; with 0.6 J.cm⁻² and 50 pulses inducing a deeper lesion than with 0.4 J.cm⁻² and 100 pulses. Chronic lesion sites were populated by loose cellular tissue akin to the trabecular meshwork tissue.

Conclusions: Multi-pulse 213 nm UV laser delivery is able to effectively ablate rat trabecular meshwork and adjoining limbus tissues *in vivo* using an intraocular fibre optic delivery system.

Keywords

Laser ablation; Trabecular meshwork; Glaucoma

Introduction

Laser technology has the potential to provide the required precision and control to transect and remove tissue, with minimal unwanted damage to surrounding tissue. Excimer lasers have been used for corneal refractive surgery for many years, the ultraviolet (UV) pulsed beam providing exceptional control of ablation depth and minimal damage to surrounding tissue [1]. Solid state systems based on the 5th Harmonic of Nd:YAG lasers have also shown excellent results for this application [2]. With corneal ablation, the laser is delivered through an air environment, with minimal energy loss. However, we are interested in intraocular surgery in a fluid environment, where UV wavelengths require fiberoptic delivery [3-8].

Our group has previously used UV lasers with wavelengths of 266 nm and 213 nm to perform experimental tissue ablation in cadaver eyes in a fluid environment [3-5]. We found that the ablation depth and laser parameters varies with tissue types, such as the trabecular meshwork, the common adventitial sheath at the A-V crossing point, retina, and subretinal tissues. We found that 213 nm laser pulses could provide a cleaner ablation with less damage to the surrounding tissue in human trabecular tissue [4].

We would also like to develop a new technique and procedure for glaucoma surgery, which creates a drainage channel through the trabecular meshwork. This new glaucoma surgery is only mildly invasive, and does not require an artificial implant. The main resistance to outflow of aqueous humour is the trabecular meshwork and inner wall of Schlemm's canal, so these should be the surgical target [9-12]. Ideally to restore normal intraocular pressure it is only required to create a localised and precise channel through the trabecular meshwork and inner wall of Schlemm's canal to match the physiological outflow resistance. It is important to note that the pathway from the trabecular meshwork and Schlemm's canal to the systemic circulation is fully lined with endothelial cells, maintaining a crucial barrier protection for the permanent outflow pathway [13]. Thus there will be no issues with conjunctival tissue reaction to aqueous humour which is a possible cause of the

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inflammation and scarring after glaucoma filtration surgery [14,15]. There is no chance of hypotony since the pressure in collector channel (assumed equal to episcleral venous pressure) is ~9 mmHg.

In addition to glaucoma surgery, UV laser ablation could benefit other intraocular surgeries which require precision at the micron level. For example, another potential application for such technology is high precision sheathotomy at the artery-vein crossing point for treating branch retinal vein occlusion. The target sheath tissue for sheathotomy is very thin (~50 μm) at the site of the occlusion. The purpose of this procedure is to relieve the compression and deformation of the retinal vein and allow normal haemodynamic conditions to be restored, but damage to the retinal artery, vein and surrounding retinal tissue should be avoided. For decades, mechanical sheathotomy has been used for dissecting the adventitial sheath at the A-V crossing site in retinal branch vein occlusion patients. Previous attempts have been made using surgical instruments such as a bent tip microsclerotomy knife or a dedicated sheathotomy knife after vitrectomy [16-18]. It is difficult to use a knife held by the surgeon's hand to perform such surgery with sufficient precision. The increased precision offered by UV laser ablation may have significant advantages over mechanical surgical techniques.

The present study examines the use of 213 nm laser pulses in an *in vivo* rat model, in both acute experiments and in survival experiments with a two-week recovery period.

Materials and Methods

Animal preparation

Sixteen adult male Brown-Norway rats were housed in a light/dark cycle of 12 hours light (50 lux) and 12 hours dark. They were fed standard laboratory rat chow with water *ad libitum*. Animal positioning, eye fixation, and intraocular manipulation, were similar to those reported in our earlier studies of intraretinal oxygen measurements using microelectrode techniques in rats [19,20].

The rat was anesthetized with an intraperitoneal injection of Ketamine (50 to 90 mg/100 g) and Xylazine (3 to 10 mg/100 g). The rat was then mounted prone in a modified Stellar stereotaxic system (model 51400; Stoelting Co., Wood Dale, IL), placed on a platform and its upper palate rested upon a smooth metal rod slightly above the level of the body to keep its mouth and airway opened. The head was securely held in place using a stereotaxic device and ear bars to prevent movement during laser treatment. The eye to be lasered was kept in position using an eye ring and four sutures. Rectal temperature was monitored and maintained at 37.5°C by a homeothermic blanket (Harvard Apparatus).

For acute experiments, only one eye was treated and the rats were euthanized through an intra-cardiac injection of Euthal at the conclusion of the experiment, and the eyes enucleated for histology processing. To minimize the number of animals used, up to five lesions were induced in the same eye for acute experiments. Only 3 rats were used for acute experiments.

For chronic experiments, only one lesion was induced in each of the experiment eye of the animal. 12 rats had only one eye lasered and 1 rat had both eyes lasered. The rats were kept warm after the experiment using a temperature controlled mat and towels and allowed to recover. Antiseptic ointment (1% Chlorsig) was applied to both eyes during recovery to prevent later infection. These rats were cared for at the animal facility for a further two weeks before they were euthanized for enucleation of the treated eye.

All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The study was approved by the University of Western Australia Animal Ethics Committee (Approval Number 03/100/189).

Laser ablation of trabecular meshwork and limbus tissue

A schematic diagram of the experiment arrangement is shown in Figure 1. The laser and associated optics enabled pulsed ultraviolet laser radiation to be delivered to the tissue surface through an optical fibre. The system is a modified version of the one previously reported [3,4]. The fifth (213 nm) harmonic of a pulsed Nd:YAG laser (Surelite II10; Continuum, Santa Clara, CA) was launched into an optical fiber using a custom-made hollow glass taper as a beam concentrator. The 213 nm laser beam was isolated by a prism, and other harmonics absorbed by a beam stop. The pulse duration was 4 to 6 nanoseconds. Beam energy was measured using calibrated laser energy meters (Gentec QE25 and ED100AUX; Gentec Electro-Optics, Quebec, Canada). A low-power 633 nm visible HeNe (Helium-Neon) laser (Uniphase, Milpitas, CA), collinear with the 213 nm beam, was also launched into the optical fibre as an alignment aid. Custom optical fibre tapers were manufactured from speciality silica/silica fibre (FDP; Polymicro Technologies), these tapers were effective in the delivery of 213 nm laser pulses to the tissue surface. Fibres lengths were cleaved, waists were formed by propane flame pulling and subsequent cleaving formed neck-down tapers. These fibres all had core diameters of 600 μm , lengths of 600 mm, and tips tapered where the tip diameter was between 31 and 45 μm . A key difference between the current and previously reported systems was the smaller diameters of the optical fibre-tapered tips used in this

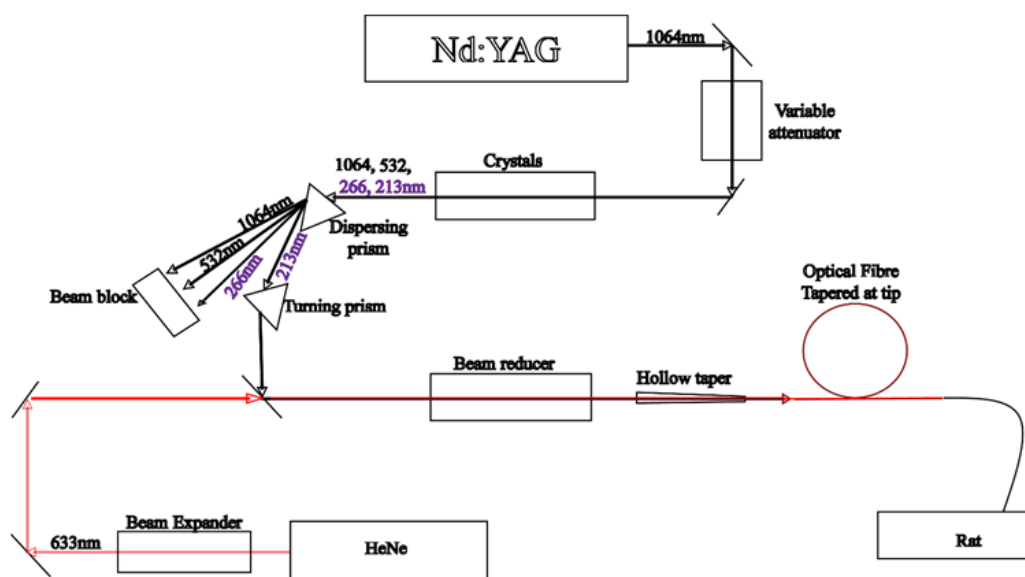


Figure 1: The schematic is a representation of the set up used for generation and delivery of the 213 nm laser beam

study. The smaller tip diameter resulted in a greater rate of fluence reduction with distance from the face of the optical fibre tip and, therefore, affected the depth profile of ablated lesions.

The angle was viewed with the aid of a gonioscopic lens (Goniolens, Ocular Instruments, USA) and high power microscope observation (Figure 2A). The tapered fibre optic entered the cornea through a freshly ablated entry hole (Figure 2B), created by the tip pushing on the entry point with the laser pulsing continuously at 10 pulses per second. This allowed the probe to penetrate the cornea with minimal leakage of aqueous humour. The corneal entry point, temporal side, ~1-1.5 mm from the limbus, was also the locus of rotation of our microsurgical system, such that rotation of the positioning system pivoted about the entry point into the eye. Translation of the fiber across the eye was achieved by a piezoelectric motor (Inchworm; Burleigh Instruments, Fishers, NY) and a hand held controller. Once near the angle, the gonioscopic lens was used to monitor positioning of the fiber tip to gently press on the trabecular meshwork in the nasal region. This process was aided by the visible laser illumination coaxial with the 213 nm beam pathway.

The trabecular meshwork lesions were generated using 50 or 100 pulses of 213 nm radiation. Fluence values of 0.4 or 0.6 J.cm⁻² were used. The laser parameters were chosen based on our previous study [4].

Histology

The angle region from the laser treated eyes were dissected into small blocks under the microscope and processed for epoxy processing and embedding. 1 µm thick epoxy sections were cut using glass knives on an ultramicrotome and stained using toluidine blue. All lesions were sectioned throughout the whole lesion, inclusive of some distance before and after the lesion region. The stained sections were imaged using the DM1200 camera on a Nikon E800 microscope and a Plan Apo x40 oil lens.

Semi-quantitation

The tissue sections for each lesion were analysed to obtain an area occupation measurements by cell debris (homogenized and damaged cellular structures), red blood cells, cellular structure that has re-populated the ablated sclera and area without any structure.

Images of the toluidine blue stained epoxy sections were converted into 8 bit grey images for analysis. The total area of ablation, area with homogenized and damaged cellular debris, red blood cells filled region were manually selected using the free hand selection tool in Image Pro Plus (v. 7.1) and area occupation collected for each category. The percentage area occupation by each category was calculated for each image analysed and averaged for each lesion with the results presented in Table 1.

Results

Appearance of lesions

Normal trabecular meshwork structures were disrupted at the ablation site (Figure 3). 9 out of 10 acute lesions were filled with red blood cells and homogenized substances, although the ablated portion in the sclera/cornea usually has clean borders (Figure 3B & 3C). On the other hand, chronic lesions tended to be cleaner, with little debris and some loose cellular contents (Figure 4).

Energy levels and Ablation depths

Both fluence levels used were able to ablate angle tissue in rat eyes. 0.6 J.cm⁻² x 50 pulses tended to induce a deeper ablation depth than 0.4 J.cm⁻² x 100 pulses. In acute lesions, the average ablation depth at the scleral-corneal junction point measured 59.3 ± 16.85 µm (5 lesions, 2 rat eyes) as induced by 0.4 J.cm⁻² x 100 pulses and 72.6 ± 33.27 µm (3 lesions, 1 rat eye) as induced by 0.6 J.cm⁻² x 50 pulses. For chronic lesions, the average ablation depth measured 73.7 ± 15.91 µm (9 lesions, 9 rats) as induced by 0.4 J.cm⁻² x 100 pulses and 81.4 ± 13.09 µm (3 lesions, 3 rats) as induced by 0.6 J.cm⁻² x 50 pulses.

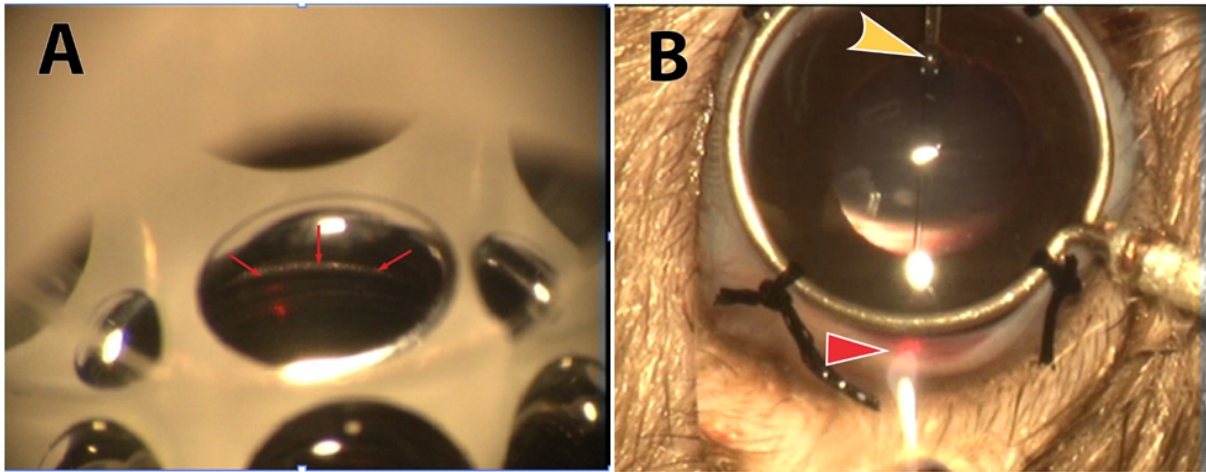


Figure 2: Angle structure observed through gonioscope for insertion of optic fibre. A) shows the magnified view of the angle structure as observed through the special gonioscope. Three red arrows point to the trabecular meshwork at the angle location and a red dot from the aiming beam indicating the location of the iris before moving to trabecular meshwork. B) shows a rat eye that has been secured in position using a special eye ring and four sutures. The optic fibre may be seen going through the cornea (curved arrow head) and a burst of laser directed by the aiming beam (red dot at the point of the straight arrow head) is being fired at the angle structure

Fluence Level and Time	Debris (% ablation area)	RBC (% ablation area)	Cellular structures (% ablation area)	Cleared (% ablation area)
0.4 J.cm ⁻² x 100p Acute	36.8 ± 20.58 (5)	32.3 ± 20.47 (6)	-	21.4 ± 10.72 (5)
0.6 J.cm ⁻² x 50p Acute	40.5 ± 12.23 (4)	48.3 ± 11.70 (4)	-	17.6 ± 6.25 (4)
0.4 J.cm ⁻² x 100p Chronic	-	15.1 ± 5.99 (9)	40.5 ± 12.83 (9)	15.1 ± 5.99 (9)
0.6 J.cm ⁻² x 50p Chronic	-	15.2 ± 10.00 (4)	54.4 ± 13.56 (4)	15.2 ± 10.00 (4)

Table 1: Semi-quantitative measurements-Percentage of area occupation by debris, red blood cells (RBC), re-populated cellular structures and area cleared. Number in brackets refers to the number of lesions analysed

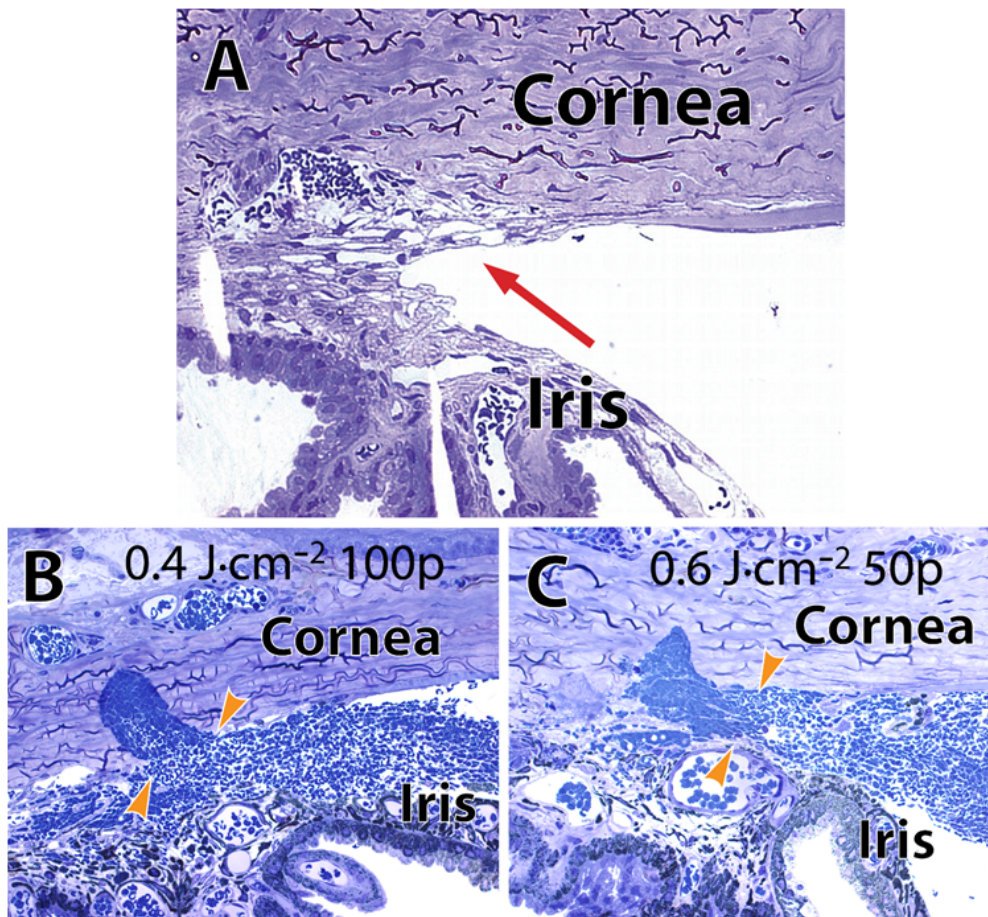


Figure 3: Histology images of a normal rat angle and two acutely lasered lesions. A) shows the angle structure of a normal untreated rat eye with loosely distributed cellular meshwork (red arrow) that is bordered by the ciliary body posteriorly and the cornea anteriorly. Blood vessels may be seen in close proximity to the trabecular meshwork surrounding the angle. B) shows an angle structure that has been acutely lasered at $0.4 \text{ J.cm}^{-2} \times 100 \text{ p}$. A blood filled void (arrow heads) may be seen in the sclera. Blood cells and cellular debris may be seen filling the gap in the meshwork where tissues have been ablated. C) shows an angle that has been lasered at $0.6 \text{ J.cm}^{-2} \times 50 \text{ p}$. Red blood cells have also filled a larger void in the cornea (arrow heads) as well as the angle region

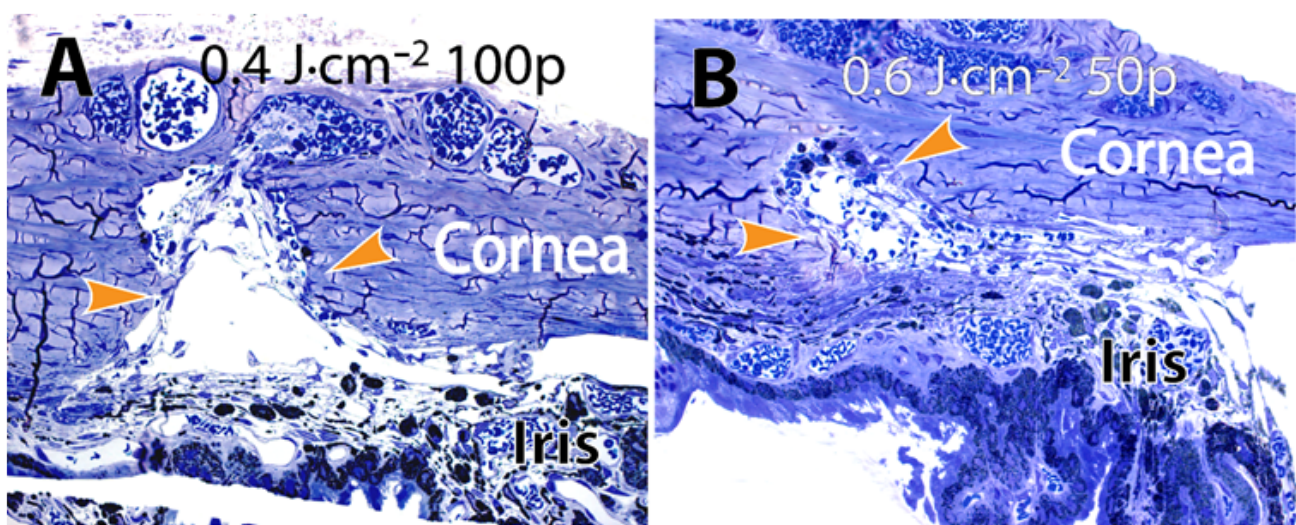


Figure 4: Histological appearance of 213 nm induced lesions 2 weeks post-ablation. The ablation borders (arrow heads) in the sclera remained well demarcated and uniform at these two energy levels applied. Image A) shows a lesion site treated with $0.4 \text{ J.cm}^{-2} \times 100 \text{ p}$. The ablated region in the sclera is now occupied by some loosely organized cellular structures and cleared of red blood cells. B) shows a lesion site treated with $0.6 \text{ J.cm}^{-2} \times 50 \text{ p}$. Similarly, the ablated region is filled with loosely arranged cellular structures with very few extraluminal red cells. The ablated region was at a greater depth induced by $0.6 \text{ J.cm}^{-2} \times 50 \text{ p}$ compared to $0.4 \text{ J.cm}^{-2} \times 100 \text{ p}$

Appearance of angle 2 weeks post-ablation

Two weeks post-ablation, the debris at the ablation site was mostly cleared. There were far fewer red blood cells and much less homogenized cellular debris remaining. Some cellular structures resembling the loose structure at trabecular meshwork populated the ablation site. Pigmented cells, possibly migrating from the iridal stroma or macrophages, were sometimes observed at the ablated region. The ablated sclera appeared to remain unfilled and open/patent.

Discussion

Our major findings from this study are:

1. 213 nm laser pulses can effectively ablate rat trabecular meshwork tissue *in vivo*,
2. The fluence required is comparable with that required to ablate trabecular meshwork of human donor tissues and
3. only limited inflammatory reaction and healing processes were evident. These results are encouraging, and lead us to consider performing similar studies in large animals more close to man, and to follow the post-surgery trabecular meshwork changes over a longer term.

The purpose of laser ablation surgery is to provide an advanced and controllable level of microsurgical precision while avoiding unwanted collateral damage. We are aware that rats are not an ideal animal model for such a study. Firstly, it is a challenge to insert the optical fibre and perform surgery in the anterior chamber in such a small eye. The rat has small size of the eyeball (6 mm in diameter) and small volume of aqueous humour (20 to 25 times smaller than that of the human eye). This means that it is critical to maintain the shape of the anterior chamber without loss of aqueous humour to allowing the tip of the optical fibre to reach the trabecular meshwork in the very narrow angle. Secondly, there is a very rich vasculature in the rat angle. It is difficult to avoid haemorrhage during the trabecular meshwork ablation. However, there are some significant advantages to use the rat as a model. The rat is one of the few experimental animals that have a trabecular meshwork and Schlemm's canal structure similar to man. Furthermore, in rodents and humans, some degree of reparative mechanisms are present in the endothelial layer. Even though human endothelial cells rarely divide physiologically, they have the ability to migrate and enlarge to compensate for small defects in the endothelial layer. We therefore selected the rat as a model to test the 213 nm laser ablation capability and the resultant healing processes.

Interestingly, we found that localised haemorrhage in the angle occurred in almost all rats immediately after UV laser ablation. Haemorrhage was also found in the histological sections from the eyes from acute experiments. Usually haemorrhage was significantly improved a few days later and almost disappeared two weeks after laser ablation. However, a few red blood cells were still found in the ablation sites in the histological sections. We noted that there were rich blood vessels in the rat angle (Figure 3A). It may help explain why haemorrhage often occurred during the laser ablation. Haemorrhage may confound the laser and tissue interaction, but trabecular meshwork and limbus tissues were effectively ablated with sharp edges. Perhaps, we have used multiple pulses in which very tiny air bubbles were produced between the tip of the optical fibre and the target tissue. These air bubbles may push the red blood cells away.

Another interesting finding is that an ablation channel was formed (Figure 4A & 4B). Some cells were seen on the surface of sclera tissue indicating some healing process. There were only minor inflammatory changes seen. Some cells and debris were found at the ablation site. These cells have relatively normal appearance and could be due to oblique cutting of the ablation channel. It was difficult to align the histology section with orientation of the optical fibre. Therefore, the results of our quantitative analysis could be influenced by the consequence of oblique histology sections.

Conclusion

In summary, our results suggest that 213 nm laser pulses can effectively ablate trabecular meshwork and limbus tissue *in vivo*, with minor inflammatory reaction and limited healing processes.

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Conflict of Interest

None of the authors have any competing interests.

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