Experimental Retinal Ablation Using a Fourth-Harmonic 266 nm Laser Coupled with an Optical Fiber Probe

Paula K. Yu, Joseph Miller, Stephen J. Cringle, and Dao-Yi Yu

PURPOSE. To explore the ablation potential of 266 nm laser pulses, with an intact porcine retina preparation.

METHODS. Segments of porcine eyes were used in an in vitro preparation in which localized areas of intact retina and choroid could be exposed to 266 nm laser irradiation. The segments of ocular tissue were bathed in fluid, to mimic the intraocular environment. Contact between the probe and the retinal surface was established before the first laser pulse. Single or multiple pulses (5–7 ns duration) at fluence levels of 0.4 to 1.2 J/cm² were delivered via a tapered fiber optic probe with a tip size of approximately 110 μm. The retinal tissue was then fixed and sectioned for histologic examination. The ablation depth and extent of damage were measured and related to fluence level and the number of pulses applied.

RESULTS. Ablation of the inner retina was achieved by single pulses at fluence levels of 0.6 J/cm² and higher. The depth of retinal ablation was highly dependent on fluence for lesions generated with a single pulse but less so for multiple pulses (5–10), particularly at lower fluence levels. Higher numbers of pulses (50–100) did not increase ablation depth in a predictable manner.

CONCLUSIONS. Pulsed laser (266 nm) irradiation at low pulse counts and high fluence levels is a possible alternative for localized retinal ablation with minimal collateral damage in a fluid environment. (Invest Ophthalmol Vis Sci. 2006;47: 1587–1593) DOI:10.1167/iovs.05-1187

Many applications exist for laser-based ophthalmic instruments that are able to perform intraocular surgeries to transect and remove fibrocellular and fibrovascular membranes in various vitreoretinal diseases. These diseases include retinal detachment with proliferative retinopathy, diabetic traction detachment, penetrating trauma, retinopathy of prematurity, and epimacular membrane.1 Surgical management of these diseases requires an advanced level of microsurgical precision and control to avoid complications such as unwanted retinal damage and hemorrhage, a common problem with current mechanical surgical procedures in which scissors, blades, or forceps are used.

Laser technology has the potential to provide the required precision and control to transect and remove tissue, with minimal unwanted damage to surrounding tissue. The 193 nm argon fluoride (ArF) excimer laser has been widely accepted for corneal refractive surgery, providing exceptional control of ablation depth and minimal damage to surrounding tissue.2 However, to be used in the intraocular environment, the excimer laser must be equipped with a specially designed articulated arm and the laser–tissue interaction is different from that in a gaseous environment.3–10 Infrared sources such as CO2, erbium-YAG, and holmium:YAG lasers have undergone trials, via optical fiber delivery, of use in intraocular surgery.11–13 However, collateral damage to surrounding tissue via thermal and shock wave effects have been reported.

In the present study, a wavelength of 266 nm was used. This wavelength is closely matched to the absorption peak of proteins in the target tissue.9–13 At this wavelength, water is not a significant absorber, the absorption coefficient of the tissue being approximately 10³ times that of water.14,15 Further advantages of this wavelength are that it can be produced by a solid state laser source and that it can be delivered through a relatively low-loss fiber-optic probe that can be readily manipulated in the intraocular environment. We investigated the first use of 266 nm laser ablation on retinal tissue and sought to determine the usefulness of this technique for the ablation of retinal tissue. Successful ablation of retinal tissue would indicate future applications in the cutting of retinal membranes or vascular sheaths in a range of retinal diseases.

METHODS

Tissue Preparation

Porcine eyes freshly obtained from a local abattoir were placed in oxygenated sodium Krebs solution and transported to the laboratory on ice. The eyes were carefully dissected in ice cold Krebs solution into segments with retina, choroid, and sclera intact. The adherent vitreous was removed. The segments were kept in oxygen-bubbled Ringer’s solution at 4°C until use. Segments were used within 3 hours of dissection. During laser exposure, the segments were pinned to a wax plate immersed in Krebs solution at room temperature.

Laser Parameters

The laser used was an Nd:YAG (Surelite III10; Continuum, Santa Clara, CA). This laser emits pulses of duration 5 to 7 ns at a wavelength of 1064 nm, with a pulse repetition rate of 10 Hz. To convert the fundamental (1064 nm) to the fourth harmonic (266 nm), we used two nonlinear β-barium borate (BBO) crystals (Fujian Castech Crystals, Fuzhou, China). The 266 nm beam was isolated with a dispersing prism and launched into a 600 μm core diameter silica–silica optical fiber (Innovaquartz, Phoenix, AZ). Several fibers were used, but all had custom-tapered tips with diameters of approximately 110 μm. The 266 nm beam was monitored at two points in the transmission pathway with calibrated joulometers (Gentec ED200 and ED100AUV; Gentec Electro-Optics, Inc., Québec, Canada). The joulometers were connected to separate channels of a digital oscilloscope (VP5730A; National Instruments, Austin, TX). The ED100AUV was used to measure the energy output directly, at the taper tip, both before and after the generation of a set of lesions on a tissue segment. This energy measurement, combined with the diameter of the tapered tip enabled the...
calculation of the fluence at the taper tip. The ED200 was also used to measure the energy of the 266 nm beam, before and after the generation of each individual lesion. This joulemeter was placed in the line of the 266 nm beam after the dispersing prism, but before the launch optics. Retinal lesions were generated using fluences of 0.4, 0.6, 0.8, 1.0, and 1.2 J/cm², combined with a varying number of pulses per lesion (1, 3, 5, 10, 50, and 100). A total of 146 retinal lesions were created.

Positioning of the Probe

The fiber-optic probe was mounted on a positioning system that enabled the probe to be positioned perpendicularly on the retinal surface. Under dissecting microscope observation, the probe tip was placed just anterior to the chosen area of retina in a region free of major retinal vessels. For final positioning, the (red) beam from a low-powered HeNe laser was emitted through the optical fiber probe, and the entire working area was obliquely illuminated with white light. Observation of the red spot combined with the shadow of the probe tip created by the oblique illumination enabled the operator to position the probe tip gently on the retinal surface. Once in contact with the retina, the HeNe beam was redirected and 266 nm laser delivery commenced. Histology of retinas contacted by the probe with this method, but without 266 nm exposure showed no anomaly or compression artifact. To demonstrate a potential surgical application in a later trial, we positioned the probe adjacent to a retinal vessel crossing point, often at an angle, to allow separation of the vascular sheath.

Histologic Studies

The location of each exposure site was carefully mapped out in relation to the pattern of visible retinal vessels. After 266 nm irradiation, the eye segments were quickly immersed in 2.5% glutaraldehyde for fixation. The tissues were fixed for at least 24 hours before being processed for epoxy embedding. One-micrometer sections were made of the lesion area and the sections stained with toluidine blue. Transverse sections of retina were obtained as accurately as possible through careful embedding and alignment of the block to the knife edge during sectioning. Lesion sites were examined and imaged with a microscope with ×20 and ×40 objectives (E800; Nikon, Tokyo, Japan) coupled with a high-resolution digital imaging device (DXM-1200; Nikon).

The depth of ablation was measured manually from printed images. Ablation depth was judged to be the maximum depth at which retinal tissue was missing. When tissue debris was observed at the lesion site, it was ignored for the purposes of ablation depth measurement.

RESULTS

Laser Ablation of Lesions

Single Pulse per Lesion. Examples of the effect of single 266 nm laser pulses at different fluence levels are shown in Figure 1. At 0.6 J/cm², a single pulse ruptured the inner limiting membrane and ablated the superficial nerve fiber layer, with some remaining coagulated nerve fiber tissue (Fig. 1A). At
The relationship between depths of retinal ablation at different fluence levels for 50 pulse repetitions is listed in Table 3 and plotted in Figure 6. There was not a definable relationship between depth of retinal ablation and fluence level used.

**Train of 100 Pulses per Lesion.** The retinal ablation and collateral damage produced by 100 pulses per lesion for a variety of fluence levels are shown in Figure 7. At all fluence levels used (0.6–1.2 J/cm²), 100 pulses were sufficient to ablate the full thickness of the retina and produce extensive damage in the choroid. Massive vacuoles are present in each lesion, had a variety of shapes, and were located in different tissue layers within the lesions. There was no clear relationship between the laser-induced tissue damage (ablation and collateral effects) and the laser parameters (number of pulses per lesion and fluence per pulse).

**Laser Ablation Applied to Retinal Arteriole and Venule Sheaths at Crossing Points**

To explore the potential of this technique to treat retinal vessel occlusion by ablation of the vascular sheath, we produced a series of lesions with a small number of relatively low fluence pulses per lesion. For these studies, the probe was positioned at the appropriate angle. Figure 8 shows two specimens with partially ablated vascular sheaths but intact vessels, indicating the potential for use of this technique to reduce the tension of the vascular sheath at arteriole and venule crossing points.

**DISCUSSION**

The purpose of laser ablation surgery is to provide an advanced and controllable level of microsurgical precision while avoiding unwanted collateral damage. From a strategic point of view, the characteristics of the target and surrounding material are critical for the selection of appropriate laser parameters, especially wavelength. In biological systems, the most common molecule is water, comprising ~70% of the tissue mass. The next most prevalent component in tissue is the organic fraction, comprising ~25% of tissue wet weight. This organic fraction consists of proteins, lipids, and acids, among others, and makes up the various cellular and extracellular structures. All these structures are rich in ultraviolet chromophores. For intraocular surgery we have to work in a fluid environment, in the vitreous cavity, the retina, or the subretinal space. The target tissues are mainly composed of proteins, particularly collagen, which has a broad absorption peak in the ultraviolet between 250 and 290 nm.12–15 It is clear that there are two approaches to wavelength selection: to match the laser wavelength to an ultraviolet absorption band of the protein (collagen) in the target tissue or to select a wavelength matched to an infrared absorption band of water.1 In the present study, we chose to investigate the effectiveness of tissue ablation with a pulsing 266 nm laser targeted at absorption by protein and sought to investigate the optimum laser parameters for this wavelength.

**Table 1. Ablation Depth for Single Pulses**

<table>
<thead>
<tr>
<th>Laser Fluence (J/cm²)</th>
<th>Ablation Depth (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>15.4 ± 1.0</td>
</tr>
<tr>
<td>0.8</td>
<td>29.2 ± 3.1</td>
</tr>
<tr>
<td>1.0</td>
<td>48.4 ± 5.6</td>
</tr>
<tr>
<td>1.2</td>
<td>52.0 ± 4.4</td>
</tr>
</tbody>
</table>

Data are the mean ± SE.
The exact mechanism of target ablation by UV laser irradiation has been more difficult to quantify than was initially thought.\textsuperscript{12,16} The photon energies of several UV laser lines are greater than the dissociation energies of many of the molecular bonds present in polymers and the organic components of tissue. This leads to the initial hypothesis of molecular fragmentation and ejection as a result of direct bond breaking after photon absorption.\textsuperscript{17} This ablative photochemical decomposition has subsequently been found to be only partially responsible for ablation, especially for UV wavelengths greater than 200 nm. The alternative hypothesis is that ablation is mediated by vibrational relaxation.\textsuperscript{18,19} For example, Oraevsky et al.\textsuperscript{20} estimate that only 2\% of the incident energy of a 308 nm excimer laser pulse contributes to photochemical decomposition of human aorta. It is likely that both of these processes play a role in the reported tissue ablation by 266 nm laser pulses. The exact contribution of each is difficult to determine.

We have tested several different protocols for ablation of retinal tissue. Although the intended surgical application may not be to ablate the retina itself, the intact retina serves as a useful model for tissue ablation. The layered nature of the retina makes ablation depth easier to assess. When single pulses were used, tissue ablation was clear, and highly dependent on the fluence of the 266 nm irradiation output from the tip of the fiber-optic probe. This opens up the possibility that single pulses of 266 nm laser irradiation could be used for transecting and removing fibrocellular and fibrovascular membranes. However, our results suggest that higher fluence rather than a large number of pulses may be more appropriate for removing or transecting thicker membranes. Unfortunately, there are drawbacks in delivering high-fluence pulses through optic fibers. A high-power laser beam can damage the input end of the optic fiber. We were careful to monitor the condition of the fiber before and after use, recleaving and recalibrating if damage occurred. However, an improved launch arrange-

![Figure 3](image_url)  
**Figure 3.** Retinal sections after exposure to multiple ($\leq$10) laser pulses per lesion. Laser-induced damage to the retina (arrowheads) was mainly located in the superficial layers. The following fluence levels and number of pulses per lesion were used: (A) At 0.4 J/cm$^2$ and 10 pulses, the inner limiting membrane was disrupted at the site of exposure. Some retinal tissue was ablated and an area of lightly stained coagulated tissue was observed. (B) At 0.6 J/cm$^2$ and 10 pulses, more tissue was removed and coagulated tissue at site of laser exposure was clearly observed. (C) At 0.8 J/cm$^2$ and 5 pulses, increasing the fluence level and reducing the number of pulses per lesion produced more effective ablation of retinal tissue. (D) At 1.0 J/cm$^2$ and 3 pulses, the depth of retinal ablation appeared to be increased. Scale bar, 50 $\mu$m.

![Figure 4](image_url)  
**Figure 4.** The relationship between the depth of retinal ablation and the number of pulses per lesion at four fluence levels. Generally, at each of the fluence levels used, the depth ablated increased with the increase in pulses per lesion. However, with the exception of the lesions generated at a fluence of 1.0 J/cm$^2$, this relationship was not linear. At any particular number of pulses, the depth of the retinal ablation increased with the increase in fluence per pulse.

<table>
<thead>
<tr>
<th>Laser Fluence (J/cm$^2$)</th>
<th>1 Pulse</th>
<th>3 Pulses</th>
<th>5 Pulses</th>
<th>10 Pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25.6 ± 4.1</td>
</tr>
<tr>
<td>0.6</td>
<td>15.4 ± 0.97</td>
<td>12.5 ± 2.3</td>
<td>—</td>
<td>38.9 ± 2.5</td>
</tr>
<tr>
<td>0.8</td>
<td>29.1 ± 3.1</td>
<td>32.5 ± 2.8</td>
<td>64.4 ± 1.4</td>
<td>214.3 ± 6.4</td>
</tr>
<tr>
<td>1.0</td>
<td>48.3 ± 5.6</td>
<td>86.9 ± 8.4</td>
<td>—</td>
<td>227.0 ± 6.9</td>
</tr>
</tbody>
</table>

Data are mean micrometers ± SE.
ment and/or technical improvements in fiber performance will reduce laser-induced changes in performance, thus enhancing long-term reliability.

One difference between lesions generated using UV- and IR-pulsed laser sources is the amount of lateral thermal damage present. Our optimal lesions, produced using a low number of pulses and a higher fluence, had minimal lateral thermal damage. This has also been reported for lesions generated using an ArF excimer UV laser source with a wavelength of 193 nm. However, reports of IR-generated lesions have regularly shown some degree of significant lateral thermal damage.1,7,8

At greater numbers of pulses per lesion, the trend of ablation versus fluence per pulse became less predictable and collateral damage was increased. The reasons for this effect are not known and require further investigation. It may be that the tissue can become less susceptible to ablation after repeated laser exposure. In addition, there is clearly a lot of ablation debris remaining within the lesions, which may be formed by the initial pulses within the pulse train and could act to alter the effect of subsequent irradiation pulses. As the lesion develops, the increasing distance from the fixed probe tip is also a factor in reducing the effective laser fluence at the tissue surface.

A remarkable difference between the lesions formed using 50 or more pulses per lesion and those formed using 1 to 10 pulses per lesion is the formation of large vacuoles. These are presumed to be formed by cavitation bubbles during ablation. It has been reported that cavitation bubbles are the main driving force of retinal ablation when using 193 nm ArF excimer laser irradiation.5 The aqueous environment in which our ablation took place is likely to have an indirect effect on the collateral damage. The effect of environment on ablation has been extensively studied, and it has been reported that, when compared with ablation in air, ablation in a liquid environment results in confinement of ablation products by the liquid. This confinement enhances the coupling of the laser energy into mechanical energy and therefore enhances the potential for mechanical collateral damage.16

It must be remembered that all UV wavelengths are mutagenic at high enough exposure levels, causing damage to the DNA. However, mutagenic changes vary with cell type, for example, quiescent cells are usually much less sensitive when compared with rapidly dividing cells. Although 266 nm is within the UV-C band (190 – 290 nm), research in the UV-C band has been central in elucidating many important features of cellular functioning.12 Further studies are needed, to gain a better understanding of the longer-term influence of UV-C radiation on the retina.

We also tested the potential of 266 nm laser pulses for the dissection of retinal arteriole and venule sheaths by using a moderate fluence level and a low number of pulses per lesion (3–10). The results are encouraging in that selective ablation of the vascular sheath without damage to the arteriole or venule was achieved.

In summary, we used an optical fiber to deliver pulses of 266 nm laser irradiation and examined the relationship between laser fluence and pulse count on the effectiveness of the ablation of retinal tissue in a fluid environment. Our results

![Figure 5](image1.png)

**Figure 5.** Retinal sections after multiple pulses (50 per lesion) at relatively low fluence levels (0.4 and 0.6 J/cm²). Tissue ablation was mainly from the superficial layers (arrowheads); however, the laser-induced damage extended over the full thickness of the retina and into the choroid. (A) In the lesion produced by 50 pulses at 0.4 J/cm², the whole thickness of retina immediately beneath the ablated area was markedly damaged, with a coagulated and disorganized residual tissue. A vacuole (short arrow) formed in the mid retina. Regional RPE showed necrotic changes, but Bruch’s membrane and the choroid are intact. (B) In the lesion produced by 50 pulses at 0.6 J/cm², the ablated area of the retina was smaller than that produced at 0.4 J/cm². A large vacuole formed in the inner plexiform and inner nuclear layers; however, the outer nuclear layer was still definable, even in the center of the laser lesion. The RPE and Bruch’s membrane were largely disrupted. Scale bar, 50 μm.

### Table 3. Ablation Depth for 50 Pulses

<table>
<thead>
<tr>
<th>Laser Fluence (J/cm²)</th>
<th>Ablation Depth (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>63.8 ± 13.6</td>
</tr>
<tr>
<td>0.6</td>
<td>44.8 ± 6.6</td>
</tr>
<tr>
<td>0.8</td>
<td>95.7 ± 30.1</td>
</tr>
<tr>
<td>1.0</td>
<td>97.6 ± 39.0</td>
</tr>
</tbody>
</table>

Data are the mean ± SE.

![Figure 6](image2.png)

**Figure 6.** The relationship between the depth of retinal ablation and fluence level per pulse in lesions created by 50 pulses. There was no definable relationship between depth of retinal ablation and fluence level per pulse.
suggest that low pulse counts at higher fluence levels may give the most predictable degree of tissue cutting with less collateral damage than do higher pulse counts at lower fluence levels.

Acknowledgments

The authors thank Dean Darcey and Judi Granger for technical assistance.

References


