Ex Vivo Exposure to Soft Biological Tissues by the 2-µm All-Fiber Ultrafast Holmium Laser System

Mariya S. Kopyeva 1,2*, Serafima A. Filatova 1, Vladimir A. Kamynin 1,*, Anton I. Trikshev 1, Elizaveta I. Kozlikina 1,3, Vadim V. Astashov 4, Victor B. Loschenov 1,3 and Vladimir B. Tsvetkov 1

Abstract: We present the results of ex vivo exposure by an ultrafast all-fiber Holmium laser system to porcine longissimus muscle tissues. A simple Ho-doped laser system generated ultrashort pulsed radiation with less than 1 ps pulse width and a repetition rate of 20 MHz at a central wavelength of 2.06 µm. Single-spot ex vivo experiments were performed at an average power of 0.3 W and different exposure times of 5, 30 and 60 s, varying the total applied energy in the range of 1.5–18 J. Evaluation of laser radiation exposure was performed according to the depth and diameter of coagulation zones, ablation craters and thermal damage zones during the morphological study. Exposure by ultrashort pulsed radiation with an average power of 0.3 W showed destructive changes in the muscle tissue after 5 s and nucleation of an ablative crater. The maximum ablation efficiency was about 28% at the ablation depth and diameter of 180 µm and 500 µm, respectively. The continuous-wave radiation impact at the same parameters resulted only in heating of the near-muscular tissue, without ablation and coagulation traces. Exposure to tissue with an average power at 0.3 W of ultrashort pulsed radiation led, within 30 and 60 s, to similar results as caused by 0.5 W of continuous-wave radiation, although with less carbonization formation.

Keywords: fiber laser; holmium laser; 2-µm radiation; ultrashort pulses; continuous-wave radiation; longissimus muscle tissue; ex vivo experiment; ablation efficiency; ablation area; coagulation; lasers in medicine

1. Introduction

In the last decade, there has been particular interest in fiber sources of the short-wave infrared spectral range (SWIR, 2–3.5 µm) due to a wide area of potential applications, including laser surgery and biomedicine [1]. Such lasers can be based on silicate, fluoride, chalcogenide, or tellurite fibers doped with rare-earth ions [2,3]. Fibers based on silicate glasses are the most successful and widely used, allowing the attainment of compact, all-fiber laser schemes with simple standard splices. At present, silica-based fibers doped with thulium (Tm 3+) or holmium (Ho 3+) ions have been extensively developed and used for various types of fiber laser systems in the 2-µm spectral range [4–7]. However, there is a limitation of the lasing wavelength in silicate glass fiber at about 2.2 µm, because the propagation losses at longer wavelengths are extremely high due to the value of the phonon energy (1100 cm⁻¹) [8]. In [9], Holmen et al. have realized a Ho-doped fiber laser with a tunable lasing wavelength from 2.025 to 2.2 µm and with a maximum slope efficiency of...
58% and 27%, respectively. These fibers, together with another glass matrix or crystalline one, are used to operate at longer lasing wavelengths [10].

The presence of strong water absorption makes 2-μm laser sources promising for medical applications [11], because a decrease in the depth of radiation penetration into water-saturated tissue would be expected, leading to their precision processing with less tissue heating. Some medical procedures, such as minimally invasive surgery, laser enucleation, skin treatment, etc. have the potential to be more accurate and reliable using SWIR sources [12,13]. Considering that one of the water absorption peaks is located at a wavelength of about 1.94 μm, the use of a holmium laser with its emission wavelength shifted into the infrared range allows one to vary the penetration depth of laser radiation [14,15]. It is worth noting that 2-μm laser radiation is eye-safe, which is also an important advantage for a number of applications [16].

The field of laser application in medicine is wide and is constantly expanding, from diagnostics and therapy to surgery, while having non-destructive and destructive effects on biological tissues [17,18]. Therefore, laser radiation parameters should be chosen based on the desired result in each specific case. It should be taken into account that the effect of laser radiation depends on the wavelength, power density, operation mode (continuous-wave or pulsed), duration of exposure and pulse repetition rate, as well as on the optical properties of biological tissues [19,20].

Frequently, medical laser systems of the 2 μm spectral range operate in continuous-wave (CW) and nanosecond or longer pulsed modes [21–23]. These laser systems are mainly based on linear absorption of laser radiation by tissues, due to the relatively low peak intensity of the radiation, which is insufficient to induce a noticeable nonlinear interaction at initial average powers. In this regard, photodamage resulting from exposure to such lasers is strongly wavelength-dependent and thermal in nature [24]. On one hand, this can have a selective effect on the tissue, but on the other hand, it can lead to a non-deterministic cutting effect in heterogeneous tissue and limit the efficiency in transparent or weakly absorbing tissues [25]. Moreover, in this case, the heat diffusion from the laser spot increases the collateral thermal damage to surrounding tissues, contributing to scarring. This fact limits the precision of laser radiation effects inside thick specimens. The development of ultrafast pulsed laser sources and amplifiers producing short picosecond or femtosecond pulses [26,27] has made it possible to improve surgical precision beyond the optical diffraction limit through new, mostly non-thermal, modes of tissue photodamage. Such a precise effect is achieved due to the nonlinear absorption that occurs when the incident power intensity is sufficient to induce simultaneous absorption of multiple photons [28]. Thus, the volume into which the laser energy is deposited decreases and, consequently, there is less damage to the surrounding tissues [29]. The first studies on the medical application of femtosecond laser pulses dealt with effects on the retina and skin [30,31]. In [32], Oraevsky et al. showed that ablation and the plasma optical breakdown threshold strongly depends on the tissue linear absorption at the laser focus for the nanosecond pulses, while for femtosecond pulses it is practically independent of the target tissue linear absorption. This, in turn, simplifies targeting of small individual cellular structures [33,34]. Thus, ultrashort pulse (USP) (10 fs–100 ps) sources allow more precise non-thermal exposure, in contrast to continuous-wave radiation [35,36]. Moreover, in [37] Amini-Nik et al. showed that the damage is noticeably less and the healing process without scarring is much faster after exposure to a picosecond infrared laser, in contrast to the conventional surgical laser or a scalpel. Thus, the use of this type of radiation can lead to a more comfortable environment for patients by reducing healing time and reducing the risk of infections during surgery.

Significant progress has been made since the first use of lasers for medical purposes as an alternative to mechanical surgical instruments [38]. The design of laser systems has been greatly improved and changed towards compactness and simplicity. Due to the numerous advantages, particular attention is paid to the medical application of fiber lasers performing precise impact with minimal collateral damage to surrounding tissues [39,40]. Additionally,
the use of fiber lasers simplifies the task of delivering laser radiation during minimally invasive operations using surgical endoscopes.

In this paper, we present the results of ex vivo exposure on soft tissues while using an ultrafast all-fiber Ho-doped laser system and compare them with the results obtained earlier in [41] with a CW Ho-doped fiber laser under the same conditions and applied energy. A simple, unique Ho-doped laser system generated ultrashort pulsed radiation with less than 1 ps pulse width, with a repetition rate of 20 MHz at a central wavelength of 2.06 μm. The main objectives were to evaluate the depth and diameter of ablation (AZ), coagulation (CZ) zones and the heat-affected zone (HAZ) after ex vivo experiments of single-spot ablation of porcine longissimus muscle tissue by 2-μm ultrashort pulsed radiation with an average power of 0.3 W in the applied energy range of 1.5–18 J. Furthermore, we aimed to quantify the ablation efficiency (AE) for the Ho-doped fiber laser used in the study.

2. Materials and Methods

2.1. Ex Vivo Tissue Preparation and Histological Procedures

Porcine longissimus muscle tissue was used as an ex vivo tissue model. The choice of the biological tissue type is due to the sufficient study of its spectral properties. Based on the results of [10], we can predict the interaction character of 2-μm laser radiation with porcine muscle tissue. After preliminary cooling to 4 °C, porcine muscle tissue was cut into small specimens up to 5 mm thick. The specimens’ temperature was restored to room temperature (22 °C) before laser exposure. The tissue surface was sprayed with a physiological solution to prevent its drying and dehydration during the experiment.

Histological sections made with a microtome Microm HM 540 (Thermo Fisher Scientific Microm International Gmbh, Walldorf, Germany) perpendicular to the exposed specimen surface were analyzed using a confocal laser scanning microscope (Zeiss LSM 710 NLO, Zeiss, Jena, Germany) with a tunable wavelength (0.8–1.5 μm). A slice with 10 μm thickness was stained with a mixture of annexin 5/acridine orange-propidium iodide. The intensity of damaged tissue staining allowed us to identify coagulation zones, and qualitatively and quantitatively evaluate deep changes in tissue caused by laser action. The approximate size of the heat-affected zones was visually estimated immediately after laser exposure using the optical microscope MBS 12 with a magnification of 20×.

2.2. Ultrafast Ho-Doped Fiber Laser System and Experimental Setup

The unique all-fiber Holmium master oscillator power amplifier (MOPA) system was used as a source of ultrashort pulses. This system consisted of a hybrid mode-locked Ho-doped fiber laser and Ho-doped fiber amplifier, pumped by Yb-doped fiber laser [42]. The experimental setup of the Holmium MOPA system is presented in Figure 1a.

The laser with ring cavity consisted of Ho-doped fiber (4 m long) and SMF-28 single-mode fiber (6 m long). The active fiber core diameter was 16 μm and cut-off wavelength of about 2 μm. The numerical aperture of Ho-doped fiber was 0.11. The holmium ion concentration in the fiber core was $5 \times 10^{19}$ cm$^{-3}$. The net cavity dispersion of the laser was estimated to be about $-1.1$ ps$^2$. The laser was pumped through a 1.125/2.1 μm wavelength division multiplexer by a CW Yb-doped fiber laser emitting at 1.125 μm, with a maximum output power of up to 8 W. The absorption coefficient of the Ho-doped fiber at the pump wavelength of 1.125 μm was about 5 dB/m. To select one direction of radiation propagation, we used a fiber isolator specialized for 2 μm. The light was out-coupled from the laser cavity with a 10/90 fiber coupler, which allowed 90% of the optical power to be out-coupled.

Hybrid mode-locking was realized by combining a fast and a slow saturable absorber in the laser cavity, namely the nonlinear polarization evolution effect and single-walled carbon nanotubes (SWCNTs). For this, a fiber polarizer and a pair of polarization controllers were placed in the laser cavity and SWCNTs were fixed between two angled polished fiber connectors FC/APC, which were placed after the isolator in order to reduce the power density. This laser produced 1 ps soliton pulsed radiation with a repetition rate of about
20 MHz at a central wavelength of 2.068 µm and average output power of 4.5 mW at pump power of 3.2 W. The output lasing spectrum is presented on the inset in Figure 1a.

The Ho-doped fiber amplifier was used to increase the laser average output power up to the maximum value of 0.5 W. Pulsed radiation of the laser was directed to the Ho-doped fiber amplifier through an isolator which was used to suppress undesirable feedback. The counter-propagating pumping of the amplifier was carried out through a multiplexer by the CW radiation of Yb-doped fiber laser at a wavelength of 1.125 µm. The pump power varied from 0 to 5 W. We used 2 m of Ho-doped fiber with holmium ions concentration of $6.5 \times 10^{19}$ cm$^{-3}$ as the active medium of the amplifier. The absorption coefficient at the pump wavelength ($\lambda = 1.125$ µm) was about 11 dB/m and the numerical aperture was 0.14. Figure 1b,c shows the lasing spectrum and autocorrelation trace at the output of the Ho-doped fiber amplifier corresponding to the average output power of 0.4 W, that corresponded to 0.3 W of power delivered to tissue. The pulse energy in this case was 15 nJ. Such a noticeable transformation of the shape of the lasing spectrum and pulse autocorrelation trace is due to the joint effect of SMF and Ho-doped fiber dispersion and nonlinearity on the USP radiation propagation. Increasing the pump power leads to the generation of higher-order solitons and then to the pulse decay on the Raman solitons [43]. Therefore, in Figure 1c, the autocorrelation trace contains three well-defined peaks, corresponding to decaying pulses. It is possible to estimate the width of the pulse central part as 250 fs from the intensity autocorrelation function.

Figure 1. (a) Experimental setup of the all-fiber Holmium MOPA system operating in the ultrashort pulsed mode: WDM—wavelength division multiplexer, Iso—isolator, SWCNTs—single-walled carbon nanotubes, PC 1,2—polarization controllers; the output spectrum of Ho-doped fiber laser is presented on the inset (MO spectrum); (b) Lasing spectrum and (c) autocorrelation trace of the output pulse corresponding to the average output power of 0.4 W.
Additionally, we carried out a comparison of pulsed and continuous-wave 2-μm radiation effects on the same soft biological tissues. Ex vivo investigation of CW Ho-doped fiber laser radiation exposure in a wide range of applied energy (1.5–66 J) at a wavelength of 2.1 μm on longissimus porcine muscle tissues was presented in our previous work [41], which also contains a detailed description of the CW Ho-doped fiber laser scheme, as well as the setup used in the experiment.

A flexible single-mode fiber cable with a polished angle connector (FC/APC) was used for the convenient delivery of radiation in both laser systems. During experiments, we controlled the bending radius of the transport fiber to prevent undesirable losses. The experimental setup for delivering laser radiation to the specimens under study was a vertical stand with a focusing system attached to it (Figure 2). Thus, the output radiation was focused through an optical objective with 8 × 0.2 NA (LOMO) and positioned perpendicularly to the surface of the biological tissue fixed on the moving platform for the focal distance adjustment. The laser spot diameter on the specimen surface was about 40 μm.

![Figure 2. Schematic diagram of the experimental setup of laser radiation delivery to the biological tissue specimens. A moving platform is used to precisely adjust the focal distance.](image)

The duration of laser radiation exposure on the tissues was chosen as 5, 30 and 60 s, as in previous work, to make the results comparable. Considering the losses in the optical system for the USP laser, the value of power delivered to the tissue was 0.3 W. Thus, we ensured that the applied energy delivered to the specimens was the same for both CW and ultrafast laser systems, in order to accurately compare the results. In the case of the ultrashort pulsed laser, the range of applied energy varied from 1.5 to 18 J. The value of applied energy was calculated by multiplying the average radiation power by the exposure time [44].

3. Results and Discussion

Figure 3a shows microphotographs of the porcine longissimus muscle tissue surface immediately after exposure of ultrashort pulsed radiation. For comparison, Figure 3b shows microphotographs after CW radiation action with the same power of 0.3 W. At the beginning of the exposure, the tissue surface is heated, which is accompanied by a change in the tissue color. Over time, the area of elevated temperature increases from the center to the periphery, forming a heat-affected zone (HAZ). In Figure 3, the edges of this zone...
are marked with a red line. The longer exposure time induces the evaporation of water contained in the near-surface tissue layers. As a result, an ablative crater (yellow line) can be observed in the center of the laser spot. Further impact causes tissue darkening around the crater and its carbonization. During the exposure, we could clearly hear clicks, which can be attributed to the characteristic sound of steam bubbles collapsing. The existence of collapsing bubbles may indicate the occurrence of cavitation effects during the action of pulsed radiation. The bubbles’ collapse could lead to tissue bursting and integrity disruption. The stress waves generated by pulsed laser radiation in absorbing materials, such as biological tissues, are due to the thermo-elastic effect [45]. Caused by the rapid temperature increase at the laser beam focus, thermoelastic stresses lead to maximum pressure increase in a finite volume of material [29]. For example, temperature rising at a wavelength of 0.8 μm and pulse duration of about 100 fs occurs over times of the order of several picoseconds, which is insufficient for acoustic relaxation [29,46]. Experimental investigation of cavitation bubble formation and its theoretical background by G. Paltauf et al. has shown that the photoacoustic damage mechanism should be especially taken into account when describing ultrashort pulsed laser radiation exposure to biological objects [47]. The reason is that the finite-size absorbing material volume experiences tensile stresses under the action of laser radiation, which leads to cavitation within the material and photomechanical damage [47]. In [48,49], it was shown that decreasing the pulse duration to picoseconds and femtoseconds values minimizes the thermal effects. Therefore, there is a decrease in the threshold energy for the optical breakdown ($E_{th} \sim \sqrt{\tau}$).

By comparing the two laser operating modes at an equal average power of 0.3 W, we can clearly see the difference in the exposure results. In the case of CW laser radiation exposure, only a heat-affected zone is formed on the tissue surface (Figure 3b). On the other hand, the ultrashort pulsed laser radiation shows a completely different picture (Figure 3a). Coagulated tissue, which can be distinguished by a dark color at the site of exposure (purple line), is observed for the pulsed laser after the first 5 s of exposure. The optical properties of the coagulated tissue are changing during laser radiation exposure. In turn, this effect may lead to the increased absorption of radiation. Formation of an ablative crater and tissue carbonization was clearly observed with prolonged exposure.

![Figure 3. Microphotographs of the porcine longissimus muscle tissue surface after exposure by (a) USP Ho-doped fiber laser system with an average power of 0.3 W; (b) CW Ho-doped fiber laser with a power of 0.3 W [41]. Scale bar—500 μm.](image-url)
Morphological studies were carried out to determine damage zones more accurately and to identify the character of these zones. Obtained slices allowed the depth of laser energy penetration into the studied tissues to be traced, and qualitative and quantitative evaluation of ablated tissues. Histological sections of tissue before the impact of laser radiation (shown in Figure 4a) were used to determine changes in tissues exposed to laser radiation. The image of the histological section (Figure 4b) after CW laser radiation with a power $p = 0.3$ W for $t = 30$ s confirms only superficial damage and the absence of irreversible damage to the cell structure (myocytes) of muscle tissue. This damage can be indicated as a heat-affected zone (3) of the tissue, which is reversible by its nature and could be repaired in the healing process. The tissue changes are accompanied by edema at the site of exposure and temporary cellular dysfunction. In contrast to the CW radiation, exposure to ultrashort pulsed radiation with the same average power of 0.3 W leads to completely different results (Figure 4c). After 30 s of exposure, there is an ablation zone (AZ), marked with a yellow line, where an ablation crater (1) with rough and charred edges has formed due to the evaporation of the intracellular fluid and the burning of the residual tissue. The ablation zone is followed by the coagulation zone (CZ), marked by the blue line. In turn, the coagulated tissue represents the burned, loose and compact layers formed along the path of laser beam penetration into the tissue depth. The carbonization of myocyte mineral components leads to the formation of a burnt edge (2A). Necrotized cells with vesicularly altered cytoplasm constitute a loose layer (2B). Finally in the coagulation zone, we distinguished a compact layer (2C), where the loss of water components in myocytes leads to their dystrophy. As in the case of CW laser exposure, a heat-affected zone (3) is observed. In both cases (Figure 4c,d), the HAZ zone is not marked with a line, since this zone is larger than the recorded area of the microphotographs.

![Figure 4](image-url)  
**Figure 4.** Section of porcine longissimus muscle tissue (a) before exposure; and after 30 s exposure by Ho-doped fiber laser systems operating in (b) CW mode with a power of 0.3 W (applied energy of 9 J) [41]; (c) USP mode with a power of 0.3 W (applied energy of 9 J); (d) CW mode with a power of 0.5 W (applied energy of 15 J) [41]. 1—Ablation zone (AZ), 2—Coagulation zone (CZ) with the elements of the coagulation crust and fragmentary charring, 3—Heat-affected zone (HAZ).
We also compared the results described above with those obtained after continuous-wave radiation action with a power of 0.5 W in the applied energy range of 2.5–30 J. Figure 4d shows a histological cross-section after 15 J of 0.5 W of CW radiation. Increasing the CW laser power and, consequently, the applied energy led to more destructive effects in the tissue. Therefore, we also observe the following three thermal damage zones: ablation, coagulation and heat-affected zone, as in the case of USP laser radiation. A comparison of the interaction effect between CW and USP radiation was described in [29]. Thus, for cell surgery, the power of an argon laser at 488 nm and 514 nm wavelengths should be more than 1 W [50], which exceeds the average power of a femtosecond laser at 800 nm wavelength by more than three times [34]. Vogel et al. explain this by the fact that interactions of CW radiation and pulsed radiation with pulse duration longer than 10 μs are based on linear absorption, whereas absorption at exposure with ultrashort pulses is nonlinear.

Figure 5 compares the diameters of visible thermal damage (HAZ) for two laser modes of exposure, USP radiation of 0.3 W and CW radiation of 0.3 W and 0.5 W. Increasing the time of exposure leads to an increase in the HAZ diameter in both cases. Exposure of USP and CW radiation with an average power of 0.3 W for 5 s (1.5 J) resulted in HAZ diameters of 1000 μm and 750 μm, respectively. In the first case, tissue coagulation and the beginning of crater formation was observed, in contrast to the case of the CW laser. After 0.3 W of USP laser radiation exposure for 60 s (18 J), a HAZ diameter of about 2000-μm was measured, which is almost two times higher than that measured after the CW radiation exposure with the same power and time. It can be noted that exposure by continuous-wave laser radiation with a power of 0.5 W for 30 s (15 J) leads to comparable values of HAZ diameter to those measured after the pulsed radiation.

![Figure 5](image_url)

**Figure 5.** Dependency diagram of the heat-affected zone (HAZ) diameter on the applied energy of USP and CW laser radiation for different exposure times (5, 30 and 60 s).

For CW laser radiation with a power of 0.3 W, as well as for USP radiation with the lowest applied energy of 1.5 J (t = 5 s and a power of 0.3 W), formation of an ablation crater and tissue carbonization were not observed. Therefore, these values are not presented in Figure 6, which shows the dependency diagrams of the depth and diameter of the ablation (AZ) and coagulation (CZ) zones on the applied exposure energy for USP with a power of 0.3 W and CW with a power of 0.5 W radiation. As can be seen in Figure 6, the dependence of ablation depth on exposure time was weak for low applied energies, for both CW and USP radiation. The diameter of the ablation crater increased after USP radiation exposure. The maximum demonstrated ablation depth was 180 μm and the diameter was 500 μm.
CW radiation exposure, the ablation crater diameter was about 300 μm, and dependence on exposure time was not observed. The depth of the coagulation zone, created by USP radiation with a power of 0.3 W for 60 s (18 J) and CW radiation with a power of 0.5 W for 60 s (30 J), was approximately the same. The diameter of the coagulation zone for such combinations of parameters differed slightly.

Based on data reported in the literature, there are five main types of laser interaction with biological tissues. The main one is photothermal, which describes numerous effects [20]. Tissue temperature is an important parameter for this type of interaction. Understanding the change in local temperature will give insights into the effects that accompany laser exposure. Tunc et al. [51] confirmed in their studies that fast delivery of laser energy resulted in a sharp temperature increase, which led to the predominance of tissue vaporization over heat conduction to surrounding tissues and, as a consequence, more effective ablation and less thermal damage. On the contrary, a slow increase in temperature was accompanied by greater thermal damage. This is due to the fact that the processes of light absorption and heat generation are fast, in contrast to the heat distribution (dissipation). Therefore, temperature monitoring during laser surgery will help to reduce irreversible thermal damage and to reduce carbonization.

For quantitative analysis of the single-spot experiments on muscle tissue ablation by ultrashort pulsed 2 μm radiation, we have calculated the values of the ablation area (AA), specifically the area of the ablation crate and the coagulation area (CA) as an area of the coagulation zone [51,52]. Both areas were measured on histological cross-sections using the publicly available software “ImageJ” (National Institutes of Health) [53]. The obtained data allowed us to find the ablation efficiency (AE), measured by the ratio of the ablation area to the total irreversible thermal damage area \( AE, \% = \frac{AA}{AA+CA} \times 100 \). In addition, the calculated values for USP radiation were compared with the values for CW radiation and are shown in Figure 7. The ablation area (AA) obtained after exposure by USP radiation of 0.3 W (green bars) and CW radiation of 0.5 W (red bars) increased with the growth of the applied energy. The ablation efficiency (AE) of the USP laser radiation was about 27% for 18 J of applied energy while for the CW laser radiation it was lower by 5% for 15 J of applied energy and higher by 5% for 30 J. Thus, it can be noted that the ablation efficiency by ultrashort pulsed radiation with lower average power (0.3 W) is comparable to the ablation efficiency by continuous-wave radiation with a higher power (0.5 W).
In this work, we studied the exposure of ultrashort pulsed laser radiation in the spectral range of 2–2.2 μm with a central wavelength of 2.06 μm to porcine longissimus tissue ex vivo. A simple, robust and compact all-fiber Ho-doped laser system generated ultrashort pulsed radiation with a duration of less than 1 ps and a repetition rate of 20 MHz. This type of ultrafast all-fiber Holmium laser system with a high pulse frequency and low energy for soft tissue irradiation has not yet been reported in the literature. After ex vivo single-spot ablation experiments on the muscle tissue in the range of applied energy 1.5–18 J, the depth and diameter of coagulation zones, ablation craters and thermal damage zones were evaluated.

Comparison of USP laser exposure at an average power of 0.3 W with a CW laser at the same power and exposure time showed a difference in results. The continuous-wave laser radiation exposure led to reversible damage, namely the formation of a heat-affected zone on the tissue surface without destructive changes in the cell structure. The ultrashort pulsed radiation with the same average power caused more pronounced destructive tissue damage, which could be associated with the mechanism of laser-tissue interaction, and possibly nonthermal. The relative power safety for this USP laser exposure, resulting in non-destructive and reversible effects, is less than 1.5 J. Comparison of ablative efficiency has shown that USP laser ablation can be achieved at lower average power values compared to CW laser ablation. In addition, morphological studies have shown that the tissue at the edge of the formed crater during USP laser ablation was less susceptible to carbonization.

It should be noted that tissue carbonization is often undesirable in surgical practice because charred tissue has a tendency to a longer healing period and, as a consequence, to a higher risk of infection [54]. We can assume that, as proposed in [36,55], cutting the high-repetition-rate pulse sequence and creating pulse bursts with a relatively low repetition rate could contribute to a decrease in thermal damage of tissues, and consequently a reduction of carbonization.

The results obtained from ex vivo experiments with tissues of postmortem animals are part of a major study of the effect on biological tissues of two-micron laser radiation. The next stage will be in vivo experiments on animals using the laser systems studied in this work and the previous one [41].

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