



Experimental confirmation of the promising selective use of “green” laser radiation for photothermolysis of hemoglobin-containing tissues

Natalia Gorbatova^{1,2} · Ella Gasanova¹ · Sergei Zolotov¹ · Anatoly Sirotkin³ · Gennady Kuzmin³ · Alexander Tertychny⁴ · Natalia Stankova⁵ · Maria Remennicova⁶ · Oleg Tikhonovich³

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Abstract

To date, the problem of treating pathological subepithelial capillary structures, in particular, capillary angiodysplasia of the skin, remains. One of the promising methods for its elimination is photothermolysis by laser radiation of the yellow-green spectral range due to the selective absorption of blood by hemoglobin in blood vessels and, subsequently, their sclerosis and regression. In experimental work in vitro on model objects, chilled liver and skin samples from minipigs of the Svetlogorsk population, a comparison was made based on morphological studies of the results of the green laser radiation effect with a wavelength of 525 nm on these objects. In the case of a combined object (the liver under the skin), the green laser radiation that is little absorbed by the skin passes through it, which provides an effect on the underlying liver, where more pronounced thermal changes are formed compared to the skin. The results of the experimental study confirmed the selectivity of the laser radiation effect with a wavelength of 525 nm on hemoglobin-containing tissues. This fact is promising for selective photothermolysis of pathological subepithelial capillary structures, which will ensure the precision of treatment with minimal damage to skin tissues.

Keywords Laser radiation in the green region of the spectrum · Selective photodestruction · Angiodysplasia of skin capillaries · Model objects liver and skin of minipigs · Morphological examination

Introduction

Today, the problem of treating capillary angiodysplasia of the skin in adults and children remains [1–6]. In recent years, the

number of publications on the use of various types of laser radiation for the treatment of this vascular skin pathology has increased [2–9].

However, many specialists note an unsatisfactory clinical and esthetic result of treatment due to photodestruction not only of capillary structures containing hemoglobin but also unpredictable thermal damage to the skin [1–6]. All this negatively affects the results of treatment and can cause the development of cicatricial deformity of the skin, hyperpigmentation, and other negative consequences in the affected area [2–6, 10].

This fact dictates the choice of such laser radiation, which will provide a selective effect on subepithelial vascular structures with minimal thermal damage to the skin, including its growth zone [10, 11]. Currently, one of the most promising and effective methods for eliminating capillary angiodysplasia of the skin is the photodestruction of laser radiation of the yellow-green spectral range due to the selective absorption of laser radiation by the pigment (hemoglobin) of the blood in the blood vessels, followed by their sclerosis and regression [10]. It is known that the result of exposure to light on biological tissue is determined by three main factors: the wavelength

✉ Oleg Tikhonovich
tichon@kapella.gpi.ru

¹ Research Institute of Emergency Pediatric Surgery and Traumatology, Moscow, Russian Federation

² National Medical Research Center for Children’s Health, Ministry of Health of the Russian Federation, Moscow, Russian Federation

³ Prokhorov General Physics Institute of the Russian Academy of Sciences, Moscow, Russian Federation

⁴ Sechenov University, Moscow, Russian Federation

⁵ “Research Center for Biomedical Technologies of Russia”, Krasnogorsk, Moscow Region, Russian Federation

⁶ PJSC “Perm Scientific and Production Instrument Making Company” Laboratory of Agrobiophotonics, Perm Federal Research Center of the Ural Branch of the Russian Academy of Sciences, Perm, Russian Federation

of radiation, the duration of exposure, and the density of absorbed energy. The most important parameter of laser radiation is its wavelength, which determines the depth of its penetration into biological tissue and the selectivity of absorption by its various elements, chromophores [3, 7, 10, 11].

Yellow-green laser radiation of the visible spectrum has absorption by hemoglobin with two local maxima, green 520–540 nm and yellow 570–580 nm. Consequently, the radiation is absorbed by the contents of the blood vessels. At the same time, it is almost the same and is less absorbed by melanin and water [12]. In turn, it is the green wavelength of 520–540 nm that almost equally has a selective effect on hemoglobin and oxyhemoglobin, which is the greatest interest for the selective photothermolysis purpose of subepithelial capillary structures, mainly containing blood of a mixed nature.

Experimental research

A comparative in vitro experimental study on liver and skin samples from minipigs from the Svetlogorsk population was carried out to confirm the selectivity of “green” laser radiation by hemoglobin-containing tissues, which is necessary for effective photodestruction of subepithelial capillary structures of the skin.

Experimental equipment, materials, and methods

Equipment and modes of laser radiation We used an experimental sample of a solid-state laser apparatus based on semiconductor diodes that generate radiation in the green spectral range with a wavelength of 525 nm, near the absorption peak of hemoglobin and oxyhemoglobin, specially developed for medical purposes at the Prokhorov General Physics Institute of the Russian Academy of Sciences [11, 13].

In the experiment, we used “green” laser radiation with a wavelength of 525 nm with constant parameters of a pulse repetition rate of 10 Hz and a laser spot diameter of 0.8 mm on the surface. The following parameters were changed in the work: four modes of average power, the first 0.5 W, the second 1 W, the third 2 W, and the fourth 3 W; pulse duration 2 ms, 5 ms, and 10 ms; and exposure 5, 10, and 30 s, as well as their various combinations.

Object of study

The objects of the experimental study were chilled samples of the liver and skin of biological models, laboratory animals of minipigs (*Sus salvanus*) of the Svetlogorsk population, provided by the Federal State Budgetary Institution Scientific Center for Biomedical Technologies of the FMBA of

Russia. Minipigs (*Sus salvanus*) have the greatest similarity in anatomical structure and physiology with humans [14]. Liver tissue with a relatively homogeneous structure and a significant hemoglobin content can be considered an optimal object for studying the selectivity of “green” laser radiation with hemoglobin, and the skin with a much lower hemoglobin content, an object of comparison [14].

Laboratory animals, minipigs, were used in the amount of 3 individuals, at the age of 8 months. The sampling of experimental material, liver and light skin from the minipigs’ abdomen for the study, was carried out at the time of slaughtering the pig, then immediately the samples were placed in a physiological solution cooled to 5 °C. No more than 2 h later, chilled tissue samples from minipigs were used in experimental studies.

Experimental research technique

In the experimental work, three series of studies were carried out to comparatively study the results of exposure to “green” laser radiation with a wavelength of 525 nm on cooled samples of the liver, skin, and a combined preparation consisting of the skin and the liver underneath it.

In the first series of experiments, laser radiation with different modes was applied to samples of liver pieces through its capsule.

In the second series, for comparison, skin samples were treated using the regimes of the previous series.

In the third series, the samples of the combined preparation were exposed to laser radiation through the skin through the liver with the same modes. In all cases, on the surface of the objects of study, the impact zones were formed by a point-contact fixed spot of laser radiation using various combinations of its parameters.

Immediately during the experiment, we studied visually detectable macroscopic changes in the zone of action on the sample surface: the severity of thermal changes, the size and shape of the negative defect, crater, width, and nature of the zone of peripheral coagulation damage. Subsequently, histomorphological examination of the corresponding preparations with morphometric assessment was performed.

Based on a comparative analysis of the results of macroscopic and histological examination of the damaged areas of the model objects, the efficiency of “green” laser radiation (525 nm) for the purpose of selective photodestruction of hemoglobin-containing tissues, in particular, subepithelial vascular structures, was assessed.

Morphological histological examination

Tissue samples with laser exposure zones for histological examination were fixed in 10% neutral formalin. Further

processing was carried out automatically in an Excelsior apparatus for histological wiring (TermoScientific, UK) and embedded in paraffin using an EC350 apparatus (Microm, Germany). From paraffin blocks, sections with a thickness of 2–3 μm were obtained using an HM355S microtome with an STS section transfer system (TermoScientific, UK). The resulting histological preparations were dewaxed, then stained with hematoxylin and eosin.

Ready stained histological preparations were examined under a cover glass in a CX41 microscope (Olympus, Japan). To obtain micrographs, the histological preparations were scanned on a PanoramicMidi microscope (3DHISTECH, Hungary) with software image processing to assess the morphometric parameters of thermal tissue damage.

Research results

In the first series of experimental studies on samples of liver pieces, the nature of the thermal effect was clarified and the range of modes of “green” laser radiation (525 nm) was determined, which is necessary for effective photodestruction of hemoglobin-containing tissue. The results of the study showed that changes in the liver begin to be well defined when using laser radiation with the most energy-intensive parameters of the first mode (power 0.5 W, pulse duration 10 ms, and exposure 30 s).

The effect of thermal photodestruction is enhanced in accordance with an increase in power, as well as a longer pulse duration and exposure of the parameters of the second, third, and is most pronounced in the fourth mode (power 3.0 W, pulse duration 10 ms, and exposure 30 s). In all cases, the determined characteristic changes in the zones of influence on the liver remain; there are areas represented by a relatively wide and deep crater with a pronounced bordering zone of coagulation of its walls.

On the corresponding histological preparations, minus destruction is noted, in the form of a crater, the surface of the walls of which is in places made with a thin layer of compact coagulation necrosis, then below it is a layer of wider cellular necrosis, under which is a relatively narrow area of homogeneously coagulated tissues with clear boundaries of transition to unchanged tissue structures.

Based on the analysis of macroscopic and histomorphological studies of the first experimental series, it was determined that the “green” laser radiation (525 nm), with all parameters used in the experiment from the second to the fourth regime, guaranteed to provide the effect of photodestruction of hemoglobin-containing liver tissue. Examples of the result of thermal photodestruction of liver samples under irradiation in modes 2 and 4 are shown in Fig. 1 (a, b, c, d).

In the second series of experiments, it was noted that thermal changes on the skin begin to be well defined only when

using the most aggressive energy parameters of the second mode (1 W; 10 ms; 30 s). With an increase in the energetic activity of the effects in the third and fourth modes, the manifestations of photodestruction increase and become well defined visually and on histological preparations. Minus tissue destruction with the formation of a crater is observed only at the most aggressive energy parameters of the fourth mode (3 W; 10 ms; 30 s).

In all the affected areas, there was a relatively wide coagulation zone without clear boundaries with the surrounding tissue structures. This testifies to the deep propagation of this laser radiation in the skin and the minimal selectivity of the absorption of “green” laser radiation (525 nm) by its tissues. Examples of the result of exposure to 3 (2 W; 10 ms; 30 s) modes are shown in Fig. 2 (a, b).

Thus, on the comparative analysis basis of the results of the first and second series of experimental studies, it was found that with the same parameters of “green” laser radiation effect (525 nm) on liver and skin samples, significantly less photodestruction of the skin was determined compared to the liver, and characteristic features were noted, their damage.

On the liver, a more locally pronounced effect was determined, which, accordingly, causes the formation of a greater minus destruction with the formation of a crater and clearly defined boundaries of coagulation layers. The presence of less pronounced minus destructive lesions was noted on the skin, but the formation of a more widespread, 3–5 times greater width and almost homogeneous nature, a common zone of coagulation changes, without a distinct border with intact tissues.

In the third series of experimental studies, the features of the “green” laser radiation effect with a wavelength of 525 nm on the liver through the skin were studied in the case of a combined sample. In the zones of exposure, a distinct effect of thermal changes on the liver with minimal damage to the skin above it was noted starting with the use of the maximum energy parameters of the second mode (1 W, 10 ms, 30 s), shown in Fig. 3 (a, b, c, d, e).

The severity of the characteristic manifestations of thermal damage to the skin and under it the corresponding part of the liver increases with a greater energy activity of the second, third, and especially the fourth regime, this laser radiation. In the case of the combined sample, in all cases on the skin, there is a significantly smaller minus destruction with a small crater, but relatively wide thermal zones; on the liver, minus destruction is more pronounced in the form of a relatively deep and narrow crater, with the presence of certain coagulation areas of thermal damage on its walls.

Examples of the results of exposure to the combined sample, with the energy parameters of the third mode 3 (2 W, 10 ms, 30 s), are shown in Fig. 4 (a, b, c, d).

Thus, the results of the third series of studies confirmed the differences in the nature of thermal destructive changes in the skin and liver, and also clarified that the skin provides

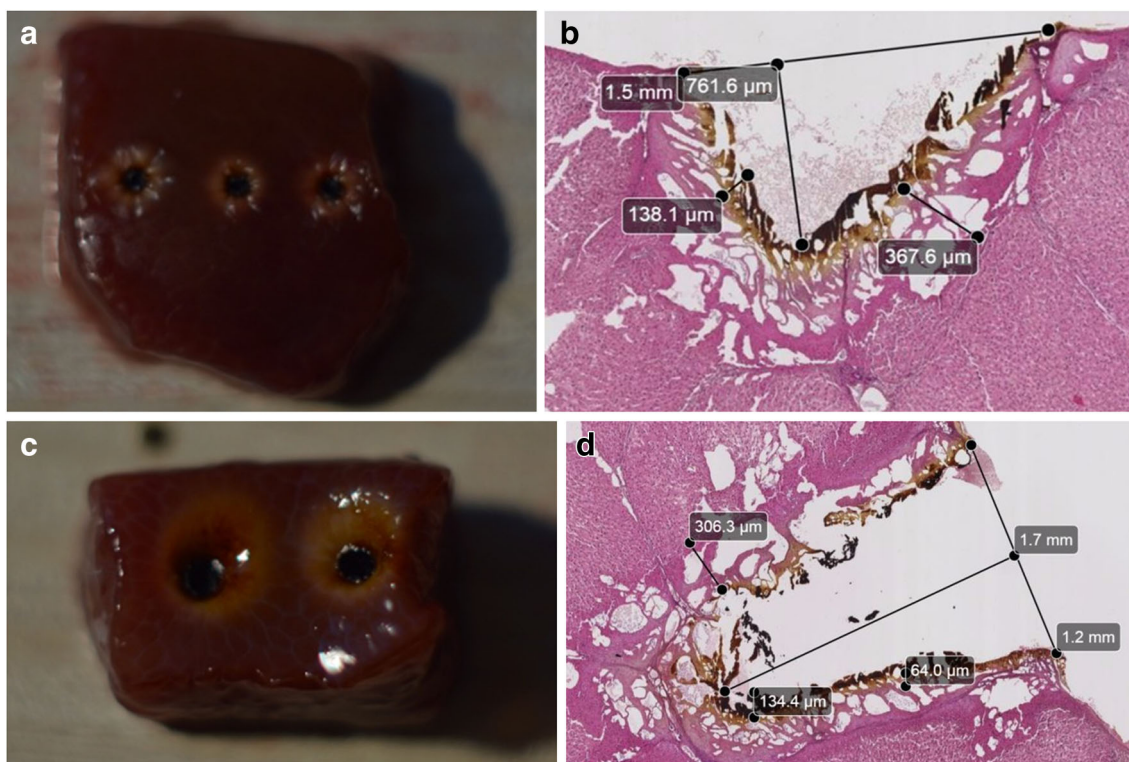


Fig. 1 **a, b, c, d** Liver photodestruction in mode 2 (1 W; 10 ms; 30 s) and in mode 4 (3 W; 10 ms; 30 s). **a** Liver macro-specimen, mode 2. Deep defects, craters, up to 1.5 mm in diameter, along the brown-yellow edge, a coagulation rim, total damage diameter up to 3.0 mm. **b** Liver histological specimen, mode 2. A deep triangular defect with a diameter of 1.5 mm on the surface and penetrating to a depth of 761.6 μm is determined in the affected zone. On the surface of the walls edges of the defect, there is a narrow layer of compact necrosis up to 138.1 μm of brown color; under it is a wide area up to 367.6 μm with pronounced vacuolization of the tissue. The girder structure is preserved in places. Magnification $\times 100$. **c** Liver macro-specimen, mode 4. Pronounced tissue destruction, in the

form of a funnel with a wide crater up to 2.0 mm in the center, edges of a brown-yellow color up to 1.5 mm wide, a general zone of thermal damage up to 5.0 mm in diameter. **d** Liver histological specimen, mode 4. In the affected zone, there is a deep defect of a pointed triangular shape, occupying an area on the surface with a diameter of up to 1.2 mm and penetrating to a depth of 1.7 mm. Along the wall edges of a dark brown defect, there is a narrow zone of compact coagulation necrosis, with a maximum thickness of 64 to 134.4 μm , under it is an area with pronounced tissue vacuolization, cellular necrosis, up to 306.3 μm thick. The girder structure is well preserved in places. Magnification $\times 100$

sufficient passage of “green” laser radiation (525 nm) for photodestruction of the underlying liver, where more

pronounced thermal changes are organized in comparison with the skin above it.

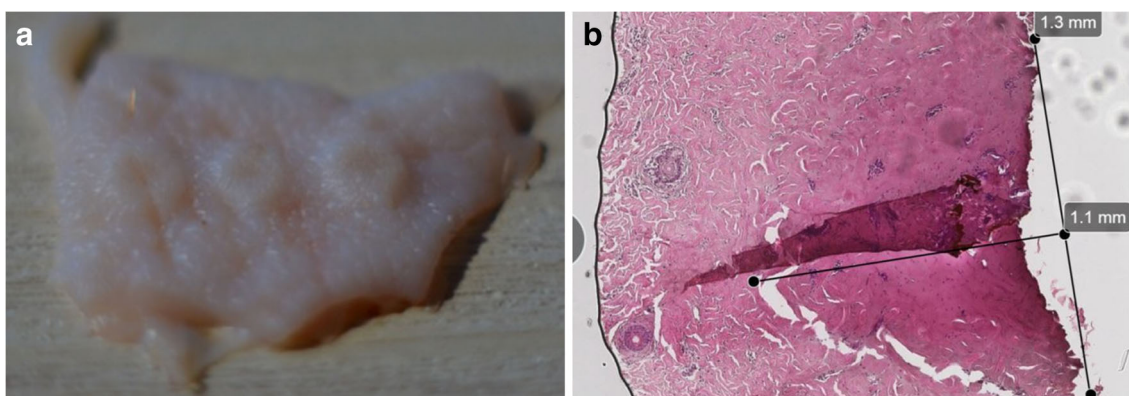


Fig. 2 **a, b** Photodestruction of the skin at 3 modes (2 W; 10 ms; 30 s). **a** Skin macro-specimen, mode 3. The skin in the affected area is raised; the areola is up to 3.0 mm in diameter; in the center of a slightly yellowish color, it is deepened. **b** Histological preparation of the skin, mode 3. In the affected area, there is a flat depression with uniform coagulation changes

in the dermis 1.3 mm wide and up to 1.1 mm deep, which gives the affected area a rounded appearance. Clear boundaries of the coagulation change transition in unchanged tissues are not visualized. Magnification $\times 100$

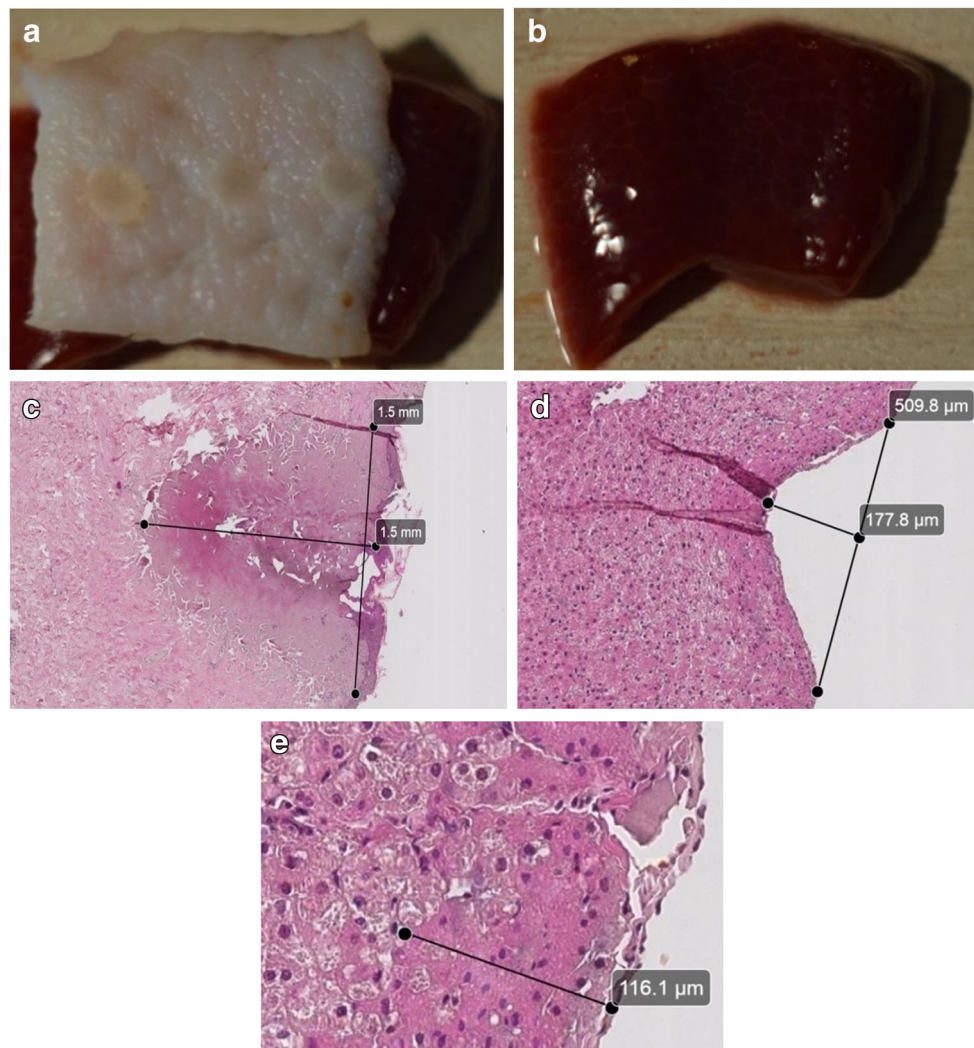


Fig. 3 **a, b, c, d, e** Combined sample skin and liver mode 2 (1.0 W, 10 ms, 30 s). **a** Macro skin preparation from a combined sample changes on the skin in the form of slight elevation and slight yellowing in the affected areas. **b** Liver macro from a combined sample. On the liver, under the skin, zones of thermal damage are also determined, in the form of small depressions with a whitish and dried surface. **c** Histological skin preparation from the combined sample. The area of influence on the skin has a regular triangular shape, with a contour rounded at the bottom, measuring 1500 by 1500 μm , represented by compacted homogenized tissues of coagulation necrosis, only in the central part is a small tissue defect. Magnification $\times 100$. **d** Liver histological specimen from the combined sample. The zone of influence on the liver has a triangular

shape with gentle edges and is located over 509.8 μm with a maximum depth of 177.8 μm . Directly under the zone of influence, there is a thin strip of liver tissue with signs of soft coagulation, tissue compaction, and violation of the beam structure, and the nuclei of hepatocytes are preserved. Magnification $\times 100$. **e** A fragment of a histological preparation of the liver from a combined sample. A fragment of the previous liver specimen at a higher magnification, showing a zone of soft coagulation with a thickness of 116 μm , with a compact arrangement of hepatocytes with preserved nuclei and not along the entire length of the traced boundaries of the cytoplasmic membrane of hepatocytes. Magnification $\times 400$

The discussion of the results

Based on the analysis of the results of the study performed, some regular features of the effect of laser radiation in the “green” spectral range with a wavelength of 525 nm were confirmed and noted. On the skin, the volume of destruction with minus tissue is much less than in the liver; however, the zone of peripheral thermal coagulation damage is wider, without the

formation of a compact and cellular structure of necrosis, without clear boundaries with the surrounding tissues.

On the liver, damage zones are formed more local with a pronounced nature of destructive changes, in the form of a wedge-shaped defect—a crater, with the formation on the walls of relatively narrow zones of compact and cellular thermal necrosis with distinct boundaries between themselves and with surrounding tissues.

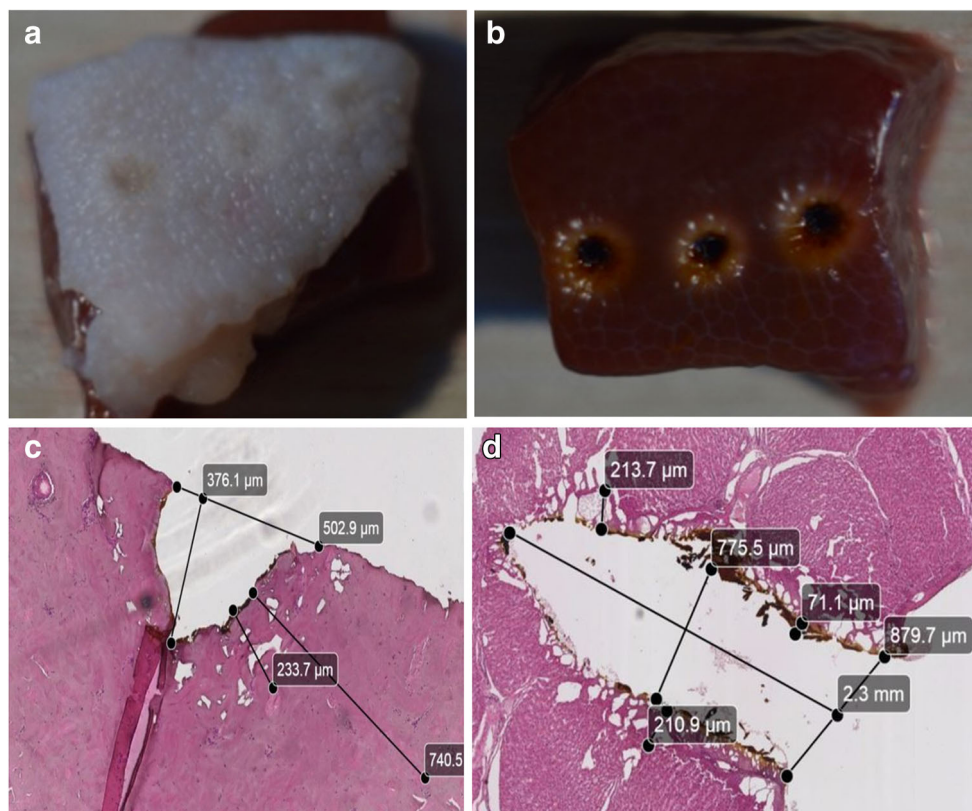


Fig. 4 **a, b, c, d** Combined sample, skin, and liver, mode 3 (2 W, 10 ms, 30 s). **a** Macro skin preparation from a combined sample. In the affected area, the skin is raised by an areola up to 2.0 mm in diameter; in the center of a yellowish color, there is a depression up to 0.5 mm in diameter, without a visible crater. **b** Liver macro from a combined sample. The liver has deep and wide craters up to 1.5 mm; the walls are dark brown, around a light brown coagulation rim; the diameter of the total lesion is up to 3.0 mm. **c** Histological preparation of the skin from the combined sample. In the affected area on the skin, a dermis defect of irregular shape with a pointed bottom is determined by a width of up to

502.9 μm and a depth of up to 376.1 μm . The edges of the defect with a wide area of coagulation changes in the dermis up to 740.5 μm . In it, in an area up to 233.7 μm wide, there are large irregularly shaped cavities. Magnification $\times 100$. **d** Liver histological specimen from the combined sample. In the affected zone, a wedge-shaped defect, deep up to 2300 μm and up to 879.7 μm wide, is determined. The edges of the defect with areas of coagulation changes, a thin layer of compact necrosis up to 71 μm , and under it, a section up to 213.7 μm wide of cellular necrosis with pronounced tissue vacuolization. Magnification $\times 100$

In the case of the combined sample, the effect of the passage of laser radiation through the skin tissue and the effect on the underlying liver, where significantly more pronounced thermal changes are formed than on the skin, were noted.

This effect is due to the greater selectivity of the “green” laser radiation with a wavelength of 525 nm to the liver tissue containing hemoglobin compared to the skin, which provides less damage to it and more pronounced photodestruction of the underlying liver.

Conclusion

The results of the experimental study confirmed the selectivity of the “green” laser radiation effect with a wavelength of 525 nm on hemoglobin-containing tissues, with significantly more pronounced thermal

damage to the liver tissue during isolated exposure and when passing through the skin, in the case of a combined sample. The nature and volume of thermal coagulation and minus destructive changes in the liver and skin were determined by the selectivity of exposure to “green” laser radiation with a wavelength of 525 nm and progressively depend on the used energy regime.

Laser radiation with a wavelength of 525 nm in the green spectral range, near the absorption peaks of hemoglobin and oxyhemoglobin, within certain energy parameters, may be promising for the purposes of selective photothermolysis of pathological subepithelial capillary structures.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval Not required.

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