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Interaction of laser radiation with avascular biological tissues depending on their thickness and absorption changes

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This article examines the interaction of near-IR laser radiation at wavelengths of 1.56 μm and 1.45 μm with avascular biological tissues of varying thicknesses, with absorption changing due to the diffusion of a clearing agent glycerol in varying percentages. The sample thicknesses are correlated with the thickness of various avascular biological tissues in the body: the range studied ranges from „thin“ samples simulating the tympanic membrane to „thick“ samples corresponding to articular and costal cartilage. A sample thickness of 500 μm represents the „borderline“ thickness, relative to which the transmitted intensity dynamics vary for two wavelengths 1.56 μm and 1.45 μm. The obtained results indicate the possibility of controlling the thermal effect by selecting the wavelength and concentration of the active clearing agent, which is essential when determining the therapeutic ranges of laser exposure parameters for biological tissue.

Keywords: laser, avascular biotissue, cartilage, tympanic membrane, glycerol.

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1. Introduction

Modification of the structure and properties of a biological tissue exposed to laser radiation is actively used for the reconstruction of biological tissues without destruction or coagulation of the biological object. Laser irradiation, characterized by coherence and monochromaticity, enables a high degree of directivity (collimation) of laser beams. This makes it possible to focus the radiation on the object, ensure high selectivity of the effect on biological tissues, and carry out localized treatment of local areas inside the biological tissue while preserving the adjacent structures. The main marker of effectiveness is preservation of the functional characteristics of biological tissue. Avascular collagenous tissues, such as cartilage and the tympanic membrane (TM), have a number of unique biomechanical and optical properties making them relevant subjects of research in the context of minimally invasive laser correction and reconstructive surgery. The depth of penetration of the near-infrared laser radiation into avascular collagen tissues depends on the absorption and scattering properties of these tissues and is a critical parameter that ensures various physical and chemical processes in different exposure conditions. In particular, understanding the interaction of infrared laser radiation with collagen structures of different thicknesses is critical for developing methods of point thermal plasticity, thermal remodeling, laser welding of biological tissues, and launch of regenerative processes.

A study of the cellular mechanisms of cartilage tissue regeneration has shown that thermomechanical exposure to laser radiation 1.56 μm has a pronounced effect on the synthesis of type II collagen and proteoglycans under conditions of normoxia and hypoxia. Previous studies in articular cartilage regeneration have convincingly demonstrated the growth of hyaline cartilage in contrast to fibrous cartilage [1–5] *in vivo* with a characteristic geometry of about a millimeter. At the same time, the optimal conditions for chondrocyte culture regeneration were found [6–8]. It is the thermomechanical laser exposure that presumably can significantly stimulate the regenerative processes. However, in order for this approach to work on thin films such as an tympanic membrane (TM), additional research and extension of the identified regimes to other wavelength ranges are required.

Wavelengths of 1.45 and 1.56 μm belong to the near-infrared range and are characterized by high absorption in water — the main component of biological tissues. However, the degree of this absorption varies: at 1.45 μm, it is 3 times greater (absorption coefficient 30 cm^{-1}) than at 1.56 μm (absorption coefficient 10 cm^{-1}), which results in different depths of penetration: 0.33 and 1 mm, respectively [9,10], which correlates with various average geometry of avascular tissues in the human body and allows for modeling of tissue effects ranging from hundreds of micrometers (corresponding to TM) to several millimeters (corresponding to articular and costal cartilage). The difference in these two wavelengths allows for the variation of the heating profile and thermal distribution in the tissue, resulting in the control of thermally dependent collagen denaturation, regeneration, and local tissue remodeling.

A comparative analysis of thermal and optical changes in tissues under laser irradiation at these wavelengths allows us to establish optimal ranges of exposure conditions for

various precision medical intervention tasks. Understanding these processes is crucial for improving the efficiency and safety of laser medical technologies, including laser cartilage reconstruction, creation of custom-shaped implants, correction of TM defects, and other clinical applications. In addition, the modification of biological tissue using biologically active agents allows for modeling both the structural features and the optical characteristics of a biological tissue. Thus, for instance, optical clearing enables to expand the scope of optical diagnostics and treatment by increasing the depth of optical probing of biological tissues and the resolution of optical methods [11–15]. One of the most studied active agents is glycerol, which has a pronounced hygroscopicity and the ability to change the tissue structure by redistributing water and interacting with collagen fibers. In paper [16], it was shown that the penetration of glycerol molecules into the collagen matrix leads to a decrease in the scattering coefficient due to partial dehydration of the tissue and a reduction in optical inhomogeneity. Additional modeling of optical clearing mechanisms [17] confirmed the key role of osmotic and molecular interactions in the processes of structural ordering. In turn, experimental data [18] showed that the effect of glycerol is accompanied by changes in the optical, weight, and geometric properties of the skin, including swelling due to its hygroscopicity.

The TM is a thin, translucent, avascular tissue that plays a key role in transmitting sound vibrations. When modeling or restoring the structure of TM in experimental and clinical conditions, materials with similar mechanical and acoustic properties are required. In this regard, a thin cartilage (with a thickness of about $100-500\,\mu$m) is considered as a promising model.

Due to its high stability and resistance to deformation, cartilage has long been used in reconstructive otology. Studies have shown that at a thickness of ≤ 0.5 mm, the acoustic characteristics of chondral grafts approach those of the native TM. Thus, in the experiment by Zahnert et al. (2000), a series of acoustic measurements were conducted on middle ear models made of chondral plates of various thickness. It was found that grafts up to $500\,\mu$m in thickness did not have any significant effect on the transfer function [19].

Moreover, a study [20] based on the laser vibrometry method confirmed that the oscillatory characteristics of $0.1-0.2$ mm thick chondral patches were almost identical to those of native TM. These results were confirmed by using the finite element method (FEM) of modeling [21,22], which showed that thin chondral elements retain an amplitude-frequency response similar to that of a membrane, especially for small perforations.

Clinically, [23] it was concluded that thin chondral grafts with a thickness of $0.1-0.5$ mm demonstrate high efficiency in tympanoplasty, providing both structural stability and preservation of auditory function. A literature review reports that when using chondral patches [24], their acoustic properties are almost as good as those of fascia grafts with an advantage of biomechanical stability, while the variation in sample thickness of less than 1 mm does not affect the acoustic gain [25,26].

Thus, thin samples of avascular cartilage (within $100-500\,\mu$m) demonstrate mechanical and acoustic properties similar to those of the TM and can be used as an experimental model of the TM in *in vitro* studies of laser exposure, vibrometry, modeling, and testing of reconstructive techniques.

This study is aimed at investigating the effect of a clearing agent (glycerol of various percentage) in the impact of laser radiation on biological tissues of different thicknesses, which correspond to the thickness of various avascular biotissues in the body: from „thin“ samples that imitate the TM to „thick“ samples that correspond to articular and costal cartilages.

2. Materials and methods

This study uses pig costal cartilage as a model of avascular collagen tissue. Its homogeneity, availability, and morphological similarity to human structures such as eardrum and articular cartilage make it an optimal experimental model. „Thin“ samples ($100-500\,\mu$m), which simulating the TM, allow for the assessment of surface effects and thermal damage, while thicker „samples“ (1 mm or more) demonstrate the behavior of bulk cartilage structures during deep heating.

Optical properties were studied on samples of biological tissues of a mature pig (aged 8–9 months). Fresh biomaterial was obtained directly from the slaughterhouse after the animals butchering and stored at a temperature of $-18\,^{\circ}$C. Before the experiment, the samples were thawed and cut into plates of various thicknesses: 100, 150, 200, 500, 1000, 1500 and $2000\,\mu$m (minimum three samples of each thickness). Using a punch, discs with a diameter of 10 mm were cut out and stored in a saline solution to prevent their drying. The tissue was cut using a cryomicrotome MCM-2850 (MT Point, Russia).

During the experiment, the tissue samples were exposed to laser radiation at wavelengths of $1.45\,\mu$m (Lahta Milon, Russia) and $1.56\,\mu$m (Ire-Polus, Russia). The radiation was supplied through a fiber optic cable (diameter $600\,\mu$m) perpendicular to the surface of the sample. The irradiation period was 15 s, power density was $5-10$ W/cm^2 for a wavelength of $1.45\,\mu$m and $20-25$ W/cm^2 for the wavelength of $1.56\,\mu$m at effective radius of the exposure area of 1 mm. The temperature of the front surface of the sample was monitored by IR camera FLIR A600 (FLIR Systems Inc, USA). The transmitted radiation entered the integrating sphere and was registered by the spectrometer ATP8000 Optosky (Optosky Spectroscopy Solutions, China).

Intact samples were exposed to laser radiation with a therapeutic power sufficient to achieve tissue heating of $30\,^{\circ}$C for samples with a thickness of $100\,\mu$m. After the first stage of exposure the samples were placed for 2 min in physiological solution, and then for 90 min in glycerol solutions of various concentration (35 and 50 % for the thin

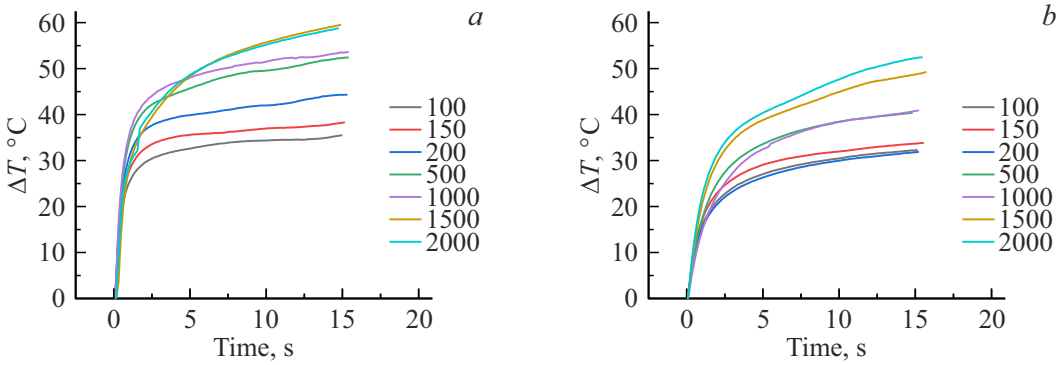

Figure 1. Average temperature response of samples of different thickness to laser irradiation with a wavelength of 1.45 (*a*) and 1.56 μm (*b*).

samples 10, 25, 35, 50 and 100 % by weight for the samples with a thickness from 1 mm). After impregnation the samples were exposed again to the same laser irradiation. These measurements enabled to assess the effect of glycerol concentration on the change in the optical properties of tissues during laser heating.

To study the reversibility of changes after the experiment, the samples were placed in a saline solution for 90 minutes. After that the samples were exposed to repeated laser irradiation. Thus, the possibility of restoring the original optical characteristics of tissues by removing glycerol using soaking was studied.

This protocol allowed to provide a comprehensive assessment on the dynamics of optical properties of tissues of various thicknesses after their exposure to glycerol and laser heating, as well as to verify the stability of such changes and their potential reversibility. The obtained data are important for developing the methods to control optical properties of tissues in laser therapy and diagnostics, as well as for selecting exposure conditions for the biological tissues of different thickness.

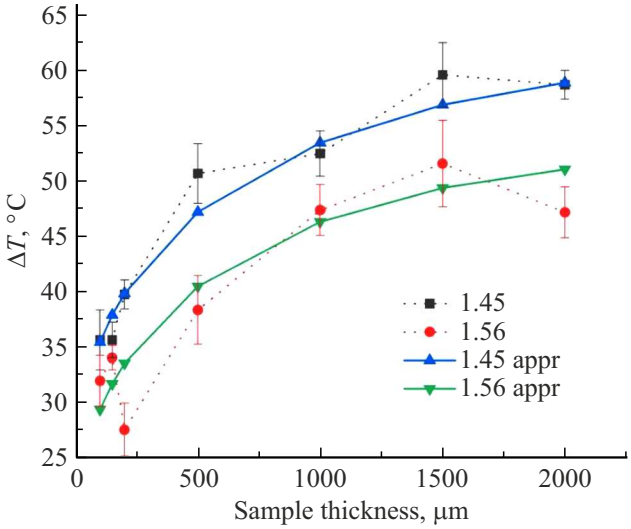

Figure 2. The difference in heating between samples of the same thickness for two wavelengths.

3. Results and discussion

3.1. Heating of intact samples

Experimental data demonstrate the temperature response of samples of different thicknesses under laser exposure at wavelengths of 1.45 and 1.56 μm. In both cases, the maximum temperature on the front surface of the sample increased as the material became thicker, but the nature of the dependence and the absolute values of the temperatures differed (Fig. 1).

For a wavelength of 1.45 μm, there is a steady increase in maximum temperature with increasing thickness. The temperature curves reach a plateau at $\sim 30-50\,^{\circ}$C, indicating a rapid achievement of a stationary thermal state. For samples with a thickness of 1500 and 2000 μm, maximum temperatures reached $\sim 60\,^{\circ}$C, and the maximum temperature growth rate was consistent within the experimental error, with a lower initial temperature growth compared to thinner samples.

For a wavelength of 1.56 μm, the general shape of the curves is similar, but the initial growth rate of the maximum temperature is flatter. The maximum surface temperature versus sample thickness curve for this wavelength is more monotonic because of the lower absorption coefficient and, hence, a more uniform heating throughout the depth. If we select the irradiation powers such that a sample with a thickness of 100 μm produces the same heating for two different wavelengths, then, similar heating of samples of other thicknesses will produce lower absolute temperatures when heated with a 1.56 μm laser compared to 1.45 μm (Fig. 2).

Thus, at a thickness of 1000 μm, the heating does not exceed 40 °C for a wavelength of 1.56 μm, while it is about 50 °C for a wavelength of 1.45 μm.

The analysis of the temperature response of collagen tissues of different thicknesses subjected to laser irradiation

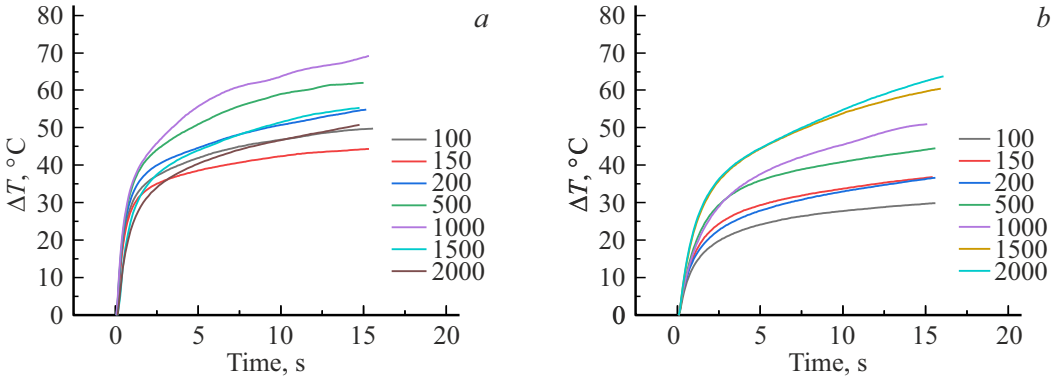

Figure 3. Averaged temperature response of samples under laser exposure at wavelengths of 1.45 (*a*) and 1.56 μm (*b*) after their soaking in 35 % glycerol solution, for the samples of various thicknesses.

with wavelengths of 1.45 and 1.56 μm showed that the experimental results can be approximated by a sum of linear and power functions with exponents of 0.45÷0.48. In the experiment *in vivo*, when it is impossible to determine the temperature non-invasively and when the thickness of the real biological sample varies (for example, TM with different thickness in the middle and at the edge of the fibrous ring), such an approximation will allow to know the degree of heating, understand the temperature range of the exposure, and prevent overheating of the tissue.

3.2. The effect of sample thickness on their heating

Glycerol impregnation has a significant effect on the temperature response of the material exposed to laser irradiation, and there is a pronounced dependence on the wavelength, sample thickness, and glycerol concentration (Table 1). At the wavelength of 1.45 μm glycerol contributes to enhanced heating within the entire thickness range, especially at a concentration of 50 %. This may be due to an increase in the absorption coefficient and a decrease in reflection and refraction at the internal boundaries of the biological tissue [27,28] because of the replacement of the intra-matrix fluid with a glycerol solution with its refractive index closer to that of the matrix itself [29].

Average change in the heating of 1500 and 2000 μm thick samples is $\sim$ 10 %÷20 %, while the effect of impregnation for thinner samples with a thickness of 500 and 1000 μm is more noticeable and the heating change will reach $\sim$ 25 %÷50 %.

At a wavelength of 1.56 μm, the effect of glycerol is weak at small thicknesses, and in some cases, there is even a decrease in heating, which is due to lower absorption and energy redistribution deeper in the tissue. Only when the thickness is more than 500 μm does the effect of glycerol impregnation lead to the temperature rise. These findings indicate that the thermal effect can be fine-tuned by adjusting the wavelength and concentration of the active optical clearing agent.

Table 1. Average change in sample heating (in % relative to the heating of the intact sample before impregnation) when the samples were soaked in a 35 and 50 % glycerol solution for 90 min

Thickness of sample, μm	Laser radiation wavelength, μm			
	1.45		1.56	
	35 %	50 %	35 %	50 %
100	38 ± 16	35 ± 5	−10 ± 2	7 ± 12
150	22 ± 5	24 ± 20	4 ± 8	5 ± 4
200	6 ± 8	22 ± 6	−10 ± 6	27 ± 20
500	22 ± 3	12 ± 3	12 ± 7	14 ± 2
1000	30 ± 14	47 ± 3	21 ± 8	40 ± 8
1500	8 ± 7	18 ± 1	21 ± 10	30 ± 8
2000	12 ± 2	20 ± 6	24.5 ± 8.6	38 ± 14

3.3. Effect of glycerol concentration on heating and optical response of „thick“ samples

The temperature response graphs for „thick“ samples illustrate a significant temperature rise reaching $\sim$ 50 °C for intact samples and $\sim$ 70 °C for samples after glycerol impregnation. This level of heat exposure can lead to changes in the physical and chemical properties of the tissue, including increased plasticity and, in some cases, initial signs of collagen denaturation. In order to exclude the second-order phase transition from further experiments, the laser power was reduced so that the maximum temperature change did not exceed 40 °C. Experiments were carried out for the two groups of samples of the same thickness (for laser with the wavelength of 1.45 μm — 1.7 ± 0.1 mm, for 1.56 μm — 2.0 ± 0.1 mm, minimum five samples in each group were used in the experiment). The following concentrations of glycerol were taken: 10, 25, 35, 50 and 100 %.

The experimental results showed a rise of the sample temperature with a corresponding increase in the glycerol concentration for a laser wavelength of 1.56 μm (Fig. 4). This effect is caused by higher absorption of radiation

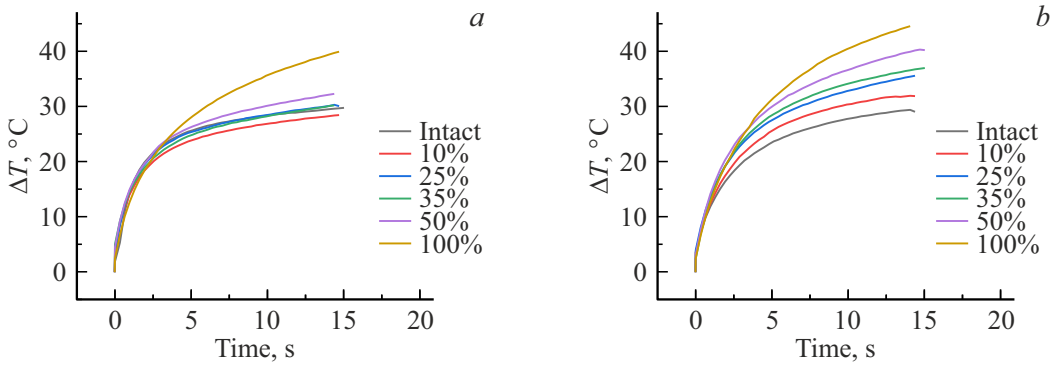

Figure 4. Averaged temperature response of samples under laser exposure at wavelengths of 1.45 (*a*) and 1.56 μm (*b*) after their soaking in glycerol solution of various concentration

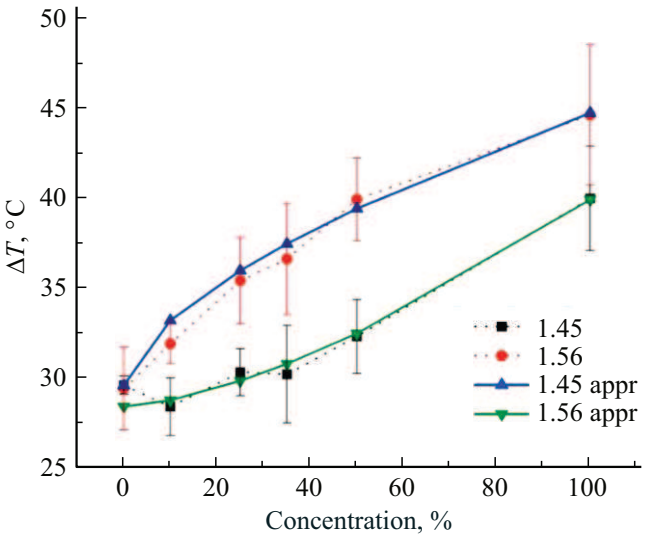

Figure 5. Dependence of heating of samples of the same thickness under laser irradiation with a wavelength of 1.45 and 1.56 μm on the concentration of glycerol during impregnation for 90 min.

in biological tissue due to changes in optical properties caused by glycerol diffusion. The experimental data are well approximated by a combination of linear and power functions with exponents of 0.63 for a wavelength of 1.56 μm and 1.5 for a wavelength of 1.45 μm (Fig. 5).

The temperature rise demonstrated in this study related to laser-irradiated samples impregnated with glycerol solutions is consistent with previously obtained data on the thermal effect when using fructose- and glycerol-containing media [30] on cartilage tissue samples with a thickness of 1 mm. It is known that the thermal conductivity of aqueous solutions of glycerol and fructose is approximately 30% lower than that of pure water [31,32]. Reduced thermal conductivity limits the efficiency of heat dissipation from the laser-exposed area, which in turn results in accumulation of thermal energy and, consequently, leads to more pronounced local tissue heating.

The exponent of the power function used for approximation is less than one for a wavelength of 1.56 μm and indicates the presence of saturation in glycerol-tissue interaction for both „thick“ and „thin“ samples: the initial temperature rise with increasing concentration is followed by a saturation trend and possible decrease at high concentrations. At the same time, for a wavelength of 1.45 μm, both positive and negative first derivatives of the temperature growth dynamics were observed (Fig. 5), which may be caused by large variation in the effective optical and thermal characteristics of the biological tissue samples at a small penetration depth 1.45 μm of laser radiation, which affects both the uneven absorption of laser radiation and thermal conductivity process in the tissue.

3.4. Changes in light transmission under laser exposure

The change in transmission of radiation with wavelengths of 1.45 μm and 1.56 μm by tissue samples during their impregnation with an aqueous solution of glycerol is shown in Fig. 6. The intensity dynamics of the transmitted laser radiation is normalized to the initial intensity measured when the laser radiation is turned on.

The experiments were conducted in „fiber-sphere“ optical configuration, where laser radiation was supplied to the sample through an optical fiber, and the collected transmitted signal was registered using an integrating sphere. This scheme provided high measurement accuracy, allowing for a complete picture of the intensity and distribution of the transmitted signal. The thickness of the studied samples and the laser power, as well as other heating parameters, were consistent with the temperature change experiment in Section 3.3.

During the experiments, a difference was found in the dynamics of the light intensity passing through the sample at sample thicknesses less than and greater than 500 μm. For samples with a thickness of less than 500 μm, there was almost no change in the transmitted radiation (ΔI) during the irradiation process. On the contrary, at a thickness of more than 500 μm, a significant rise of ΔI was observed

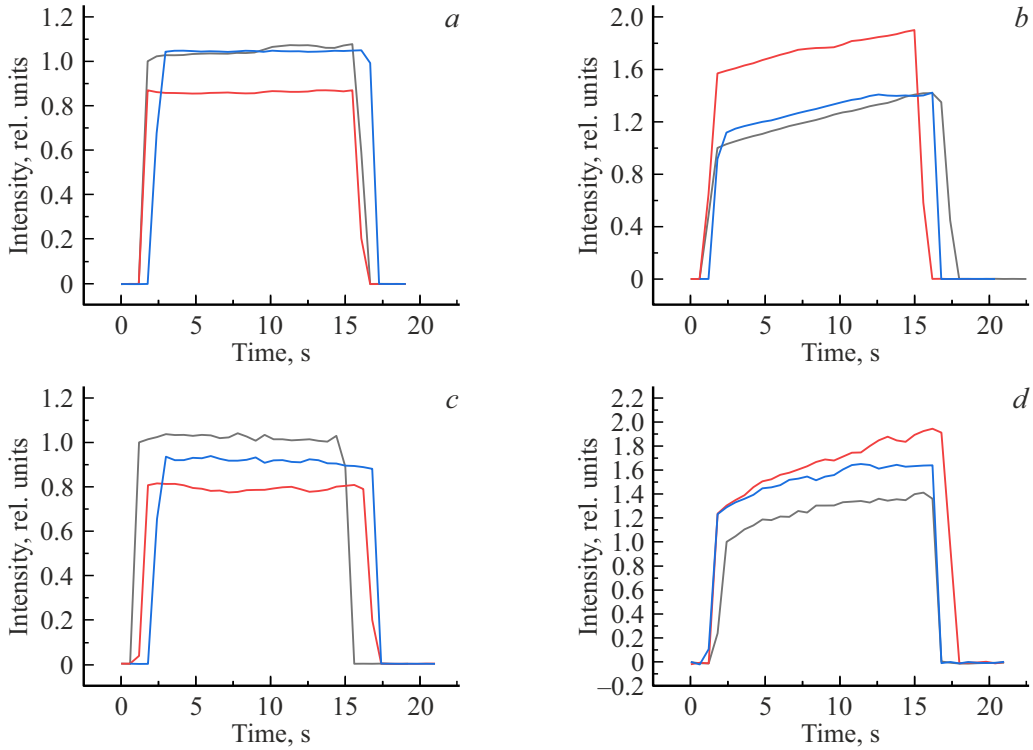

Figure 6. Changes in the light transmittance with the wavelength 1.45 (*a, b*) and 1.56 μm (*c, d*) 100 (*a, c*) or 1000 μm (*b, d*) after impregnation with 50 % aqueous solution of glycerol. Black curve — intensity of intact sample, red curve — intensity after optical clearing, blue curve — after removing the clearing agent. All curves are normalized to the initial intensity value when the laser is turned on.

Table 2. Change in intensity (ΔI) of light transmitted through the sample during laser exposure as a percentage for two wavelengths before and after impregnation with aqueous solutions of glycerol of two concentrations (35 and 50 %)

Thickness of sample, μm	Laser radiation wavelength, μm					
	1.45			1.56		
	Before impregnation	35 %	50 %	Before impregnation	35 %	50 %
100	5.6 ± 1.7	3 ± 2	0 ± 5	-1.5 ± 2.4	0.1 ± 3.7	-0.5 ± 0.7
150	6 ± 3	7 ± 5	3 ± 2	-1.5 ± 3.4	2.2 ± 2.9	1.6 ± 5.6
200	7 ± 8	0 ± 9	1 ± 1	-3.5 ± 6.0	-3.8 ± 7.5	2.4 ± 4.7
500	27 ± 11	11 ± 1	3 ± 4	20 ± 10	17 ± 6	17 ± 6
1000	33 ± 9	25 ± 4	16 ± 3	31 ± 8	27 ± 12	50 ± 9
1500	59 ± 6	24 ± 8	16 ± 3	32.5 ± 6	49 ± 7	33 ± 7
2000	61 ± 14	31 ± 2	19 ± 2	29 ± 3	35 ± 4	38 ± 2

during exposure, and the nature of the change differed for wavelengths of 1.45 μm and 1.56 μm. The exact values of the intensity change (ΔI) as a percentage of the initial value during laser exposure time are outlined in Table 2.

The analysis of the data in Table 2 shows that the value of ΔI after the samples impregnation with glycerol solutions demonstrates a different dependence on the wavelength of laser radiation. In particular, for samples irradiated at a wavelength of 1.45 μm, there was a decline in ΔI compared to the initial state when the tissue was impregnated with glycerol . At the same time, for a wavelength of 1.56 μm, ΔI the intensity either increases or remains within the experimental error.

Conclusions

The obtained results indicate the possibility of controlling the thermal effect by selecting the wavelength and the concentration of the active optical clearing agent. The thickness 500 μm of samples is the „boundary“ thickness when evolution of the passed intensity changes for the two wavelengths 1.56 and 1.45 μm in the near-infrared

range. Thus, the dynamics of changes in both intensity and maximum temperature are determined by the optical properties, which can be modulated by active optical clearing agents, as well as by the thickness of the irradiated sample. This is particularly important when defining the therapeutic ranges of laser exposure parameters for biological tissues comparable in thickness to articular and costal cartilage, and for thin cartilage-like films — analogs of the tympanic membrane.

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Conflict of interest

The authors declare that they have no conflict of interest.

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