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Control of the optical properties of gum and dentin tissue of a human tooth at laser spectral lines in the range of 200–800 nm

A.A. Selifonov, V.V. Tuchin

Abstract. **A change in the optical properties of the tissue of the gums and dentin of a human tooth is experimentally investigated *in vitro* with the application of a 99.5% glycerol solution. The kinetics of the total transmittance in the spectral range of 200–800 nm on characteristic laser lines is studied. It is shown that the effectiveness of optical clearing, expressed as a relative increase in the total transmittance, for gum tissue in the laser radiation range of 200–800 nm can vary significantly (by an order of magnitude). In this case, the absolute values of optical transmittance in the UV region are relatively small due to the strong absorption by the endogenous chromophores of the studied tissues, and in the visible and NIR regions, on the contrary, they are significant, which is due to the absence of strongly absorbing chromophores. The effectiveness of dentin clearing in the entire visible and NIR spectral regions is on average 25% for 300 minutes of immersion of the sample in a 99.5% glycerol solution. The presented results are necessary for increasing the efficiency of laser therapy and methods for optical monitoring of morphological changes in biological tissues for the purpose of early diagnosis of diseases.**

Keywords: *gum, dentin, glycerol, optical clearing, total transmittance spectra, excimer lasers, harmonics of an Nd:YAG laser, Ti:sapphire laser.*

1. Introduction

In recent decades, laser technologies are increasingly used in many fields of medicine, in particular in dentistry and cosmetology, as well as for providing various impacts on soft and hard tissues [1, 2]. The use of laser techniques gives good clinical results when removing calculi in hard-to-reach areas of the human body [3], in the treatment of otolaryngologic diseases [4], in physiotherapy for the correction of neurological diseases [5], etc.

When radiation is absorbed by biological tissues, the basic physical processes are reduced to local heating of the absorbers (chromophores) distributed in them and to the manifestation of an internal photoelectric effect and electrolytic dissociation of molecules and complexes. In addition, there is a weakening of ionic bonds and ion–dipole interactions of biomolecules. Laser radiation in the wavelength range of 850–1300 nm is absorbed mainly by water, biological structures (primarily lipids, including cell membranes) and oxygen dissolved in tissues. The latter leads to the formation of highly reactive singlet oxygen, the energy of which is realised in the form of an indiscriminate photodynamic effect without the participation of exogenous photosensitisers [6, 7]. Primary free radical reactions induce activation of the cells (leukocytes, fibroblasts, keratinocytes, etc.), which is expressed in an increase in bactericidal activity of laser therapy. The lifetime of singlet oxygen in water is about 3 μs, and in cells and tissues it can be an order of magnitude shorter due to the high reactivity of oxygen, and so the average distance travelled by its molecule can be very small. To achieve subsequent biological effects, it is very important to localise photosensitisers, as well as laser radiation, at the site of the expected impact [8, 9].

Using laser radiation, it is possible to selectively affect, e.g., hair roots, pigment spots, to treat acne, and to perform laser fractional ablation of the skin (collagen rejuvenation), improving its appearance and increasing permeability to microparticles, drugs, etc. [10–12].

Bashkatov et al. [13] studied the optical properties of the human colon tissue *in vitro* in the spectral range of 350–2500 nm. Based on the measured diffuse reflection spectra, as well as the total and collimated transmission, they calculated the absorption and scattering coefficients, and the scattering anisotropy factor using the inverse Monte Carlo method. Genin et al. [14] studied the kinetics of collimated transmission of stomach wall tissues under the action of an aqueous 40% glucose solution. They found that the use of this optical clearing agent (OCA) increases the depth of light penetration from 5% to 15% (depending on the selected spectral range). A change in the kinetics of collimated transmission of the human dura mater *in vitro* in the range 400–700 nm under the action of a 40% glucose solution was described in [15]. From these measurements, the permeability coefficient of the dura mater for glucose was determined, which amounted to $(1.3 \pm 0.13) \times 10^{-4}$ cm s^{-1}. In the study of the optical properties in the range of 200–1000 nm of the rectum wall tissue treated with water-glycerol solutions, Carneiro et al. [16] found the

A.A. Selifonov Saratov State University, ul. Astrakhanskaya 83, 410012 Saratov, Russia; V.I. Razumovsky Saratov State Medical University, ul. Bol'shaya Kazach'ya 112, 410012 Saratov, Russia; e-mail: selif-ei@yandex.ru;
V.V. Tuchin Saratov State University, ul. Astrakhanskaya 83, 410012 Saratov, Russia; National Research Tomsk State University, prosp. Lenina 36, Tomsk, Russia 634050; Institute of Precision Mechanics and Control, Russian Academy of Sciences, ul. Rabochaya 24, 410028 Saratov, Russia; Bach Institute of Biochemistry, Federal Research Centre 'Fundamentals of Biotechnology', Russian Academy of Sciences, Leninsky prosp. 33, 119071 Moscow, Russia; e-mail: tuchinvv@mail.ru

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formation of 'dynamic' transparency windows of biological tissue with high efficiency in the UV spectral region, which are formed upon immersion clearing and only during the application of the clearing agent. Processing the tissue of the rectal wall creates two new optical windows with transmittance peaks at 230 and 300 nm, which opens up the possibility of developing clinical procedures for diagnosis or treatment in the ultraviolet range.

Effective is the use of lasers in surgery. Surgical incisions performed using radiation from diode lasers (λ = 808 – 980 nm) and a solid-state Nd:YAG laser (1064 nm) differ from traditional ones in the absence of bleeding, the preservation of the microvasculature around the surgical field, the sterility, the preservation of viability of the incision edges, reduced pain and, as a result, a significantly shorter rehabilitation period [17].

In dentistry, laser radiation can be used as a catalyst to activate peroxide compounds during tooth whitening. Laser irradiation causes homogeneous photoinduced thermolysis in tissues in the form of a pronounced photothermal effect, in which the endogenous chromophores are various forms of haemoglobin, water, protein structures, and tissue detritus [18] or exogenous dyes [19] and photocatalytic nanoparticles [20]. The use of laser or bright LED radiation is advisable for any surgical and therapeutic interventions on the soft tissues of the maxillofacial area [21].

The interaction of intense pulsed laser radiation with hard tooth tissues leads to the destruction of the hydroxyapatite crystal lattice due to absorption of radiation and a sharp increase in kinetic energy hydroxyapatite OH groups, as well as evaporation of the hydration shell of the crystal during microseconds, the so-called 'microexplosion' [18]. This is a significant advantage of laser technology for the preparation of hard tooth tissue over traditional preparation with a contact drill. As a result of multiple microexplosions, many craters are formed on the prepared surface, which leads to a significant increase in the area of contact between the tooth tissues and the filling material. At the same time, enamel prisms are preserved at the treatment boundary, there are no microcracks, dentinal tubules remain open, and odontoblasts remain active in the treatment zone, which leads to a significant acceleration in the development of secondary and tertiary dentin at the rehabilitation stage after filling [22]. Laser therapy is also widely used in the treatment of periodontal soft tissues [23]. For non-surgical periodontal therapy with the manifestation of antibacterial effects, it is recommended to use an Er:YAG laser (2940 nm) with a pulse energy of 140–160 mJ and a pulse repetition rate of 10 Hz [24]. Clinical studies of 25 randomly selected patients have shown that a combination of Er:YAG laser (2940 nm) and Nd:YAG laser (355 nm) therapy may be useful in the treatment of inflammatory periodontal diseases, especially in hard-to-reach places such as deep gingival pockets [25].

It was found that a wide variety of used lasers, experimental facilities, and methods for delivering radiation leads to various clinical results in treating patients, which makes it urgent to study the interaction of laser radiation with hard and soft tissues both in dentistry and in other areas of medicine, including quantitative studies of optical properties at laser wavelengths [26]. In their review [27], Jing et al. examined the general principles of optical clearing of biological tissues, as well as the application of this method in the study of biological processes in bone and dental tissues with the expectation of future three-dimensional visualisation.

Significant scattering of UV, visible and NIR radiation by biological tissues, including gum and tooth dentin, combined with strong absorption at individual wavelengths limits the transport of probe radiation through the surface layers of biological tissue to a sufficient depth, which is one of the problems of laser medicine. Reducing scattering by immersion clearing of biological tissues is one of the possible ways to solve this problem, providing an increase in the efficiency of optical methods for diagnosing and treating diseases [28]. For immersion clearing of biological tissues, both hyperosmotic agents (glucose, sorbitol, glycerol, polyethylene glycol, propylene glycol, dimethyl sulfoxide) and solutions having normal osmolarity (radiopaque substance iohexol, etc.) are used [29–33].

In this work, we studied the kinetics of the total transmittance of tissue samples of the gum and dentin of a human tooth *in vitro* upon immersion of tissue samples in a 99.5% glycerol solution, and determined the effectiveness of optical tissue clearing in the spectral range of 200–800 nm at characteristic laser wavelengths.

2. Materials and methods

To measure the transmittance of biological tissue samples in the spectral range from 200 to 800 nm, we used a Shimadzu UV-2550 dual-beam spectrophotometer (Japan) with an integrating sphere. A halogen lamp with radiation filtering in the studied spectral range served as a radiation source. The limiting resolution of the spectrometer was 0.1 nm. Spectra were normalised before measurements using a $BaSO_4$ reference reflector.

Figure 1 shows the location of the sample when recording the total transmittance spectra. All experiments were carried out at room temperature (~25 °C) and normal atmospheric pressure.

The material for the *in vitro* study was teeth (molars), removed in patients aged 15 to 25 years in the dental clinic according to orthodontic indications. The extracted teeth were stored in saline in the refrigerator. Wet teeth were cut with a diamond disk into sections about 1 mm thick along the growth axis and etched with 35% phosphoric acid for 15 s, and then the acid was removed with a stream of water. Then, using a brush and a 95% solution of ethyl alcohol, the surface was cleaned of sawing products and other external contaminants, for which the saw cut (sample) was placed in a Techsonic UD100 SH-45L ultrasonic bath for 10 min, after which it was wiped with a lint-free cloth moistened with alcohol. Samples were air dried for one day. Microphotographs of dentin sections were obtained using a MIRA 2 LMU scanning electron microscope (SEM). The thickness of the cuts (samples) of the biological tissue was measured with a micrometer at several points of the sample and averaged. The accuracy of each measurement was ±10 μm. In total, ten samples taken from different teeth were studied in the experiment.

Sections of the gum tissue of a 68-year-old man who died from acute coronary insufficiency and did not have pathological changes in the tissues of the oral cavity also served as material for *in vitro* studies. The resulting biopsies were stored frozen until the start of the experiment. *In vitro* experiments were performed on 10 samples of gingival tissue sections. The thickness of the biological tissue samples was measured with a micrometer, for which the gum tissue was placed between two glass slides (the thickness of which was then subtracted);

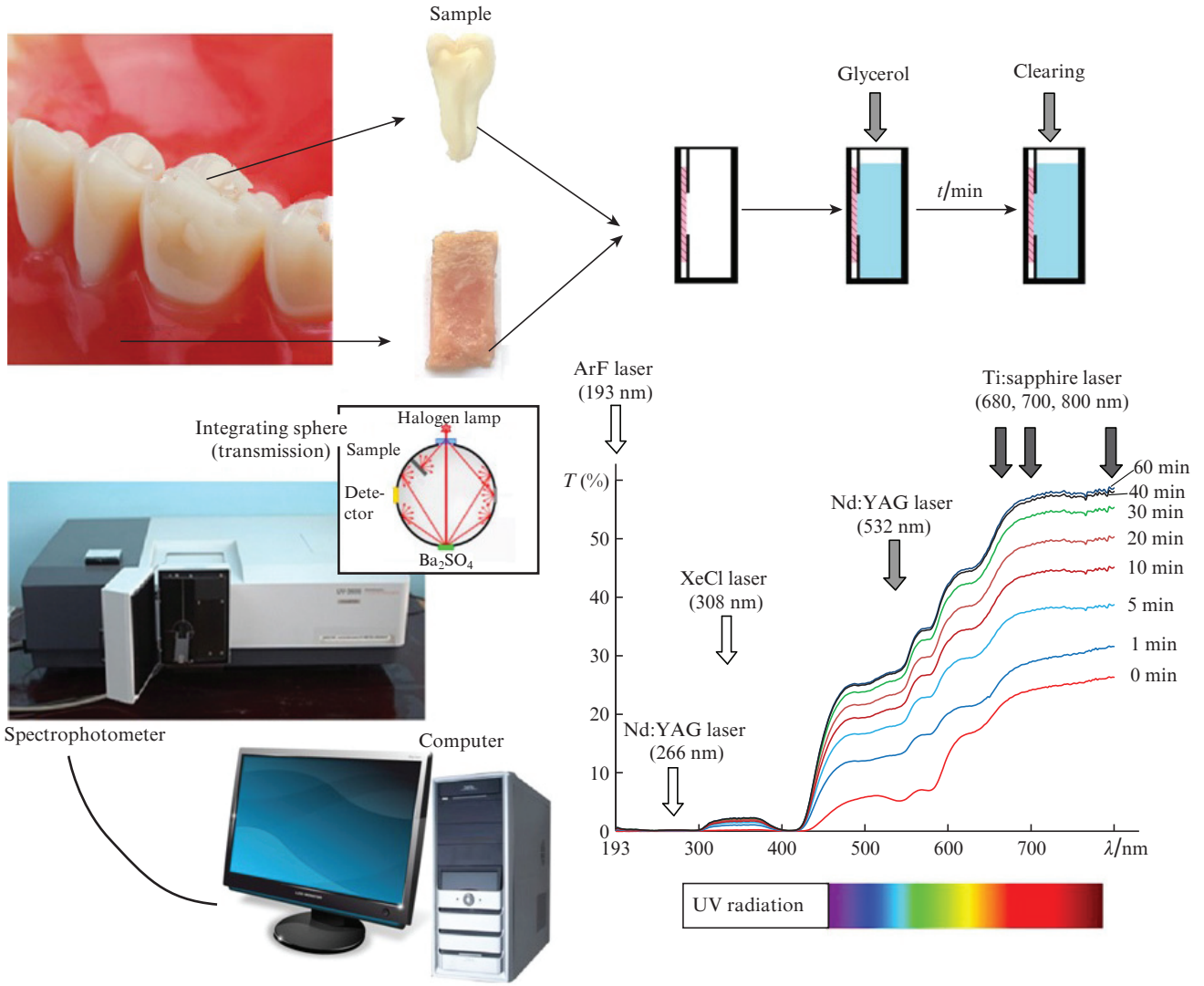

Figure 1. Experimental scheme for *in vitro* measurements of the total transmittance of human gum and dentin samples upon immersion in a 99.5% glycerol solution.

the measurements were carried out at several points of the sample and averaged. The accuracy of each measurement was ±10 μm. On average, the thickness of the samples of sections of gingival tissue was 0.48 ± 0.06 cm. The standard deviation (SD) was calculated by the formula

$$\mathrm{SD} = \sqrt{\sum_{i=1}^{n} (\bar{l} - l_i)^2 / n(n-1)}, \qquad (1)$$

where n is the number of examined samples; l_i is the thickness of the ith sample of biological tissue; and $\bar{l}$ is the average value of the thickness. All experiments were carried out at room temperature (~25 °C).

As an immersion agent, a 99.5% solution of glycerol ChDA (Russia) was used. Glycerol ($C_3H_5(OH)_3$) is a colourless viscous liquid with a molecular mass of $M = 92.1$ g mol^{-1}. It is a representative of trihydric alcohols, hygroscopic, nontoxic and odourless. Glycerol is widely distributed in wildlife and plays an important role in metabolic processes in animals; it is a component of many foods, creams, and cosmetics and is used as a clearing agent [29].

3. Results and discussion

A human tooth consists of a root, neck and crown covered with enamel, i.e. the hardest tooth tissue. Dentin makes up the bulk of the tooth, has a light yellow colour and has some elasticity; it is harder than bone and cement, but 4–5 times softer than enamel. It consists of intercellular substance penetrated by dentin tubules that determine its trophism with an inner diameter of 0.5 to 4 μm (depending on the tooth region) (Fig. 2). The average number of dentinal tubules per unit volume (density) in the middle part of the dentin of the tooth root is much less than in the middle part of the dentin of the tooth crown. The density of the tubules in the dentin, located closer to the outer part of the tooth, also differs significantly from the density near the occlusal fissure. It can be considered that the tooth presents bundles of coordinated natural optical fibres. The twisted shape of the optical waveguides determines the high efficiency of lighting the pulp practically regardless of how the light hits the external surface of the tooth. The waveguide effect is significantly more pronounced in dentin than in enamel, where enamel prisms are light-conducting elements [34].

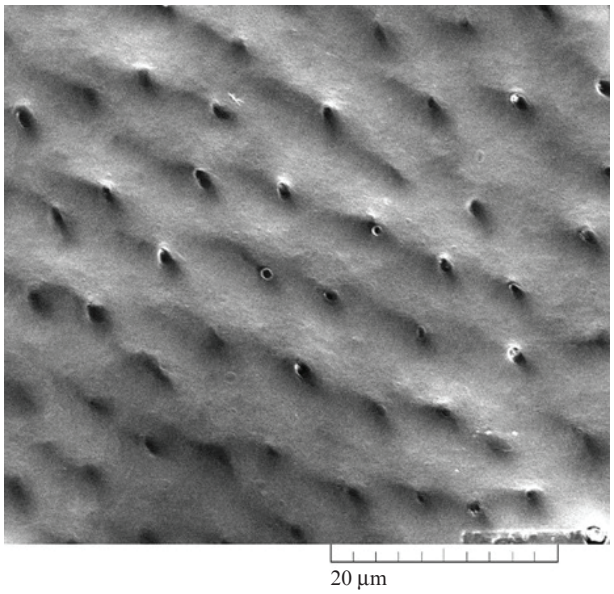

Figure 2. SEM image of one of the test samples, a transverse section of a human tooth in the region of the middle part of the dentin of the tooth crown (5000× magnification).

The human gum consists of a tissue membrane covering the upper and lower jaw and surrounding the teeth. The protective function of the gum tissue is determined by the presence in its composition of hyaluronic acid, macro- and microphages, plasma, etc. Due to its collagen structure formed from fibroblasts, the gum tissue has a fairly high density. From an optical point of view, the gum tissue can be attributed to fibrous tissues, mainly consisting of collagen fibres repeatedly intertwined with each other, around which there is an interstitial fluid containing proteoglycans, glycoproteins, proteins, polysaccharide complexes, etc. [35].

Glycerol is a highly effective means for the optical clearing of biological tissues. As a strong hygroscopic agent, glycerol first draws water from the tissue due to osmosis, and then, penetrating into the tissue, binds interstitial intercellular water, thereby increasing the concentration of soluble components in the remaining water and increasing its refractive index [2, 16].

The average values of the thickness and weight of the samples before and after clearing are presented in Table 1. It can be seen that after immersion of samples of soft tissue of human gums in a 99.5% glycerol solution, their thickness decreases on average, which is explained by tissue dehydration and its corresponding shrinkage. When glycerol acts on solid dentin samples, their thickness does not noticeably change.

Table 1. Average values of thickness and weight of gum ($n = 10$) and dentin samples ($n = 10$) before and after immersion in a 99.5% glycerol solution.

Characteristics of the sample	Sample	
	Gum	Dentin
Initial thickness/mm	0.59 ± 0.06	0.81 ± 0.06
Final thickness/mm	0.48 ± 0.06	0.80 ± 0.06
Initial weight/mg	467 ± 6	189 ± 2
Final weight/mg	421 ± 7	190 ± 2

The reduction in size, some compression and weight loss of the samples when using hyperosmotic agents for optical clearing of soft biological tissues were observed by the authors of Refs [36, 37].

The total transmittance spectra (TTS) of the gum and dentin samples are shown in Fig. 3. In the UV range of the gum TTS, there are pronounced dips characteristic of protein absorption bands, as well as blood haemoglobin. In the regions at 415–420 nm and 540–580 nm, dips are observed corresponding to the absorption bands of oxyhaemoglobin (415, 542 and 576 nm) [38]. Water absorption in the studied range of 200–800 nm is negligible. In the UV range, TTS for both types of tissue also have a decline characteristic of the absorption bands of connective tissue proteins in the form of collagen and reticular fibres. After immersion of gum tissue samples in a 99.5% glycerol solution, the absorption bands of endogenous gum chromophores (oxyhaemoglobin) become less pronounced, which is associated with a lower probability of effective absorption of photons when they pass through a cleared (less scattering) biotissue sample. The values of the total transmittance coefficients of both the gums and dentin increase in the entire wavelength range (Fig. 3) with respect to the initial state of the samples (before immersion in glycerol), which indicates a decrease in light scattering by the samples as a result of their immersion in glycerol. Since glycerol has no absorption bands in the entire studied range of 200–800 nm [2], the increase in transmission is not accompanied by changes in the shape of the spectra.

The kinetic dependences of the total transmittance coefficient of the studied samples of biological tissues, measured for a number of wavelengths used in laser medical systems, are presented in Fig. 4. It is seen that the interaction of glycerol with the samples leads to a gradual increase in the transmittance in the range of 200–800 nm.

The main source of light scattering in biological tissues is the nonuniformity of the refractive index due to the difference in its values for different components of biological tissues, i.e., mitochondria, nuclei, other organelles and cytoplasm of cells or for interstitial fluid and structural elements of connective (fibrous) tissue (collagen and elastin fibres). When an immersion fluid with a higher refractive index than that of the interstitial fluid is introduced into the biological tissue, the interstitial fluid is partially replaced by the immersion agent, which equalises the refractive indices of the tissue scatterers (for example, collagen fibres) and their environment and, as a result, significantly reduces light scattering [27, 29–32]. An important parameter for quantifying the achieved tissue clearing is the clearing efficiency [32]

$$Q(T) = \frac{T(t) - T(t=0)}{T(t=0)} \times 100\%, \qquad (2)$$

where $T(t = 0)$ is the transmittance at the initial moment of time; and $T(t)$ is the transmittance at time t. The results of calculating the effectiveness of clearing gum and dentin tissue at wavelengths characteristic of medical lasers are presented in Fig. 5 and Table 2. In medical practice, excimer lasers (ArF, 193 nm; XeCl, 308 nm; XeF, 351 nm), a nitrogen laser (337.1 nm), an argon laser (351.1, 363.8, 488.0 and 514.5 nm), a helium–neon laser (543.3, 632.8 nm), a Ti:sapphire laser (680–1100 nm), an Nd:YAG laser (1064 nm) and its second, third and fourth harmonics (532, 355 and 266 nm, respectively [2]) are used most frequently.

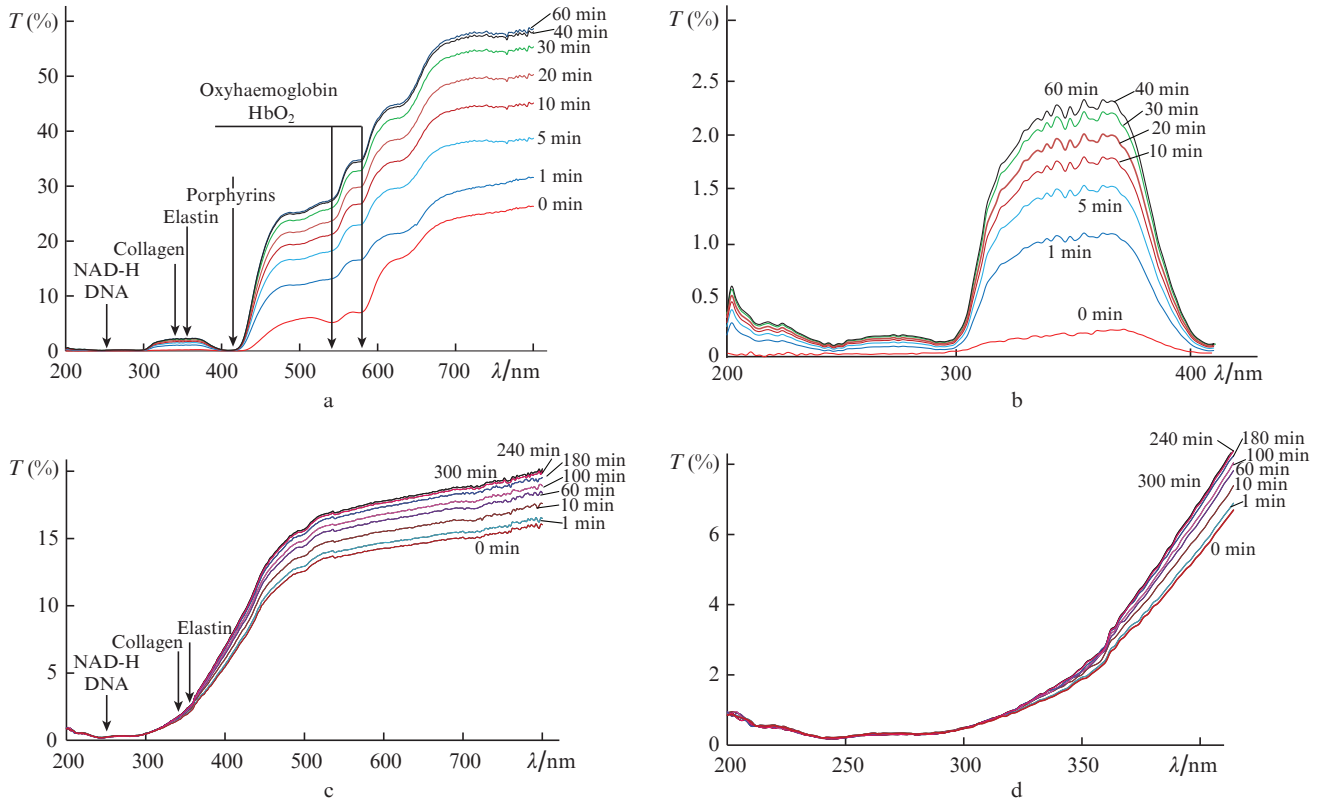

Figure 3. Kinetics of the total transmittance spectra during immersion with glycerol of (a, b) gum samples and (c, d) dentin of a human tooth. Spectra are shown both in the entire wavelength range, (a, c) 200–800 nm, and (b, d) with magnification in the UV range of 200–400 nm; the figure schematically presents the absorption regions of the main endogenous chromophores of biological tissues.

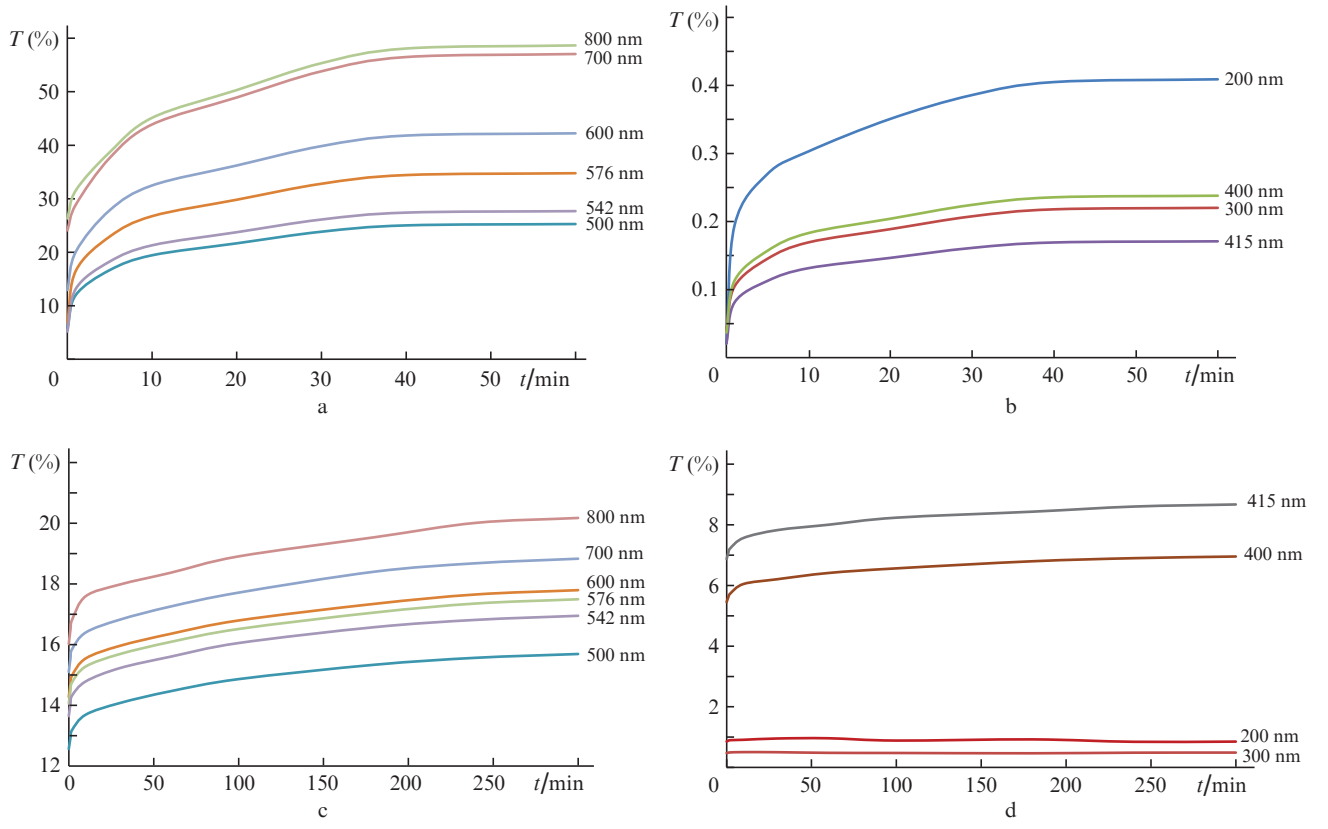

Figure 4. Kinetic dependences of the total transmittance $T(t)$ of (a, b) gum tissue and (c, d) dentin samples for different wavelengths when exposed to a 99.5% glycerol solution.

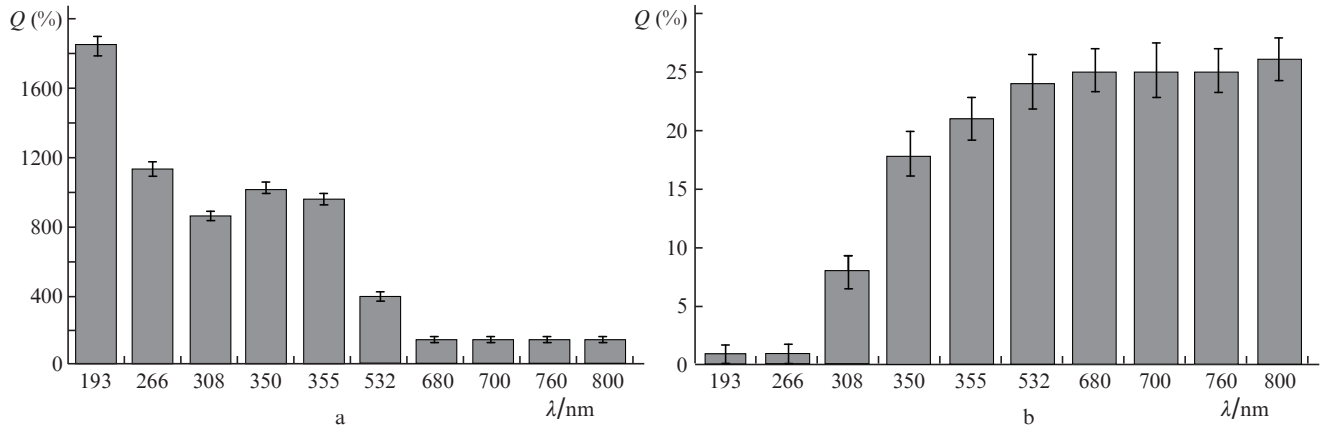

Figure 5. Diagram of the effectiveness of optical clearing at different wavelengths of (a) the gum and (b) dentin after immersion in a 99.5% glycerol solution.

From the results of calculating the effectiveness of clearing from experimental data, it follows that the greatest efficiency is achieved at a wavelength of 200 nm, close to the wavelength of an excimer ArF laser (193 nm) for human gum tissue with 60 minutes of glycerol action and amounts to 1847%. Although the absolute transmittance at this wavelength is small due to strong absorption and amounts to only 0.4%, the increase in the number of transmitted photons is significant, which contributes to the successful detection of UV signals from large depths of tissue. In the visible and near-IR regions, within the so-called 'first therapeutic transparency window' [2], the clearing efficiency is significantly lower and averages slightly more than 100%–200%; however, due to the absence of strong absorption bands of endogenous chromophores in this spectral region, the absolute transmission values are quite large and reach 60%.

Note that since on average the thickness of the studied samples of human gum tissue during immersion in a 99.5% glycerol solution slightly decreases as a result of dehydration and osmotic compression, the clearing efficiency was also evaluated by changing the effective attenuation coefficient. The transmittance of the tissue sample was determined by the approximate relation [29]

$$T \approx \exp(-\mu_{\text{eff}} l),$$

where $\mu_{\text{eff}} = \sqrt{3\mu_a(\mu_a + \mu_s')}$ is the effective attenuation coefficient (cm^{-1}); l is the thickness of the biotissue sample (cm); μ_a is the absorption coefficient (cm^{-1}); $\mu_s' = (1-g)\mu_s$ is the reduced scattering coefficient (cm^{-1}); g is the scattering anisotropy parameter; and μ_s is the scattering coefficient (cm^{-1}).

Then, the clearing efficiency, with the influence of the sample thickness taken into account, can be introduced as the relative decrease in μ_{eff}:

$$Q(\mu_{\text{eff}}) = \frac{\mu_{\text{eff}}(t=0) - \mu_{\text{eff}}(t)}{\mu_{\text{eff}}(t)} \times 100\%. \quad (3)$$

The clearing efficiency, estimated from a decrease in the attenuation coefficient μ_{eff}, for excimer laser wavelengths was ~20% (193 nm), 17% (308 nm) and 25% (351 nm); for Nd:YAG laser harmonics, 12% (266 nm), 24% (355 nm) and 45% (532 nm); and for wavelengths of Ti:sapphire lasers, 51% (680 nm), 52% (700 nm) and 51% (760–800 nm).

The efficiency of dentin clearing in the UV region for 300 min of immersion of the sample in a 99.5% glycerol solution varies from 1% to 20% (Fig. 5b), and the absolute transmittance varies from 0.5% to 6% (Fig. 4d) as the wavelength grows. In the visible and near-IR regions, the dentin clearing efficiency is on average 25% (Fig. 5b), while the absolute transmittance is from 15% to 20% (Fig. 4c). These results confirm the possibility of forming 'dynamic' tissue transparency windows with very high efficiency in the UV spectral region during immersion clearing and only for the duration of the clearing agent action [16].

Using the results of other authors obtained for other tissues, we can indicate the prospects for increasing the efficiency of clearing. In a number of studies, the effectiveness of optical clearing of biological tissues (in particular, skin and skull bones of small animals, human skin) is determined by recording the spectra of collimated transmission (*ex vivo*) or diffuse reflection (*in vivo*) [30, 32, 36]. Thus, Tuchina et al. [30] found an increase in the collimated transmission of rat skin in *ex vivo* experiments when it was cleared with a 40% glucose solution. Similar dependences were obtained by Genina et al. [39] when studying the optical properties of the skin of a laboratory mouse after optical clearing using an 84.4% glycerol solution in the measurement of collimated transmission spectra, and an increase in signal by 20–40 times (*ex vivo*) and by 16% (*in vivo*) for specific wavelengths.

Table 2. Effectiveness of the clearing of the gum tissue for 60 minutes and dentin for 300 minutes with immersion in a 99.5% glycerol solution.

Clearing efficiency (%) (average value, $n = 10$)	Wavelength/nm									
	Excimer lasers			Harmonics of Nd:YAG laser			Ti:sapphire laser			
	193	308	350	266	355	532	680	700	760	800
Gum	1847±60	857±25	1016±30	1133±40	952±30	396±25	141±19	137±18	128±17	134±19
Dentin	1±1	8±2	18±3	1±1	21±3	24±4	25±3	25±4	25±3	26±3

It can be seen that the interaction of glycerol with human gum and dentin samples leads to a gradual increase in transmittance both in the UV range and in the visible region of the spectrum. In the range of 300–400 nm, radiation scattering predominates, since collagen and elastin absorb radiation in this region. After immersion in a 99.5% glycerol solution, the refractive indices of scattering components in these biological tissues are equalised and scattering is significantly reduced. In this wavelength range, a greater efficiency of gum tissue clearing is observed. At 400 nm, absorption predominates due to the presence of porphyrins and oxyhaemoglobin in the gum tissue, which are endogenous chromophores of biological tissue.

Thus, for *in vivo* studies of the effectiveness of gingival clearing, diffuse reflection spectroscopy can be used, and in some pathological cases, for example, if the patient has a gingival pocket, tissue transmittance can also be measured using optical fibres.

4. Conclusions

In the present work, the optical characteristics of the tissues of the gums and dentin of a human tooth *in vitro* exposed to a 99.5% glycerol solution were studied. The saturation of 10 gum tissue samples (~0.6 mm thick) with this solution takes place completely within 30–55 min, which increases the depth of penetration of optical radiation into biological tissue with different efficiencies depending on the wavelength. It was revealed that the highest efficiency of *in vitro* gum tissue clarification in humans is observed in the UV region, and its maximum value exceeds 1800%. The maximum clarification efficiency of dentin samples turned out to be much lower, 15%–25% in the near-UV, visible and near-IR spectral regions. The results obtained indicate the effectiveness of the use of a highly concentrated solution of glycerol (99.5%) as an optical clearing agent for controlling the optical characteristics of the studied biological tissues. It is especially effective for human gum tissue. An increase in the total transmittance of radiation transmitted through the sample is observed, which indicates a decrease in the scattering properties of the sample. The presented results can be used in dentistry for laser therapy of both periodontal soft tissues and hard tooth tissues, including photodynamic and photothermal therapy, as well as for optical diagnosis of dental diseases.

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In this work, no studies have been conducted involving people as objects of research.

The authors declare no conflict of interest.

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