ISSN 0030-400X, Optics and Spectroscopy, 2010, Vol. 109, No. 2, pp. 225–231. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © E.A. Genina, A.N. Bashkatov, Yu.P. Sinichkin, V.V. Tuchin, 2010, published in Optika i Spektroskopiya, 2010, Vol. 109, No. 2, pp. 256–263.

Optical Clearing of Skin under Action of Glycerol: Ex Vivo and In Vivo Investigations

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Received February 11, 2010

Abstract—The behavior of optical parameters of the skin of a laboratory rat under the action of an aqueous solution of glycerol is studied ex vivo and in vivo. It is found that the collimated transmission coefficient of ex vivo skin samples increases by a factor of 20–40-fold depending on the wavelength in the studied spectral range, and the diffuse reflection coefficient of skin in vivo decreases on the average by 16%. The results presented can be useful for many methods of laser therapy and optical diagnostics of skin diseases and localization of subcutaneous neoplasms.

DOI: 10.1134/S0030400X10080126

INTRODUCTION

Interest in the use of optical methods for diagnostics and therapy of various diseases constantly increases because they are relatively simple, rather inexpensive, and patient-friendly. However, the transport of probing light through surface layers of biotissue still remains a significant problem in modern laser medicine. In particular, upon the diagnostics of subcutaneous and intracutaneous diseases of optical methods, the considerable scattering of light in the visible and near-IR spectral ranges by skin tissues restricts the spatial resolution and probing depth [1–3]. Reducing light scattering by immersion clearing of biotissues is a possible way of solving this problem.

It is well known that the main reason for scattering of optical light in cellular structures and biotissues is the difference between the refractive indices of structural components of a tissue (collagen and elastin fibers) and interstitial medium, as well as between cellular organelles and cytoplasm of cells [1–3]. In the method of optical clearing of a biotissue, it is exposed to the action of a biocompatible immersion compound, the so-called clearing agent, whose osmolarity and refractive index are, as a rule, higher than those of the interstitial fluid [4]. At present, the following three mechanisms by which the light scattering of biotissues is reduced under the action of a clearing agent have been proposed [5–11]:

(i) osmotic dehydration of a biotissue;

(ii) partial replacement of an interstitial fluid by the clearing agent, as a result of which the refractive indices of structural components of a biotissue become matched;

(iii) structural modification or dissociation of collagen of a biotissue.

The first mechanism is only characteristic of hyperosmotic clearing agents and consists of removing water from the interstitial or intercellular space. The concentration of soluble components that enter the composition of the interstitial fluid increases and, correspondingly, its refractive index increases. Apart from matching the refractive indices of fibrils or the organelles and surrounding medium, as a result of dehydration, the packing density and the degree of order of scatterers (cellular structures, collagen and elastin fibers) increase, which also facilitates the optical clearing of biotissue [9–11].

The second mechanism is mainly observed for fibrous tissues, particularly the skin dermis, because molecules of the clearing agent are considerably smaller in size than the average diameter of the cross section of interfibrillar gaps, which is about 185–200 nm, whereas, e.g., the diameter of a large polyethylene glycol molecule (molecular weight is 20000) is smaller than 5 nm [4, 5, 8, 9, 12]. Both the first and the second mechanisms lead to the matching of the refractive indices of main scattering objects and cytoplasm (for cellular structures) or interstitial fluid (for fibrous tissues).

The structural modification of collagen also changes the scattering of a biotissue. As is known, collagen is soluble in sugars (glucose, fructose, etc.) and sugar alcohols (ethylene glycol, glycerol, sorbitol, etc.) [6, 7]. It was noted that these clearing agents cause the swelling of biotissues and the dissociation of collagen fibrils into microfibrils [6, 7]. This destabilization of the collagen structure can additionally reduce the scattering of light by a biotissue due to a decrease in the size of scatterers [6]. However, it was noted in [13] that, although the diameter of fibrils decreases, glycerol solutions cause the considerable swelling of a

biotissue, in particular the skin, which leads to a decrease in its transparency. At the same time, in works, optical clearing of some biotissues was observed (dura mater, sclera), which was accompanied by their certain swelling [14–16].

It should also be noted that, in reality, the first two mechanisms can act simultaneously, differing only in the magnitude of the contribution to the clearing effect depending on the types of the clearing agent and biotissue. The third mechanism apparently only affects the degree of clearing upon the prolonged action of hyperosmotic immersion liquids on a biotissue. In [13], it was shown that, within 30 min after the in vivo injection of glycerol solutions into the rat skin, no dissociation of collagen fibrils was observed.

Glycerol is one of the most widely used agents for optical clearing skin. Numerous experiments in vitro showed that aqueous solutions of glycerol of different concentrations increase the transparency of samples of skin because of a decrease in its scattering characteristics [5, 6, 9, 11, 17–20]. However, the mechanism by which glycerol increases the degree of clearing of a biotissue has a complicated character and has not been completely understood. In addition, upon the use of this method for clearing skin in vivo, additional factors arise, such as the metabolic reaction of a living biotissue to a hyperosmotic immersion agent, peculiarities of functioning of a biotissue and effect of the physiological temperature to the rate of the process, which should be taken into account because they can considerably change both the rate and the magnitude of the clearing effect. If high-concentration glycerol solutions are used as a clearing agent, side effects can occur, such as distortions of the structure of a biotissue and stasis of small blood vessels [17].

This work presents results of ex vivo and in vivo studies of changes of optical properties of skin exposed to the action of an aqueous solution of glycerol.

MATERIALS AND METHODS

Preparation of Skin Samples for ex vivo Spectral Measurements

Ex vivo experiments were performed using ten samples of skin of laboratory albino rat, which were taken from different animals 1 h post mortem. The time interval between autopsy and measurements did not exceed 15 min. Prior to experiments, hair coverings and subcutaneous fat layers were carefully removed from the surfaces of samples. The skin was cut into samples with an area of about 1 × 1.5 cm. To measure the thickness of the biotissue samples, they were placed between two slides; the measurements were performed with a micrometer at several points of each sample. The error of each measurement was ±50 μm. The obtained values were averaged. Before treatment with glycerol, the average thickness of the studied samples was 0.57 ± 0.16 mm; after the spectral measurements, this parameter was 0.58 ± 0.2 mm. The standard deviation (SD) was calculated by the formula

$$SD = \sqrt{\sum_{i=1}^{N} \left(\bar{A} - A_i\right)^2 \Big/ N(N-1)},$$

where N is the number of studied samples, A_i is the thickness of the ith sample of the biotissue, and $\bar{A}$ is the average thickness found by the formula $\sum_{i=1}^{N} A_i/N$. All experiments were performed at room temperature.

Preparation of Animals for in vivo Spectral Measurements

Diffuse reflection spectra were measured in vivo for three laboratory albino rats. The age of the animals was about nine months, and their mass was about 200 g. The animals were anesthetized by an intraperitoneal injection of a 1% solution of sodium ethaminal with a dose of 40 mg per kg animal body mass. After immobilization, the hair covering the measurement area (thigh) was removed. To overcome the protective barrier of epidermis, the clearing agent was introduced by subcutaneous injection. The volume of the introduced preparation was 0.1 ml.

As a clearing agent, we used a standard 84.4% aqueous solution of glycerol (ZAO ÉKOlab, Russia). The refractive index of the solution, measured on an Abbe refractometer (at a wavelength of 589 nm), was 1.452, pH 6.5.

Experimental Setup and Measurement Methods

Collimated transmission spectra were measured on a USB4000 multichannel spectrometer (Ocean Optics, United States) in the range 400–1000 nm. The schema of the experimental setup is shown in Fig. 1 (A). As a light source, we used an HL-2000 halogen lamp (Ocean Optics, United States).

To measure the collimated transmission, skin samples were fixed on a frame with an aperture of 7 × 7 mm and were placed in a cell between two specialized fiberoptic cables (a P400-1-UV-VIS patch cord optical fiber assembly, Ocean Optics, United States) with an inner diameter of 400 μm. One fiberoptic cable served to deliver light to the sample, while the other cable was used to collect transmitted light. To ensure the collimation of the beam, 74-ACR collimators were fixed at the ends of the fiberoptic cables using SMA-905 connectors (all equipment from Ocean Optics, United States).

As a reference signal, we used the signal from a 5-ml cell filled with an agent under study. After that, a sample of skin was fixed on the frame, and the frame was placed in the cell. In this geometry, the clearing agent interacted with the skin sample mainly through

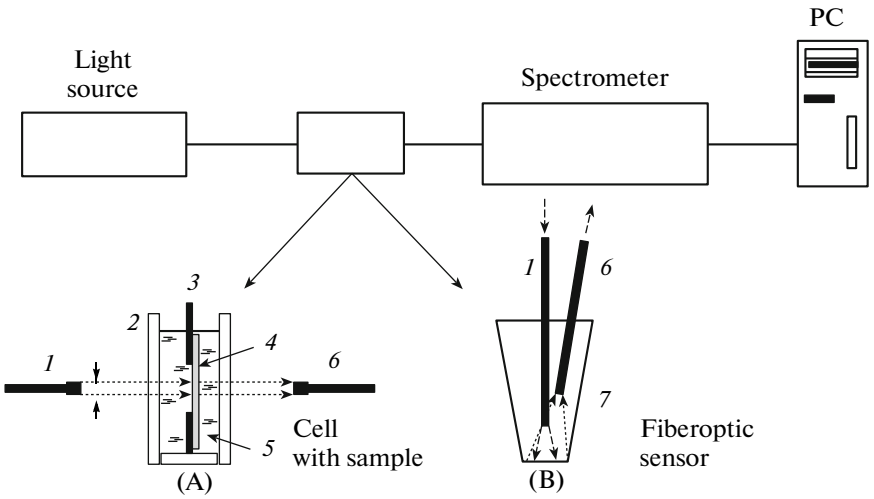

Fig. 1. Schema of experimental setup for recording spectra of collimated transmission (A) and diffuse reflection (B): (*1*) light guides delivering light to biotissue sample, (*2*) cell, (*3*) frame for fixing skin sample, (*4*) skin sample, (*5*) immersion liquid, (*6*) light guide collecting light transmitted through sample, and (*7*) housing for fixing optical fibers.

dermis. The spectra were recorded every 2–5 min for 2 h.

The diffuse reflection spectra were measured on an LÉSA-6med multichannel spectrometer (BioSpec, Russia) in the range 400–700 nm. schema of the experimental setup is shown in Fig. 1 (B). As a light source, we used a xenon lamp with a power of 250 W. Measurements were performed using a fiberoptic sensor that consisted of two fibers with an inner diameter of 400 μm and numerical aperture of 0.2. The central fiber delivered light to the skin surface, while the side fiber collected diffusely reflected light. The fibers were rigidly packed in a housing to fix the distance between the fiber ends and sample surface. The distance between the illuminating fiber and the skin surface was 12 mm. The configuration of the fiberoptic sensor that was used in our experiments ensured normal illumination of the skin surface by a spot with a diameter of 4 mm and collection of light reflected at an angle of 20° relative to the illuminating fiber from the skin area with a diameter of 8 mm. The ratio between the illuminated area and light-collection area was about 1 : 4. This geometry of the sensor made it possible to detect only the light that was diffusely reflected by the skin, to eliminate the specularly reflected component, and to minimize losses of backward scattered light in the long-wavelength spectral range.

The sensor measured the signal averaged over the area of collection of light. The reproducibility of measurement results was within 5%. To detect the reflected signal, the sensor was placed in the area of injection of the clearing agent directly against the skin surface. As a reflection reference, we used a WS-1-SL reflection standard (Ocean Optics, United States). Initially, measurements were performed for the intact skin, then, after the injection of the clearing agent, every 30 s for 25 min.

RESULTS AND DISCUSSION

The spectra of the collimated transmission coefficient T_c and the kinetics of its behavior at several wavelengths measured for one of the samples of whole skin exposed to the action of glycerol are presented in Figs. 2 and 3, respectively. These figures demonstrate changes in the optical properties of skin in the course of its interaction with the glycerol solution. Differences between the spectra of samples were about 20% and were caused by different thicknesses of samples and the individual peculiarities of their structure (occurrence of more densely packed collagen fibers, blood vessels, etc.).

The shape of the transmission spectrum of skin is determined by the spectral dependences of the scattering and absorption coefficients of the biotissue [21]. The skin has a complex and inhomogeneous structure, which contains cellular and fibrous layers; in addition, blood and various chromophores and pigments are inhomogeneously distributed over the skin volume [22, 23]. A particular feature of the skin of the rat is that it does not contain melanin.

The main distinguishable layers of the skin of the rat in the direction from the surface are epidermis (a bloodless layer) and dermis (contains vascular plexuses). Since the dermis is considerably thicker than the epidermis, it makes determining the contributions to the formation of the absorption and scattering spectra of the skin [3, 21]. The skin is a typical fibrous biotissue, the main components of which are collagen and elastin fibers, which are grouped into randomly intertwined bundles, and interstitial fluid, which contains

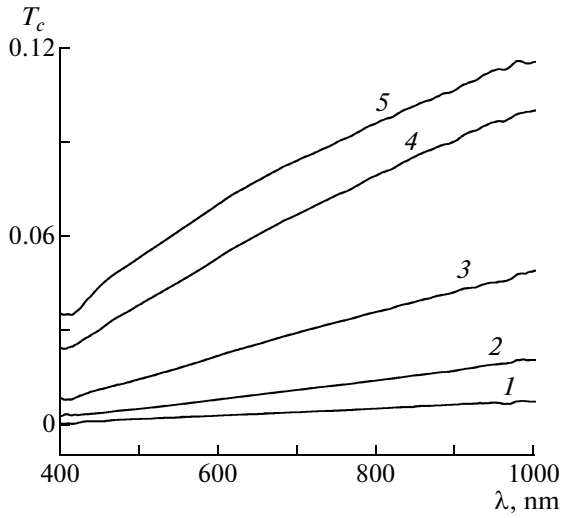

Fig. 2. Spectra of collimated transmission coefficient of sample of whole skin of rat measured ex vivo after different time periods of the action of glycerol solution on sample: (*1*) 0, (*2*) 8, (*3*) 16, (*4*) 30, and (*5*) 120 min.

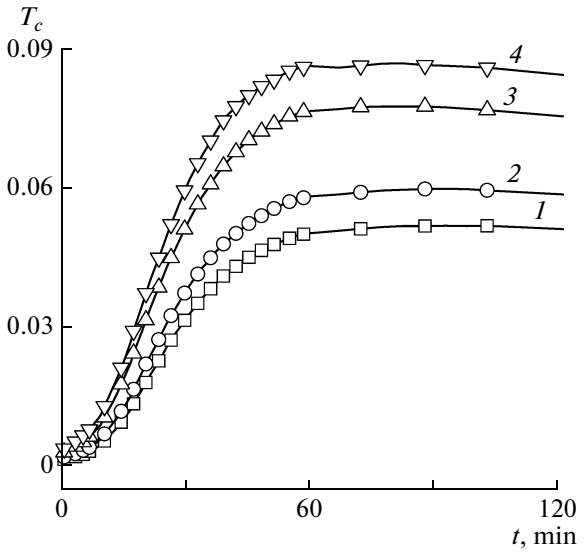

Fig. 3. Kinetics of behavior of collimated transmission coefficient of sample of whole skin of rat subjected to the action of glycerol solution and measured ex vivo at different wavelengths: (*1*) 488, (*2*) 532, (*3*) 633, and (*4*) 700 nm.

salts and proteins dissolved in water [3]. Inside the tissue, collagen and elastin fibers are in a hydrated state [24].

It is well known that the scattering properties of fibrous tissues are determined by their structure and the difference in the refractive indices of structural components (hydrated collagen and elastin fibers) ($n_c = 1.47$) and interstitial fluid ($n_I = 1.345$) [3]. Since the dermis contains a well-developed capillary network, the main absorber in the visible wavelength range is hemoglobin of blood. The absorption bands of oxygenated hemoglobin have maxima at 420, 547, and 577 nm. However, Fig. 2 clearly shows that, at the initial moment of time (*1*), absorption bands of blood have no effect on the shape of the spectrum. It is likely that this is related to the fact that they are masked against the background of strong scattering of the sample. It follows from Figs. 2 and 3 that, at the initial moment of time, the skin sample is weakly transparent for the optical light (the transmission coefficient for different wavelengths is no more than 0.005%). It can be clearly seen from Fig. 2 that, as a result of optical clearing of the skin (*5*), the spectrum corresponding to the 120-min action of the clearing agent markedly exhibits the Soret band (420 nm). The absence of less pronounced absorption bands of blood in the range 500–600 nm is related to a small probability of absorption of photons upon their direct propagation through the sample of the biotissue subjected to clearing. One should also take into account low blood filling of skin samples upon ex vivo measurements.

In the course of optical clearing of skin, the collimated transmission coefficient of the sample monotonically increases in the entire considered wavelength range. The curves of Fig. 3 correspond to the transmission coefficient of skin at wavelengths of several laser light sources that are frequently used in laser therapy (ruby/alexandrite (694/700 nm), helium–neon (632.8 nm), and argon (488 nm) lasers and the second harmonic of light of a Nd:YAG laser (532 nm)). It can clearly be seen that, within 1 h, T_c increases 20–40-fold depending on the wavelength. In this case, we can assume that the diffusion of glycerol into skin along

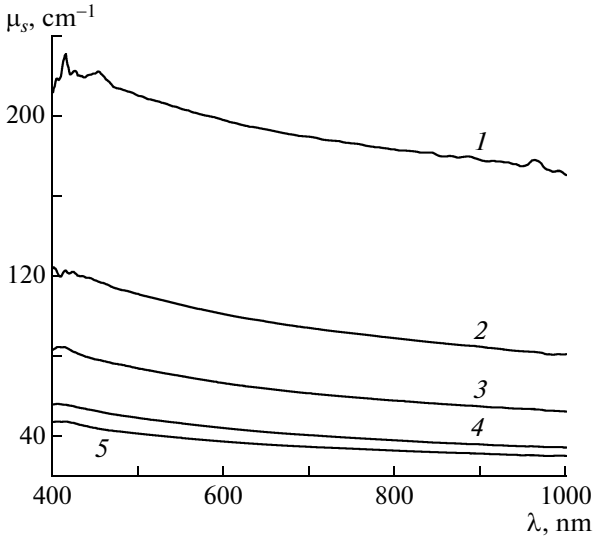

Fig. 4. Spectra of scattering coefficient of ex vivo skin calculated based on the Bouguer–Beer law for different time periods of the action of glycerol solution on sample: (*1*) 0, (*2*) 8, (*3*) 16, (*4*) 30, and (*5*) 120 min.

the concentration gradient occurs simultaneously with the diffusion of water from the interstitial space to the surrounding solution. The two processes contribute to the increase in the collimated transmission coefficient within first 60 min. However, at 60–120 min, the collimated transmission coefficient T_c saturates and even somewhat decreases. This behavior can seemingly be explained by the interaction between the renewed interstitial fluid (with a certain amount of glycerol and reduced amount of water) and hydrated collagen and elastin fibers, whose refractive index begins to increase due to the water removal. In this case, the refractive index of the interstitial fluid somewhat decreases because of the passage of water from fibers to the interfibrillar space. Both processes cause a certain increase in light scattering by fibers and, correspondingly, a certain darkening of the sample.

As is known, a collimated beam propagating through a biotissue is attenuated according to an exponential law, and the intensity of collimated light passed through a thin sample of a tissue can be estimated based on the Bouguer–Beer law [1]. Therefore, the attenuation coefficient μ_t of light in a biotissue is approximately determined by the formula

$$\mu_t = (-1/z)\ln T_c(t),$$

where z is the thickness of the sample and $T_c(t)$ is the collimated transmission coefficient of the sample during the clearing process. The attenuation coefficient of a biotissue is determined by its absorption and scattering, $\mu_t = \mu_a + \mu_s$, where μ_a and μ_s are the absorption and scattering coefficients, respectively. Because the average absorption coefficient of skin in this spectral range is considerably smaller than the scattering coefficient $\mu_a \ll \mu_s$ [21], we can assume that the attenuation coefficient is mainly determined by the scattering coefficient. The spectrum of μ_s calculated by the described method is presented in Fig. 4. This figure shows that, depending on the wavelength, the scattering coefficient decreases 4.5–6-fold.

In vivo diffuse reflection spectra R_d of intact skin (Fig. 5a) clearly show the effect of three absorption bands of oxygenated hemoglobin. As follows from Fig. 5, within first 3.5–4 min (Fig. 5a), R_d of skin decreases on the average by 16%. In this case, the shape of the spectra remains nearly the same. In the next 5–6 min, the reverse phenomenon is observed; namely, the diffuse reflection coefficient increases approximately by 20%, with the shape of the spectra also remaining unchanged (Fig. 5b). Subsequently, until the end of measurements, the level of the signal remains; however, it is observed that, in spectral intervals corresponding to the absorption of blood, the reflection spectra become deformed (Fig. 5c).

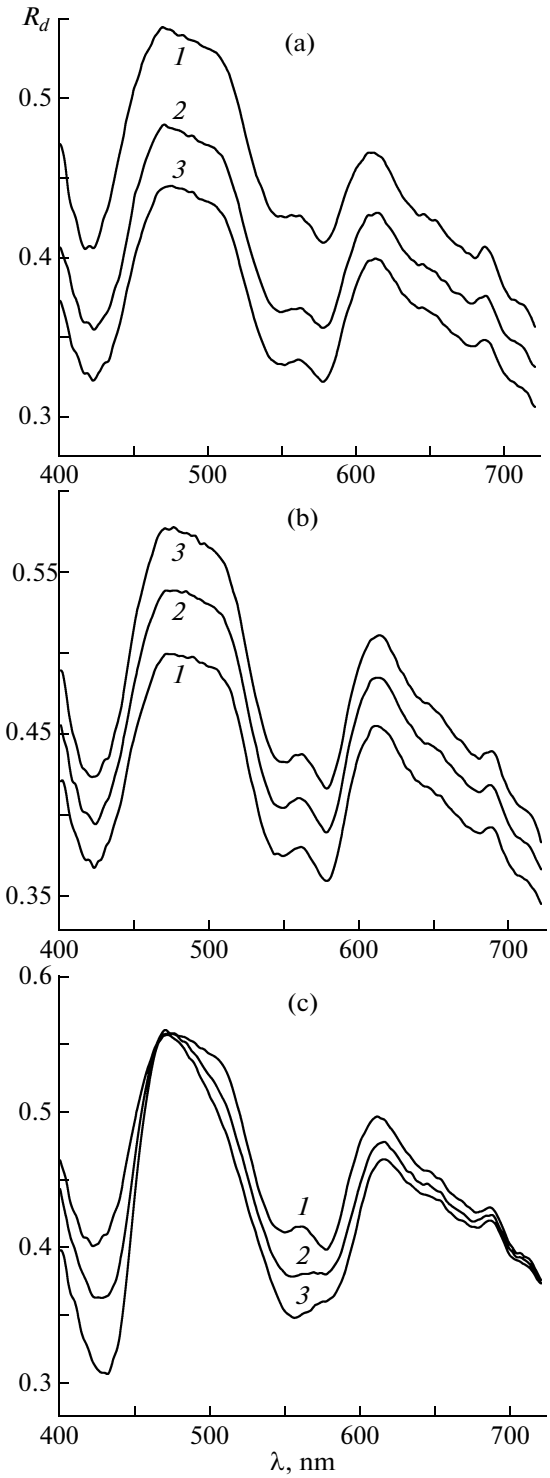

Fig. 5. Spectra of diffuse reflection coefficient of skin of rat measured in vivo at different time moments after subcutaneous injection of 84.4% glycerol solution. (a, first stage of clearing): (*1*) 0, (*2*) 1.5, and (*3*) 3.5 min; (b, second stage of clearing): (*1*) 5.5, (*2*) 7.5, and (*3*) 10.5 min; and (c, third stage of clearing): (*1*) 11, (*2*) 15, and (*3*) 20 min.

Figure 6 shows the dynamics of the change in the diffuse reflection coefficient of skin at the same wavelengths that were presented in Fig. 3. Symbols denote experimental data.

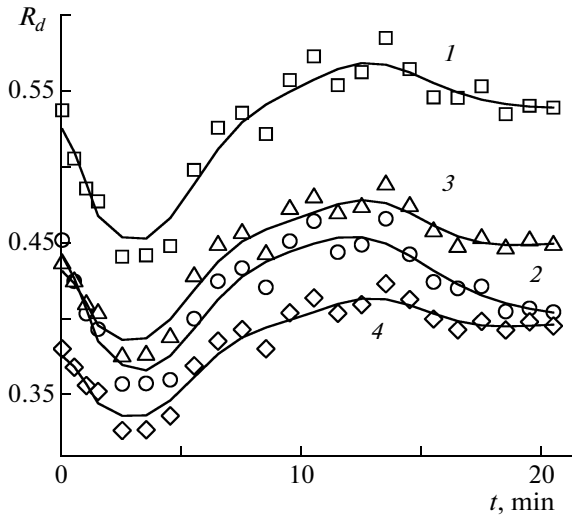

Fig. 6. Kinetics of behavior of diffuse reflection coefficient of skin of rat measured in vivo at several wavelengths: (*1*) 488, (*2*) 532, (*3*) 633, and (*4*) 700 nm.

An analysis of the obtained spectra and dynamic curves allows us to assume that the direct in vivo interaction of the high-concentration glycerol solution with the skin dermis can approximately be divided into three stages. At the initial stage, the introduction of glycerol into the dermis facilitates the optical immersion, i.e., the matching of the refractive indices of scatterers and the surrounding medium. This process reduces the scattering of skin and, therefore, reduces the reflection coefficient (Fig. 5a). At the same time, glycerol, which is a hyperosmotic agent, causes the diffusion of free water from the tissue surrounding the injection region to the zone of localization of glycerol. In this case, glycerol binds water molecules in the interfibrillar space. As is known, glycerol is a strongly hygroscopic compound and, being placed in a humid environment, it adds water molecules up to achieving the saturation level (55 vol %). It was shown that each glycerol molecule can add six water molecules [25]. Consequently, at the second stage, the region of the dermis that interacts with glycerol swells due to the added water. In this case, the refractive index of the glycerol solution in the interstitial space decreases compared to its value at the first stage, which manifests itself as an increase in the reflection coefficient of skin (Fig. 5b).

The deformation of reflection spectra at the third stage of clearing is related to the interaction of glycerol with skin vessels. The shift of the absorption maximum of hemoglobin in the Soret band from 420 to 428 nm, the decrease in the absorption at 547 and 577 nm, and the occurrence of the absorption maximum at a wavelength of 555 nm corresponds to the transition of hemoglobin from the oxygenated form to the deoxygenated one [26]. This process can be caused by stasis in microvessels and capillaries of the dermis under the action of glycerol. In [17], it was shown that the in vivo application of a 75% glycerol solution to mesenteric vessels of the rat caused dilatation and stasis of vessels, as well as the local intravascular hemolysis restricted by the region of action of glycerol.

Therefore, the kinetics and degree of clearing skin ex vivo and in vivo differ considerably from each other. The time period during which the transparency of the biotissue achieved a maximum in ex vivo experiments considerably exceeded the clearing time of the skin area investigated in vivo. Upon the ex vivo action of the glycerol solution on the sample of the skin, this time period was longer than 1 h, whereas, in the case of the action of the same solution on the living tissue, the clearing process proceeded only 3–4 min. It is likely that this is a consequence of metabolism of the living tissue.

In addition, in contrast to the ex vivo clearing of skin samples, the in vivo scattering characteristics of skin do not change monotonically. In our opinion, this is caused by the differences in the in vivo and ex vivo clearing mechanisms. Upon ex vivo clearing, the water contained in the interstitial space leaves the region of observation, while glycerol penetrates this region in accordance with the concentration gradient. Conversely, upon in vivo investigation, water diffuses into the localization region of glycerol, i.e., into the region of observation, while glycerol tends to penetrate into surrounding regions. As opposed to results of [13], where it was observed that the reflection coefficient of skin increased immediately after the injection of glycerol into it; i.e., the biotissue became more turbid and, in our work, we observed that the reflection coefficient of the skin decreased visibly due to the diffusion of glycerol inside the dermis from the injection region both toward the surface of the skin and along it.

CONCLUSIONS

Our results showed that the use of an aqueous solution of glycerol as a clearing agent makes it possible to quite efficiently control the optical properties of skin. In particular, the collimated transmission coefficient of ex vivo skin samples increases 20–40-fold depending on the wavelength, and the diffuse reflection coefficient of in vivo skin decreases, on the average, by 16% in the spectral range of 400–700 nm, which will facilitate an increase in the penetration depth of the optical light into the biotissue. The change in the shape of diffuse reflection spectra is indicative of stasis of microvessels and capillaries of skin in the region of the local action of glycerol. We proposed an explanation of the mechanisms by which the glycerol solution interacts with the dermis of ex vivo and in vivo skin samples. Our results can prove to be useful for many methods of laser therapy and optical diagnostics of skin diseases and localization of subcutaneous neoplasms.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project no. 08-02-92224), by the program of developing the potential of higher schools of the Russian Federation (2009–2010) (project nos. 2.1.1/4989 and 2.2.1.1/2950), by the Saratov State University project no. 1.4.09 of the Federal Agency for Education of the Russian Federation (2009–2010), and by the State Contract no. 02.740.11.0484.

REFERENCES

1. V. V. Tuchin, *Lasers and Fiber Optics in Biomedical Studies* (Sarat. Gos. Univ., Saratov, 1998) [in Russian].
2. *Biomedical Photonics Handbook*, Ed. by T. Vo-Dinh (CRC Press, Boca Raton, 2003).
3. V. V. Tuchin, *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis* (SPIE Press, Bellingham, 2007).
4. V. V. Tuchin, I. L. Maksimova, D. A. Zimnyakov, I. L. Kon, A. H. Mavlutov, and A. A. Mishin, J. Biomed. Opt. **2**, 401 (1997).
5. G. Vargas, E. K. Chan, J. K. Barton, H. G. Rylander III, and A. J. Welch, Las. Surg. Med. **24**, 133 (1999).
6. A. T. Yeh, B. Choi, J. S. Nelson, and B. J. Tromberg, J. Invest. Dermatol. **121**, 1332 (2003).
7. A. T. Yeh and J. Hirshburg, J. Biomed. Opt. **11**, 014 003 (2006).
8. A. N. Bashkatov, A. N. Korolevich, V. V. Tuchin, Y. P. Sinichkin, E. A. Genina, M. M. Stolnitz, N. S. Dubina, S. I. Vecherinski, and M. S. Belsley, Asian J. Phys. **15** (1), 1 (2006).
9. V. V. Tuchin, *Optical Clearing of Tissues and Blood* (SPIE Press, Bellingham, 2006).
10. C. G. Rylander, O. F. Stumpp, T. E. Milner, N. J. Kemp, J. M. Mendenhall, K. R. Diller, and A. J. Welch, J. Biomed. Opt. **11** (4), 041 117 (2006).
11. E. A. Genina, A. N. Bashkatov, A. A. Korobko, E. A. Zubkova, V. V. Tuchin, I. V. Yaroslavsky, and G. B. Altshuler, J. Biomed. Opt. **13** (2), 021 102 (2008).
12. E. A. Genina, A. N. Bashkatov, E. A. Zubkova, T. G. Kamenskikh, and V. V. Tuchin, Opt. Las. Engin. **46**, 915 (2008).
13. X. Wen, Z. Mao, Zh. Han, V. V. Tuchin, and D. Zhu, J. Biophoton. **3** (1–2), 44 (2010).
14. A. N. Bashkatov, E. A. Genina, Yu. P. Sinichkin, V. I. Kochubey, N. A. Lakodina, and V. V. Tuchin, Biophys. J. **85** (5), 3310 (2003).
15. É. A. Genina, A. N. Bashkatov, V. I. Kochubeĭ, and V. V. Tuchin, Opt. Spektrosk. **98** (3), 515 (2005) [Opt. Spectrosc. **98** (3), 470 (2005)].
16. E. A. Genina, A. N. Bashkatov, N. A. Lakodina, S. A. Murikhina, Yu. P. Sinichkin, and V. V. Tuchin, Proc. SPIE **4001**, 255 (2000).
17. E. I. Galanzha, V. V. Tuchin, A. V. Solovieva, T. V. Stepanova, Q. Luo, and H. Cheng, J. Phys. D **36** (14), 1739 (2003).
18. R. Cicchi, F. S. Pavone, D. Massi, and D. D. Sampson, Opt. Expr. **13** (7), 2337 (2005).
19. S. Plotnikov, V. Juneja, A. B. Isaacson, W. A. Mohler, and P. J. Campagnola, Biophys. J. **90**, 328 (2006).
20. Z. Mao, D. Zhu, Y. Hu, X. Wen, and Z. Han, J. Biomed. Opt. **13** (2), 021 104 (2008).
21. A. N. Bashkatov, E. A. Genina, V. I. Kochubey, and V. V. Tuchin, J. Phys. D **38**, 2543 (2005).
22. G. F. Odland, in *Physiology, Biochemestry, and Molecular Biology of the Skin*, Ed. by L. A. Goldsmith (Oxford Univ. Press, Oxford, 1991), pp. 3–62.
23. A. R. Young, Phys. Med. Biol. **42**, 789 (1997).
24. E. M. Culav, C. H. Clark, and M. J. Merrilees, Phys. Therapy **79**, 308 (1999).
25. J. W. Wiechers, J. C. Dederen, and A. V. Rawlings, in *Skin Moisturization*, Ed. by A. V. Rawlings and J. J. Leyden (Taylor and Francis, London, 2009), pp. 309–321.
26. S.A. Prahl, http://www.omlc.ogi.edu/spectra/.

Translated by V. Rogovoi