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Optical clearing of the eye sclera *in vivo* caused by glucose

E.A. Genina, A.N. Bashkatov, Yu.P. Sinichkin, V.V. Tuchin

***Abstract*. Changes in the optical properties of the eye sclera of a laboratory animal (rabbit) caused by the noninvasive administration of an immersion agent (aqueous glucose solution) into sclera are studied experimentally *in vivo* by the method of reflection spectroscopy and simulated numerically. The diffusion coefficients of glucose and water in sclera are estimated from simulations of optical clearing within the framework of the developed model as $(5.4 \pm 0.1) \times 10^{-7}$ and $(5.8 \pm 0.2) \times 10^{-7}$ cm^2 s^{-1}, respectively. Experiments showed that the application of the glucose solution on the sclera allows the efficient control of its optical properties, which makes it possible to deliver the laser energy to internal eye tissues without considerable attenuation and distortions of its spatial distribution in laser diagnostics and therapy.**

Keywords: *multiple light scattering in biological tissues, reflection spectroscopy, immersion optical clearing, sclera, glucose.*

1. Introduction

In the last decade optical methods have found increasing applications in medicine. Along with numerous diagnostic methods such as optical coherence tomography [1], confocal microscopy [2], fluorescence spectroscopy [3], and optical diffusion tomography [4], the methods of laser therapy and surgery are growing in importance. In particular, transscleral laser operation, in which retina and ciliary body are irradiated by a laser beam directly through sclera, is promising in ophthalmology for medical treatment of glaucoma, myopia, and other diseases [5 – 7]. Because the transport of radiation to the region under study through surface fibrous tissues, in particular, the eye sclera is accompanied by considerable losses caused by multiple light scattering, the problem of increasing the transparency of these tissues remains of current interest for optical methods of diagnostics and medical treatment.

It is well known that the scattering properties of fibrous tissues are determined by their structure and a difference in the refractive indices of structural components (collagenic and elastin fibres) and the interstitial liquid [8, 9]. It was shown [10 – 15] that the optical properties of biological tissues can be efficiently controlled by treating them with biologically compatible hyperosmotic liquids. In this case, the basic control mechanism is the matching of the refractive indices of scatterers (for example, collagenic fibres) and the interstitial liquid due to the osmotic dehydration of a biological tissue and diffusion of immersion liquids to the tissue. The tissue dehydration also results in a tighter and, hence, more regular packing of scatterers, which causes an additional clearing of the tissue due to coherence effects [16].

Such a control aimed at the reduction of light scattering in biological tissues is extremely important both for increasing the efficiency of diagnostic methods and more accurate dosimetry of the laser action. In particular, the optical clearing of sclera should increase the probe depth and resolution of the optical tomography of eyes. In the case of transsceral surgery, this method should reduce the sclera damage and improve the accuracy of laser beam focusing on retina and ciliary body [10, 11, 14].

The eye sclera mainly consists of collagenic fibres packed into flat bundles immersed into an amorphous basic substance. Under sclera, a layer of retinal pigment epithelium (RPE) is located, which is formed by thin collagenic bundles of fibres of internal sclera layers. It contains a great amount of a pigment, mainly melanin, which is predominantly localised between collagenic fibre bundles. The eye choroid (EC) located under the RPE layer contains a network of bloods vessels and capillaries [5, 17, 18]. The sclera itself is virtually devoid of the blood network. Inhomogeneities in the structure and differences between the refractive indices of hydrated collagenic fibres ($n_c = 1.474$) and the interstitial liquid ($n_{liq} = 1.345$) cause multiple light scattering in sclera, making it opaque in the normal state [18].

Numerous *in vitro* experiments have shown that immersion liquids such as Trazograph 60 and 76 [10, 19], Hypaque 60 and 76 [11], polyethylene glycol [19], and aqueous glucose solutions [14, 20] increase the transparency of sclera by reducing light scattering in it without the tissue damage if they are used for a short time. However, the data on optical clearing of sclera *in vivo* with the help of immersion liquids are virtually absent. It is reasonable to assume that the use of this method for sclera clearing *in vivo* involves additional factors such as the metabolic reaction of a living biological tissue to a hyperosmotic immersion agent, the specific features of the tissue functioning, and the influence of the physiological temperature on the rate of the process,

E.A. Genina, A.N. Bashkatov, Yu.P. Sinichkin, V.V. Tuchin Department of Optics and Biomedical Physics, N.G. Chernyshevskii Saratov State University, ul. Astrakhanskaya 83, 410012 Saratov, Russia;
e-mail: eagenina@optics.sgu.ru

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which should be taken into account because they can considerably change the dynamic characteristics and magnitude of the clearing effect.

In this paper, we present the results of *in vivo* experiments on the control of optical properties of the rabbit eye sclera by administering noninvasively the 40 % aqueous glucose solution used as an immersion agent. This solution was chosen based on *in vitro* investigations of sclera clearing under the action of solutions of various immersion agents [10, 11, 14, 19, 20]. These experiments have demonstrated the higher efficiency of the 40 % glucose solution compared to that of glucose solutions of lower concentrations [14, 20]. At the same time, more concentrated glucose solutions, as solutions of some other immersion agents, cause a strong osmotic shock and considerable dehydration of biological tissues [20], which can violate the metabolism of sclera and eye as a whole in *in vivo* application of these solutions. In addition, the 40 % aqueous glucose solution is inexpensive and readily available.

2. Materials and methods

We studied an eye of a five-month old albino rabbit. The rabbit was anaesthetised by the injection of the 0.1 % sodium pentobarbital solution. The dose of the administered preparation was 40 mL kg^{-1}. The measurements of the reflectance were started within 30 min after injection. To eliminate involuntary blinking during experiments, a standard eyelid holder was used.

As an immersion agent for optical clearing of sclera, we used the 40 % aqueous glucose solution for injections (Microgen RPA, Russia). The refractive index n_a of the agent measured with an Abbe refractometer was 1.39 at a wavelength of 589 nm. The agent was applied on the sclera surface by drops. The total volume of the administered solution was 0.1 mL.

The dynamics of reflection spectra was measured during optical clearing of sclera with a fibre multichannel spectrometer (LESA-6med, BioSpek, Russia) equipped with a specially developed detector. Figure 1 presents the scheme of the experimental setup with a fibreoptic detector. The detector consisted of two optical fibres with the internal diameter 400 μm and the numerical aperture 0.2. Radiation was delivered through the central fibre oriented perpendicular to the sclera surface. The distance between the illuminating fibre and sclera surface was 12 mm. The design of the fibreoptic detector provided normal illumination of the sclera surface by a light beam of diameter 4 mm and collection of radiation reflected from the sclera surface area of diameter 8 mm at an angle of 20° with respect to the illuminating fibre. The ratio of the illuminated area to the light-collection area was approximately one to four. Such geometry of the detector provided the detection of only diffusion reflection of light from sclera, eliminating specular reflection, by minimising losses due to backscattered radiation in the long-wavelength spectral region.

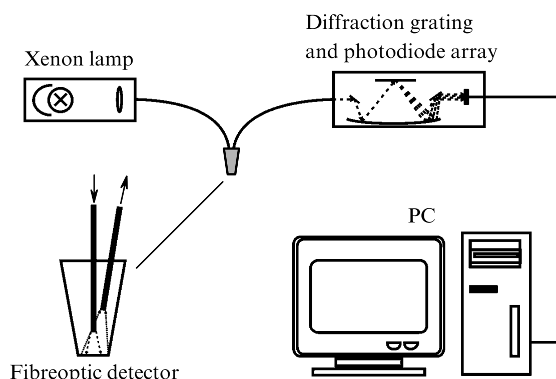

Figure 1. Scheme of the experimental setup for *in vivo* measurements of the reflection spectra of the eye sclera.

The detector recorded a signal averaged over the light-collection region with a reproducibility of ±5 %. The signal intensity changed within ±10 % on passing from probing one sclera region to another, while the dynamics of variation in the diffusion reflection from sclera caused by the immersion agent was the same irrespective of the light-collection region.

Reflection spectroscopy belongs to noninvasive methods and is contactless, i.e. the ends of fibres used in measurements do not touch the sclera surface.

The spectrometer was calibrated by using a $BaSO_4$ plate. A radiation source was a 250-W xenon lamp. The reflection spectra were recorded in the spectral range from 400 to 750 nm each 30 s for 30 min.

The reflection spectra of sclera were simulated by the Monte-Carlo (MC) method. The algorithm used in MC simulations is presented in [21]. This method is based on monitoring the random trajectories of photon packets inside a biological tissue from the instant of photon incidence on the tissue until photon absorption in the tissue or escape from it. A specific feature of this algorithm is the use of the so-called Russian roulette procedure. This procedure is employed to equalise the incident radiation energy and radiation energy absorbed by the medium and escaped from the region under study due to multiple scattering. The Russian roulette procedure involves a periodic increase in the statistical weight of individual photon packets selected randomly instead of the total weight of a group of photon packets lost in the medium, i.e. photons neglected in the further consideration because of their small statistical weight. Thus, an increase in the statistical weight of a photon enhances the probability of its 'survival' (successful escape from the medium at the given point). Although a disadvantage of the Russian roulette procedure is the increase in the photon path length, this method is well tested and can be used in the problems of radiation dosimetry in biological tissues [21 – 25], whereas refined MC methods [12, 26, 27] provide a more accurate visualisation of optical inhomogeneities inside biological tissues.

Monte-Carlo simulations were performed by using 50000 photon packets. The propagation direction of a photon packet after each scattering event was selected according to the Henyey – Greenstein phase function. The statistical weight of the photon packet for which the Russian roulette procedure was performed was 10^{-4}.

The reflection spectra of the eye sclera obtained by numerical simulations were compared with experimental spectra to obtain their best agreement. As a result, the optical parameters of sclera varying during its optical clearing were determined.

The optical model of sclera was a three-layer system consisting of sclera, RPE, and EC. The parameters of the layers were specified by using data from [23]. The thickness

of sclera, RPE, and EC was set equal to 500, 10, and 250 μm, respectively.

3. Results and discussion

Figure 2 presents the reflection spectra of sclera measured at different instants of time during the action of aqueous glucose solution on it. The shape of the spectra is determined by the absorption bands of blood hemoglobin with maxima at 415, 540, and 568 nm. Because the content of blood in sclera is low, absorption in hemoglobin almost does not affect the spectral shape during *in vitro* studies [10, 19, 20]. The absorption bands of hemoglobin observed in the reflection spectrum of sclera measured *in vivo* are related to the EC, which is usually removed during *in vitro* investigations [19, 20], and also to the functioning capillary network inside sclera. In addition, a hyperosmotic substance such as glucose acting on the eye surface stimulates the biological tissue, which is accompanied by the additional supply of blood through capillaries to the region under study. As a result, the reflectance of the tissue decreases due to increasing absorption. This leads to considerable differences in the dynamics of the sclera reflectance measured *in vivo* and *in vitro* in the wavelength range from 400 to 600 nm.

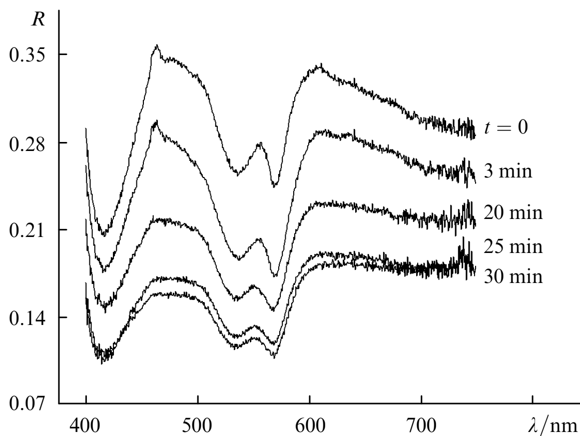

Figure 2. Reflection spectra of the rabbit eye sclera measured *in vivo* during optical clearing under the action of the 40 % aqueous glucose solution.

Figure 3 shows the time dependences of the reflectance at the two wavelengths during *in vivo* optical clearing of sclera. One can see that the sclera reflectance decreases under the action of glucose, which means that the sclera transparency increases. However, this process is not monotonic. The oscillations of dynamical curves is caused by the alternating of processes of clearing, which appears after the application of glucose solution on the eye surface, and recovery of the optical properties of sclera after diffusion of glucose from the detection region to surrounding tissues and diffusion of water from surrounding tissues to the detection region. The assumption about the presence of oscillations of dynamic curves is based on the coincidence of the instant of a drastic decrease in the sclera reflectance R with the instant of addition of a new portion of the immersion solution to sclera. However, the decrease in R is not prolonged. Within 2 – 3 min after the next addition of solution drops, the reflectance increased again, indicating the decrease in the glucose content in sclera.

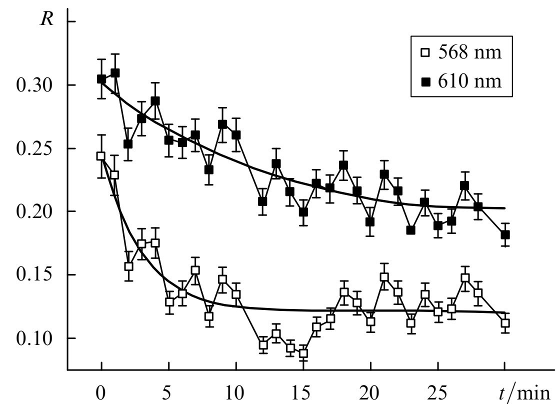

Figure 3. Dynamics of the sclera reflectance at two wavelengths during optical clearing. Squares are experimental values, solid curves are approximations of experimental data.

The two wavelengths in Fig. 3 lie within (568 nm) and outside (610 nm) the absorption band of blood. The dynamics of the reflectance at these wavelengths during optical clearing of sclera is considerably different. In the native sclera, multiple scattering of light in the visible and near-IR regions occurs. The reflectance of sclera at 610 nm gradually decreased after glucose administration. The characteristic decrease time of the reflectance at this wavelength can be estimated from the approximation solid curves in Fig. 3. The decrease in the reflectance terminated after 25 – 27 min. The additional administration of glucose resulted only in oscillations of the reflectance with respect to its average value, which is demonstrated in Fig. 3. The reflectance at a wavelength of 568 nm lying within the absorption band of blood decreased much faster (for 10 – 12 min), which is explained by the response of the eye to intense illumination during measurements and by the osmotic action of glucose increasing the local concentration of hemoglobin due to blood inflow through vessels. A further small increase in R in this wavelength range can be explained by a decrease in the absorption of light in sclera due to the stasis of capillaries and microvessels caused by the hyperosmotic action of glucose inside sclera [28].

The relative decrease in the reflectance in the spectral regions under study is also different. The reflectance in the spectral region 610 – 750 nm decreased on average by a factor of 1.5 – 1.7 and more than by half in the blood absorption region. A change from multiple scattering of photons to low-order scattering during optical sclera clearing results in an increase in the photon mean free path. Therefore, a greater number of photons propagate through a sclera layer without scattering and is absorbed in a vascular layer. This explains a greater decrease in the reflectance of sclera in the region of blood absorption bands compared to the region 610 – 750 nm.

It was shown in [20] that the reflectance of sclera samples *in vitro* monotonically decreased under the action of the 40 % glucose solution in the visible and near-IR ranges, unlike *in vivo* measurements.,

The durations of the sclera clearing process *in vivo* and *in vitro* also considerably differ. The time during which the maximum transparency of sclera was achieved *in vivo* considerably exceeds the clearing time of sclera *in vitro*.

While this time was 8 – 10 min upon the action of the 40 % glucose solution on sclera samples *in vitro* [14, 20], the clearing processes during *in vivo* experiments proceeded for no less than 20 min. This is probably explained by the geometry of *in vitro* experiments because the diffusion of glucose to sclera occurred from both surfaces and side sections of a sample.

By simulating numerically optical sclera clearing under the action of the 40 % glucose solution, we estimated the diffusion coefficients of glucose and water in sclera as $(5.4 \pm 0.1) \times 10^{-7}$ and $(5.8 \pm 0.2) \times 10^{-7}$ cm^2 s^{-1}. The obtained value of the diffusion coefficient of glucose is lower than that for sclera clearing *in vitro* [14]. It can be explained by the influence of Tenon's capsule and conjunctive covering a greater part of the external surface of the intact sclera and preventing the penetration of the immersion liquid inside sclera (Tenon's capsule and conjunctive were removed in experiments *in vitro*). In addition, an immersion agent in a living tissue is constantly removed from the observation region due to tissue metabolism. The diffusion of water from surrounding tissues to the region treated by glucose prevents the complete clearing of sclera and tends to dilute the immersion solution, resulting in the slowing down of the clearing process and rapid recovery of the optical properties of sclera after the termination of the glucose action. Therefore, it is impossible to provide the permanent presence of the immersion agent inside sclera and, hence, to achieve the same clearing rate as in measurements *in vitro* [10, 14, 19, 20].

Figures 4 – 6 present the results of numerical simulations of the optical clearing of sclera obtained within the framework of our model. Figure 4 shows the transmission spectra of a three-layer system consisting of sclera, RPE, and EC obtained at different instants in the spectral range from 620 to 750 nm. The influence of blood on the absorption of light in sclera in this spectral range is insignificant. One can see that the total transmittance of this system at the initial instant is a few percent, and light does not reach in fact the internal tissue of the eye. The transmittance is increased approximately by a factor of 1.2 after clearing.

These results differ somewhat from the data obtained upon sclera clearing *in vitro* [20]. According to [20], the total transmission of sclera samples at 620 nm increased by a factor of 1.5. This is explained by the fact that the RPE, which mainly determines the absorption of light in sclera, is removed together with EC from sclera in studies *in vitro*, as mentioned above, which results in a considerable increase in transmission of sclera samples.

This is confirmed by the simulation results. Figure 5 demonstrates the time dependences of the fraction of absorbed photons A in each of the layers of the system under study. One can see from Fig. 5a that the fraction of photons absorbed in sclera A_{scl} decreases with time, on average, by 10%, in accordance with sclera clearing. The fraction of photons absorbed in the RPE A_{RPE} increases with time (Fig. 5b). This is explained by the fact that upon clearing of the upper sclera layers, an increasing number of photons reach the pigment layer and is absorbed in it. The value of A_{RPE} achieves maximum during 20 min. The fraction of photons absorbed in this layer increases, on average, by 30 %. The fraction of photons absorbed in the EC A_{Ch} increases by 40 % (Fig. 5c). This means that, despite sclera clearing, the main part of light transmitted through sclera is absorbed in pigment and vascular layers. As a

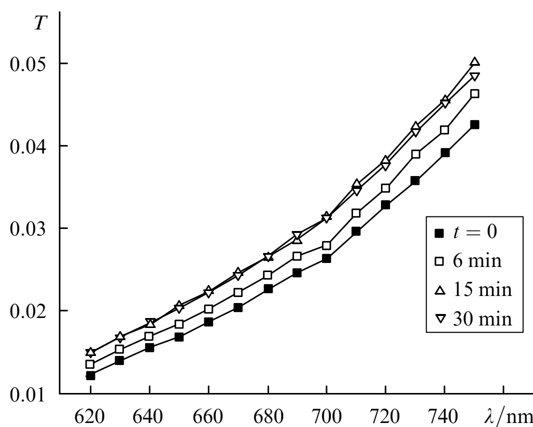

Figure 4. Transmission spectra of a three-layer structure, consisting of sclera, RPE, and EC, at different instants upon action of the 40 % glucose solution.

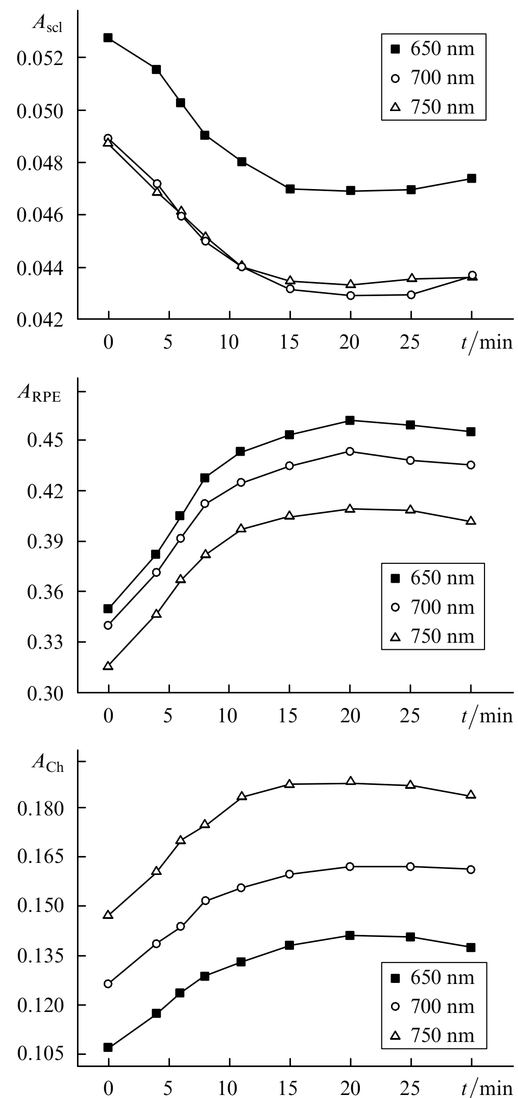

Figure 5. Time dependences of the fraction of photons absorbed in each of the three layers of the system: scleral layer (a), RPE (b), and EC (c) at three wavelengths during optical clearing.

result, the intensity of light incident on the internal tissues of the eye increases insignificantly (see Fig. 4). This should be taken into account in the dosimetry of laser radiation in surgery because a considerable increase in the absorption of light in RPE and EC layers during sclera clearing can cause their overheating and damage.

Figure 6 demonstrates the time dependences of the sclera scattering coefficient at three wavelengths. The scattering coefficient decreased for 20 min, on average, by a factor of ~ 1.7. A comparison of the results of *in vitro* and *in vivo* investigations shows good agreement between the values of sclera scattering coefficients during sclera clearing [20].

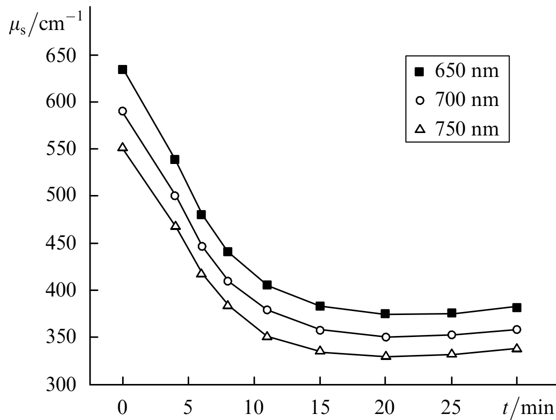

Figure 6. Time dependences of the sclera scattering coefficient μ_s at three wavelengths during optical clearing.

Due to a different behaviour of the reflectances at the two wavelengths, we can estimate the contrast C of the absorption bands of blood (hemoglobin) in sclera from the expression

$$C = \frac{R_{\lambda_1} - R_{\lambda_2}}{R_{\lambda_1} + R_{\lambda_2}},$$

where R_{λ_1} and R_{λ_2} are the sclera reflectances at $\lambda_1 \approx 610$ nm and $\lambda_1 \approx 568$ nm. One can see from Fig. 7 that contrast reaches a maximum within 12–13 min after the administration of the glucose solution. This means that for the given detector geometry and location depth of blood vessels, this time is the optimal clearance time at which the presence of blood (hemoglobin) in the tissue is visualised with the highest contrast (which increases more than twice). This can be explained by the fact that a decrease in the scattering coefficient at the initial stage of clearing occurs only in the surface layers of the biological tissue where the blood content is low. However, after the prolonged action of a clearing agent on sclera, the penetration depth of light in sclera increases and light is strongly absorbed in the RPE and EC. A further clearing of sclera weakly affects the reflectance in the absorption region of the blood hemoglobin; however, outside this region ($\lambda > 600$ nm) the sclera reflectance continues to decrease, thereby reducing the contrast of the absorption bands of hemoglobin. The dynamics of contrast of absorption bands in this case also depends on the physiological response of the tissue on the action of the hyperosmotic agent at which the blood volume in the tissue increases.

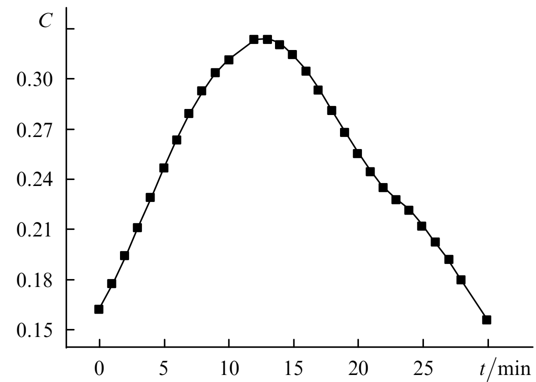

Figure 7. Contrast dynamics of the absorption bands of blood contained in sclera *in vivo* during optical clearing.

4. Conclusions

The control of optical properties of the eye sclera has been studied *in vivo* by the noninvasive administration of the aqueous glucose solution into sclera. The experiments have shown that the time of sclera clearing caused under the action of the 40 % glucose solution is 20–25 min. Sclera clearing, i.e. a passage from the regime of multiple scattering of photons in sclera to the low-order scattering regime, occurs due to a partial equating of the refractive indices of collagen fibres and the interstitial liquid under the action of a hyperosmotic immersion agent.

Although the response of organism to wash out the immersion agent from sclera prevents sclera clearing, the clearing degree is high enough and comparable with that *in vitro*.

The diffusion coefficients of glucose and water in sclera estimated in MC simulations of optical clearing of sclera within the framework of the model developed in the paper are $(5.4 \pm 0.1) \times 10^{-7}$ and $(5.8 \pm 0.2) \times 10^{-7}$ cm^2 s^{-1}. It has been shown that the administration of the 40 % glucose solution into the eye sclera provides the efficient control of its scattering properties. The scattering coefficient of sclera in the spectral range between 620 and 750 nm has decreased by a factor of 1.7. At the same time, the faction of photons absorbed in the inner sclera layers has increased considerably during clearing.

In addition, absorption in sclera has increased considerably in the region of absorption bands of blood. The estimate of the contrast of absorption bands of blood has shown that the dynamic curve of the contrast achieves an extremum within 12–13 min after the beginning of glucose action, the contrast being increased by more than twice.

The results obtained in this study can be used for the development of noninvasive methods of optical tomography of eye and in transscleral surgery.

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References

1. Knüttel A., Bonev S., Knaak W. *J. Biomed. Opt.*, **9**, 265 (2004).

2. Gerger A., Koller S., Kern T., Massone C., Steiger K., Richtig E., Kerl H., Smolle J. *J. Invest. Dermatol.*, **124**, 493 (2005).
3. Zhu B., Jaffer F., Ntziachristos V., Weissleder R. *J. Phys. D: Appl. Phys.*, **38**, 2701 (2005).
4. Prince S., Kolehmainen V., Kaipio J.P., Franceschini M.A., Boas D., Arridge S.R. *Phys. Med. Biol.*, **48**, 1491 (2003).
5. Rol P.O. *Optics for Transscleral Laser Applications* (PhD Tesises) (Zurich: Institute of Biomedical Engineering, 1992).
6. Sacks Z.S., Loesel F., Durfee C., Kurtz R.M., Juhasz T., Mourou G. *Proc. SPIE Int. Soc. Opt. Eng.*, **3726**, 516 (1998).
7. Kuo P.-Ch., Peyman G.A., Men G., Bezerra Y., Torres F. *Lasers Surg. Med.*, **35**, 157 (2004).
8. Tuchin V.V. *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis* (Bellingham: SPIE Press, 2000) Vol. TT38.
9. Schmitt J.M., Kumar G. *Appl. Opt.*, **37**, 2788 (1998).
10. Maksimova I.L., Zimnyakov D.A., Tuchin V.V. *Opt. Spektrosk.*, **89**, 86 (2000).
11. Sacks Z.S., Kurtz R.M., Juhasz T., Mourau G.A. *J. Biomed. Opt.*, **7**, 442 (2002).
12. Meglinsky I.V., Bashkatov A.N., Genina E.A., Churmakov D.Yu., Tuchin V.V. *Kvantovaya Elektron.*, **32**, 875 (2002) [*Quantum Electron.*, **32**, 875 (2002)].
13. Bashkatov A.N., Genina E.A., Sinichkin Yu.P., Kochubey V.I., Lakodina N.A., Tuchin V.V. *Biophys. J.*, **85**, 3310 (2003).
14. Bashkatov A.N., Genina E.A., Sinichkin Yu.P., Kochubei V.I., Lakodina N.A., Tuchin V.V. *Biofizika*, **48**, 309 (2003).
15. Bashkatov A.N., Zhestkov D.M., Genina E.A., Tuchin V.V. *Opt. Spektrosk.*, **98**, 695 (2005).
16. Tuchin V.V. *Optical Clearing of Tissues and Blood* (Bellingham: SPIE Press, 2005) Vol. PM15.
17. Komai Y., Ushiki T. *Invest. Ophthal. Vis. Sci.*, **32**, 2244 (1991).
18. Nemati B., Dunn A., Welch A.J., Rylander III H.G. *Appl. Opt.*, **37**, 764 (1998).
19. Tuchin V.V., Maksimova I.L., Zimnyakov D.A., Kon I.L., Mavlutov A.H., Mishin A.A. *J. Biomed. Opt.*, **2**, 401 (1997).
20. Bashkatov A.N., Genina E.A., Kochubey V.I., Lakodina N.A., Tuchin V.V. *Proc. SPIE Int. Soc. Opt. Eng.*, **3908**, 266 (2000).
21. Wang L., Jacques S.L. *Monte Carlo Modeling of Light Transport in Multi-Layered Tissues in Standard C* (http://ece.ogi.edu/omlc).
22. Wang L., Jacques S.L., Zheng L. *Computer Methods and Programs in Biomedicine*, **47**, 131 (1995).
23. Hammer M., Roggan A., Schweitzer D., Muller G. *Phys. Med. Biol.*, **40**, 963 (1995).
24. Drezek R., Sokolov K., Utzinger U., Boiko I., Malpica A., Follen M., Richards-Kortum R. *J. Biomed. Opt.*, **6**, 385 (2001).
25. Friebel M., Roggan A., Muller G., Meinke M. *J. Biomed. Opt.*, **11**, 034021 (2006).
26. Meglinsky I.V. *Kvantovaya Elektron.*, **31**, 1101 (2001) [*Quantum Electron.*, **31**, 1101 (2001)].
27. Meglinsky I.V., Churmakov D.Yu. *Opt. Spektrosk.*, **96**, 1025 (2004).
28. Galanzha E.I., Tuchin V.V., Solovieva A.V., Stepanova T.V., Luo Q., Cheng H. *J. Phys. D: Appl. Phys.*, **36**, 1739 (2003).