Optical clearing of human eye sclera

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ABSTRACT

We present experimental results of investigation of the optical properties of the human eye sclera controlled by administration of osmotically active chemical, such as 40%-glucose solution. Administration of the chemical agent induces diffusion of matter and as a result equalization of the refractive indices of collagen and ground material. Results of the experimental study of influence of the glucose solution on the absorption and scattering properties of human sclera are presented. *In vitro* reflectance and transmittance spectra of the human sclera samples were measured by commercially available spectrophotometer CARY-2415 in the spectral range from 400 to 1800 nm. The reduced scattering coefficient of human sclera samples is significantly decreased under action of the osmotical solution were demonstrated.

Keywords: optical clearing, glucose, sclera, optical properties, absorption coefficient, reduced scattering coefficient

1. INTRODUCTION

Transscleral diagnostics, therapy and surgery are important for laser ophthalmology. Recent technological advancements in the photonics industry have led to a resurgence of interest in optical imaging technologies and real progress toward the development of non-invasive clinical functional imaging systems. Over the last decade, non-invasive or minimally invasive spectroscopy and imaging techniques have witnessed widespread exciting applications in biomedical diagnostics. Spectroscopic techniques are capable of deep-imaging of tissues that could provide information of blood oxygenation and detect cutaneous, brain and breast tumors, whereas confocal microscopy, OCT and multi-photon excitation imaging have been used to show cellular and sub-cellular details of superficial living tissues. Spectroscopic and OCT techniques are applicable for blood glucose monitoring with diabetic patients. Besides diagnostic applications, optical methods are widely used in modern medicine, for example, for photodynamic therapy and for laser surgery of different diseases. Interest in using optical methods for physiological-condition monitoring and cancer diagnostics and therapies has been increased due to their simplicity, safety, low cost, contrast and resolution features in contrast to conventional X-ray computed tomography and ultrasound imaging.

The main limitations of the majority of the imaging techniques, including OCT and near-infrared (NIR) spectroscopy deal with the strong light scattering in superficial tissues[1-5], which cause decrease of spatial resolution, low contrast, and small penetration depth. Solution of the problem, i.e. reducing light scattering, and thus improving image quality and precision of spectroscopic information, can be connected with control of tissue optical properties. It is well-known that the major source of scattering in tissues and cell structures is the refractive index mismatch between cell organelles, like mitochondria, and cytoplasm, extracellular media and tissue structural components such as collagen and elastin fibers[1,6-8]. The tissue scattering properties can be significantly changed due to action of osmotically active immersion liquids[1,5,9-21]. Similar results have been obtained for optical clearing of whole blood[4,22-24]. Administration of the immersion liquid having a refractive index higher than that of tissue interstitial fluid induces a partial replacement of the interstitial fluids by immersion substance and hence, matching of refractive indices of tissue scatterers and the interstitial fluid. Osmotic activity of the immersion agents may cause tissue dehydration that also equalizes refractive indices within a tissue. The matching, correspondingly, causes the decrease of scattering. As osmotic immersion liquids, aqueous solutions of glucose and mannitol, propylene glycol, glycerol and other biocompatible chemicals are used.

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Recently a number of results on noninvasive *in vivo* monitoring of glucose concentration using visible and near infrared (NIR) light scattering techniques were reported [10,12,13,15,21,25,26]. The main idea of such measurements is based on the dependence of tissue scattering properties on the refractive index mismatch between collagen fibers (and/or cellular membrane) and the extrafiber (extracellular) substance. In general, an increase in tissue glucose concentration reduces index of mismatch and correspondingly decreases the scattering coefficient. Therefore, measurement of scattering coefficient allows to estimate the glucose concentration in the tissue. Osmotic effects play an essential role in such measurements and can dramatically change tissue optical response on glucose concentration. The possibility of scleral reflectance measurements for *in vivo* monitoring of glucose concentration was discussed in our previous papers [9-11,15,16,21]. It was shown that turbidity of sclera could be effectively controlled using above discussed immersion effect and that osmotic effects are very important.

In this paper we present the results of experimental study of absorption and scattering properties of human sclera controlled by administration of 40%-glucose solution. In contrast to our previous investigations, performed in the visible spectral range, in this study we investigated the optical clearing effect in the wide wavelength range from 400 to 1800 nm.

2. MATERIALS AND METHODS

In this study we used a commercially available computer-controlled CARY-2415 ("Varian", Australia) spectrophotometer with integrating sphere to make total transmittance and diffuse reflectance measurements in the 400-1800 nm wavelength range for human eye sclera. Inner diameter of the sphere is 100 mm, size of the entrance port is 20×20 mm and the diameter of the exit port is 16 mm. As a light source, a halogen lamp with filtering of the radiation in the studied spectral range is used in the measurements. The diameter of incident light beam on the tissue sample is 3 mm. Scan rate is 2 nm/sec. The spectral bandwidth of spectrophotometer was set at 1 nm. The measurements were carried out at room temperature about 20°C. The diffuse reflectance was calibrated on the basis of reflectance value from standard reflectance plate ($BaSO_4$).

The sequence of obtaining of the scleral optical properties was the following: 1) recording of the experimental spectra of total transmittance T_t and diffuse reflectance R_d of the native sample which had not be treated with glucose solution; 2) clearing of the scleral sample (the sample was immersed in Petri dish filled the glucose solution) during of the time interval 5 min; 3) recording of the total transmittance and diffuse reflectance spectra of the cleared sample of sclera; 4) clearing of the scleral sample during of time interval 5 min again (total time of the clearing is 30 min); 5) processing of the reflectance and transmittance spectra and obtaining of absorption and scattering properties of the scleral sample.

For each tissue sample the thickness in each moment was measured. 40%-aqueous solution of glucose with refractive indices n = 1.39 (λ = 589 nm) was used for scleral optical clearing.

The samples of the sclera were extracted from the human eye. The dissection and measurements on the eye were performed within 24 h *postmortem*. After enucleation, the eye was placed in saline. Before *in vitro* measurements, the conjunctiva and the ciliary body as well as the retina with choroid were removed. The human scleral samples were cleaned and cut into pieces of about 20×20 mm^2. The mean thickness of the human scleral samples was about 1.0 mm.

For processing the experimental data and determination of the optical properties of tissue, the inverse adding-doubling (IAD) method developed by Prahl *et al*[27] has been used. The IAD method is widely used in tissue optics for processing the experimental data of spectrophotometry with integrating spheres[27-33]. This method allows one to determine the absorption (μ_a) and the reduced scattering coefficients $(\mu_s' = \mu_s(1-g))$ of a tissue from the measured values of the total transmittance and the diffuse reflectance. Here μ_s is the scattering coefficient, and g is the anisotropy factor of scattering. In these calculations the anisotropy factor has been fixed as 0.9, since this value is typical for the tissue in the visible and NIR spectral ranges[1]. The main advantage of the IAD method in comparison with many other methods of solution of the radiative transfer equation is connected with its validity for the arbitrary ratio of the absorption and scattering coefficients[27]. The property of the IAD method becomes essentially important in the case of determination of the optical properties of tissues within strong absorption bands, when the values of the absorption and scattering

coefficients become comparable. Other methods, for example, diffusion approximation[34] or Kubelka-Munk method[35], for their applicability require a fulfillment of the condition $\mu_a / \mu_s \gg 1$. The inverse Monte Carlo technique[36] can also be used for arbitrary ratio of μ_a and μ_s, but requires very extensive calculations. The main limitation of IAD method is connected with the possible loss of scattering radiation through lateral sides of a sample at calculations[37]. Loss of light through the sides of the sample and sample holder may erroneously increase the calculated value of the absorption coefficient. These losses depend on the physical size and geometry of the sample, i.e., the losses existing in the case, when the sizes of a sample do not exceed significantly the diameter of the incident beam. The size of the exit and the entrance ports of the integrating sphere are also important for errorless measurements of the total transmittance and the diffuse reflectance[37]. The tissue sample should completely cover the port in the integrating sphere, and the distance from the edge of irradiating beam on the sample to the edge of the port should be much larger than the lateral light propagation distance, which is determined as $1/(\mu_a + \mu_s')$. If this is not satisfied, then light will be lost out from the sides of the sample and the loss will be attributed to absorption, and, so the absorption coefficient will be overestimated. These requirements have been met in our experiments, since maximal size of the sphere port does not exceed 20 mm, while the minimal size of the samples of sclera is 20 mm. Besides, Pickering *et al*[37] have reported that area of tissue sample has to be smaller than the area of the inner surface of the integrating sphere. This requirement has also been met in our experiments, since the area of the inner surface of integrating sphere used in the measurements was 314.16 cm^2, while the area of the tissue samples does not exceed 6.0 cm^2.

Calculation of the tissue absorption and reduced scattering coefficients was performed for each wavelength point. The algorithm consists of the following steps: (a) the estimation of a set of the optical properties; (b) the calculation of the reflectance and transmittance with the adding-doubling iterative method; (c) the comparison of the calculated with the measured values of the diffuse reflectance and the total transmittance; (d) iteration of the above steps until matching (within the specified acceptance margin) is reached. With this iterative process, the set of optical properties that yields the closest match to the measured values of reflectance and transmittance are taken as the optical properties of the tissue.

3. RESULTS AND DISCUSSION

Figures 1 and 2 show the measured optical properties of the samples of sclera calculated by IAD method on the basis of measured values of the total transmittance and the diffuse reflectance. Figure 1 presents the wavelength dependence of the tissue absorption coefficient in the visible spectral range from 400 to 800 nm. In the figure it is seen that absorption coefficient monotonically decreased with increasing of wavelength. In the spectrum, absorption band of blood oxyhemoglobin (415 nm[38]) it is seen. However, the absorption bands of oxyhemoglobin (540 and 575 nm[38]) and water (970 nm[39,40]) are not observed. In the figure it is also seen that absorption coefficient of the tissue samples is not changed during the clearing process.

Figure 2 presents the spectral dependence of the reduced scattering coefficient of the scleral tissue. It is clearly seen that, with increase of wavelength, the reduced scattering coefficient decreases smoothly, which corresponds to the general spectral behavior of the scattering characteristics of biological tissues[6,7]. Figure 3 presents dynamics of decreasing of reduced scattering coefficient of the sclera in dependence on time of clearing and corresponded increasing of glucose concentration within the tissue. In the figure it is seen that penetration of glucose into the tissue and thus clearing process prolonged up to 10-15 min in dependence on wavelength.

Figure 4 presents the wavelength dependence of the tissue absorption coefficient in the infrared spectral range from 800 to 1800 nm. In the spectrum, strong absorption band of water (1450 nm[26,27]) is clearly seen. The absorption bands of water located at 980 and 1200 nm[26,27] are considerably less observed. In the figure it is also seen that absorption coefficient of the tissue samples is practically not changed during the clearing process excluded spectral range corresponded to water absorption band with maximum at 1450 nm. As has been seen in the figure, intensity of the absorption band significantly decreased during the clearing process that is connected with osmotical dehydration of the tissue.

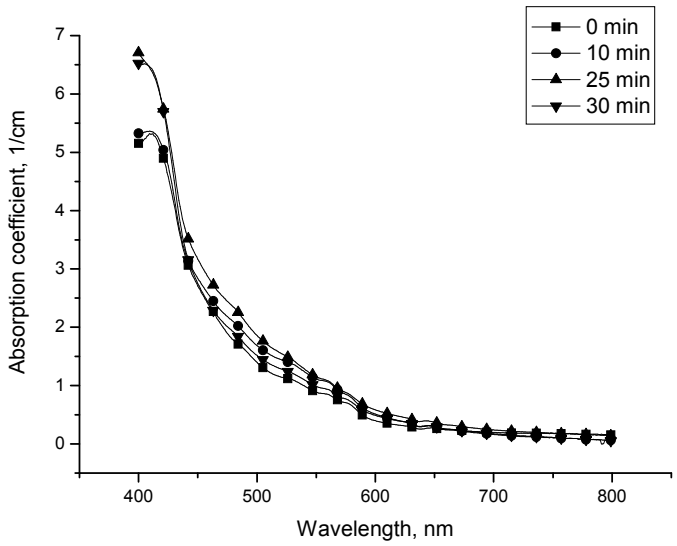

Fig. 1. The absorption coefficient spectra of human eye sclera measured at different time intervals in the visible spectral range. The symbols correspond to the experimental data.

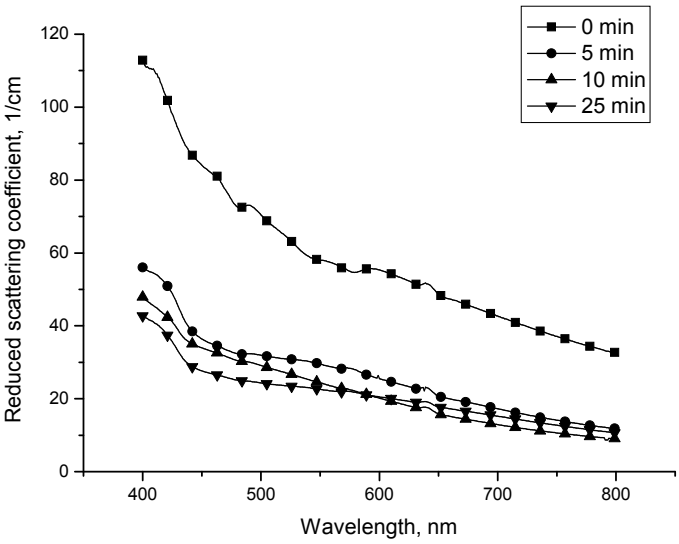

Fig. 2. The spectra of reduced scattering coefficient of human eye sclera measured at different time intervals in the visible spectral range. The symbols correspond to the experimental data.

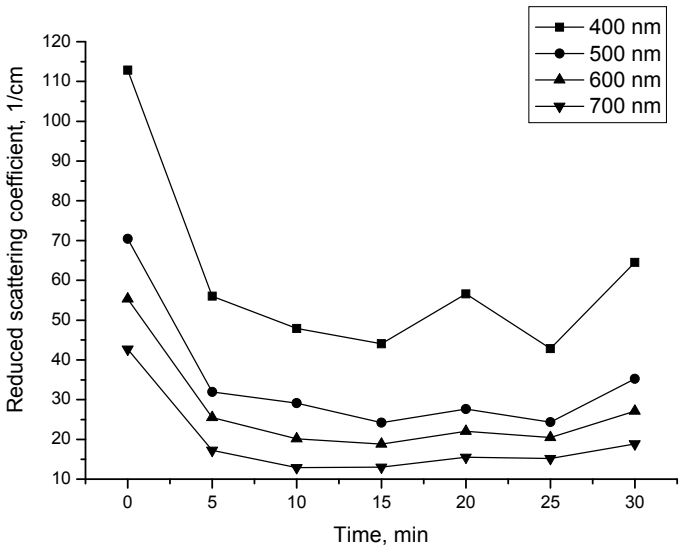

Fig. 3. The time-dependence of reduced scattering coefficient of human eye sclera measured at different wavelengths in the visible spectral range concurrently with administration of the 40%-glucose solution. The symbols correspond to the experimental data.

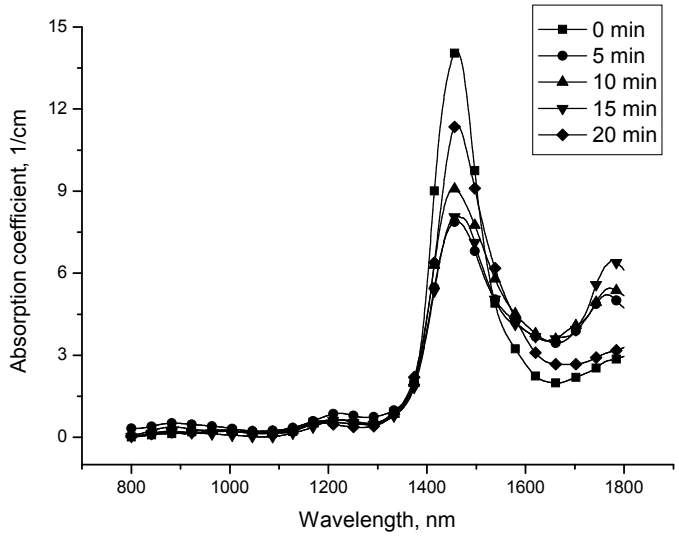

Fig. 4. The absorption coefficient spectra of human eye sclera measured at different time intervals in the infrared spectral range. The symbols correspond to the experimental data.

Figure 5 presents the spectral dependence of the reduced scattering coefficient of the scleral tissue measured in the infrared spectral range from 800 to 1800 nm. It is clearly seen that, at initial moment, with increase of wavelength, the reduced scattering coefficient decreases smoothly, which corresponds to the general spectral behavior of the scattering characteristics of biological tissues[6,7]. However, in the range of the strong absorption band of water (1450 nm), the shape of the scattering spectrum deviates from the monotonic dependence. At the same time, in the range of the water absorption bands with maximums at 980, 1189 and 1787 nm the effect is not observed. Figure 6 presents dynamics of

decreasing of the reduced scattering coefficient of the sclera in dependence from time of clearing and corresponding increasing of glucose concentration within the tissue. In the figure it is seen that the reduced scattering coefficient significantly decreased during penetration of glucose into the tissue, and, so the clearing process prolonged up to 10-15 min in dependence from wavelengths.

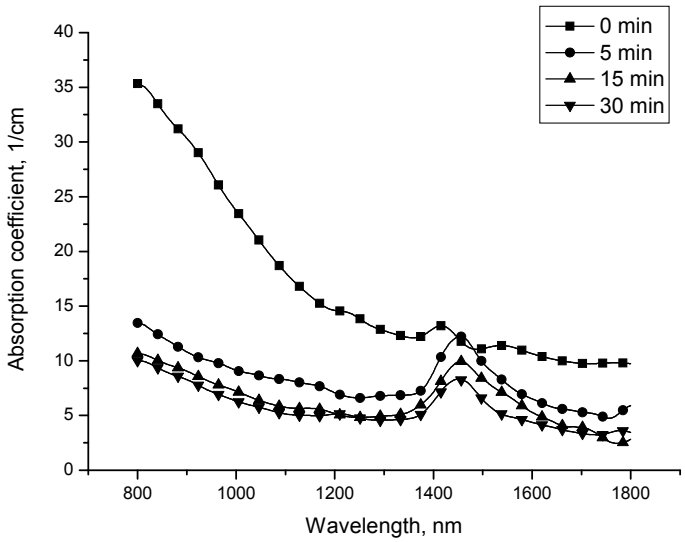

Fig. 5. The spectra of reduced scattering coefficient of human eye sclera measured at different time intervals in the infrared spectral range. The symbols correspond to the experimental data.

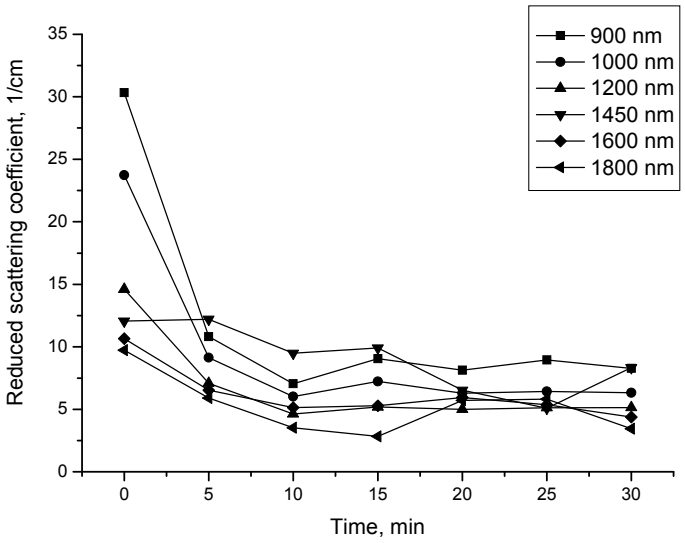

Fig. 6. The time-dependence of reduced scattering coefficient of human eye sclera measured at different wavelengths in the infrared spectral range concurrently with administration of the 40%-glucose solution. The symbols correspond to the experimental data.

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