Eye tissues study

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ABSTRACT

Theoretical and *in vitro* and *in vivo* experimental study of spectral and polarization characteristics of the human and rabbit eye tissues are presented. The possibility of control of optical properties of eye cornea, lens and sclera is discussed and realized experimentally for glucose solution as the refractive index matching factor.

Keywords: Absorption, turbid media, multiple scattering, spectroscopy

1. INTRODUCTION

Healthy tissues of the anterior human eye chamber, e.g. the cornea and lens, are highly transparent for visible light due to their ordered structure and the absence of strongly absorbing chromophors [1–5]. Scattering is an important feature of light propagation in eye tissues. The size of the scatterers and the distance between them are smaller than or comparable with the wavelength of visible light and the relative refractive index of the scattering matter is equally small (soft particles). Typical eye tissue models are long round dielectric cylinders (corneal and scleral collagen fibers) or spherical particles (lens protein structures) having refractive index n_C and chaotically (sclera, opaque lens) or orderly (transparent cornea and lens) distributed in the isotropic base matter with refractive index $n_I < n_C$. Light scattering analysis in eye tissues often is possible using a single scattering model owing to the small scattering cross-section.

2. OPTICAL MODEL OF EYE TISSUES

The structural elements that give the cornea the strength to preserve its proper curvature while withstanding the intraocular pressure are located within its stromal layer, which constitutes 0.9 of the cornea's thickness [1,6]. The stroma is composed of many successively stacked layers of lamellae, which varied in width (0.5-250 μm) and thickness (0.2–0.5 μm) in dependence of tissue region. Each lamella is composed of a parallel array of collagen fibrils surrounded by a homogeneous solution consisting of water, mucoproteins, and various salts. The collagen fibrils in the human cornea have a uniform diameter of about 25–30 nm with a periodicity close to two diameters and there appears to be some regularity in the organization of fibril axes about one another. Thus, the stroma has at least two levels of structure: lamellae that lie parallel to the cornea's surface; and an ultrastructure within each lamella consisting of small, uniform diameter, parallel collagen fibrils that have some degree of order in their spatial positions.

The human sclera is a turbid, nontransparent medium that covers about 80% of the eyeball and serves as a protective membrane [3,6]. Together with cornea, it allows for the eye to withstand both internal and external forces to maintain its shape. It is a strong, fibrous tissue that mainly consists of conjunctive collagen fibers packed in lamellar bundles that are immersed within an amorphous ground (interstitial) substance. The stroma is the thickest layer of the sclera. In the scleral stroma, collagen fibrils exhibit a wide range of diameters, from 25 to 230 nm. The average diameter of the collagen fibrils increases gradually from about 65 nm in the innermost part to about 125 nm in the outermost part of the sclera; the mean distance between fibrils centers is about 285 nm. These fibrils are arranged in the individual bundles in a parallel fashion but more randomly than that in the cornea. Hydration of the human sclera can be estimated as 68%. About 75% of its dry weight is due to collagen, 10% is due to other proteins, and 1% to mucopolysaccharides.

The healthy human lens is a coherent structure containing about 60 % water and 38 % proteins [5,7]. The lens consists of many fiber cells. The predominant dry components of a mammalian lens are three kinds of structural proteins named α-, β-, and γ - crystallins and their combined weight accounts for about 33 % of the total weight of the lens. Photo-oxidation of lens

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proteins by chronic UV, UVA, or visible light results in oxidized forms of these proteins, which cross link to other proteins, causing opacities or pigment formation. The light scattering is caused by random fluctuations in the refractive index.

The types of fiber cell disruption due to cataract formation include intracellular globules, clusters of globules, vacuoles with the contents wholly or partially removed, clusters of highly curved cell membranes, and odd-shaped domains of high or low density [7]. These spherical objects are variable in size (often in range 100 to 250 nm) and occur in clusters, that creates potential scattering centers.

In designing an optical model of the eye tissue, in addition to form, sizes, and density of the scatterers and the tissue thickness, it is important to have information on the refractive indices of the tissue components. For inhomogeneous materials, such as tissues, the refractive indices of fibrils, interstitial medium, and tissue can be derived using the law of Gladstone and Dale, which states that the resulting value represents an average of the refractive indices of the components related to their volume fractions. One can estimate the refractive index of the scatterers (for example, scleral fibrils - hydrated collagen) using the expression for the average refractive index of the tissue

$$n_s = n_c \cdot V_c + n_I \cdot V_I , \tag{1}$$

where n_C, n_I, and V_C, V_I are the refractive indices and volume fractions of the scatterers and interstitial fluid, respectively.

Because of similar fibrous nature of the cornea and sclera (the differences are mainly due to distinctions in the fibrils arrangement and their diameters) it is expected that the refractive indices of scleral collagen and its interstitial fluid should be close to those of the cornea. The refractive indices measured for the dry corneal collagen and for the interstitial fluid are the following: 1.547 and 1.342, respectively. For the dry collagen it was measured as n_C= 1.55 and calculated for the interstitial substance as n_I=1.345.

In case of disordered (randomly distributed) scatterers, the resultant field intensity is the total intensity of fields scattered by individual scatterer (fibril or particles). For ordered scatterers, fields, rather than intensities, should be summed to take into account effects of interference arising in the presence of the near order of scatterers.

3. TRANSMISSION AND SCATTERING SPECTRA OF EYE TISSUES

Corneal transmittance was calculated using a model monodisperse system of long dielectric non-absorbing cylinders (fibrils) of 26 nm in diameter and refractive index n_C = 1.470. The cylinders were orderly oriented parallel to the corneal surface in the base matter (n_I = 1.345). The transmittance anisotropy for linearly polarized radiation and the marked effect of scattering on corneal transmittance in the UV spectral region was demonstrated. The corneal transparence in the visible range is explained by the high degree of its fibrils arrangement, so the diffuse light intensity decreases due to interference along all directions (destructive interference) except the incident light direction (constructive interference). The effect of scattering is the most essential in a short wavelength region and defines small UV radiation transmittance of the cornea, approximately 50% for 320 nm.

Disordering of the fibrils arrangement (for example after keratomy) results in decreasing of cornea transmission, especially for the short wavelengths. Another essential feature of the cornea is the presence of a preferable direction of the fibrils alignment. The results of such anisotropy are the form birefringence and dichroism of the cornea. The transmission spectrum substantially depends on the orientation of polarization vector of the linear polarized light relative to the collagen fibrils; the light polarized along fibrils is scattered more effectively. As it follows from calculations and measurements the peripheral cornea polarization sensitivity in UV (320 nm) is about 5-fold higher than in the red (633 nm). In the Rayleigh limit ($\lambda >> 2a$), the form birefringence is defined as [8]

$$n_e - n_o = V_1 V_2 \left(n_1 - n_2\right)^2 \Big/ \left(V_1 n_1 + V_2 n_2\right), \tag{2}$$

where V_1 is the fractional volume of the cylinders which are arranged in parallel to each other; V_2 is the ground substance volume; and n_1, n_2 are the respective indices of refraction. The birefringence can be high for a small diameter cylinders and goes to zero for a system consists of parallel cylinders with large diameters ($2a \geq \lambda$)

The lens is less transparent than the cornea. The visible light passing through the human lens undergoes an appreciable degree of both scattering and absorption by different chromophores including protein-bound tryptophane, 3-hydroxy-L-kynurenine-*O*-β-glucoside (3-HKG), and age-related protein (responsible for lens yellowing in aged subjects) [5]. Age-related changes in the lens optical properties are as a rule due to the appearance of scatterers having increased diameters and refractive index and also to the enhanced content of age-related protein. A remarkable difference between the short-wave portions of the two profiles of transmittance spectra calculated for 'young' and 'old' lens models is readily apparent. Experimentally obtained the total transmittance spectra for senile and cataractous lenses showed that age-related variations in the composition of scatterers and absorbers lead to significant differences in scattering spectra. There is a qualitative correlation between the experimental findings and the calculated values for both backscattering and scattering at 90^0.

The transition from a completely diffusion to a partially coherent transmission of the sclera caused by refractive index matching was demonstrated theoretically and experimentally. Transformation of polarization properties of sclera was also studied. For example, for the translucent human scleral sample by its impregnation by a highly concentrated glucose solution (about 70 %) the optical anisotropy, defined by Eq. (2), $n_e - n_o = 0.001$. The additional measurement of the collimated transmittance allowed for estimation of the refractive index of the ground substance of the translucent tissue, n_2=1.39. Using a well known value of the refractive index of hydrated collagen, n_1=1.47, the mean diameter and mean length of collagen fibrils were evaluated, respectively, as 100 nm and 0.5 mm. These data well correlate with electronic microscopy study [3].

It should be noted that in spite of a high turbidity of the sclera it has a quite ordered fibrils structure within separate layers [6]. Using electronic micrographs of scleral samples, presented in the paper [6], the radial distribution function of collagen fiber centers for monodisperse system (a layer of sclera) was reconstructed (see Fig. 1). Such function allowed us to calculate corrections for values of the cross section of light extinction and the angular distribution of the scattered light, caused by fibers ordering due to their dense package.

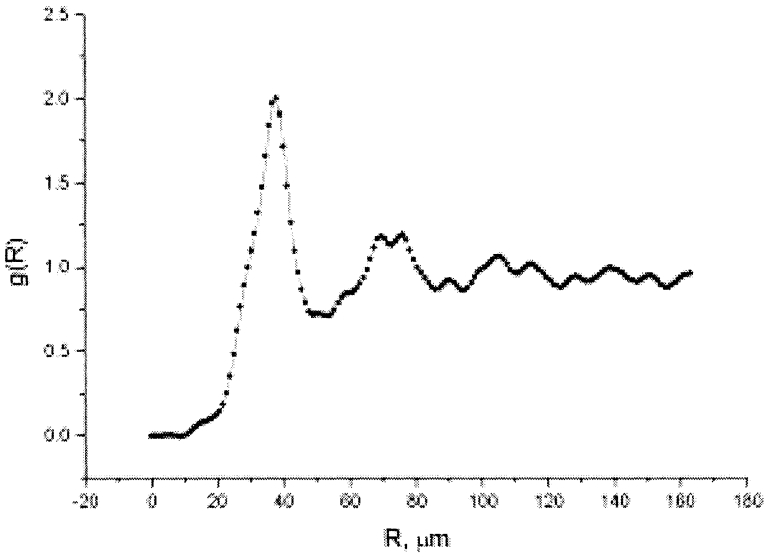

Fig. 1. The reconstructed radial distribution function of collagen fiber centers for monodisperse system (a layer of sclera).

The experimental and theoretical results show that the human scleral transmission and the rabbit eye reflection spectra can be substantially changed by administration of osmotically active chemical agent (*glucose* solution). From experimental data presented in Figs. 2,3 one can see that for *in vivo* measurements reflectance decreases up to 1.44–2.09 folds that is considerably less than that for *in vitro* measurements of collimated transmittance, increasing of transmittance up to 14.6–25.8 folds. From the optical point of view we have to compare the same quantities. Unfortunately, it is not easy to perform *in vivo* measurements of collimated and especially total transmittance. Nevertheless, our Monte Carlo simulations based on *in*

vivo and *in vitro* measurements allows one to provide more correct comparison of *in vitro* and *in vivo* results. The calculated values of diffuse reflectance ratio for untreated and treated by *glucose* objects correspondingly for *in vitro* and *in vivo* measurements show the same tendency of their changes with the wavelength (Table). Differences in absolute values of this ratio, which are higher for *in vivo* case, can be explained by a multi-layered structure of the living tissue (consisting of the conjunctiva, the Tenon's capsule, the sclera, the ciliary muscle, and the ciliary pigment epithelium), part of which are extremely absorbing. The living tissue seems to be more effectively controlled by immersion phenomenon due to more strong influence of absorbing layers which reduce the fluence rate of the back scattered photons as the light penetrates more deeply inside tissue (due to reduction of scattering) where absorption is maximal. Another reason for more effective control is the blood perfusion and metabolic activity of leaving tissue, which allows for more effective impregnation of tissue in spite of that the osmolyte effects only the exterior surface of the tissue.

This is also remarkable that the total transmittance for *in vivo* case is 3–6 folds more effectively controlled by tissue immersion than that for separated scleral samples (Table). The total transmittance of the anterior eye layers measured at the posterior interface of the human sclera determines the laser energy applied to the ciliary body. The collimated transmittance in its turn determines the laser irradiation of the local area of the eye bottom.

Table. Calculated and measured (*in vivo* and *in vitro*) ratios of changes of optical parameters caused by *glucose* administration

Wavelength, nm	$R_{d\,max}/R_{d\,min}$ *in vivo*	$R_{d\,max}/R_{d\,min}$ *in vitro*	T_{max}/T_{min} *in vivo*	T_{max}/T_{min} *in vitro*	R_{max}/R_{min} *in vivo*	$T_{c\,max}/T_{c\,min}$ *in vitro*
420	1.66	1.13	6.35	1.12	2.09	14.6
630	1.87	1.18	3.28	1.31	1.44	25.8
700	1.99	1.22	3.03	1.10	1.38	17.8

Measurements of the time-dependent collimated transmittance allowed us, basing on presented tissue refractive index matching model, to estimate the diffusion coefficients of the chemical agent (*glucose – 40%*). The mean value of the diffusion coefficient for *glucose* solution transport in the human scleral sample is equal to $3.45 \cdot 10^{6} \pm 4.59 \cdot 10^{7}$ cm^2/sec. This value is not far from values for small molecules (like sucrose, glucose, etc.) diffusion in water at zero concentrations $(3.6–5.2) \cdot 10^{-6}$ cm^2/sec at 12–15 ^0C). We believe that this experimental result confirms the correctness of the refractive index matching model [4].

In vitro experiments show the intensive optical clearing at first few minutes, then transmittance saturates and even goes down after approximately 8 min after a sample placed in solution. Besides refractive index matching the intensive clearing at first minutes is determined by tissue shrinkage due to osmotic stress of glucose solution. In its turn, the saturation of refractive index matching with time leads to saturation of tissue transmittance, but for a long time of sample kept in solution its swelling causes growing of tissue thickness and corresponding reduction of the transmittance.

Experimental data for collimated transmittance, estimated time-dependent changes of refractive index of interstitial space and sample thickness, and some predictions of initial scattering and absorption properties of tissue allowed us to provide Monte Carlo simulation of the light absorption fraction, the diffuse reflectance, and the total transmittance. These data predict the behavior of optical and thermal properties of scleral tissue under laser irradiation. As it was expected the light absorbed fraction is reduced with time (decreasing of tissue scattering properties due to refractive index matching effect). Less scattering causes shorter photon migration paths and less probability for photon to be absorbed.

Moreover, results of *in vitro* measurements, especially determined diffusion coefficient for glucose in tissue, allowed us to perform the Monte Carlo simulation of the light propagation in the rabbit eye. Initial values of scattering coefficient and values of absorption coefficient for various tissue layers were taken from [19]. The time-dependent behavior of the diffuse reflectance, the total transmittance, and the light absorbed fraction was analyzed. Such dependencies can be used for prediction of optical and thermal properties of the living eye treated by the osmotic solution. For example, it was shown that the light absorbed fraction rapidly increases with the time. That is connected to a significant translucence of the upper layers of the rabbit eye and well irradiation of lower layers of the eye membrane having a high absorption, like ciliary body components. It established that as far as the light absorption fraction in the conjunctiva and the sclera is decreased in the ciliary body it is considerably increased. This confirms the possibility of using of osmotically active substances for selective photodestruction of the ciliary body.

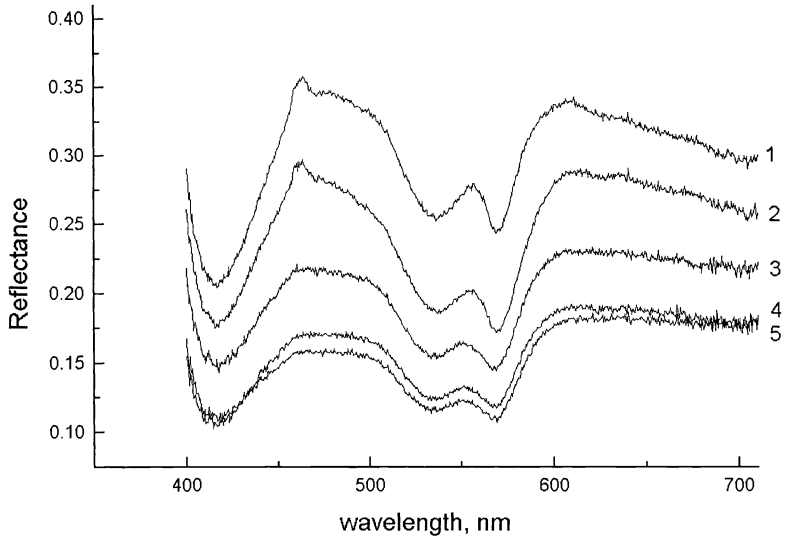

Fig. 2 The *in vivo* time-dependent reflectance spectra of the rabbit eye sclera measured concurrently with administration of *glucose-40%* solution: 1 - 60 sec; 2 - 240 sec; 3 - 1260 sec; 4 - 1500 sec; and 5 - 1800 sec after drop of *glucose-40%* solution into the rabbit eye.

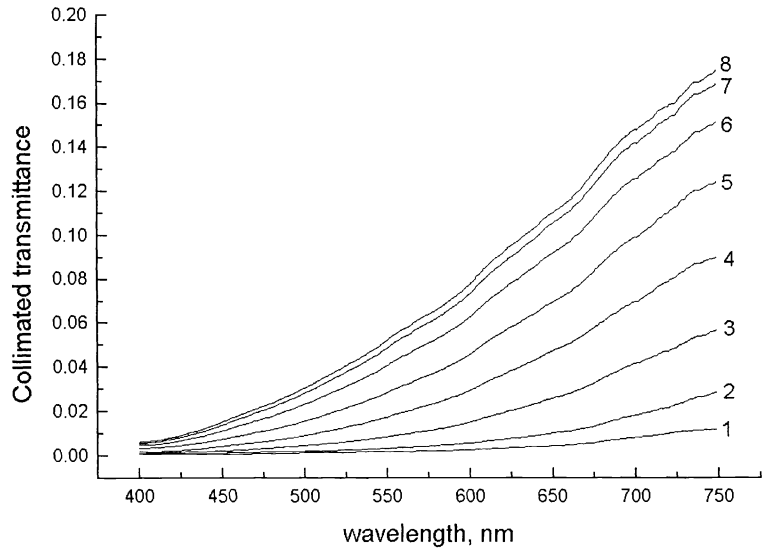

Fig. 3. The time-dependent collimated transmittance of the human sclera sample of initial thiscness 0.5 mm impregnated by a *glucose-40%*: 1- 10 sec; 2 – 60 sec; 3 – 120 sec; 4 – 180 sec; 5 – 240 sec; 6 – 300 sec; 7 – 390 sec; 8 – 510 sec after the scleral sample was immersed in a *glucose-40%* .

4. CONCLUSION

This study shows that transmittance and backscattering spectra of eye tissues and their polarization properties depend on tissue structure ordering and refractive index matching of scatterers and ground matter. Natural or artificial controlling of tissue structure properties allows for substantial changes of optical properties of eye tissues: cornea, sclera and lens.

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