The influence of osmotically active chemical agents on the transport of light in the scleral tissue

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ABSTRACT

Experimental study and computer simulation were used to analyze tissue optics during a process of optical clearing due to refractive index matching. Tissue samples of the human and bovine sclera and the collagen sponge as a phantom were investigated. Osmotically active solutions, such as *verografin* and *propylene glycol*, were used as chemicals. A characteristic time response of human scleral optical clearing in the range 1 to 20 min was determined. The matter diffusion coefficient for the scleral samples to impregnated by *verografin* solution were experimentally estimated; the average value is $1.27 \cdot 10^{-5} \pm 3.77 \cdot 10^{-6}$ cm^2/sec. The results are general and can be used to describe many other fibrous tissues.

Keywords: light scattering, sclera, osmolytes, refractive index matching

1. INTRODUCTION

The possibility of application of transscleral diagnostic and therapeutic methods is one of the important tasks in the laser ophthalmology. Solution of the problem is connected with control of the optical properties of the human sclera. This control means change of the scattering or absorption properties of the media. Absorption and scattering can be changed by using various physical and chemical reactions (e.g. compression, dehydration, coagulation and others). The optical properties of a tissue can be effectively controlled using osmotically active solutions [1]. Administrated chemical agent with a higher refractive index than that of tissue ground substance diffuses into a tissue, and water diffuses from a tissue to the surrounding solution, affects correspondingly changes of the interstitial substance, and equalizes the refractive indices of scatterers and the base material. As a result tissue optical clearing is observed.

The purpose of the present research is the experimental study of scleral optical transmittance, controlled by administration of osmotically active chemicals and the estimation of the matter diffusion coefficient in a living tissue and tissue-like-phantom.

2. PHYSICAL PROPERTIES AND STRUCTURE OF THE HUMAN SCLERA

The sclera has a compound structure. It is a nontransparent fibrous tissue and mainly consists of collagen fibers (fibrils) packed in lamellar bundles that are immersed in an amorphous base substance containing glycosaminoglicans, proteins, and protein-polysaccharide complexes.

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. These fibrils are arranged in individual bundles in parallel fashion. Within each bundle the groups of fibers are separated from each other by large empty lacunae randomly distributed in space. Collagen bundles show a wide range of widths (1 to 50 μm) and thickness (0.5 to 6 μm) and tend to be wider and thicker toward the inner layers. They cross each other in all directions but remain parallel to the scleral surface [1,2].

The scattering properties of the sclera are defined not only by its structure but by the ratio of refractive indices of collagen fibers and base substance. Following Refs. 1, 2, we can estimate the refractive index of the scleral sample using the law of Gladstone and Dale

$$n = \sum_{i=1}^{N} n_i V_i, \quad \sum_i V_i = 1, \tag{1}$$

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SPIE Vol. 3726 • 0277-786X/99/$10.00

where n is the resulting average value of the tissue refractive index, n_i and V_i are the refractive index and volume fraction of an individual component, respectively, and N is the number of components.

For the average refractive index of the sclera the expression can be represented as

$$n_s = n_{col}V_{col} + n_{base}V_{base}, \quad (2)$$

where n_{col}, n_{base} and V_{col}, V_{base} are the refractive indices and volume fractions of collagen and base material, respectively.

Using data that the average refractive index of sclera is $n_s = 1.385$, the volume fraction of hydrated collagen is $V_{col} = 0.31$, and refractive index of base substance is $n_{base} = 1.345$, on the basis of Eq. (2) can be obtained a refractive index of the scleral fibrils $n_{col} = 1.474$. All data were measured for $\lambda = 589$ nm [1,2].

3. OPTICAL AND MATTER DIFFUSION MODELS OF THE HUMAN SCLERA

The transmission of collimated light by a tissue layer of thickness l is defined as [1]

$$T_c = I/I_0 = exp(-\mu_t l), \quad (3)$$

where I_0 and I are the intensities of the collimated incident and detected light, respectively; $\mu_t = \mu_a + \mu_s$ is the extinction coefficient, where μ_a and μ_s are the absorption and scattering coefficients, respectively. For the human sclera the absorption and scattering coefficients are $\mu_a \cong 0.008$ mm^{-1} and $\mu_s \cong 25$ mm^{-1} at the wavelength $\lambda = 650$ nm [3].

Optical model of the sclera in a local region can be represented as a plane plate with a thickness l that is filled by thin and long dielectric cylinders (collagen fibers) with average diameter ~100 nm and refractive index $n_{col} = 1.474$. The cylinders are located in planes that are parallel to the plate surface, but within each plane their orientations are random. The interstitial space is filled by homogeneous base substance with the refractive index $n_{base} = 1{,}345$. This refractive index is a controlled parameter and can be changed from 1.345 to 1.474. For $n_c = n_{base} = 1.474$ the medium becomes totally homogeneous and optically transparent [1].

In an approximation of noninteracting dielectric thin cylinders the ratio of two values of scattering coefficients μ_s which correspond to the definite wavelength and different values of n_{base} is given by [1]

$$\mu_{s2} \cong \mu_{s1}[(m_2-1)(m_1-1)]^2, \quad (4)$$

where $m = n_{col}/n_{base}$ is the ratio of refractive indices of the cylinders and base materials. The value $m_1 = n_{col}/n_{base1} \cong 1.096$ is corresponded to the normal sclera in this model. It is expected the matching degree up to $m_2 \cong 1.001$, $\mu_{s2} \sim 10^{-4}\mu_{s1}$.

To estimate the diffusion coefficient of chemical agent when it diffuses within the interstitial substance of the sclera we may consider a diffusion through a membrane. Following model was used for solution of the task. It is two spaces separated by a partially permeable membrane. Internal volume is filled by interstitial substance of the sclera and external one is filled by agent solution. We accounted the fluxes of the chemical agent and water containing in interstitial substance through a thin membrane. Membranes degree of permeability, accounts the structure of tissue collagen fibrils arrangement, spaces between fibrils and etc., and defines the value of the diffusion coefficient.

Assuming that both water and agent have the same paths for diffusion we can find the diffusion coefficient. From Refs. 1, 3 follows that for a large external volume when concentration C_0 of matter which surrounds tissue, can be considered as a constant, the time dependent concentration of substance under study $C(t)$ can be expressed by

$$C(t) \cong C_0\,[1-exp(-t/\tau)], \quad (5)$$

where $\tau = l^2/D$, D is the diffusion coefficient, l is the thickness of the membrane (thickness of the scleral sample).

4. METHODS AND MATERIALS

Our experiments were performed *in vitro* with the human sclera. The conjunctiva and the ciliary body, as well as the retina with choroid were removed. The scleral samples were cleaned and cut into pieces of about 10×10 mm^2. The thickness of the human scleral sample was ~ 0.5 mm.

Bovine sclera and collagen sponge as models of the sclera were used. The size of the bovine scleral samples was the same as for the human ones, but the thickness was ~ 1mm. The size of the sponge was 15×20 mm^2, the thickness of about 1.43 mm. Average diameter of fibers structure which we seen by a light microscope was ~ 8.5 μm.

The bovine scleral samples were fixed on a plastic plate with square hole 5×5 mm^2 and placed in a 5-ml cuvette filled with the chemical agent. The collagen sponge was placed in the cuvette without fixation.

Two types of chemical agents were used for scleral optical clearing, such as *verografin-76%* and *propylene glycol*. For the present study it was important to know the refractive indices of osmolytes. Experimental values of the refractive indices measured by Abbe refractometer ($\lambda = 589$ nm) were n = 1.485 and 1.431 for *verografin-76%* and *propylene glycol*, respectively.

The spectrophotometric measurements of the collimated light transmission in the wavelength range 600 to 800 nm were obtained with commercially available spectrophotometer CARY-2415. These measurements were carried for samples of the human sclera in the solution of *verografin-76%*.

To obtain time-dependent measurements at the selected wavelength 650 nm for the samples of bovine sclera and sponge, a portable photocolorimeter was used.

5. RESULTS AND DISCUSSION

The human scleral transmission spectra measured by spectrophotometer for different periods of *verografin*-76% administration are presented in Figure 1. *Verografin-76%* administration makes this tissue highly transparent up to 15% at 800 nm. These spectra show that the transmission degree of the human sclera for different wavelengths can be controlled. The absolute error of a measurement of experimental spectra collimated transmittance (for a commercially available spectrophotometer CARY-2415) is 0.15 %.

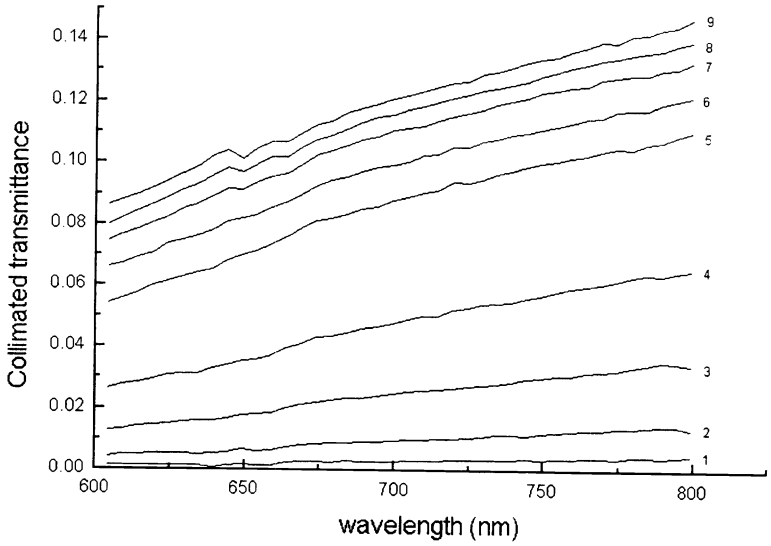

Figure 1. The time dependent collimated transmittance of the human sclera sample impregnated by a *verografin*-76% solution. Each spectrum was measured during 45 sec. The represented graphs correspond to 1 - 25 sec., 2 - 110 sec., 3 - 195 sec., 4 - 280 sec., 5 - 365 sec., 6 - 450 sec., 7 - 535 sec., 8 - 820 sec., 9 - 1215 sec. after scleral sample was immersed in a *verografin*-76% solution.

The time-dependent collimated transmittance of the human scleral sample impregnated by a *verografin-76%* solution was measured at 650 nm. The results are presented in Figure 2.

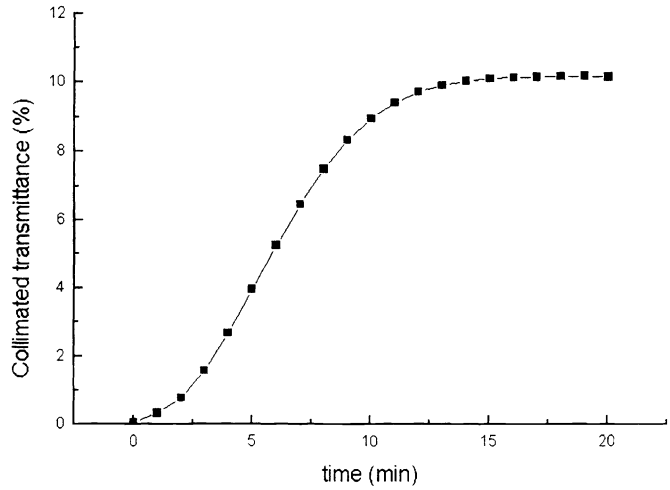

Figure 2. The time dependent collimated transmittance of the 0.5-mm-thick human scleral sample measured at 650 nm concurrently with administration *verografin*-76% solution. The measurements were carried out on spectrophotometer CARY-2415.

The same measurements were performed for model samples (the bovine sclera and sponge) with *propylene glycol* administration using photocolorimetric method. Figures 3 and 4 show the dynamics of tissue clearing in *propylene glycol.* The curves allow to define a characteristic time response. The absolute error of a measurements of time-dependent collimated transmittance is 0.25 %.

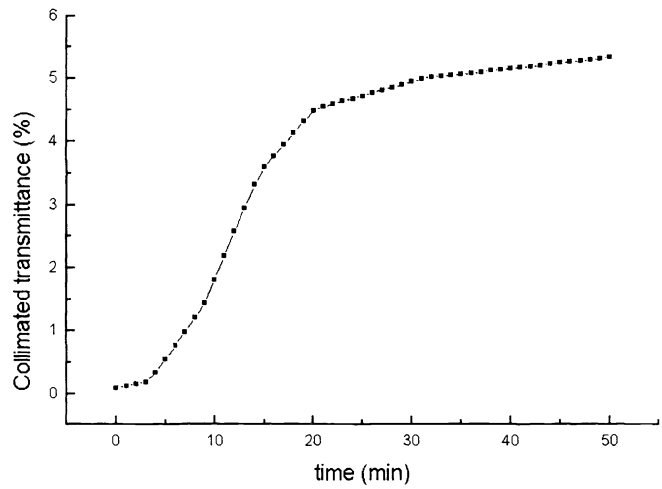

Figure 3. The time dependent collimated transmittance of a 1-mm-thick bovine scleral sample measured at 650 nm concurrently with administration *propylene glycol* solution.

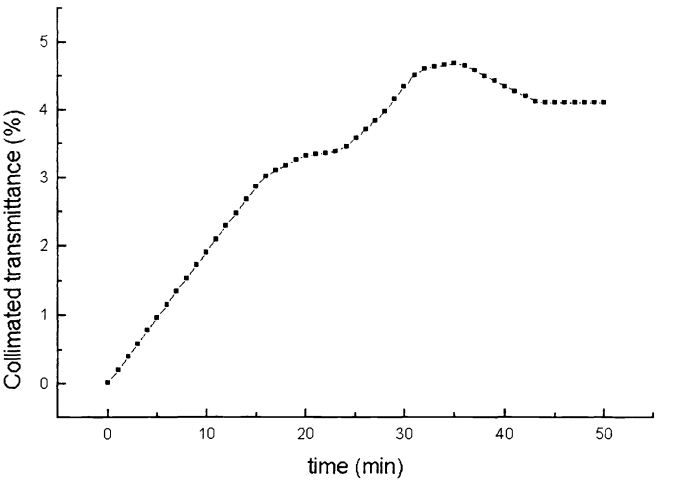

Figure 4. The time dependent collimated transmittance of a 1.43-mm-thick collagen sponge sample measured at 650 nm concurrently with administration *propylene glycol* solution.

Measurements of the time-dependent collimated transmittance allowed us, basing on presented tissue refractive index matching model, to estimate the diffusion coefficients of chemical agents [see Eqs. (1) – (4)]. The refractive indices of *verografin-76%* and *propylene glycol* were obtained by experimentally. The time-dependent diffusion coefficient of *verografin-76%* in the human sclera is shown on Figure 5. The mean value of the diffusion coefficient for 76% *verografin* solution transport in the human scleral sample is equal to $1.27 \cdot 10^{-5} \pm 3.77 \cdot 10^{-6}$ cm^2/sec.

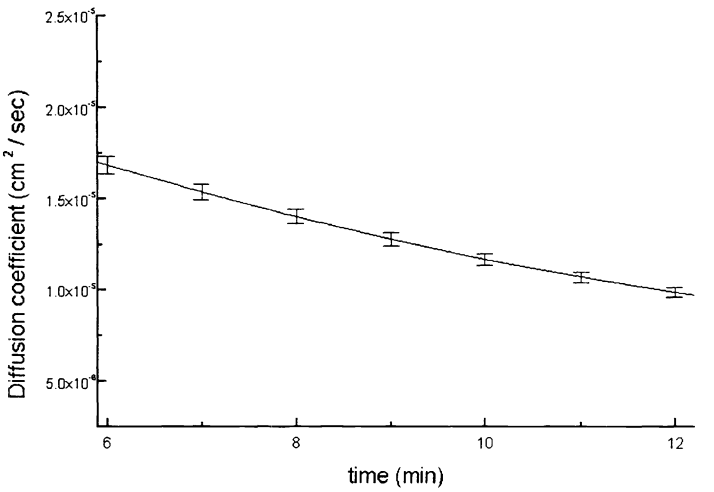

Figure 5. The time dependence of the diffusion coefficient for the 76%-*verografin* solution in the 0.5-mm-thick human scleral sample. Vertical lines represent rms values

Figures 6 and 7 illustrate dependence *D(t)* for *propylene glycol* solution, respectively for the bovine sclera and collagen sponge sample. Mean value of the diffusion coefficient for *propylene glycol* solution transport in bovine scleral sample is equal to $1.88 \cdot 10^{-5} \pm 7.98 \cdot 10^{-6}$ cm^2/sec. Mean value of the diffusion coefficient for *propylene glycol* solution transport in phantom – collagen sponge sample $1.75 \cdot 10^{-5} \pm 1.24 \cdot 10^{-5}$ cm^2/sec.

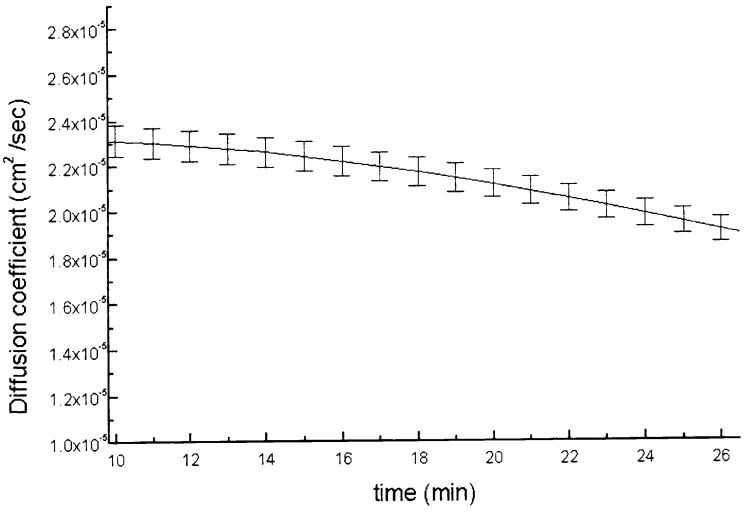

Figure 6. The time dependent diffusion coefficient solution *propylene glycol* in 1-mm-thick bovine scleral sample. Vertical lines represent rms values

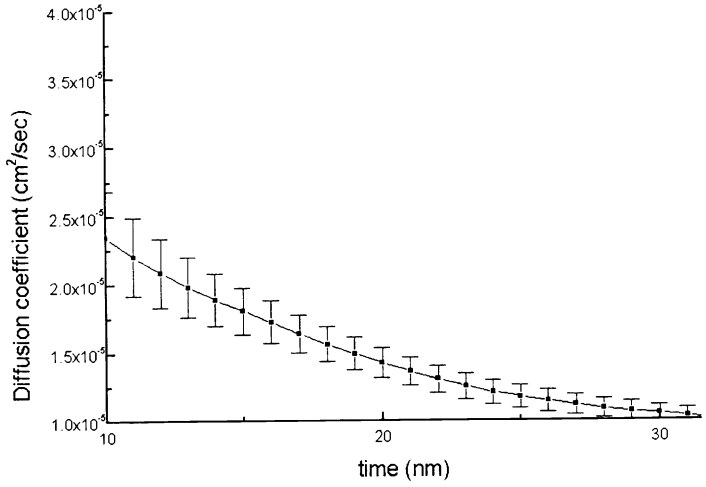

Figure 7. The time dependence for the diffusion coefficient of the *propylene glycol* solution in 1.43-mm-thick phantom – collagen sponge. Vertical lines represent rms values

6. CONCLUSION

The results of this paper show that administration of osmolytes to a fibrous tissue allows for effectively control of its optical characteristics. The scattering properties of the sclera are effectively reduced by the refractive indices matching of the collagen fibrils and interstitial substance.

The dynamics of tissue optical clearing using osmolytes is defined by a characteristic time response of about 15 – 50 min in dependence on of the used tissue and osmolyte. Dynamic characteristics were successfully used for estimation of diffusion coefficients of studied chemicals (*76%-verografin* and *propylene glycol* solutions) in the human and bovine sclera as well as in a collagen's sponge. These values are well match to values of diffusion coefficient of small molecules diffusion in water [4].

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