Estimation of wavelength dependence of refractive index of collagen fibers of scleral tissue

Alexey N. Bashkatov, Elina A. Genina, Vyacheslav I. Kochubey, and Valery V. Tuchin

Saratov State University, Astrakhanskaya 83, Saratov 410026, Russia

ABSTRACT

We present experimental results and computer modeling on the optical properties of the human scleral tissue. The wavelength dependence of the refractive index of collagen fibers in the human scleral tissue was estimated on the base of Mie theory. Dispersion formula describing this dependence was derived. The results are general and can be used to describe optical properties of many other fibrous tissues.

Keywords: optical properties, sclera, light scattering, refractive index

1. INTRODUCTION

Knowledge of tissue optical properties in the visible range is of great importance for biomedical optics. Recently, many research groups reported the optical properties of various tissues.[1-5] Such data are very important for laser diagnostics, surgery and therapy. In the mentioned above papers, the optical properties were defined on the basis of rigorous or approximate solution of the radiative transfer equation for a turbid medium. In this case the optical properties are described by the following phenomenological coefficients, like absorption and scattering coefficients, and anisotropy factor. However, for describing of light propagation in tissues using of Mie theory [6] it is necessary to know the scatterer sizes and dependence of refractive index on the wavelength of both scatterers and surrounding medium. Similar problem arises in biomedical optics when control of tissue optical properties is analyzed.[7-15]

Human sclera is a typical fibrous tissue. The structure and properties of fibrous tissues (for example sclera and cornea of eye, dermis of human skin etc.) are described in details in Refs 9, 10, 13, 15-25. Unfortunately, there are only a few papers reporting the values of refractive indices of fibrils (the ground scatterers in fibrous tissues). [10, 18, 19, 25-28]

For estimation of refractive indices of scleral tissue components at various wavelengths, it is necessary to replace one of the components defining scattering by the substance with known value of refractive index. For example, the interstitial fluid can be replaced by a saline. Knowing the scatterer sizes, refractive indices of the surrounding medium, scattering properties of the investigated sample it is possible to estimate the value of refractive index of collagen fibers.

In this paper, we have presented the results of experimental study and computer modeling of the human scleral tissue optical properties. The values of refractive index of collagen fibers in scleral tissue at various wavelengths were estimated.

2. MATERIALS AND METHODS

The experiments were performed *in vitro* with the samples of the human scleral tissue. Tissue samples were obtained by autopsy within a day *post mortem*. The scleral tissue was kept in saline during 24 hours. Before experiments, the scleral tissue was cut into pieces with the area about 1 cm^2. Tissue thickness was 0.66 mm. All experiments were performed at room temperature.

The refractive index of collagen fibers (at wavelength 589 nm) can be estimated as 1.474.[10] Wavelength dependence of the refractive indices of water is described as[10]:

$$n_{H_2O}(\lambda) = 1.31848 + \frac{6.662}{\lambda[nm] - 129.2}. \tag{1}$$

Controlling Tissue Optical Properties: Applications in Clinical Study, Valery V. Tuchin, Editor,
Proceedings of SPIE Vol. 4162 (2000) © 2000 SPIE · 0277-786X/00/$15.00

In Ref. 13, it was shown that volume of the scatterers of the scleral tissue is about 3.9 μm^3. In Ref. 19 it was reported that majority of collagen fibers have diameter near 200 nm. It allows us to estimate their mean length as 125 μm.

We used a commercially available computer-controlled CARY-2415 spectrophotometer with integrating sphere for investigation of the scleral tissue optical properties. Total transmittance and diffuse reflectance were measured in the 400-800 nm wavelength range using standard methodology.

We have used the inverse adding-doubling method developed by *Prahl et al.*[29] to calculate the absorption and reduced scattering coefficients of the scleral tissue from the measured values of the total transmittance and diffuse reflectance. To obtain optical properties of the investigated samples we have used a computer program of *S.A. Prahl* (Oregon Medical Laser Center, USA; www.omlc.ogi.edu).

For collagen fibers refractive index reconstruction, we used the method described in Ref. 4. We used the values of refractive indices of water (Eq. 1) as values of refractive indices of the surrounding medium. For modeling, we used the spherical scatterers with diameter of 1.96 μm.[13]

3. RESULTS AND DISCUSSION

Figure 1 presents the spectra of diffuse reflectance (squares) and total transmittance (circles) of the human scleral tissue. Using inverse adding-doubling method, we have calculated optical properties of the human scleral sample. Results of this calculation are presented in Figs. 2, 3. Figure 2 presents the absorption coefficient of the human scleral sample treated by saline during 24 hours. Figure 3 presents the reduced scattering coefficient of the human scleral sample treated by saline. The values of the absorption coefficients and the reduced scattering coefficients obtained by us are closed to the data presented in Refs. 2 and 30 for the rabbit sclera and porcine sclera, respectively.

Using the method described in Ref. 4 we have calculated the wavelength dependence of the refractive index of collagen fibers of scleral tissue (Fig. 4). In this study, we used ensemble of the randomly distributed spheres with a mean diameter 1.96 μm as a model of scattering system. This representation is based on the results of Refs. 31 and 32, where it was shown that the scattering properties of a system of non-orientated scatterers with a shape like a finite cylinder can be modeled using simpler model of a system of spherical particles with the equivalent volume of an individual scatterer. We also check this calculations to ensemble of the randomly distributed used spherical scatterers with mean diameter from 0.8 to 3 μm. It is interested to say that obtained wavelength dependencies of refractive indices of scatterers do not depend from scatterers sizes. Analogous results can be obtained if the scatterers are presented as infinite cylinders with diameters about 200 nm. It must be noted that in this calculations we used "effective" scatterers and they do not show polydisperse nature of real scleral scatterers.

The wavelength dependence of the refractive index of collagen fibers, presented in Fig. 4, is well described by Cauchy's formula. This formula is prevailing for approximation of wavelength dependence of refractive index for various substances including tissues.[27, 33] We have obtained the following dispersion formula using the least-squares method:

$$n_{col}(\lambda) = 1.426 + \frac{19476}{\lambda^2[nm]} - \frac{1131066900}{\lambda^4[nm]} \quad (2)$$

4. CONCLUSION

In this study we have measured and calculated the optical properties of the scleral tissue. Using Mie theory we have estimated the wavelength dependence of refractive index of collagen fibers material. The corresponding dispersion formula was derived. The results are general and can be used to describe optical properties of other fibrous tissues.

ACKNOWLEDGEMENTS

The investigation described in this publication was made possible in part by grant "Leading Scientific Schools" # 00-15-96667 of the Russian Basic Research Foundation.

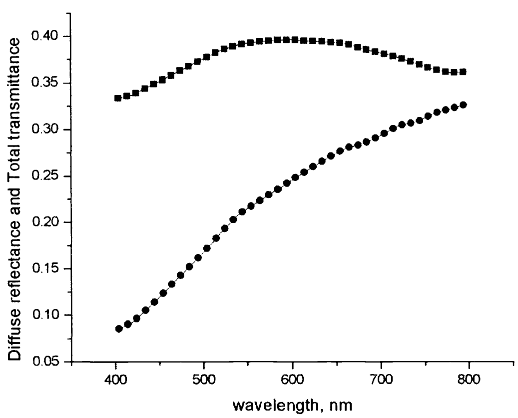

Figure 1. The diffuse reflectance (squares) and the total transmittance (circles) of the human scleral tissue sample.

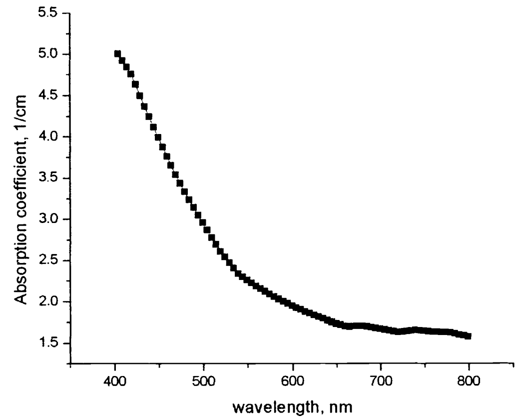

Figure 2. The absorption coefficient of the human scleral tissue sample.

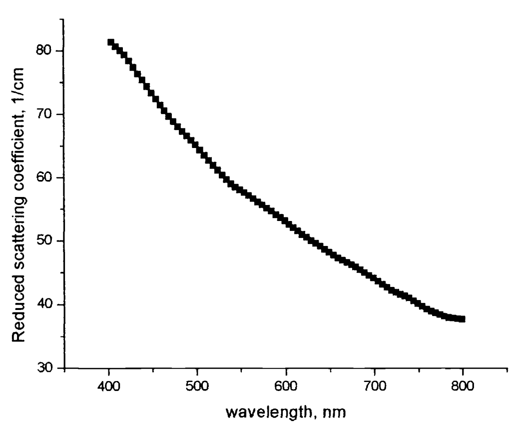

Figure 3. The reduced scattering coefficient of the human scleral tissue sample.

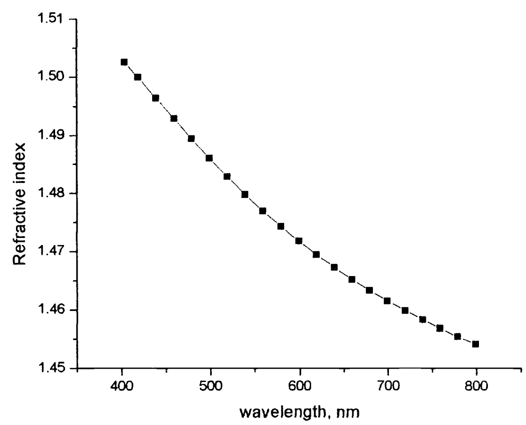

Figure 4. The wavelength-dependence of the refractive indices of the collagen fibers on scleral tissue sample.

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