Diffusion of glucose solution through fibrous tissues: *in vitro* optical and weight measurements

Elina A. Genina, Alexey N. Bashkatov, Nina A. Lakodina, Snezhana A. Murikhina, Yuri. P. Sinichkin, Valery V. Tuchin

Saratov State University, Astrakhanskaya 83, Saratov 410026, Russia

ABSTRACT

The diffusion of glucose solutions with various concentrations through human sclera and cerebral membrane *in vitro* was studied. The dynamics of this process was monitored by time-dependent weight and optical measurements. Glucose administration induces the diffusion of matter and as a result the equalization of the refractive indices of collagen fibrils and ground material, and corresponding changes of transmittance spectra of fibrous tissue. Transmittance spectra of the human scleral and cerebral membrane samples impregnated by glucose were measured. Investigation of diffusion process in scleral samples, previously dried and swelled in distillated water was performed. Experimental results are presented.

Keywords: glucose; sclera; cerebral membrane; clearing; swelling.

1. INTRODUCTION

The possibility of application of transscleral and cerebral diagnostics, therapy and surgery is one of importance for modern laser medicine. The solution of the problem is connected with control of the optical properties of the fibrous tissue. Such control means the change of the scattering or absorption properties of a tissue[1-7]. Using of various physical and chemical reactions (*e.g.* compression, dehydration, coagulation and others) can change absorption and scattering. The optical properties of tissue can be effectively controlled using osmotically active solutions. The main idea of the use of such solutions is based on the dependence of tissue scattering properties on the refractive index mismatch between collagen fibers and the extrafiber substance. In general, an increase in tissue glucose concentration reduces index mismatch and correspondingly decreases the scattering coefficient.

The possibility of scleral reflectance measurements for *in vivo* monitoring of glucose concentration was discussed in our previous papers [4-6]. It was shown that turbidity of sclera could be effectively controlled using above discussed immersion and that osmotic effects are very important.

In this paper *in vitro* diffusion of glucose solutions into fibrous tissue has been investigated by both optical and weight methods for samples of human sclera and cerebral membrane. The results show that both clearing and swelling of tissue take place during process of diffusion. Because of these, it is important to study the process of diffusion by these two methods simultaneously.

2. MATERIALS AND METHODS

The experiments were performed *in vitro* with the samples of human sclera and cerebral membrane. Tissue samples were obtained by autopsy within a day *post mortem*. After enucleation eyes were inflated with saline. The temperature of storage was +1°C. The cerebral membrane tissue was kept under temperature -12°C.

Address all correspondence to Elina A. Genina and Valery V. Tuchin. Tel: 8452 514693; E-mail: eagenina@optics.sgu.ru and tuchin@sgu.ssu.runnet.ru

In *Saratov Fall Meeting '99: Optical Technologies in Biophysics and Medicine*, Valery V. Tuchin, Dmitry A. Zimnyakov, Alexander B. Pravdin, Editors, Proceedings of SPIE Vol. 4001 (2000) • 0277-786X/00/$15.00

Before the measurements the conjuctiva and the ciliary body of eye as well as retina with choroid were removed. The human sclera and cerebral membrane were cut into pieces with the area about 1 cm^2. The thickness of the samples of sclera and cerebral membrane was ~0.4 mm and ~0.6 mm, respectively.

The samples of both sclera and cerebral membrane were placed into cuvette filled by immersion liquid (1.5 cm^3). As immersion liquids glucose solutions with different concentrations (1M, 1.5M, 2.1M and 3M) were used. The refractive indices of the solutions measured by Abbe refractometer were 1.348, 1.367, 1.391, and 1.398, respectively. Dynamics of mass changes was determined by torsion balance.

We also investigated swelling of dry scleral samples. The samples were dehydrated to dry weight, immersed into distillated water and then into glucose solution. The dynamics of mass changes of samples were also measured. The measurements were performed every 1 min for 30 min. Sizes of the samples were measured before and after swelling. All experiments were performed at room temperature.

The measurements of the collimated light transmittance spectra were performed using OMA (fiber optic spectrometer LESA-6med, BioSpec, Russia). The scheme of the experimental setup is shown in Fig. 1. A 250 W xenon arc lamp with filtering of the radiation in the 400 - 800 nm spectral range was used in these measurements.

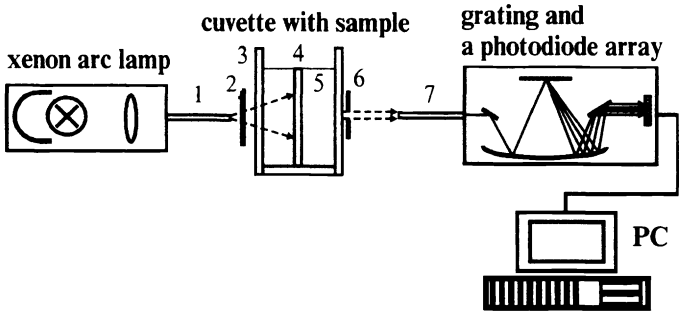

Figure 1. Experimental setup for measurements of the collimated light transmittance spectra: 1 – optical irradiating fiber; 2 – neutral filter; 3 – cuvette; 4 – plate with scleral sample; 5 – glucose solution; 6 – the 0.5 mm – diaphragm; 7 – receiving fiber.

The cuvette with sample was placed between two optical fibers (400 μm core diameter). One fiber transmitted the excitation radiation to the sample, and the other collected the radiation transmitted by the sample. The distance between the fibers was 145 mm. The 0.5 mm – diaphragm placed 9 mm apart from the tip of the receiving fiber was used to provide collimated transmittance measurements. Neutral filters were used to attenuate the incident radiation.

For there experiments samples were fixed on plastic plate with a square aperture 5×5 mm^2 and placed in 5-ml cuvette filled with glucose solution. The measurements were performed every 30 sec for 30 min.

3. RESULTS AND DISCUSSION

The time-dependent collimated transmittance of the human scleral and cerebral membrane samples measured at different wavelengths with administration of 2.1M glucose solution is presented in Figs 2 and 3, respectively. Glucose administration makes these tissues highly transparent. The maximum value of transmittance is achieved in 5-7 min. Then decreasing of tissue transparence is observed.

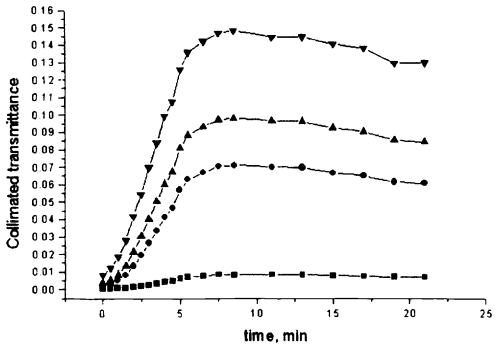

Figure 2. The time-dependent collimated transmittance of the human scleral sample measured at 420 (squares), 590 (circles), 630 (up triangles), and 700 (down triangles) nm concurrently with administration of 2.1M glucose solution.

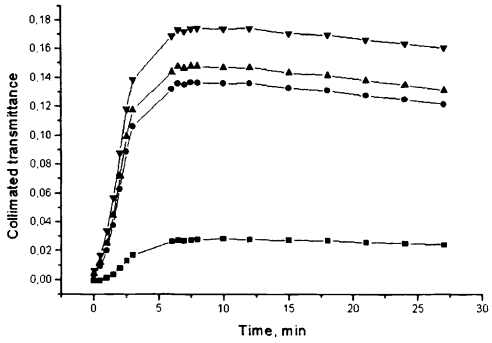

Figure 3. The time-dependent collimated transmittance of the human cerebral membrane sample measured at 420 (squares), 590 (circles), 630 (up triangles), and 700 (down triangles) nm concurrently with administration of 2.1M glucose solution.

The results of time-dependent measurements of mass change dynamics for scleral and cerebral membrane samples are shown in the Figs. 4 and 5, respectively. Normalized weight means ratio of sample weight in the process of swelling to initial weight.

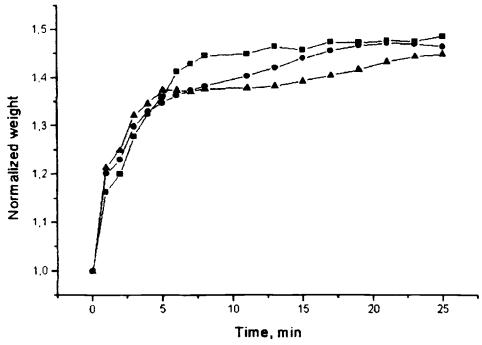

Figure 4. The time-dependent normalized mass change dynamics of scleral samples in glucose solutions with different concentrations: 1M (squares), 2.1M (circles), and 3M (up triangles).

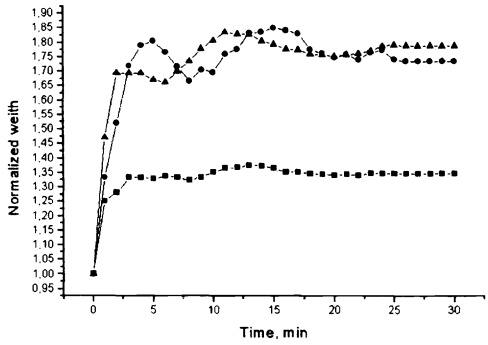

Figure 5. The time-dependent normalized mass change dynamics of cerebral membrane samples in glucose solutions with different concentrations: 1M (squares), 2.1M (circles), and 3M (up triangles).

Comparison of the curves in Figs. 2 and 3 shows that cerebral membrane has approximately the same enlightenment degree with sclera. Thus, the maximum change of scleral and cerebral membrane transmittances at wavelength λ = 700 nm is 15 % and 17 %, respectively. Comparison of the curves in Figs. 4 and 5 shows that the changes weight of the sample increased in 1.5-1.7 times relatively to initial weight for both tissue. Thus, the interaction of various fibrous tissues (sclera and cerebral membrane) with osmotically active liquids (glucose solutions) is defined by identical laws. However, water content in the sclera and in the cerebral membrane is differed from each other. Water volume in the both sclera and cerebral membrane is 69 % and 36 %, respectively. It is well known that in describing of diffusion in porous substances, the diffusion velocity is defined by porosity coefficient [8]. The value of sclera porosity coefficient is larger that one of cerebral membrane. It is probably to suggest that glucose diffusion into sclera goes slower then one into cerebral membrane. However, appreciable

differences in both clearing dynamics and mass changes of the samples are not observed. It means that differences between scatterers in both sclera and cerebral membrane take place. In generally, there are collagen fibers, but sizes of the fibers in hard cerebral membrane, probably, are higher then ones in sclera. The clearing degree of the samples are approximately equal at considerable differences in scatterers volume fractions (0.31 for sclera and 0.64 for cerebral membrane). From other hand, there are differences in hydration degree of collagen fibers of sclera and cerebral membrane. Volume fraction of water, connected with collagen fibers for sclera is 0.35 (from total volume of fibers). But that for cerebral membrane, probably, has less value (the refractive index of collagen fibrils, in contrast, is higher). It decreases the rate of cerebral membrane clearing. Probably, it is necessary to account to the packing of scatterers. All there processes take place simultaneously and play important role in the interaction of tissue and osmotically active liquids. Coincidence of mass change dynamics for sclera and cerebral membrane is well explained by present scheme. Although the glucose diffusion into cerebral membrane goes more quickly then into sclera, the quantity of glucose penetrating into interstitial space of cerebral membrane is less then into sclera. In contrast, the adding mass connected with swelling of collagen fibers is larger for cerebral membrane then for sclera. Thus, although the differences in the initial structures of sclera and cerebral membrane take place, the interaction of the tissues with osmotically active liquids (glucose solution) is the same. It allows us to conclude that the obtained results are generally and can be used for describing of other fibrous tissues.

During the experiment two competitive processes take place simultaneously. In the first 5 minutes generally glucose diffusion into tissue diffuses. In this stage increase of tissue clearing is observed. Diffusing into interstitial space glucose solution shifts pH of the sample ground substance to more acid area. It is well known that the change of solution pH into more acid or more alkaline area from colloid isoelectric point increases the swelling degree of tissue (Fig. 6). It is explained by appearing of positive or negative charge of colloid particles and therefore increasing of hydration degree [9].

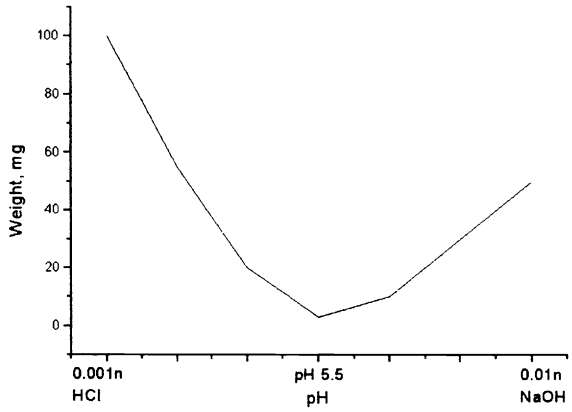

Figure 6. Dependence of human cardiac muscle swelling on environmental pH in 30 min [9].

The swelling of collagen fibers leads to the increase of their size and change of packing. The space between the fibrils rises and the scattering coefficient increases. Therefore the decrease of tissue transparence is observed.

The acid reaction of solutions is increased with rise of glucose concentration. The swelling degree of scleral and cerebral membrane sample increases with rise of glucose concentration. Fig. 7 shows dependence of swelling degree on glucose solution concentration for scleral and cerebral membrane samples.

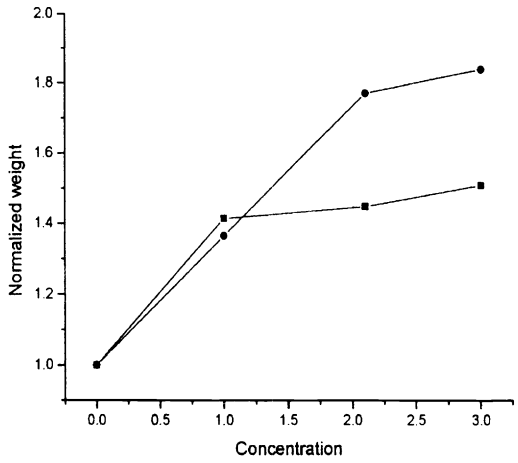

Figure 7. Dependence of swelling degree on glucose solutions concentrations for scleral (squares) and hard cerebral membrane (circles) samples in 25 min after tissue immersion into glucose solution.

Fig. 8 shows time-dependent collimated transmittance of hard cerebral membrane for glucose solutions with different concentrations. The least clearing corresponds 1.5M glucose solution. It is connected with small refractive index of this solution. That is why the matching of collagen fibers and base substance refractive indices is inconsequent. The best enlightenment of the sample is observed for 2.1M glucose solution. We suggest the follow explanation of this fact. Osmotical effect of 3M glucose solution is higher than one of 2.1M glucose solution. As a result at impregnation of the sample into 3M glucose solution bark effect is observed. This effect consists in dehydration of scattering fibers and increasing of their refractive index. The mismatching of refractive indices of scatterers and base substance takes place. As a result the clearing degree of cerebral membrane sample in 3M glucose solution is less then in 2.1M glucose solution. Thus 2.1M glucose solution (from used by us) is the most optimal solution for clearing of living tissue.

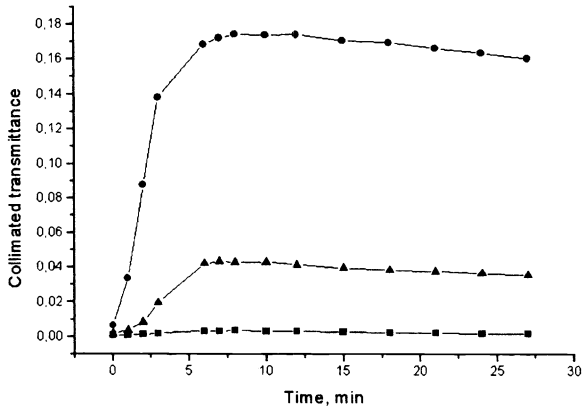

Figure 8. The time-dependent collimated transmittance of the human hard cerebral membrane sample measured at 700 nm concurrently with administration of 1.5M (squares), 2.1M (circles), and 3M (up triangles) glucose solution.

The swelling of dry scleral samples in water and then in 3M glucose solution are presented in Figs. 9 and 10, respectively. There were investigated three scleral samples and standard deviation of weight values is shown.

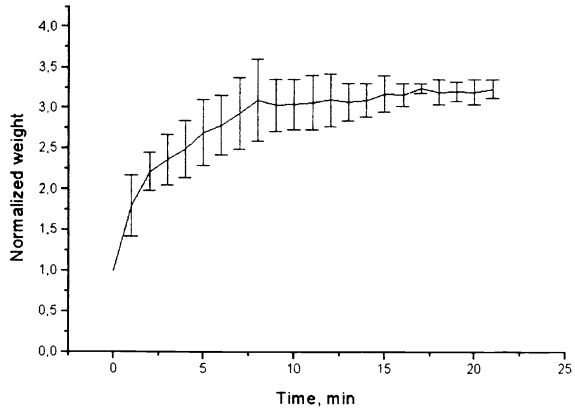

Figure 9. The time-dependent normalized mass change for three scleral samples in distillated water.

In this case dynamics of swelling of the samples is dramatically different. The enlightenment of the tissue is induced by matching effect. However, we can see considerable decrease of sample weight in first minutes after the samples were immersed in glucose solution. It is due to water diffusion from tissue into environment solution under the action of osmotic pressure difference. This process is known as shrinkage [10]. Further rise of sample mass is explained by action of swelling. This process is described above. Nevertheless, despite considerable swelling of the samples, the weight after swelling was less then their initial weight. Possibility it is connected with change of inner structure of fibrous tissue under dehydration (*e.g.* decreasing of interstitial space).

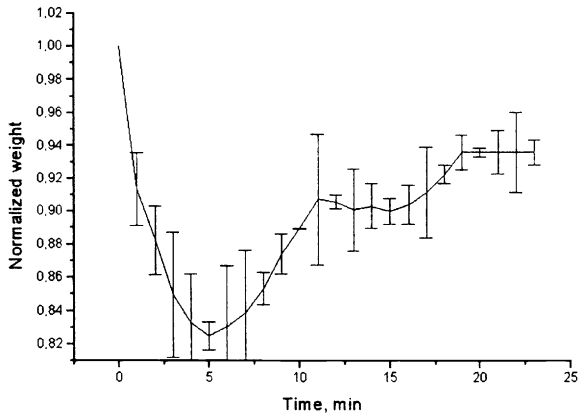

Figure 10. The time-dependent normalized mass changes for three scleral samples in 3 M glucose solution.

4. CONCLUSION

The weigh and optical study of glucose diffusion into sclera and cerebral membrane performed *in vitro* is important in understanding the mechanism of diffusion of osmolytes into fibrous tissue. The results of weight and optical measurements are well matched. The dynamics of tissue optical clearing under action of glucose solutions of various concentrations exibits a characteristic time response of about 5-7 min. For fibrous tissues (sclera and cerebral membrane) the action of osmotically active liquid can be saturated on two stages. In the first stage glucose diffuses into tissue. As a result, a rise of the sample

weight is observed. Simultaneously considerable enlightenment of tissue due to matching of scatterers and base substance refractive indices takes place. In more prolonged administration of the osmolyte (from 8 to 20 min for 2.1 M glucose solution) tissue swelling saturates and even slightly reduces the tissue transparency. In summary, administration of osmolytes with optimal concentration to a fibrous tissue allows us to control its optical characteristics more effectively.

ACKNOWLEDGEMENTS

The research described in this publication was made possible in part by the grant "Leading Scientific Schools" (No. 96-15-96389) of the Russian Foundation of Basic Researches.

REFERENCES

1. P.O. Rol, "Optics for Transscleral Laser Applications," D. N. Sci diss., Institute of Biomedical Engineering, Zurich, Switzerland, 1992.
2. Z.S. Sacks, D.L. Craig, R.M. Kurtz, T. Juhasz, G. Mourou, "Spatially resolved transmission of highly focused beams through the cornea and sclera between 1400 and 1800 nm," *Proc.* SPIE, **3726**, pp. 522-527, 1998.
3. Z.S. Sacks, F. Loesel, C. Durfee, R.M. Kurtz, T. Juhasz, G. Mourou, "Transscleral photodisruption for the treatment of glaucoma," *Proc.* SPIE, **3726**, pp. 516-521, 1998.
4. V.V. Tuchin, I.L. Maksimova, D.A. Zimnyakov, I.L. Kon, A.H. Mavlutov, A.A. Mishin, "Light propagation in tissues with controlled optical properties," *J. Biomed. Opt.*, **2** (4), pp. 401-417, 1997.
5. A.N. Bashkatov, E.A. Genina, V.I. Kochubey, V.V. Tuchin, Yu.P. Sinichkin, "The influence of osmotically active chemical agents on the transport of light in the scleral tissue," *Proc.* SPIE, **3726**, pp. 403-409, 1998.
6. A.N. Bashkatov, V.V. Tuchin, E.A. Genina, Yu.P. Sinichkin, N.A. Lakodina, V.I. Kochubey, "The human sclera dynamic spectra: in vitro and in vivo measurements," *Proc.* SPIE, **3591**, pp. 311-319, 1999.
7. V.V. Tuchin, "Light scattering study of tissues," *Physics-Uspekhi*, **40**, pp. 495-515, 1997.
8. A.N. Tikhonov, A.A. Samarskiy, "Equations of the mathematical physics," Moscow, Nauka, 1977.
9. M.I. Ravich-Scherbo, V.V. Novikov, "Physical and colloidal chemistry", Moscow, Visshaya Shkola, 1975.
10. A.V. Lykov, "Effects of the transport in capillary-porous bodies", Moscow, Gosudarstvennoe izdatel'stvo technical-theoretical literaturi, 1954.