Doppler spectroscopy of blood in capillary plexus under the action of 40% glucose solution

A. N. Korolevich[a,b,*], A. Yelshin[b], M. Belsley[b], M. Mota[c]
[a]Institute of Physics National Academy of Sciences of Belarus,
Skarina Ave., 68, 220072 Minsk, Belarus.
[b]Physics Department, Minho University, Campus Gualtar, 4709 Braga, Portugal,
[c]Bioengineering Department, Minho University, Campus Gualtar, 4709 Braga, Portugal

ABSTRACT

The investigation of blood dynamical characteristics in the skin under the action of 40% glucose solution was performed *in vivo* by the laser Doppler technique. Experiments demonstrate that glucose solution affects significantly the blood perfusion and concentration. Qualitative explanation was made for observed perfusion dynamic effect in the skin dermis based on the following factors: tissue cells shrinkage and additional capillaries opening under osmotic stress. The size of glucose vesicle lens was measured under the skin by ultrasonography. The analysis of vesicle sizes monitoring leads to the conclusion that glucose lens spread, basically, along the skin than in the perpendicular to the skin surface direction. Obtained results show the significant anisotropic perturbation of the dynamic characteristics of blood in vascular plexus under the optical active solution influence that must be taken into consideration during optical clarification of biological tissues.

Keywords: light scattering, blood, skin, optical clarification.

1. INTRODUCTION

At the present time the growing interest is observed to the increasing a penetration depth of optical radiation by means immersion liquids application. This idea has been recently proposed simultaneously in Russia[1] and USA[2]. The main idea is that using of osmotically active liquids decreases scattering in tissues and thus potentially increases the depth of penetration of the radiation.

Vargas et. al.[2] showed that diffusion of glucose solutions cause significant increase of optical transmittance of skin. Later by Galanzha et al[3] the effect of glucose solution on the rat skin optical clearing was demonstrated.

Numerous investigations show that immersion liquids are using on the practice change not only optical but, also, structural parameters of the tissues[4] diameter of blood vessel[3], and value of perfusion[5]. Therefore, the dynamic of glucose diffusion in the biological tissues should be investigated carefully for the practical application of this effect in the clinical practice.

The diffusion process is already investigated in a brain[6,7]. However, the structure the biological tissues not always uniform. For example, the skin structure uniform along the surface but essentially heterogeneous in depth[8]. Obviously, the diffusion coefficient of immersion liquid and optical parameters, respectively, will be dependent on the diffusion direction. The information about diffusion of such type of liquids in the skin is limited.

Therefore, the aim of this paper is the more detail monitoring of blood dynamical characteristic, during the immersion liquid diffusion in the skin over the glucose solution injection.

2. EXPERIMENTAL DETAILS

Depending on the purpose, for the skin optical transparency is used different hyperosmotic solutions of chemical agents:

* akorolevich@yahoo.com; phone 375 172 840 948

Saratov Fall Meeting 2005: Optical Technologies in Biophysics and Medicine VII,
edited by Valery V. Tuchin, Proc. of SPIE Vol. 6163, 61630I, (2006)
1605-7422/06/$15 · doi: 10.1117/12.696961

glycerol, mannitol, glucose, dimetil sulfoxide, dextran and so on. Hyperosmotic solutions of glucose is more investigated among others and produce more prolonged and stable optical effect than other substances[3], therefore the commercially available 40%-aqueous glucose solution (pH=3.5, n=1.39) has been used as a clearing agent.

2.1 Method of blood parameters measurement

We used a laser Doppler technique because it offers several advantages (easy to handle, versatile, high reproducibility, and good sensitivity).

Bonner and Nossal[9] showed that the first moment of the spectrum, i.e. the mean Doppler frequency <f>, should be proportional to the producting the root mean square velocity (ν) and volume fraction of the red blood cells (N), i.e. flux: $F = k\bar{\nu}N$, where k is the arbitrary constant. Therefore, the flux is expressed in terms of arbitrary perfusion units allowing making the relative comparison, for example, before and after stimuli.

The loops of capillaries in the human skin are mainly oriented mainly perpendicular to the dermis surface. They resemble a hairpin in shape and consequently they have rising and descending knees[10]. However, the scattering, occurring in the dermal tissue, more or less randomizes the direction of a photon reaching a red blood cell. Theoretically, the probability of a certain scattering angle and the probability of a certain frequency shift decrease roughly exponentially with increasing scatter angle and with the amount of the frequency shift. The average measurable Doppler frequency shift remains proportional to the average velocity of blood cells and is roughly independent of the observation angle[11].

2.2 Experimental set-up and measured values

The measurements were carried out using a commercially available instrument, the PeriFlux 4001 from Perimed (Stockholm, Sweden). This instrument uses a single mode laser radiation with a wavelength of 780 nm and a power of 0.8mW to provide the light used for measuring the perfusion.

From the power spectrum $P(f)$, obtained from the analysis of the temporal fluctuations of intensity scattered light $I(t)$, it is possible to obtain a value of blood perfusion, which is determined as the first moment (M_1) of spectrum: $\int_{-\infty}^{+\infty} fP(f)df$. In other words, the perfusion ($\langle N\rangle\nu$) is defined as the product of quantity of the particles in the detecting volume multiplied by their average speed [ml/min100gram].

Accordingly, the concentration = $\langle N\rangle \propto M_0 = \int_{-\infty}^{+\infty} P(f)df$, where M_0 is zero-moment. The values of perfusion rate are different for the various human tissues[12] but in general, case the perfusion rate value may occur as result of vasodilation that is a thermoregulatory mechanism or, as in our case, can be induced pharmacologically.

2.3 Skin structure

Skin is highly complicated organ that performing a complex role within the body's equally complex activities. Skin permeability is an important property controlling the body life and serves as a gateway for delivering some drugs or improving and restoring the skin behaviours[13,14]. Skin and dermal blood vessels behaviours are an informative source of the human health condition.

The skin itself has a complicate structure. A cross-sectional sketch of normal human skin is shown in figure 1. As follow from this sketch, the skin consists of three main layers from the surface: epidermis (100 μm thick, the blood-free layer), dermis (1-2 mm thick, vascularised layer), and subcutaneous fat.

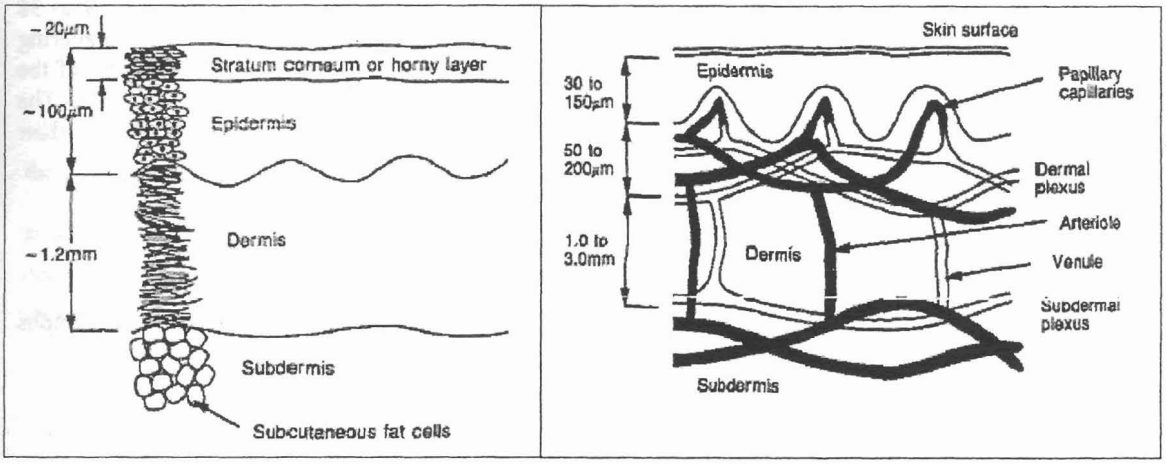

Fig. 1. The cross-section through skin is illustrating the cellular nature of the epidermis and the fibrous nature of dermis. Typical dimensions for the "thin" skin are shown by [15].

2.4 Experimental protocol

The radiation from the semiconductor diode laser passed through lens to be coupled into the light-guiding fibre with the help of which it is guided to the surface of the object being investigated. Flexible fibre-optics laser probe was attached to the patient's body so that the laser radiation was occurred perpendicularly to the skin's surface (see fig. 2).

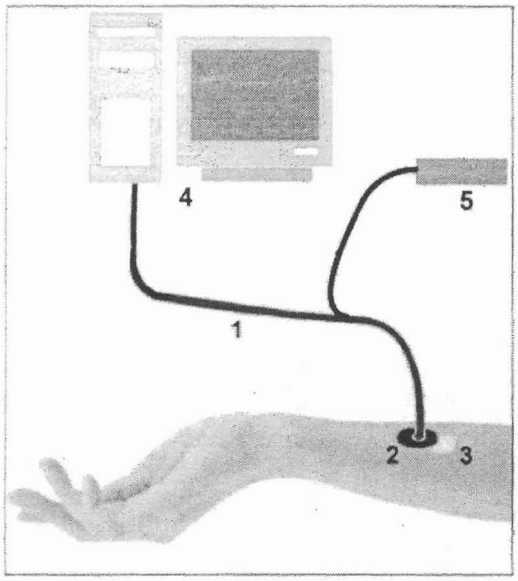

Fig. 2. Scheme of experimental setup for measurement of blood perfusion: 1 – optical cable with two optical fibres; 2 – the double sided adhesive disc to hold the position of the optical cable; 3 – the place of glucose injection (the light circle is the swelling of the skin); 4 – computer; 5 – diode laser.

In this study, we do not measure the absolute value of the blood velocity, but only a change in the average value of the velocity. For the relative measurements, two detectors have been used. The first one was positioned on the left and the second on the right forearm of the healthy volunteer as a comparison channel.

The measurements carried out on the practically healthy volunteers of both sexes with ages from 19 to 38 years. The intradermal injection of the osmotically active agent is a more acceptable way to decrease scattering properties of skin. Glucose solution was injected slowly into the dermal layer of skin (approximately 0.1 ml) of the volunteers forearm. In this case, the protective properties of the stratum corneum barrier will be avoided. The measurement of dynamics of the optical clearing was started at 60 sec. after the injection. All volunteers gave their informed consent for participation and the study protocol.

3. EXPERIMENTAL RESULTS

The large differences observed for different volunteers, normalized values of the perfusion represent on the graphs below, figure 4.

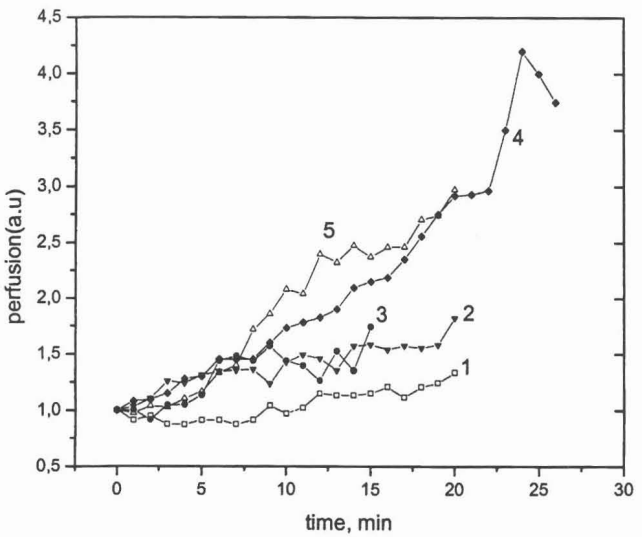

Fig.3. The normalized value of the blood perfusion versus time after the injection of 40% glucose solution for the different healthy volunteers (curves 1-6).

As can be seen from Fig. 4, the injection of glucose causes an increase in the perfusion of the blood; nevertheless, the dynamics of a change in the perfusion is different for the different volunteers. Bashkatov et al.[5] show that in the case of *in vivo* measurements at intradermal glucose injection the glucose solution localized only within skin and does not penetrate into subcutaneous muscle tissue. Respectively, we can assume the glucose will be spread after injection lengthwise the surface of skin.

4. THEORETICAL ESTIMATIONS

The mass transfer in dermis implicated to the glucose diffusion has complex nature and even in simplified physical form includes several stages. Part of glucose diffuses trough extracellular void and by osmotic transfer into tissue cells and later, when glucose during the time washed out, left cells. Other part of glucose, by extracellular space, permeates in blood capillaries and perfuse with the blood flow out from zone affected by injection. As was mentioned above, both tissue and blood systems are subjected to hyperosmotic effect due to high glucose solution concentration. Under hyperosmotic condition in tissue, the cell volume is shrinking with time and extracellular void increases. In the vascular system, glucose affects the shape of blood cells that results in rheology of blood flow.

If the gradient in permeant activity is localized within dermis layer, the steady-state flux, J, is then given by

$$J = -D_e \frac{dC}{dx}, \tag{1}$$

where D_e is the glucose effective diffusion coefficient, C is the glucose concentration, and x is the distance through the dermis (to the blood network), respectively.

We can assume the glucose concentration in the papula constant whereas the glucose concentration in the dermis is varying with time: from zero value to the maximum with further reducing. Moreover, the effective diffusion coefficient becomes a function of glucose concentration and time. The effective diffusion coefficient, D_e can be represented in the form[7]

$$D_e = D_0 \frac{\varepsilon}{\tau}, \tag{2}$$

where D_0 is the glucose diffusion coefficient in bulk liquid, ε is the extracellular void fraction, and τ is the tortuosity factor characterising the average tortuosity of the solute pathway.

For the dense living cell, packing at steady-state condition it was found that[7]

$$\sqrt{\tau} = 1/(0.23 + 0.3\varepsilon + \varepsilon^2) \tag{3}$$

Under osmotic condition ε becomes dependent on time, hence, effective diffusion coefficient in equation (1) is the function of time. The dependence of D_e / D_0 on ε in condition of equation (3) is given in figure 6, where the extracellular void fraction may be consider as the latent function of time.

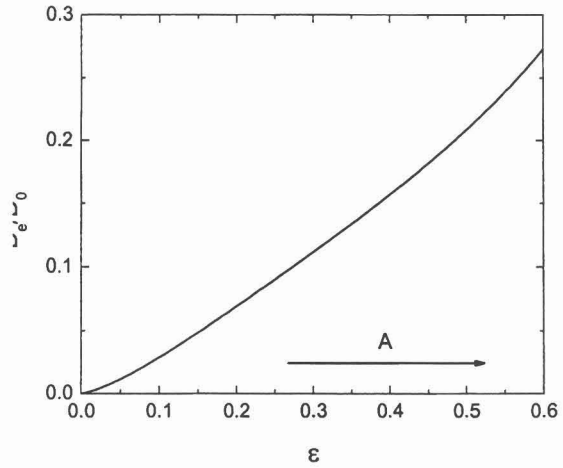

Fig. 4. Dependence D_e / D_0 vs. ε of the model (2) – (3). Arrow (A) shows the direction of extracellular void fraction increases under osmotic condition.

Based on figure 6 we can, estimate a non-linear effect of diffusion and mass transfer linked with the effective diffusion coefficient variation. In the range of the extracellular void fraction variation under osmotic stress from $\varepsilon \sim 0.25$ up to $\varepsilon \sim 0.5$, we have, respectively, increasing in diffusion from D_e / D_0 from 0.09 up to 0.21.

Obtained results and made estimations show the significant influence of dynamic characteristics on the dermis tissue when the optical active solution is used for the skin transporance control.

5. DISCUSSIONS AND CONCLUSIONS

Experiments demonstrate that the glucose solution affects significantly the blood perfusion and red cells concentration increase, whereas the mean blood velocity does not change significantly. Qualitative explanation was made for observed perfusion dynamic effect in the skin dermis based on the: tissue cells shrinkage and additional capillaries opening under osmotic stress. Based on the diffusion approach was shown that the cells shrinkage effect can be responsible up to half of the perfusion increase and the rest may be related with the capillaries opening phenomena. It was admitted, that the extracellular glucose diffusion is changing with time because of cells shrinkage under osmotic stress. Measured by ultrasonography the dynamic of glucose vesicle size and skin transparency window shows the glucose diffusion in the direction along the skin. The diffusion in the direction perpendicular to the skin surface was unelectable. Obtained results and estimations show the significant influence of the dynamic characteristics on the dermis tissue when the optical active solution is used for skin transparency control that must be taken into consideration.

ACKNOWLEDGEMENTS

This work supported in part by the Portuguese Foundation for Science and Technology, grants SERH/BPD/5556/2001, and the Byelorussians Foundations for Basic Research, project F04-050.

REFERENCES

1. V.V. Tuchin, A.N. Baskatov, E.A. Genina, Yu.P. Sinichkin, N.A.Lakodina, "In vivo investigation of the immersion-liquid-induced human skin clearing dynamics," *Tech. Physics Lett.*, **27**, pp. 489-490, 2001.
2. G.Vargas, K.F. Chan, S.L. Thomsen, A.J. Welch, "Use of osmotically active chemical agents to alter the optical properties of tissue: Effects on the detected fluorescence signal measured through skin," *Laser in Surgery and Medicine*, **29**, pp. 213-220, 2001.
3. E. I. Galanzha, V. V. Tuchin, A. V. Solovieva, T. V. Stepanova, Q. Luo and H. Cheng, "Skin backreflectance and microvascular system functioning at the action of osmotic agents," *J. Phys. D: Appl. Phys.*, **36**, pp. 1739–1746, 2003.
4. K.P. Ananthapadmanabhan, A.Lips, C. Vincent, F. Meyer, S. Caso, A. Johnson, K. Subramanyan, M. Vethamuthu, G. Rattinger, D.J. Moore, "pH-induced alterations in stratum corneum properties," *International journal of cosmetic science*, **25**, pp. 103-112, 2003.
5. A. N. Bashkatov, A. N. Korolevich, V. V. Tuchin, Y. P. Sinichkin, E. A. Genina, M. M. Stolnitz, N. S. Dubina, S. I. Vecherinski, M. S. Belsley," In vivo investigation of human skin optical clearing and blood microcirculation under the action of glucose solution," *Asian Journal of Physics*, **14(1)**, 2005 (will printed).
6. A.N. Baskatov, E.A.Genina, Yu.P. Sinichkin, V.I. Kochubey, N.A. Lakodina, V.V. Tuchin," Glucose and mannitol diffusion in human *Dura Mete," Biophysical Journal*, **85**, pp. 3310-3318, 2003.
7. M. Mota, J. A. Teixeira, J. B. Keating and A. Yelshin," Changes in diffusion through the brain extracellular space," *Biotechnol. Appl. Biochem.* ,**39**, 223–232, 2004.
8. G.J. Tortora, and S.R. Grabowski. *Structure of the Skin. Principles of Anatomy and Physiology*, John Wiley & Sons, Inc., pp 140-145, 2000.
9. R. Bonner, R. Nossal. Model for the laser Doppler measurements of blood flow in tissue. Applied Optics, **20**, 2097-2107, 1981.
10. R.F. Schmidt and G. Thews, *Human Physiology*, Berlin, Germany: Springer-Verlag, 1989.
11. R. F. Bonner, and R. Nossal," Principles of laser Doppler flowmetry," in: *Laser Doppler blood flowmetry*, ed. A.P. Sheperd, P.A.Oberg, Kluwer Academic Publisher, Boston , 1990.
12. J.A. Davidson, S. Gir, J. Paul," Heat transfer analysis of frictional heat dissipation during articulation of femoral implants,"*J. Biomed. Mat. Res.*, **22**, pp. 281-309, 1988.
13. G. Cevc, "Drug delivery across the skin", *Expert Opin. Invest. Drug.*, **6(12)**, pp. 1887-1937, 1997.
14. Y.M. Lee, S. H. Lee, G. Ko, J. B. Kim, D. H. Sohn, "Effect of Benzalkonium Chloride on Percutaneous Absorption of Antisense Phoshorothioate Oligonucleotides," *Arch. Pharm. Res.*, **19(5)**, pp. 435-440, 1996.
15. P. A. Payne," Measurement of properties and function of skin, "*Clin. Phys. Physiol. Was.*, **12(2)**, pp. 105-129, 1991.