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In vivo Investigation of the Immersion-Liquid-Induced Human Skin Clearing Dynamics

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Abstract—The results of an *in vivo* investigation of human skin clearing caused by the immersion-liquid-induced matching of the refractive indices are reported for the first time. It was established that subcutaneous injections of a glucose solution produce a significant long-term suppression of the light scattering in the skin dermis, which is an important factor ensuring an increase in efficacy of the optical tomography, photodynamic therapy, and photodestruction of deep objects. © *2001 MAIK "Nauka/Interperiodica".*

The possibility of controlling the optical properties of biological tissues is an important factor for the development of optical tomography, photodynamic therapy, and photodestruction methods [1–13]. An effective means of changing the optical properties of biological tissues is based on using osmotically active liquids such as glucose, glycerol, propylene glycol. Below we report for the first time on the results of an *in vivo* investigation of the human skin clearing effect, whereby a long-term suppression of the light scattering in the skin dermis is caused by subcutaneous injections of a glucose solution.

The optical characteristics of the human skin are mostly determined by the properties of the dermis, which accounts for ~95% of the total skin thickness and has a refractive index comparable to that of the epidermis [8, 9]. In the optical transparency interval of the skin (600–1400 nm), the absorption coefficients of both dermis and epidermis amount to less than one-tenth of the corresponding light scattering coefficients.

The results of the previous *in vitro* investigations revealed a number of features in the interaction of osmotically active liquids with biological tissues [1–13]. Introduced into a biological tissue (usually by diffusion through the sample surface), an immersion liquid with a refractive index greater than that of the interstitial and intercellular substances would decrease the difference in refractive indices between the scatterers (collagenic and elastin fibrils, melanin granules, cell membranes, etc. [9, 14]) and their environment. This matching is manifested by optical clearing of the tissue. The matching of refractive indices is also favored by the osmotic migration of water (shrinkage), which increases the concentration of organic substances in the interstitial space and, hence, the refractive index of the interstitial substance. Although the above interactions also affect the tissue layer thickness and the scatterer size and concentration, the immersion-liquid-induced matching effect dominates and leads to a significant optical clearing of the tissues: the transmission coefficient increases from several times (for the human skin) to several tens of times (for the human sclera) [4–6, 9, 11–13]. For the sclera, the clearing effect begins 8–10 min after immersion, while the skin clearing development requires about one hour.

It was expected that, on the whole, the dynamics of skin clearing *in vivo* would follow the pattern established *in vitro*. However, significant quantitative changes were expected in the time response as determined by the method of immersion liquid introduction (subcutaneous injection) and by the living organism's reaction to the treatment.

A 40% glucose solution was introduced into a volunteer in a volume of 0.1 ml by single subcutaneous injection into the inner side of the forearm. The light reflection spectra in the 400–800 nm range were measured with an optical multichannel analyzer of the LESA-6Med type (BioMed, Russia) linked to a fiber transducer. The transducer, comprising a system of optical fibers, can be represented by an effective system of two fibers (source and detector) with an equivalent distance of $r_{sd} = 2.5$ mm and an average probing depth of $0.35r_{sd} = 0.9$ mm [9].

Figures 1 and 2 show the reflection spectra and the time variation of the human skin reflectance measured at various light wavelengths. As is seen, the reflection spectra show a scattering background determined by the diffuse reflection of the skin with well-pronounced bands due to the blood optical absorption. Within one hour after injection of the glucose solution, the skin reflection coefficient K decreases on average by a factor of 3.8 and then exhibits a slow increase, which indicates that glucose is eliminated from the observation area and the skin reflectance tends to restore itself to the initial level. Based on the results of *in vitro* measurements and the proposed skin clearing model, we may

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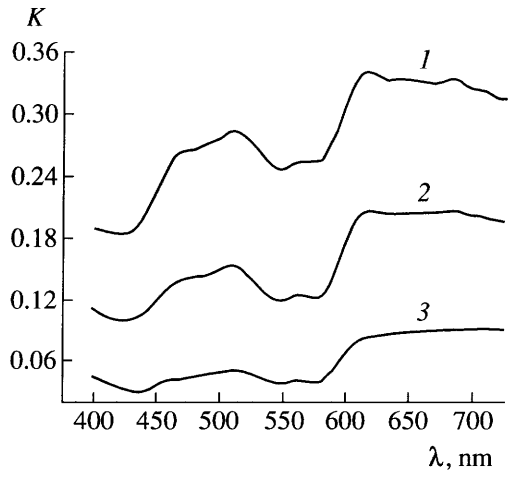

Fig. 1. Optical reflection spectra of human skin measured (*1*) before and (*2*, *3*) 23 and 60 min after subcutaneous injection of a 40% glucose solution.

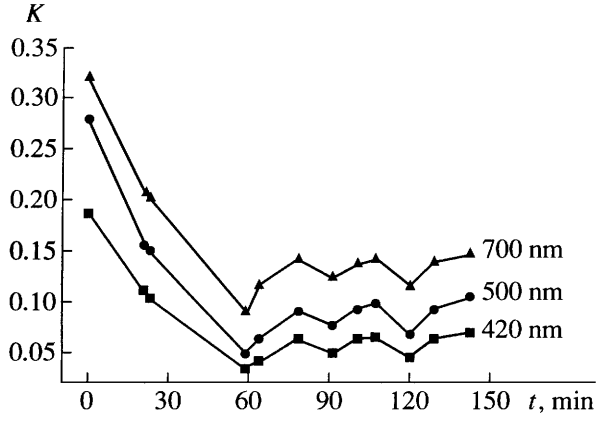

Fig. 2. Time variation of the reflection coefficient K of the human skin measured at various wavelengths after subcutaneous injections of a 40% glucose solution.

suggest that the main contribution to clearing in the first stage (first hour) is due to the refractive index matching between collagenic fibrils of the dermis (n = 1.46) and the interfibril space (n = 1.36) [9, 14] to which glucose (n = 1.39) diffuses. Using a method described in [15], we may estimate the coefficient of glucose diffusion in the human dermis from our experimental data: $D_G = (2.56 \pm 0.13) \times 10^{-6}$ cm^2/s. This value is 3.6 times lower than the coefficient of glucose diffusion in water at 37°C ($D_G \approx 9.2 \times 10^{-6}$ cm^2/s) estimated by extrapolating the data reported in [16]. The difference naturally reflects the character of the skin tissue permeability for glucose.

It should be noted that the skin clearing is retained for several hours, the initial reflectance level restoration being slow and exhibiting oscillations with a period of about 30 min. Using the above glucose diffusion coefficient $D_G = (2.56 \pm 0.13) \times 10^{-6}$ cm^2/s, we may readily estimate the time required for glucose to impregnate a 0.9-mm-thick dermis layer: $\tau = l^2/D_G \approx 53$ min [4, 5]. This is the time of glucose outdiffusion from the region of injection to the epidermis (the time of clearing development). In the stage of the initial reflectance level restoration (clearing removal), the major role belongs to the lateral diffusion in the skin and the downward diffusion. For a significant decrease in the skin clearing, it is necessary that glucose diffuse out of the region sensed by the transducer by a distance of least $l = (0.5–0.7)r_{sd} = 1.25–1.75$ mm, which requires approximately from 1.7 to 3.3 h in agreement with experiment (Fig. 2).

The oscillatory character of reflectance in the clearing removal stage is related to the complex dynamic character of the process of diffusion in a multicomponent biological tissue. Explaining this behavior would require additional investigation.

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