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The Interaction of Indocyanine Green Dye with the Human Skin Epiderm Studied *In Vivo*

É. A. Genina[a]*, A. N. Bashkatov[a], V. I. Kochubei[a], V. V. Tuchin[a], and G. B. Al'tshuler[b]

[a]*Saratov State University, Saratov, Russia;*

**@e-mail: eagenina@optics.sgu.ru*

[b]*Laser Research Center, Institute of High-Precision Mechanics and Optics, St. Petersburg, Russia*

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Abstract—A shift of the light wavelength corresponding to the optical absorption peak of Indocyanine Green used for staining the horny layer of the human epiderm was measured *in vivo*. It is established that the dye molecules occur in both free and chemically bound state at the skin surface and only in the bound state at a depth of ~5 μm. *© 2001 MAIK "Nauka/Interperiodica".*

Indocyanine Green is a biologically active dye which is widely used in various fields of medicine owing to a strong optical absorption in the near IR range, low toxicity, and rapid elimination from the human organism. For a long time this compound was used only for diagnostic purposes [1–6]. However, in recent years the field of application increased to include the phototherapy of tumors [7–11], photocoagulation [12] and repair (welding) [13] of tissues, and the treatment of skin and hair folliculi disorders [14], which is related to a significant optical absorption in the region of wavelengths generated by high- power diode lasers.

Indocyanine Green is a tricarbocyanine dye exhibiting a clearly pronounced absorption peak in the near IR range (~790 nm) and virtually not absorbing in the visible range. The compound corresponds to the empirical formula $C_{43}H_{47}N_2O_6S_2Na$ and has a molecular weight of 775. A relatively small width of the absorption peak allows this dye to be used in the selective laser thermolysis process, but the interaction with biological tissues leads to a change in the absorption spectrum of Indocyanine Green.

The interaction of Indocyanine Green dye (IGD) with cell proteins was experimentally studied. It was established that binding of the dye molecules to various organic molecules in biological tissues leads to a shift of the absorption peak toward longer wavelengths. The absorption peak maximum was observed at 805 nm in the human blood [15] and human skin *in vivo* [16] and at 810 nm in the epiderm cell cultures [17]. It should be noted that the reflected signal observed in [16] was formed predominantly in a deep layer of the skin characterized by a high blood supply. This circumstance explains identical positions of the IGD absorption peak observed in the human blood and skin. Refinement of the wavelength corresponding to the maximum absorption of IGD interacting with the human skin *in vivo* is very important for correctly choosing the operating laser wavelength used for the selective laser thermolysis.

The purpose of this study was to determine *in vivo* a shift of the absorption maximum of IGD solutions used in the laser thermolysis process, which takes place as a result of the dye interaction with a horny layer of the human epiderm.

The experiments were performed with thin pieces of the human skin stained with IGD solutions. The samples were obtained by sequentially (layer-by-layer) tearing the skin off with the aid of a sticky ribbon (Multi-Film, Tesa, Biersdorf, Hamburg) from the same area (on the inner forearm surface). The absorption spectra of the samples attached to microscope glasses were measured in a wavelength range from 350 to 1200 nm using a CARY-2415 spectrophotometer equipped with an integrating sphere. We employed two dye solutions based on glycerol and ethyl alcohol. The first solution contained an additional component—dimethylsulfoxide (DMSO); the second solution contained a greater proportion of ethyl alcohol.

Figures 1 and 2 show the absorption (optical density) spectra of three sequentially separated skin layers stained with IGD solutions. The thickness of each layer was about 3–5 μm. As is seen, the IGD absorption peak maximum in the skin is shifted toward longer wavelengths as compared to the peak in solution (the latter is shown for comparison in each figure with an intensity reduced by a factor of five). The shift is indicative of the fact that IGD molecules occur in a horny layer of the human epiderm in a chemically bound state.

In the spectra of samples prepared using the first solution (Fig. 1), the absorption peak shift amounts to 11 nm. In this experiment, the dye solution was thoroughly washed from the skin prior to sampling. Coincidence of the peak positions in the spectra of the first and second layers indicates that no free IGD molecules were present on the skin surface. In Fig. 2, the positions

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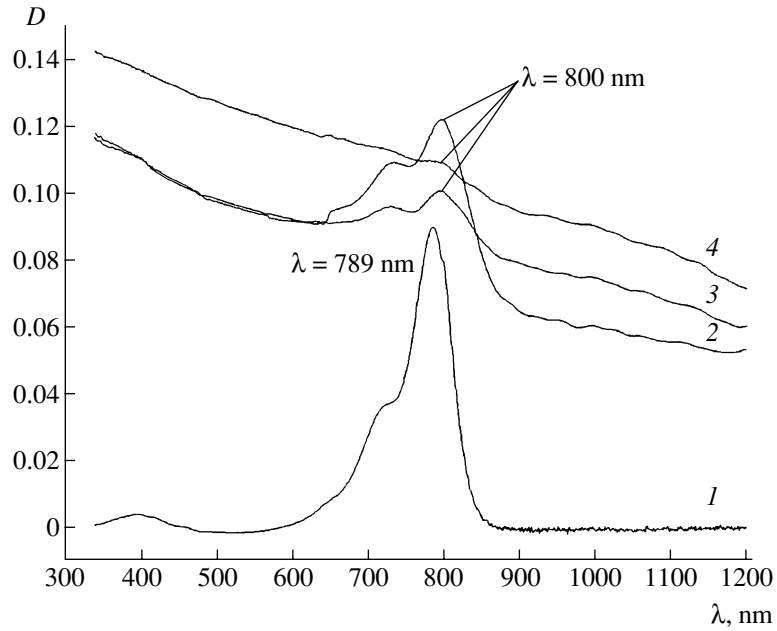

Fig. 1. Optical density spectra of Indocyanine Green (*1*) in a glycerol–ethanol–DMSO solution and (*2–4*) in three sequentially separated layers (first, second, and third, respectively) of the human skin stained with this solution.

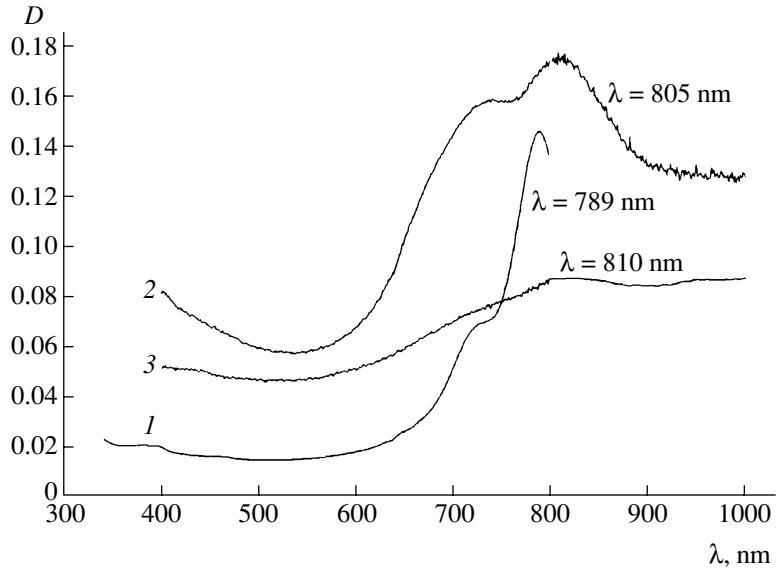

Fig. 2. Optical density spectra of Indocyanine Green (*1*) in a glycerol–ethanol solution and (*2, 3*) in two sequentially separated layers (first and second, respectively) of the human skin stained with this solution.

of maximum absorption in the spectra of the first and second layers are different: the latter peak is still more shifted toward longer wavelengths (by 21 nm) than the former one (16 nm). This difference suggests that the surface layer contains both free and bound dye molecules, whereas only the bound IGD is present at a depth of ~5 μm.

An analysis of the absorption spectra of IGD in solutions and tissues also revealed a change in shape of the spectra. The main peak significantly decreases in relative intensity and becomes comparable with the second peak observed at ~715 nm. This shape of the IGD spectrum is typical of the dye molecules in water, blood plasma, and albumin solutions [18]. Since a major protein in the human epiderm is keratin, the shift of the absorption peak position and the change in shape of the spectrum are most likely explained by the dye binding to keratin.

Thus, we have determined for the first time *in vivo* a shift of the absorption maximum of Indocyanine Green

dye used to stain a horny layer of the human epiderm, This change in the optical absorption spectrum of the dye widely used in selective laser thermolysis must be taken into account in choosing radiation sources ensuring the most effective removal of skin tumors, treatment of skin disorders, hair depilation, etc.

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SPELL: folliculi, depilation