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Use of Optical-Spectral Methods for *in vivo* Noninvasive Assessment of Nanoparticles Accumulation in Biological Tissues

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Abstract—Methods for noninvasive estimation of the nanoparticles concentration in biological tissues *in vivo* were developed with a view to laying the scientific groundwork for diagnosis and treatment of malignant tumors. The methods were tested in experimental animals (mice inoculated with Ehrlich carcinoma). Luminescent spectroscopy and diffuse reflectance spectroscopy were applied for examination of biotissues. A special algorithm of spectral data analysis with the use of the model solution simulating the optical properties of biotissues was devised. It was shown that the optical-spectral methods developed are effective in high-accuracy noninvasive real-time quantitative analysis of the nanoparticles accumulation in biotissues.

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INTRODUCTION

Nanotechnology-based approaches are being actively promoted now in various sectors of biomedicine. One of the main medicinal applications of nanotechnologies is oncology; even a new direction referred to as "cancer nanotechnology" in the English-language scientific literature arose [1]. It is supposed that nanotechnologies will be able to provide solutions to problems of early diagnosis and determination of the location of malignant tumors and performance of targeted drug delivery thereto and will enable development of new methods of selective therapy. The most promising approaches to addressing these problems are based on nanotechnologies thriving from modern advances in biophotonics. These include, above all, visualization of tissues with nanoobjects acting as luminescent markers, as well as development of nanoscale optical biosensors, phototherapy of tumors with the use of nanoparticles as active light-absorbing agents, and photoinduced drug delivery in nanocapsules.

Introduction of nanotechnologies in biomedicine had posed a number of fundamental problems whose overcoming would contribute to successful development of new methods of diagnosis and therapy. For example, clinical trials of several nanoparticles-based substances are already underway now, and such substances will only increase in number further [2, 3]. In this context, estimation of the nanoparticles accumulation directly in organs and tissues *in vivo* is of vital importance. The task of studying nanoparticles at the cellular level may be considered as successfully resolved, while noninvasive studies of biodistribution of the nanoparticles directly in a living organism still cause difficulties. Methods used for this purpose are typically nonuniversal and suitable for studying a fairly narrow range of nanoparticles possessing specific properties [4, 5].

For solving nanobiomedicine tasks, including that of examination of nanoparticles in biological tissues *in vivo*, optical-spectroscopic techniques, diffuse light scattering spectroscopy and luminescent spectroscopy, offer an attractive opportunity. These methods have long been used for estimating the concentration of molecular agents, photosensitizers, in biotissues as part of fluorescence diagnosis and photodynamic therapy monitoring.

Diffuse reflectance spectroscopy is widely applied for diagnosis and monitoring of physiological processes in tissues. The pioneering work by Jöbsis [6] dedicated to application of diffuse reflectance IR spectroscopy for noninvasive monitoring of oxygenation was published more than 30 years ago. Since then, the

spectroscopic analysis technique has been perfected, and its application scope has considerably expanded [7], from monitoring of oxygenation of hemoglobin and respiratory chain enzymes (cytochromes) to cancer diagnosis [8, 9] and estimation of the concentration of medicinal substances in the molecular form in biological tissues [10–12].

Another practical application of diffuse reflectance spectroscopy is estimation of the nanoparticles concentration in biological tissues. Metal (gold, silver, etc.) nanoparticles, along with dielectric nanoparticles coated with metal layers, have a pronounced plasmon resonance absorption peak. Its position depends on the particle size and shape and on the metallic coating thickness, as well as on the nearest surrounding of the nanoparticles in tissues and the degree of their aggregation. The optical absorption coefficient at the plasmon resonance frequency of the nanoparticles is fairly high, which enables recording their contribution to the total light absorption by biotissues using the diffuse reflectance spectroscopy technique. By analyzing the intensity, position, and half-width of the plasmon resonance peak of the nanoparticles in tissues it is possible to estimate their concentration and the degree of aggregation, as well as to elucidate the nature of their local environment.

Laser-induced fluorescence spectroscopy is a method that has extensive nanotechnological applications for several decades already. In medicine it is used for examination of the nanoparticles by fluorescence microscopy [13] and for visualization of tumors [14]. A disadvantage of the former technique is that, being typically concerned with distant tissues, it is not noninvasive and, moreover, entails a complex procedure which takes much time and requires expensive equipment. The latter technique involves a difficulty with gaining a quantitative result. The fiber-optic fluorescence spectroscopy method has been widely used in studies of luminescent proteins and various dyes *in vivo* [15], but its use for nanoobjects has not become sufficiently widespread as yet.

Here, we report the results of a study into the possibilities of estimating the nanoparticles concentration in biotissues *in vivo* by luminescent spectroscopy and diffuse reflectance spectroscopy.

EXPERIMENTAL

The spectral data were recorded and analyzed using a LESA-01 BIOSPEC fiber-optic spectrum analyzer [16]. Fluorescence was excited by red radiation using an He–Ne laser (wavelength 632.8 nm, power 20 mW) or by green radiation using an Nd : YAG laser (wavelength 532 nm, power 20 mW). The absorption/reflectance spectra were recorded with the aid of a halogen light source.

The technique developed was tested in animal experiments with mice (female, 25–30 g in weight, 9–10 weeks of age) inoculated intramuscularly in the right hind leg with Ehrlich carcinoma. The other hind leg was regarded as normal tissue. The wool in the areas examined was removed 24 h before the experiment using Opilca special hair removal cream.

The test animals were administered the following nanoparticles:

(1) Carbon-coated nickel nanoparticles Ni@C (synthesized with the use of an original method by Prof. A.E. Ermakov, Institute of Metal Physics, Ural Branch, Russian Academy of Sciences). Before introduction, the nanoparticles were ultrasonically dispersed with a biocompatible polymer (Proxanol-268) in distilled water. The resulting colloidal solution was injected into the tail vein of mice at the dose of 100 mg kg^{-1} of body weight.

(2) Gold nanorods. A colloidal solution (solvent saline) was injected into the tail vein of the animal.

(3) CdTe quantum dots. The solution was injected into the tail vein at the dose of 20 mg kg^{-1} of body weight.

(4) Iron(III) oxide nanoparticles (NanoArc®) coated with photoactive zinc phthalocyanine monoanhydride ZnPc (ZnPc was synthesized by Prof. E.A. Luk'yanets, Research Institute of Organic Intermediates and Dyes, State Scientific Center, Federal State Unitary Enterprise; conjugation of the iron(III) oxide nanoparticles to ZnPc was performed with the use of an original technique by A.I. Klimov, Department of Chemistry, Lomonosov Moscow State University). The weight ratio of iron(III) oxide to phthalocyanine was 40/1. The colloidal nanoparticles solution (solvent saline) was injected into the tail vein of the animal at the dose of 40 mg kg^{-1} of body weight (1 mg kg^{-1} of body weight, based on the ZnPc content).

The choice of the nanoparticles for testing the technique was dictated, first, by their application prospects for diagnosis and/or treatment of malignant tumors and, second, by the differences in their optical properties (ability for luminescence, specific light absorption features, etc.).

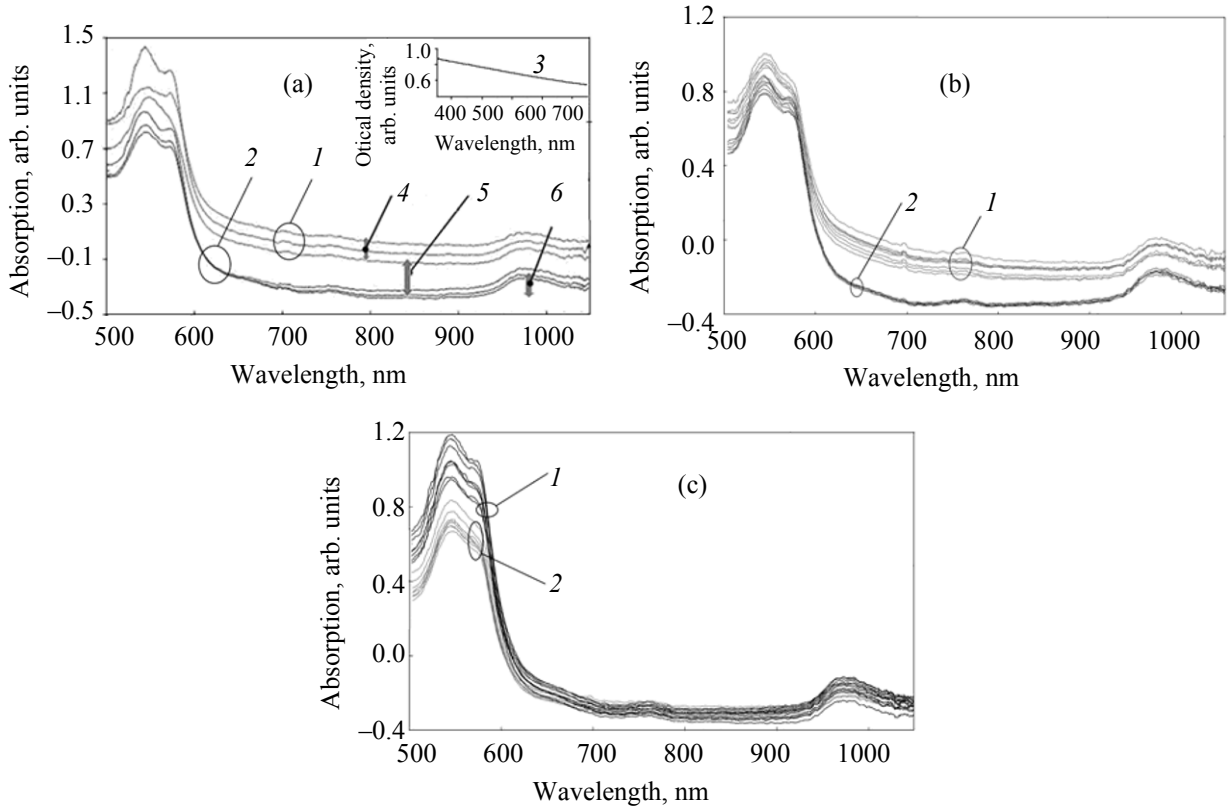

Fig. 1. (*1*) Diffuse reflectance spectra measured in the (a, b) tumor tissue (a) 1 h and (b) 20 h and (c) normal tissue 20 h after intravenous injection of the Ni@C nanoparticles at the dose of 100 mg kg^{-1} of body weight; (*2*) absorption spectra measured in the tumor tissue without nanoparticles; (*3*) optical density of the colloidal solution of the Ni@C nanoparticles (concentration 100 mg L^{-1}, sample thickness 5 mm); (*4*) inhomogeneity of biotissue and distribution of nanoparticles, (*5*) contribution from nanoparticles, and (*6*) water.

The nanoparticles accumulation in biotissues was quantitatively assessed using standard samples, specifically, substances simulating the scattering properties of the biological tissue examined and containing nanoparticles in known concentrations. Standard samples were prepared on the basis of Lipofundin MCT/LCT fat emulsion (B. Braun Melsungen). The nanoparticles examined were added into the emulsion adjusted to the concentration of 1.6% with saline, whereupon spectral measurements were performed. The measured data were employed for plotting a calibration curve which demonstrates how the fluorescence or absorption varies with the nanoparticles concentration and which was further used for estimating the nanoparticles concentration based on the signal coming from the biotissue.

RESULTS AND DISCUSSION

The diffuse reflectance spectra measured in the tumor tissue 1 and 20 h after intravenous injection of the Ni@C nanoparticles to animals at the dose of 100 mg kg^{-1} (Fig. 1) were compared with the spectra characterizing the nanoparticles accumulation in the normal muscle tissue. On this basis, the contribution from the nanoparticles to the total light absorption by

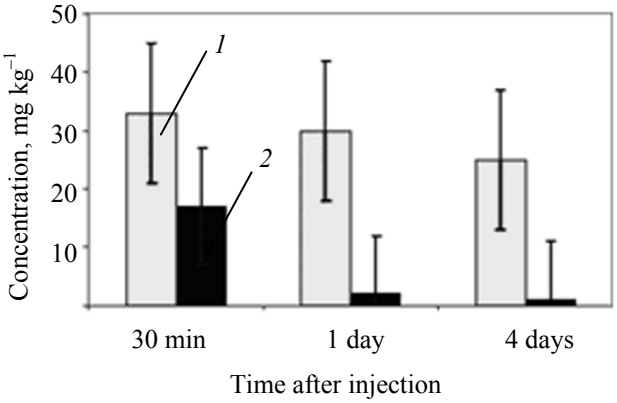

Fig. 2. Concentration of the Ni@C nanoparticles in the tumor and normal muscle tissue at different time intervals after injection of the nanoparticles: (*1*) tumor and (*2*) norm.

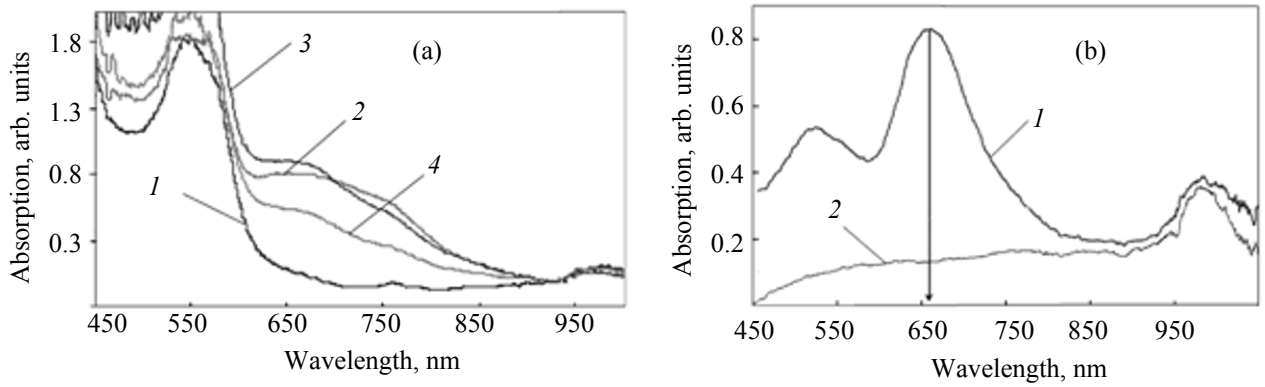

Fig. 3. Diffuse reflectance spectra measured in the tumor tissue before and after intravenous injection of (a) gold nanorods and (b) model solution (Lipofundin MCT/LCT, 1.6%). (a): Spectrum recorded (*1*) before and (*2*) 5 min, (*3*) 24 h and (*4*) 48 h after injection of the nanorods. (b): Spectrum recorded for solution (*1*) with 20 mg L^{-1} nanorods and (*2*) without nanorods injected.

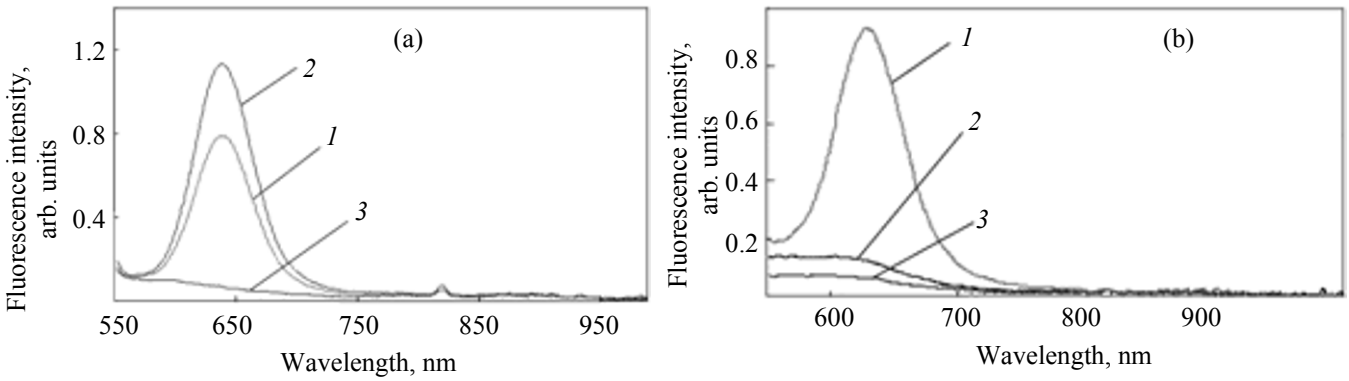

Fig. 4. Fluorescence spectra of the tumor and normal muscle tissue, measured (a) 5 min and (b) 20 h after intravenous injection of the CdTe quantum dots (dose 20 mg kg^{-1} of body weight): (*1, 3*) spectrum of tumor recorded (*1*) after and (*3*) before injection of the quantum dots and (*2*) spectrum of the normal tissue. Fluorescence was excited by 532 nm light.

the biological tissue was estimated, and the nanoparticles accumulation was quantitatively assessed (Fig. 2).

Importantly, the Ni@C nanoparticles do not have a pronounced absorption peak in the visible region of the spectrum.

Figure 3 shows the diffuse reflectance spectra measured in the tumor before and after intravenous injection of the gold nanorods, recorded at different time intervals. By contrast to the Ni@C nanoparticles, the gold nanorods have a pronounced plasmon resonance absorption peak in the visible spectral region (see Fig. 3b); they are stably retained in the tumor for at least two days.

The fluorescence spectra of the tumor and normal muscle tissue, measured after intravenous injection of the CdTe quantum dots at the dose of 20 mg kg^{-1} of body weight, are presented in Fig. 4. It is seen that, despite the lack of the nanoparticles accumulation contrast for the tumor and normal tissue immediately after introduction of the quantum dots, the accumulation contrast of 5 is reached after one day, with the nanoparticles being almost entirely eliminated from the normal tissue.

Figure 5 presents the fluorescence spectra of the tumor and the normal muscle tissue, measured 24 h after introduction of the ZnPc-coated iron(III) oxide nanoparticles. It is seen that the substance is accumulated both in the tumor and normal tissue; the accumulation contrast of 3 is reached.

CONCLUSIONS

The fluorescence spectroscopy and diffuse reflectance spectroscopy methods are efficient in studying the nanoparticles accumulation and distribution in

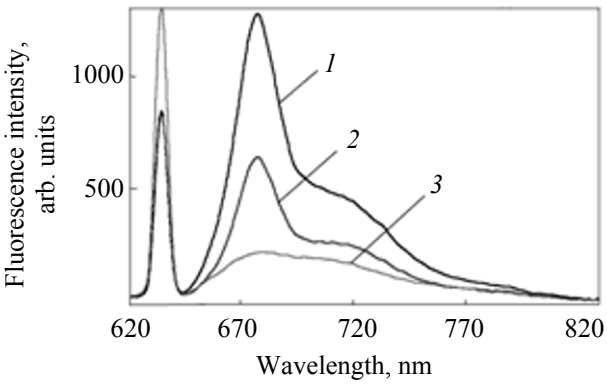

Fig. 5. Fluorescence spectra measured in the tumor and normal muscle tissue 24 h after injection of the ZnPc-coated iron(III) oxide nanoparticles: (*1*) tumor, (*2*) normal tissue, and (*3*) healthy skin of a human hand. Fluorescence was excited by 632.8 nm light.

living organisms. The advantages offered by these approaches include noninvasiveness (the desired information can be derived without affecting the dynamics of biological processes), the possibility of obtaining real-time results and thereby adjusting the treatment regime, and flexibility (detectable are most of the nanoparticles promising for medical applications).

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