Investigation of the change of tumor optical properties after laser-induced plasmon-resonant photothermal treatment of transplanted tumors in rats

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ABSTRACT

The paper presents the investigation of change of tumor optical properties of the rat tumor doped by gold nanoparticles after laser-induced plasmon-resonant photothermal treatment. To obtain the model tumors the rats have been implanted by suspension of alveolar kidney cancer cells. An hour before the experiment the animals have been injected by the suspension of gold nanorods intratumorally. For irradiation a diode laser with wavelength 808 nm has been used. After the irradiation the tumor has been removed and sliced. Spectra of total and collimated transmission and diffuse reflectance of the samples of different layers of the tumors have been measured in the wavelength range 350-2500 nm. Absorption, scattering, reduced scattering coefficients and scattering anisotropy factor of tumor tissues have been calculated with inverse adding-doubling method. The results of the experiment have shown that after doping the tumor tissue by the plasmon resonant nanoparticles and NIR laser irradiating, there is the decreases of absorption as well as scattering properties of the tumor and surrounding tissues. However, despite the sufficiently high temperature on the surface (about 80°C), the changes in the center of the tumor are insignificant.

Keywords: gold nanorods, plasmon-resonant photothermal treatment, optical properties

1. INTRODUCTION

The laser irradiation of tissues is accompanied by the hyperthermia that is used for a therapy of cancer tumors at the temperature 41-45°C[1]. Today nanoparticles are used for the increase of the laser radiation selectivity[2-4]. Plasmon-resonant nanoparticles are capable to excite local surface plasmon resonance in visible and NIR ranges of spectrum[5]. Such nanoparticles are capable for generating a thermal energy, which makes it possible to reduce the dose of laser radiation. It was shown that gold nanorods (GNRs) are suitable for photothermal therapy due to their extended lifetime in the bloodstream, colloidal stability, easy tuning of their plasmonic resonance by changing the aspect ratio of nanorods[6] and effective converting the light radiation into thermal energy[7,8].

Successful phototherapy of tumors sensitized with nanoparticles requires the solution of additional problems associated with the introduction of nanoparticles, their biodistribution in the tumor, the delivery of laser radiation to the interior of the tumor, and optimization of the concentration of nanoparticles and the dose of irradiation[9].

The effectiveness of thermal effects on tumor tissues at different depths can be assessed by measuring the optical parameters of irradiated and non-irradiated tissue. However, this problem remains insufficiently studied.

The objective of the study is investigation of the change of tumor optical properties of the rat tumor doped by GNRs after laser-induced plasmon-resonant photothermal treatment.

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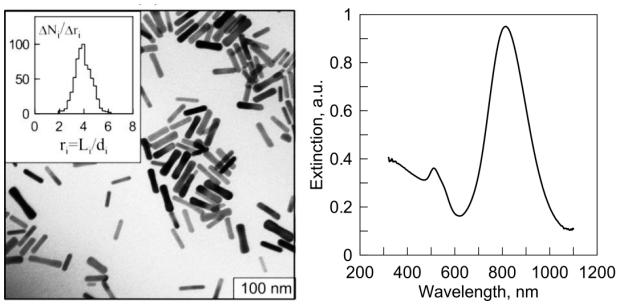
2. MATERIALS AND METHODS

2.1 Nanoparticles

The gold nanorods were synthesized in Laboratory of Nanoscale Biosensors of Institute of Biochemistry and Physiology of Plants and Microorganisms RAS. For aggregation preventing, the GNRs were functionalized by thiolated polyethylene glycol (molecular weight 5000 Da, Nektar, USA).

Figure 1(a) shows the image of the particles. The sizes of the GNRs were: 41±8 nm (length) and 10±2 nm (diameter); concentration in suspension was 400 pg/mL (optical density 20 on the wavelength 800 nm). Before measuring the extinction coefficient, the suspension was diluted 1:10. The spectrum of the extinction coefficient is shown in Fig. 1(b).



a b

Figure 1. The TEM image of gold nanoparticles and their size distribution (a) and the extinction spectrum of a suspension of gold nanorods with an optical density of 2 (b). The insert in (a) shows a histogram of the particle distribution $\Delta N_i / \Delta r_i$ with respect to $r_i = L_i / d_i$ with an average value of $r_i = 4.03 \pm 0.7$[10].

2.2 Animal preparation

Outbred albino male rats with the weight 160-200 g were used. To obtain the model tumors the rats were implanted by 0.5 mL of 25% suspension of alveolar kidney cancer cells. The experimental model of rat cancer was reproduced by transplantation of tumor cell suspension of cancer, obtained from the bank of tumor strains of Russian Cancer Research Center n.a. N.N. Blokhin.

When the tumor reached the diameter 3.0±0.3 cm^2 the animals were randomly divided into two groups: control and experimental ones. Before the experiment, the animals were anesthetized with Zoletil 50 (Virbac, France). The dose was 0.5 mg/kg.

An hour before the experiment the animals from experimental group were injected by the suspension of the GNRs from 3 points intratumorally with injection speed 0.1 mL/min. This method of injection leads to nanoparticles accumulation and retention in the tumor.

2.3 Design of experiment

The rats from experimental group were subjected to the plasmonic photothermal therapy (PPTT). For irradiation we used diode laser LS-2-N-808-10000 (wavelength 808 nm, power density 2.3 W/cm^2, the area of the irradiation spot ~0.5 $см^2$, time of radiation 7.5 min) (Laser Systems, Ltd., S.Petersburg, Russia). The temperature of skin surface was registered with IR vizualizer IRI4010 (IRYSYS, UK) every 0.5 min.

The temperature kinetics can be described well by two-exponential dependence:

$$y = A_1 \exp\left(-t / \tau_1\right) + A_2 \exp\left(-t / \tau_2\right) + y_0, \quad (1)$$

where A_1 and A_2 are the empirical constants, τ_1 и τ_2 are the characteristic times of tissue heating processes, y_0 is the maximum signal level. The characteristic times are $\tau_1 = 0.24 \pm 0.05$ min and $\tau_2 = 4.07 \pm 0.6$ min, y_0 reaches 85.8°C.

In the control group the rats did not injected the GNR suspension and did not exposed.

Next day all rats withdrawal from the experiment. The tumors were removed and frozen at the temperature -20°C during 24 hrs. Then the tumors were sliced by the following samples: skin above the tumor, tumor capsule, tumor body on the periphery, tumor body in the centre.

The samples were put between two slides and fixed. The thickness of the samples was measured with a micrometer with an accuracy of ±10 μm at five points at the periphery and center of the sample, the results averaged, and the standard deviation was calculated. The length and width of the sample were measured with a ruler with an accuracy of ±0.5 mm.

Spectra of total and collimated transmission and diffuse reflectance of the samples of different layers of the tumors were measured at the wavelength range 350-2200 nm. For the spectral measurements spectrophotometer UV-3600 (Shimatzu, Japan) with integrating sphere LISR-3100 (Shimatzu, Japan) was used. Absorption, scattering, reduced scattering coefficients and scattering anisotropy factor of tumor tissues were calculated with inverse adding-doubling method[11-13].

3. RESULTS AND DISCUSSION

The experimental group of the animals was underwent laser irradiation (at a wavelength of 808 nm) after the nanoparticle intratumoral injection. As a result, the temperature of the skin surface above the tumor was increased in the irradiation region due to the heating of plasmon nanoparticles absorbing radiation. The kinetics of the temperature change is shown in Figure 2. The figure shows clearly that within 7 minutes the temperature increased by 2.6 folds.

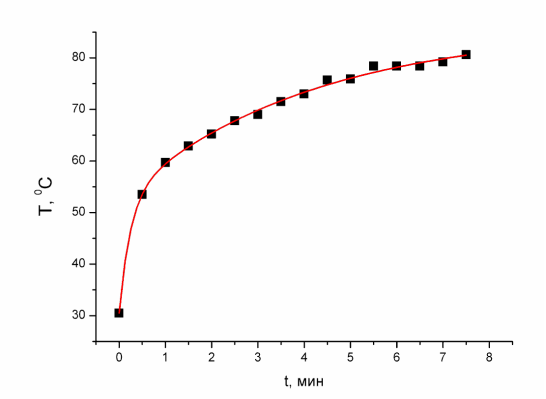


Figure 2. Temperature kinetics of skin surface above the tumor.

Eq. (1) allows obtaining characteristics times of the heating process. The characteristic times take the values $\tau_1 = 0.24 \pm 0.05$ min and $\tau_2 = 4.07 \pm 0.6$ min.

The existence of two characteristic times of tissue heating indicates that the heating process can be divided into two: rapid heating of the particles inside the tissue and a slower heating of the tissue itself, which has a weaker thermal conductivity.

Figures 3-6 show the calculated spectra of the following optical parameters of different layers of tumor and surrounding tissue after photosensibilization and PPTT: the absorption coefficient μ_a, and the reduced scattering coefficient μ_s', scattering coefficient μ_s, and scattering anisotropy factor g. Symbols correspond to averaged values of the coefficients, and vertical bars correspond to standard deviation. The spectra clearly show water absorption bands with maxima at 1452 and 1948 nm[14] and hemoglobin with maxima at 420 and 552 nm, indicating its deoxygenated form[15]. The observed increase in absorption in the region above 2200 nm is the short-wavelength shoulder of the absorption band of water with a maximum at 2950 nm[14]. The increase in the standard deviation of the absorption coefficient, observed in the region of the absorption bands, indicates a difference in the water and hemoglobin content for different samples of the tissues.

The effect of the deviation of the spectral dependence of the scattering characteristics from the monotonous one is explained by the increase of the influence of the imaginary part of the complex refractive index of the scattering centers (hydrated collagen fibers) in the region of water and hemoglobin absorption bands[16,17].

An increase in the imaginary part of the complex refractive index of the scatterers and the surrounding medium causes a significant decrease in the scattering anisotropy factor g, which together with the tissue scattering coefficient μ_s forms the spectrum of the transport scattering coefficient: $\mu_s' = \mu_s(1-g)$.

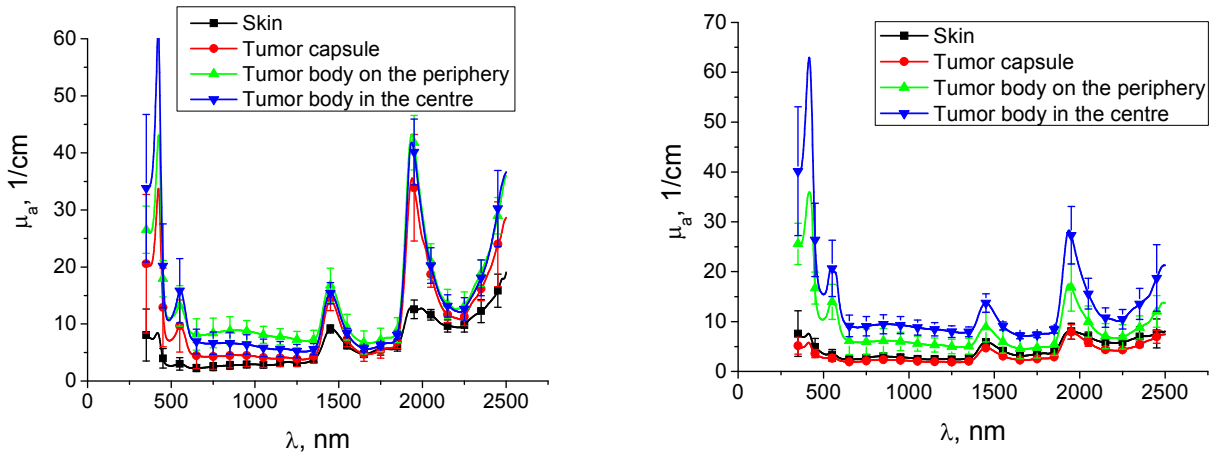


Figure 3. The absorption spectra of intact tumor and surrounding tissues (a) and after the nanoparticles injection and laser irradiating (b).

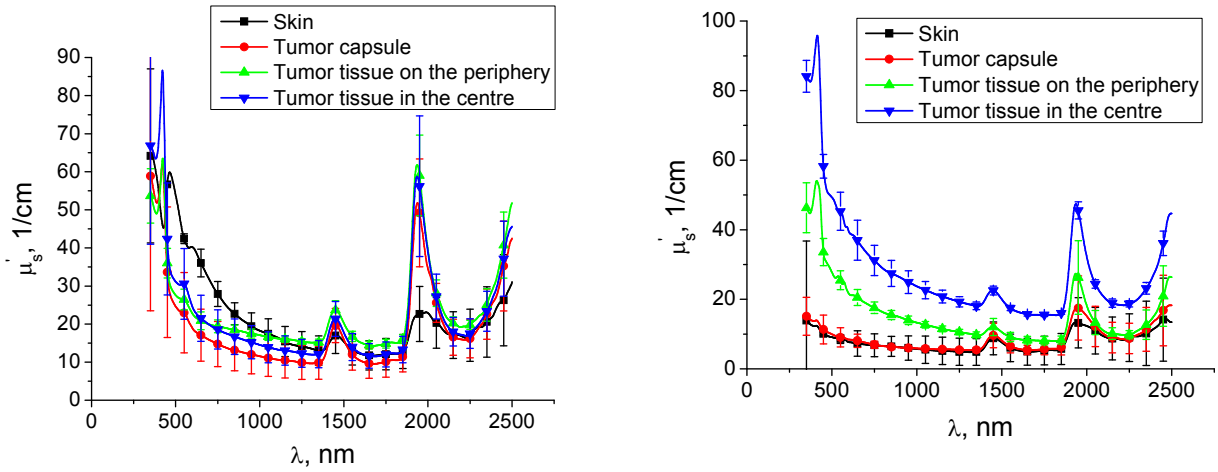


Figure 4. The spectra of reduced scattering coefficient of intact tumor and surrounding tissues (a) and after the nanoparticles injection and laser irradiating (b).

The figures demonstrate the changes in absorption and scattering properties of tumor and surrounding tissues caused by the PPTT. In particular, the change in the shape of the spectra is associated with coagulation and carbonization of the

tissue. The main target of heat exposure is the surface layers of the tissue - the skin and the capsule of the tumor. However, significant changes have also been observed in the body of the tumor, which are manifested in a significant decrease in water absorption bands, which indicates overheating of the tissue. Temperature rise near 80°C causes coagulation of proteins and thus, the change of tissue structure. At this the water content in the tissue decreases.

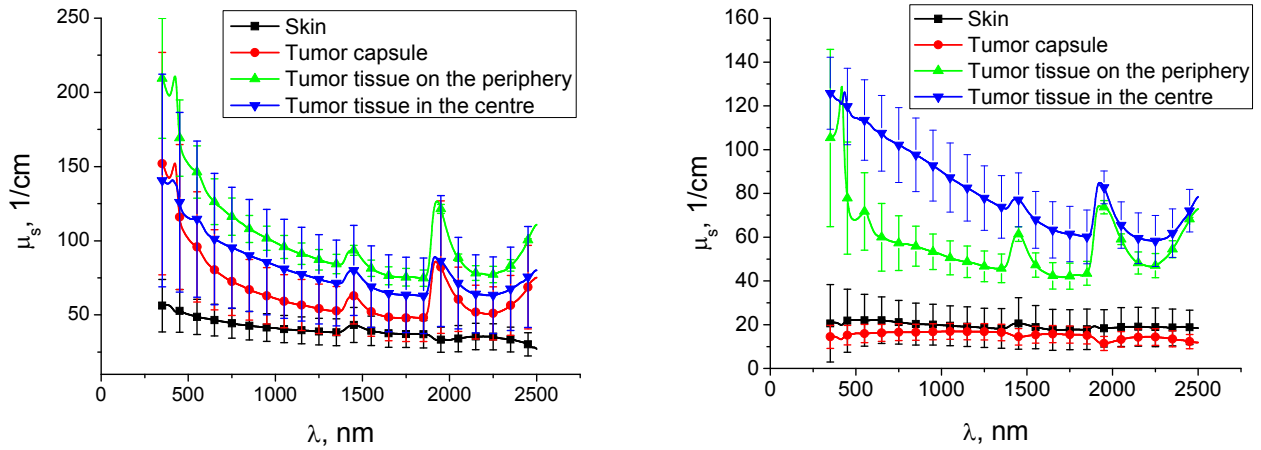


Figure 5. The spectra of scattering coefficient of intact tumor and surrounding tissues (a) and after the nanoparticles injection and laser irradiating (b).

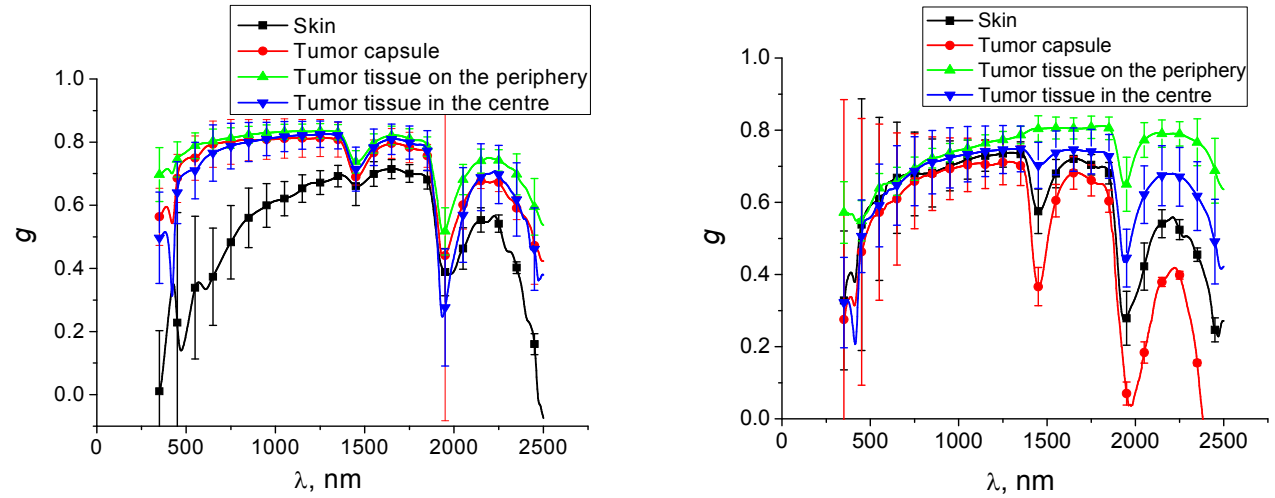


Figure 6. The spectra of scattering anisotropy factor of intact tumor and surrounding tissues (a) and after the nanoparticles injection and laser irradiating (b).

Besides we can see the decrease the tissue scattering in visible spectral range for the samples of skin, tumor capsule and upper part of tumor. That also can be connected with tissue coagulation. The coagulation leads to enlargement of main tissue scatterers. It is well known that the reduced scattering coefficient spectrum is formed by at least two types of scatterers: sufficiently small scatterers, which can be, for example, individual collagen fibers, etc., and sufficiently large

scatterers, the so-called Mie scatterers, which may be fiber bundles or their plexuses, as well as cell nuclei or other large components of tissue. Therefore, the decrease in the proportion of small scatterers and the increase of the proportion of large scatterers as a result of tissue coagulation causes light scattering decreasing at the shorter wavelengths.

At that the centre of tumor does not show the scattering decrease. It is clear that, despite the sufficiently high temperature on the surface (about 80°C), the changes in the center of the tumor are insignificant.

4. CONCLUSION

The results of the experiment have shown that after doping the tumor tissue by the plasmon resonant nanoparticles and NIR laser irradiating, there is the decreases of absorption as well as scattering properties of the tumor and surrounding tissues. However, despite the sufficiently high temperature on the surface, the changes in the center of the tumor are insignificant. Apparently, for better therapeutic effect, it is need either to increase the power of the incident radiation, or to increase the irradiation time, or to decrease the scattering of tissue covering the tumor.

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