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DIELECTROMETRIC STUDY OF RADIATION PROTECTION ABILITY OF ULTRA-DISPERSED NANODIAMONDS

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Summary. Radiation protection ability of the ultra-disperse nanodiamonds (UDD) is studied based on the measurements of dielectric permittivity of red blood cells (RBC) affected by cancer. Wistar rats with Guerin's carcinoma were treated by X-ray 5.8 Gy. Some rats received UDD with food during 5 days prior to the X-ray. The groups with UDD, X-ray, and both UDD and X-ray treatments were compared to the control group. The complex dielectric permittivity of the RBC was measured by microwave dielectrometry. It was shown, tumor development leads to the increase in the dielectric permittivity and relaxation frequency. The irradiation promotes further growth of the parameters, while UDD uptake leads to insignificant changes in comparison to the control group. Therefore, UDD occur the radioprotective effect promoting repair, compensation and restoration of body tissues that is demonstrated by normalization of the dielectric parameters of RBC.
Keywords: Nanodiamonds; Red Blood Cells; Cancer; X-ray; Dielectric permittivity, Dielectric relaxation.

1. Introduction

Modern therapy of malignant tumors is based on knowledge of the biological effect of ionizing radiation, the nature of tumor growth and the need for modern prevention of the cascade of adaptation reactions, in which all systems of the body participate, and primarily the blood system [1-4]. The established convenient model for studying tumor exposure and radiotherapy in vivo/in vitro are red blood cells (RBC) [5-7], which, in addition to the main function of the oxygen carrier, carry out transport functions by sorption both low-molecular and high-molecular compounds at their surfaces [8,9], aminoacids and lipids, performing a protective function, adsorbing toxins and antibodies [10,11]. In addition to the convenient model of cells for research purposes, RBC can be used as natural containers, loaded with certain way of drugs, which allows prolonging the effect of drugs in therapeutic doses for radiation therapy [12-14]. Structural rearrangements in the erythrocyte membrane under the influence of malignant disease and radiation lead to quantitative and qualitative changes in the composition of erythrocyte membranes [15-17]. These processes are accompanied by a change in the permeability of the cell, a violation of the function of membrane glycoproteins, changes in the content of lipid protein and protein-protein interactions, which is associated with free radical oxidation of molecules in membranes of the cells [18] and requires the use of both synthetic and natural antioxidants to correct these conditions [19-20]. Among synthetic antioxidants, prospective preparations are ultradispersed diamonds of detonation synthesis (UDD) obtained under the impact of high explosion energy [21]. Currently nanodiamonds with d~4-8 nm are widely used in biological research, r medical diagnosis and treatment [22]. Anomalously high adsorption capacity, large specific surface area, abundance of free electrons on the surface (multiple radical-donor), a large number of oxygen-containing functional groups on the surface of crystals, chemical inertness of grain UDD makes it possible to use them as a possible therapeutic facilities in oncology [20-23].

In this study the protective action of UDD on red blood cells is studied by the method of microwave dielectrometry in the frequency range corresponding to the dispersion region of water molecules and make it possible to use measurements of the dielectric constant of biological objects as a physical criterion in evaluating structural disturbances caused by various damaging factors. The complex of disorders found in RBC is associated with structural rearrangements of membranes and should be accompanied by a change in the degree of hydration of membrane components. The change in hydration in turn affects the structure and functional activity of the biological system as a whole. Thus, based on the literature data, one may couclude, that the properties of the UDD allow considering the advisability of conducting the nanodiamonds studies on the evaluation of their radioprotective properties. To a certain extent, these issues are addressed in this paper. In this work the dielectric permittivity of the RBC membranes has been studied on the experimental model of Guerin's carcinoma.

2. Materials and Methods

Forty mature Wistar rats, weighing 160-180 g with subcutaneously transferred 20% suspension of Guerin's carcinoma cells were used for the studies. Rats were kept in vivarium on a standard diet fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) [24]. The experimental group with Guerin's carcinoma of 20 rats received 1.0 ml of a diluted suspension of UDD once per day with food during 5 days prior to the radiation treatment. The suspension has C=0.01 % of dry weight of UDD in saline. The control group of 20 rats has not received UDD with food. Therefore, four groups of 10 rats have been studied: with both UDD and X-ray (I); with UDD without X-ray (II); without UDD with X-ray (III); with neither UDD nor X-ray (IV). In that way, 20 rats from the groups I and III were treated by X-ray with a dose of 5.8 Gy. Biological control included 10 rats. Blood samples were collected in 25 days after the irradiation. The irradiation of rats was carried out under the following conditions: the device RUM-3M, the voltage on the tube of 190 kV, the current of 12 mA, filters 0,5 Cu + 1,0 Al, tubes - 40 cm, the dose rate of 0,52 Gy/min, doses of 5.8 Gy. The study of changes in dielectric properties of permeability and hydration of RBC has been carried out by the microwave dielectrometry at a frequency f=9.2 GHz, based on the relative measurements of the real (ε') and imaginary (ε'') parts of the complex permittivity ε^*.

The water molecule has a constant dipole moment (1.84 Debye units, where 1 Debye = $3.33\cdot10^{-30} C\cdot m$) and in an alternating electric field undergoes orientation motions. The frequency dependence of the complex dielectric constant in liquids is described by the Debye equation

$$\varepsilon(f)=\varepsilon_\infty+(\varepsilon_s-\varepsilon_\infty)/(1+if\ /\ f_d), \quad (1)$$

where ε_S *is the permittivity at low frequency (static region) and* ε_∞ *is the dielectric permittivity at high frequency (optical permittivity),* $\varepsilon_\infty=5.5$*,* f_d *and* f *are the frequencies of the dielectric relaxation of water molecules and the microwave field, respectively. Separating the real and imaginary parts of equation (1) in accordance with the equation of complex dielectric permittivity* $\varepsilon^*=\varepsilon'-i\varepsilon''$*, where* $i=\sqrt{-1}$*, we obtain*

$$\varepsilon_r'(f)=\varepsilon_\infty+\frac{\varepsilon_S-\varepsilon_\infty}{1+(f\ /\ f_d)^2}, \quad (2)$$

$$\varepsilon''(f)=\frac{(\varepsilon_S-\varepsilon_\infty)f\ /\ f_d}{1+(f\ /\ f_d)^2}. \quad (3)$$

From the equations (2), (3) for the real and imaginary parts of the dielectric permittivity, we obtain the equation for the static dielectric permittivity ε_S and the dielectric relaxation frequency of water molecules f_d in the solutions under study as

$$\varepsilon_S=\frac{\varepsilon_r'+(\varepsilon'')^2}{\varepsilon'-\varepsilon_\infty}, \quad (4)$$

$$f_d=f(\varepsilon'-\varepsilon_\infty)/\varepsilon''. \quad (5)$$

The value of the dielectric constant ε' was determined by changing the resonance frequency (Δf) of the resonator with the sample relative to the empty resonator, and the value of ε'' is the magnitude of the damping of the power of the microwave field, due to the introduction into the resonator [14]. The temperature of the test sample was measured by thermocouple copper-constantan with an accuracy of ± 0.1° C. The relative accuracy of the measurements of ε' and ε'' for the samples were 0.05% and - 0.1%, accordingly.

The frequency $f=9.2GHz$ corresponds to the gamma-dispersion range which is determined by mobility of water molecules. Molecules of free water have higher rotational mobility while the water molecules bound by the RBC membranes are bound and form hydration shells over the membranes. The RBC suspension (0.1 ml) has been placed in a quartz capillary (inner diameter d=1.0 mm) and located in the resonator of the dielectrometer at a fixed temperature.

The investigated medium has significant low-frequency losses due to the presence of ions, therefore, to eliminate the contribution of ionic conductivity to the measured value ε'', a correction for the electrical conductivity

$$\varepsilon'' = \varepsilon''_{ch} - \frac{\sigma}{2\pi\varepsilon_0 f}, \qquad (6)$$

where σ is the electrical conductivity of the sample, $Om^{-1} \cdot m^{-1}$, ε_0 is the electric field constant equal to $8.854 \cdot 10^{-12} F \cdot m^{-1}$, f is the frequency, Hz.

Since the ionic conductivity is very weakly dependent on the wavelength, its value was measured at a frequency of 10 kHz using an AC bridge P586 in a cell with platinum electrodes. The error in the determination was 5% [14].

The Mann–Whitney U-test was used, with $p<0.05$ as the criterion for significance for all statistical comparisons. A post hoc power calculation was completed for each statistical comparison.

3. Results and discussion

The temperature dependences of σ, ε' and f_d in the RBC suspensions for different groups of rats are presented in Tables 1,2 and Fig.1(a-f). The tumor has a significant impact on membrane destruction processes of the erythrocytes. RBC have the ability to adsorb the endotoxins of tumors from plasma and, thus, ensure blood detoxication [5-7]. It is also known that the extent of erythrocyte membrane disorganization depends on the concentration of necrosis tumors factor and endotoxins, and on the time of their presence in the blood [5-7,14].

Table 1 shows that for the RBC suspension the temperature dependence of the specific conductivity is not linear and characterized by a step-like change of σ values. In the rats with Guerin's tumor (group IV) the values of σ are bigger than those in the control group. The observed changes are associated with the presence of tumor toxins in the blood and changes in the viscosity and permeability of the erythrocyte plasma membranes in the presence of Guerin's carcinoma [5,25] and, as a consequence, indicate a change in the amount of the free/bound water ratio in the erythrocytes membranes. The change in the free/bound water ratio is an integral feature that reflects, among other things, the characteristics of lipid and carbohydrate metabolism, since membrane fluidity (the value inverse to viscosity) depends on the membrane structure and interactions of protein-lipid and lipid-lipid complexes in it.

Comparison of the electric conductivity of RBC suspensions from Table 2 in Group II with Group III and Group IV shows a tendency to decrease in this index over the entire temperature range. This trend is most pronounced for group II, that finds confirmation in the changes in the dielectric permittivity index (Fig. 1 a,c,e) and the index of the dielectric relaxation frequency of water molecules f_d in the erythrocyte suspension (Fig. 1 b,d,f). Probably, destruction of the boundary layers of water in the erythrocyte membranes is observed, and the destruction is even amplified by the temperature growths that are accompanied by an increase in the electrophoretic mobility of UDD. UDD is a cation exchange system with a wide range of functional groups, forming an ion-permeable structure. The observed nature of changes is probably connected with the presence near the UDD surface thin water layers with a changed structure, and with the immobilization of the dispersion medium inside the UDD, which contributes to the change in the free/bound water ratio. The water immobilized inside the secondary aggregate of UDD may not participate in the movement, and the mobility of the ion exchange is preserved.

Table 1

The dependences $\sigma(T)$ for rats of group IV and control group

T,°C	Control group. $\sigma, Ohm^{-1} \cdot m^{-1}$	Group IV. $\sigma, Ohm^{-1} \cdot m^{-1}$
5	0.88±0.06	1.22±0.09*
10	1.02±0.01	1.30±0.03*
20	1.147 ±0.03	1.47±0.04*
30	1.17±0.03	1.49±0.03*
40	1.27±0.04	1.55±0.01*

** – reliability of differences in comparison with the biological control, p≤0.05*

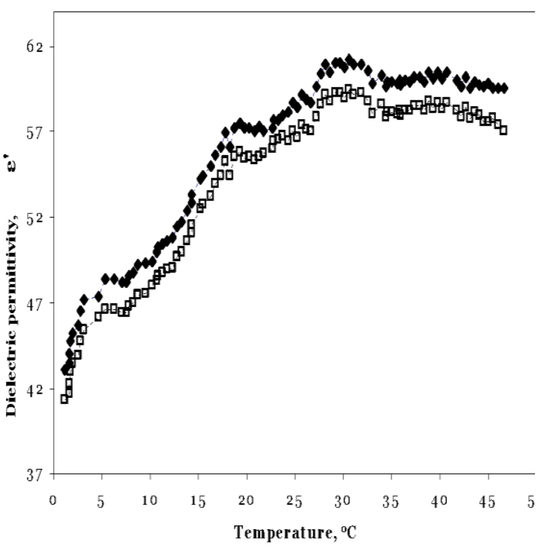
Table 2

The dependences $\sigma(T)$ for rats of group II and group III

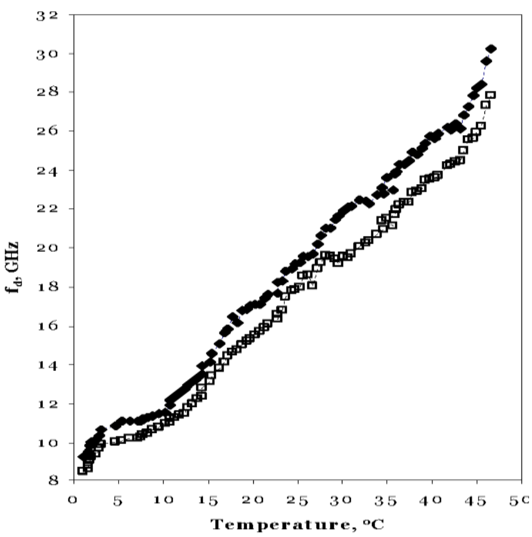
T,°C	Group II. $\sigma, Ohm^{-1} \cdot m^{-1}$	Group III. $\sigma, Ohm^{-1} \cdot m^{-1}$
5	0,98±0.03**	1.12±0.03**
10	1,20±0.03**	1.20±0.03**
20	1.31±0.04**	1.36±0.05**
30	1.34±0.03**	1.39±0.03**
40	1.4±0.08**	1.45±0.04**

*** – reliability of differences in comparison with the Group IV, p≤0.05*

As it can be seen from Fig. 1a,c,e that the values ε' of the RBC suspensions in group IV and group III are bigger in comparison with the same values in the control group. The nature of changes in the ε' values of RBC suspensions in the groups under study is most likely due to the non-monotonicity in the dependence of the amount of water bound by the cells influenced by the tumours, UDD, and X-ray.



a



b

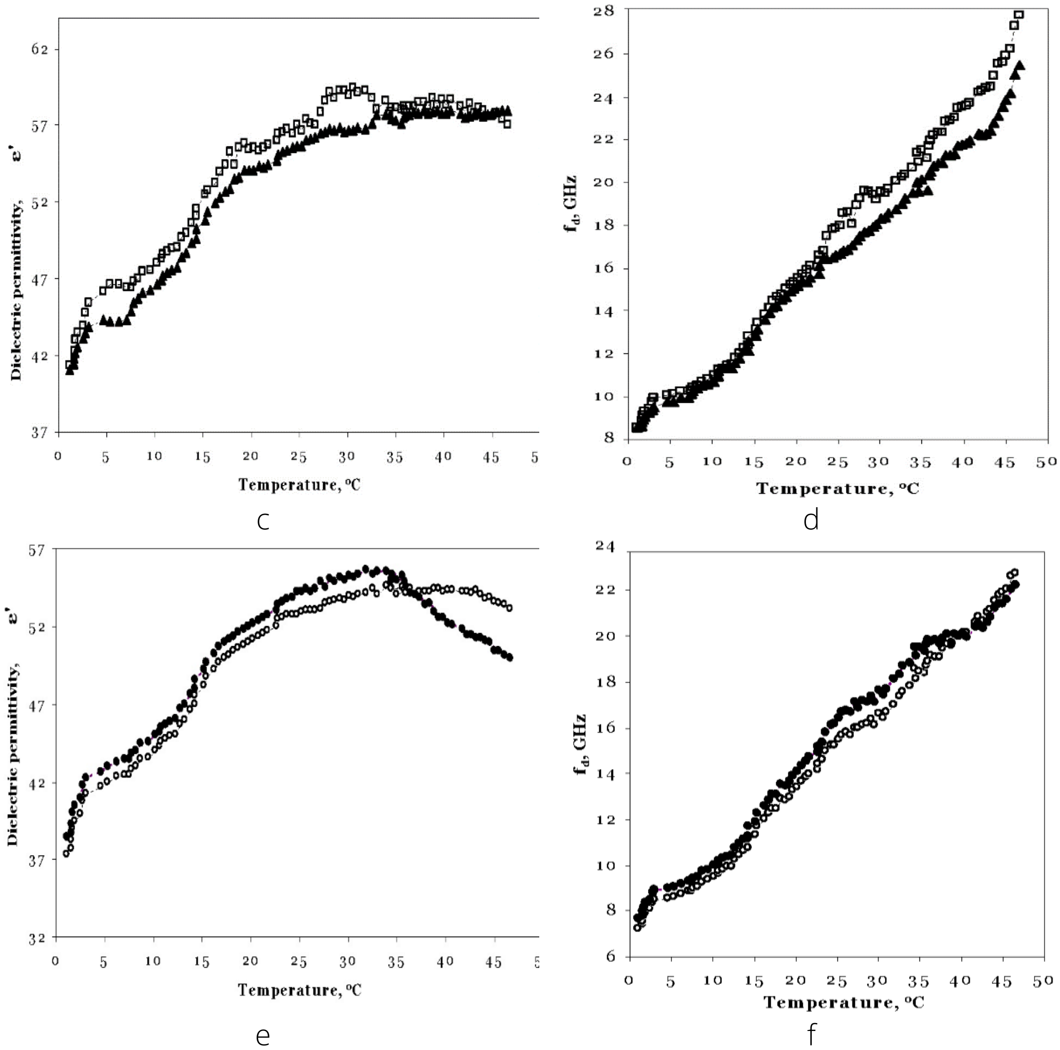


Fig.1. The dependences $\varepsilon'(T)$ (a,c,e) and $f_d(T)$ (b,d,f) for the RBC suspensions of rats: ● – group I ; ▲ – group II; ■ – group III; □ – group IV

At the present time, a lot of facts have accumulated, indicating the destructive effect of the ionizing radiation on various functional systems of the organism. At the same time, subtle mechanisms of pathological changes occurring at the cellular level require additional studies. The biological membranes of erythrocytes are assigned the role of a critical superstructure, any of its main functions being vital for the cell, and the damage to these functions can lead the cell to death. It is known that the biological feature of the action of the ionizing radiation is manifested, first of all, in the property of a "total stimulus". It is believed that at a dose of 5.8 Gy the leading role in determining the radiation responses of the cells is played not by irreversible damage to the genetic apparatus, but by reversible violations of the membrane structures that support the vital activity of the cell and its regulation. It is known that the physical processes underlying radiation damage to membranes can be either a direct effect of radiation, which leads to the absorption of the energy of ionizing radiation by membrane components or the effect of an indirect action associated with damage to protein and lipid cell molecules by free radical radiolysis products of the medium [3-5].

In the group I, after irradiating the animals received UDD with food, the values of the frequency of the dielectric relaxation of water molecules of RBC suspensions are significantly decreased in comparison with the control group (Fig. 1f). Comparing the values in group I and in the control group, it can be assumed that radiotherapy, together with UDD, promotes the transition of water from the bound state to free, which leads to an increase in its mobility. The results observed in group I, when taking UDD before irradiation show approximation of the values of dielectric permittivity in the RBC suspensions to the values of dielectric permittivity in the control group (Fig. 1e). This could be due to the fact that parallel to the processes of tissue damage under the influence of X-ray irradiation occur repair, compensation and restoration of body tissues after radiation damage, i.e. it becomes possible to implement protective adaptive reactions in the irradiated organism, which is reflected in the normalized indices of permittivity of the RBC. The radioprotective effect of UDD at the cellular level, apparently, is not due to the inhibition of lipid peroxidation (LPO) in the membranes, but to the interception of OH- radicals formed during radiolysis of water. The involvement of LPO processes in the violation of the barrier properties of the RBC membranes under the influence of accelerated electrons seems unlikely for two reasons. First, the presence of a number of antioxidant systems is typical for erythrocytes, which complicates the course of LPO reactions. Secondly, numerous studies have shown that the intensity of radiation-induced LPO is inversely proportional to the radiation dose rate. At the irradiation mode used in the present work, the average dose rate reached 0.52 Gy/min, so one should expect that the level of oxidative processes in the lipid phase of the membranes will be low. The radioprotective effect of UDD may be due to repair of lesions affecting SH- groups of proteins, or interception of free radicals arising from radiolysis of the medium. UDD having high chemically active fringe of functional groups on its surface (oxy, carboxy, carbonyl, etc.), increase the resistance of protein molecules to the effects of radiation, thus contributing to the restoration of radiation damage.

4. Conclusions

The indirect radiation influence attack on the cell membranes may induce the membrane failure. Among the membrane disturbances are increase in rigidity (viscosity) of the lipid bilayer and aggregation of the membrane proteins. Based on the experimental data presented, one can conclude that the effect of a small dose of ionizing radiation under the conditions of Guerin's carcinoma model caused nonmonotonic changes in the erythrocyte permittivity indices, which indirectly indicates deeper structural and functional changes in RBC membranes under X-ray irradiation of rats. The multidirectional nature of the changes in the real and imaginary parts of the complex dielectric permittivity of RBC is probably due to the influence of intermolecular interactions, namely, a destruction of the grid of hydrogen bonds created by water molecules or UDD particles, that may be a consequence of the X-ray exposure in the conditions of Guerin's carcinoma model.

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