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Different Sensitivity of Normal and Tumor Cells to Pulsed Radiofrequency Exposure

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***Abstract*—The effect of nanosecond radiofrequency pulses (ns RF) on tumor and normal cells has been studied. To determine the viability of cells, an MTT test was used, as well as a real time system for analyzing cell cultures-iCELLigence. It has been shown that ns RF pulses under certain combinations of operating conditions reduce cell proliferation of both tumor and normal cells. Double exposure to 1000 pulses leads to the most effective inhibition of tumor cell proliferation and was 40% after 5 days. Inhibition of the proliferative activity of normal cells was 10% and was maximum after 3 days, then cell growth resumed. The results obtained allow to consider ns RF pulses with different parameters as a promising effective factor for controlling cellular processes for biomedical purposes.**

Keywords—radio frequency nanosecond pulses; tumor cells; hela, cell proliferation, normal cells.

I. INTRODUCTION

For many years, research has been conducted on the effects of electromagnetic radiation on biological objects. Of particular interest are the effects of radio frequency pulses on rapidly dividing cells, which can control their physiological state. This is relevant for biomedical research aimed at finding therapies for various diseases, such as cancer. Minimally invasive methods of cancer treatment are the most relevant, since they allow to minimize the negative influence on normal tissues [1]. Therefore, methods for inhibiting tumor growth and subsequent death of tumor cells are actively developed using nanosecond pulse-periodic microwaves and x-ray radiation [2], as well as micro- and nanosecond electric field pulses [3–5]. Important feature of the biological effectiveness of such pulses is their non-thermal effect on the irradiated object, which is achieved by their short duration (from micro-to nanoseconds). This ensures a fairly low average absorbed energy per session, even at high values of electric field in the pulse. In this context, the high efficiency of nanosecond pulses is primarily related to the physical (modulation) parameters of the impact. Of great importance are the time parameters of exposure, in particular the pulse duration, the pulse repetition frequency, number of pulses per session and number of sessions of exposure and interval between the sessions. To select the pulse duration, it is necessary to consider the regularities established by Schoenbach et al., which take into account the charge reorientation time in the eukaryotic cell membrane, which can vary from 0.5 ns to 100 ns [5]. To select the pulse repetition frequency, we can rely on the results of V.R. Adey's work, which shows that the most biologically effective are pulse repetition frequencies or modulation frequencies from 6 to 20 Hz [6]. As for the selected exposure duration, it is known that the effect depends on the number of pulses. Changes in the functional state of mitochondria were observed even after a short exposure (10 and 50 pulses) to nanosecond microwave pulses (10 GHz), while 100 and 500 pulses were ineffective [6, 7]. Thus, the dependence of the effect on the number of pulses

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is disproportionate. In addition, it is known that pretreatment of tumor tissue cells with nanosecond electrical pulses increases the efficiency of repeated treatment (repeated exposure after 2–8 minutes) by 2.5–6 times. In addition, the effect of repeated training depends on the time between sessions of exposure [8].

The advantage of nanosecond radio frequency pulses is also that they can be filed to the target without using electrodes [9]. Previously, it was shown that exposure to radio frequency pulses (4–25 ns) can depress the functional state of mitochondria and disrupt the integrity of their membranes [10]. There is also evidence of such a well-known phenomenon as electroporation, caused by high-intensity electrical impulses, which leads to an increase in the permeability of cell membranes [11, 12]. The applied electroporation can be reversible with the repair of membrane permeability or irreversible, depending on the amplitude of electrical pulses and their number. Reversible electroporation is used to introduce biologically active substances or genetic material into the cell without affecting cell viability [13–15]. Based on the above, this study evaluated the effect of the pulsed radio frequency field on the proliferative activity of tumor and normal cells *in vitro*.

II. Materials and Methods

Experiments were performed on cervical cancer tumor cells (HeLa) and normal rat fibroblasts (3T3). The cells were incubated in Petri dishes in a humid environment containing 5% of CO_2 at a temperature of 37 °C. Irradiated cells located in test tubes with a nutrient medium in a concentration of 1 million/ml.

A. Experimental Setup

The cells were irradiated using a pulsed high-power microwave generator based on gyromagnetic nonlinear transmission line (NLTL). The microwaves generation in such a device occurs due to the excitation of a damped precession of the magnetization vector of the saturated ferrimagnetic filling of the line [9]. The experimental setup is shown in Fig. 1. A high-voltage video pulse is fed to the input of the NLTL, which is then modulated by the high-frequency component when passing through the NLTL. Then, a constant component is deleted from the pulse and absorbed in the matched load of the bandpass filter. After this, the high-frequency pulse, by means of a waveguide to coaxial adapter, is fed into the waveguide, where the irradiated medium is located, interacts with the medium (DUT) and then is absorbed at the RF load. The electromagnetic field in the waveguide corresponds to the TE_{10} mode of a rectangular waveguide.

The advantage of using a microwave oscillator based on NLTL is the fact that, they can produce microwave pulses with relatively high electric field strength value and unlike relativistic microwave oscillators, there is no accompanying x-ray radiation during its operation, which makes the use of relativistic generators impossible in biological research. Another advantage of the NLTL-based generator is the possibility of changing the electric field strength in the waveguide by several orders of magnitude, practically without changing the center frequency of the microwave pulse. In the experiments, the electric field strength varied in the range from 100 V/cm to 30 kV/cm. In this case, the central frequency of the microwave pulses was in the range from 0.6 GHz to 1 GHz, and the pulse duration was in the range from 5 to 20 ns. The pulse repetition rate at such parameters could reach 22 Hz. The central frequency and pulse duration were determined by the selected peak electric field strength in the waveguide.

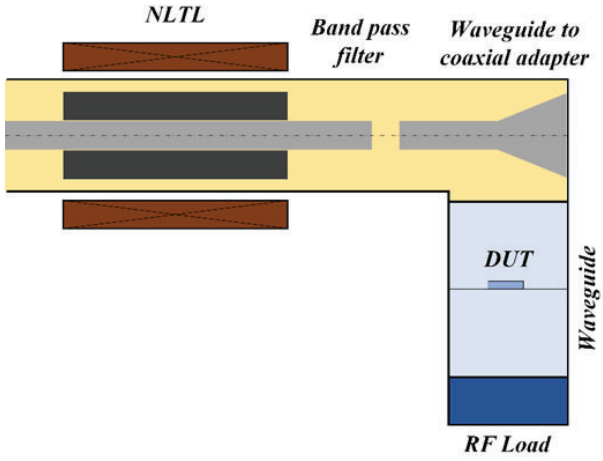

Fig. 1. Scheme of the experimental setup.

B. Methods of Exposure

Since the electromagnetic field in the waveguide has the TE_{01} structure, a limitation is imposed on the number of samples irradiated at once since the field in the waveguide is inhomogeneous along the wide wall. The irradiated medium containing tumor cells was placed inside test tubes having its length of 120 mm and a diameter of 17 mm. Thus, in order to ensure a quasihomogeneous effect of the pulse on the medium, only two tubes were located in the center of the waveguide.

In order to evaluate the electric field strength inside the medium and the amount of energy absorbed in it, the frequency dependence of its dielectric permittivity components was measured. The measurement results are presented in Fig. 2.

Using these data, numerical simulation of the irradiation process was carried out using the Ansys HFSS package [16]. The simulation results showed that the field in the samples is less than the field measured in the center of the waveguide by 10–20%. The portion of the absorbed pulse energy does not exceed 3% of the energy of the pulse. This corresponds to a maximum temperature increase of the medium being irradiated by 0.45°C per 1000 pulses at the maximum electric field strength in the wave.

C. Assessment of Viability of Cells

The proliferative activity of cells was assessed using the MTT-test. It is based on the addition of a yellow tetrazolium dye (MTT), the color of the solution changes as it is restored by cellular oxidoreductase enzymes [17]. The final product of MTT recovery is purple-colored pharmazone, which is easily soluble in DMSO. The analysis of cell viability in this method is related to the quantitative determination of formazan using a

spectrophotometer (Multiscan, USA) at 620 nm, which is linearly related to the activity of the enzyme and indirectly to the number of viable cells.

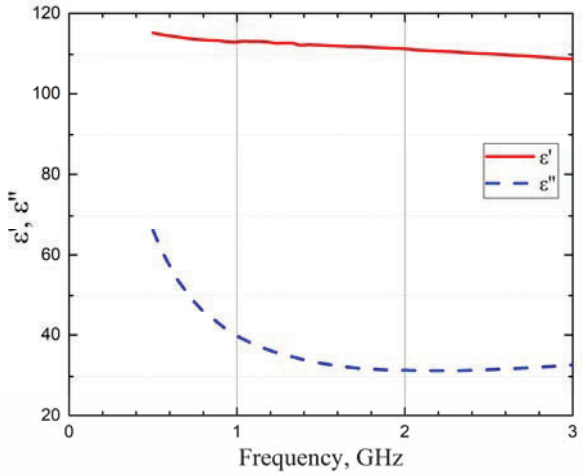

Fig. 2. Frequency dependence of the components of the dielectric permittivity of the irradiated medium.

The non-invasive cell culture analysis system iCELLigence (ACEA Biosciences Inc., USA) [18] was used to study the kinetics of cell proliferation in real time after exposure to nanosecond radiofrequency pulses. It is based on measuring the resistance using special electrodes. The cells contact the sensor surface of the electrode and act as an insulator, which leads to an increase in resistance. After irradiation, the cells were seeded into special plates, where they were incubated for 120 hours. Fixation of the cellular index was carried out continuously every hour.

Irradiated and sham groups of cells were used as controls in all experiments. The sham cells were subjected to the same manipulations as the irradiated ones, but without activating the radiation source. Nine repetitions were performed for each exposure and falsification experiment. Statistical comparisons were made using the nonparametric Mann-Whitney test in the Statistica 8.0 software.

III. Experimental Results

A. Proliferative Activity of Tumor Cells after Nanosecond Radio Frequency Pulses

In the first part of the experiment, we searched for the most effective and optimal working conditions. To do this, the proliferative activity of tumor cells was evaluated by the MTT-test.

Experiments have shown that exposure to nanosecond RF pulses with pulse repetition rate of 13 Hz and central frequency 1 GHz leads to inhibition of the proliferative activity of HeLa tumor cells (Fig. 3). Maximum electric field strength inside the waveguide in this case was in the range of 25–30 kV/cm. The effect depends on the duration (number of pulses per session) and repeatability of exposure sessions. Exposure with a shorter duration of 1000 pulses was more effective than the mode with 4000 pulses. In addition, double sequential exposure (1000 pulses + time between sessions of about 80 seconds + 1000 pulses) had a greater inhibitory effect (up to 40%) than a single (up to 30%). However, increasing the number of repetitions of sessions of exposure to three and four did not lead to an increase in the effect, it was as effective as double exposure. It is important to note that the effect is most pronounced on the fifth day after irradiation. However, on the seventh day, the proliferative activity of tumor cells begins to increase, although it does not reach the level of sham.

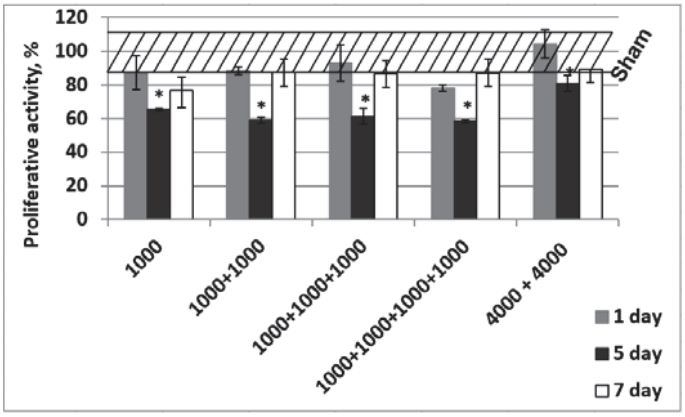

Fig. 3. The decrease in the proliferative activity of Hela cell after exposure to nanosecond radio frequency pulses. The abscissa indicates different variants of the effect on the number of pulses and repetition of the session, the ordinate indices the proliferative activity of irradiated cells relative to the sham group. The shaded region refers to the 95% confidence interval of the average proliferative activity of sham cells taken as 100%, * means that the difference from the sham is significant ($p \leqslant 0.05$).

A real-time analysis was performed to determine the kinetics of cell growth after exposure to nanosecond RF pulses. For the study, the most effective exposure mode was selected with a pulse repetition rate of 13 Hz and double sequential exposure (1000+1000 pulses). It was found that this effect can inhibit the proliferation of tumor cells, but this effect begins to be fully realized only after 100 hours after exposure (Fig. 4). It is important to note that in the first days after irradiation, the proliferation of tumor cells increases. However, the effect of inhibition after 5th day was up to 60% compared to the sham one. These data confirm the data obtained by MTT-test analysis.

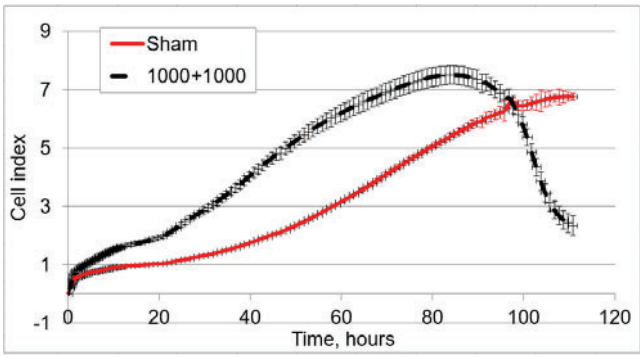

Fig. 4. The Cell Index kinetics of HeLa cells were monitored for 120 hours after nanosecond radio frequency pulses exposure (1000+1000 pulses sessions with a 80 second interval between sessions, with a repetition rate of 13 Hz) with iCELLigence system.

B. *Proliferative Activity of Normal Cells after Nanosecond Radio Frequency Pulses*

The response of normal cells (3T3) differed from the response of cervical cancer cells to nanosecond radio frequency pulses with a pulse repetition rate of 13 Hz and an electric field strength of up to 30 kV/cm. As in the case of HeLa, there was an inhibition of the proliferative activity of 3T3 cells, but it was manifested only on day 3 and reached a maximum of 10% compared to the sham group (Fig. 5). In this case, the effect did not depend on the duration of exposure (the number of pulses). However, by 5th day the proliferative activity began to increase again, which indicates the restoration of the functional state of the cells.

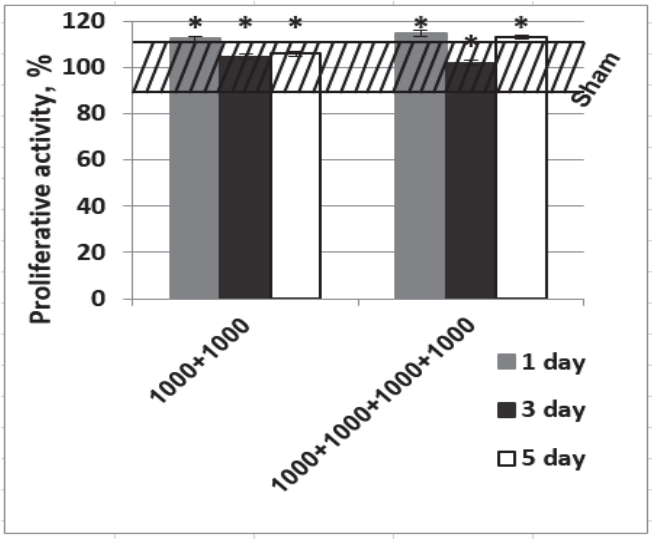

Fig. 5. The decrease in the proliferative activity of 3T3 cell after exposure to nanosecond radio frequency pulses. The abscissa indicates different variants of the effect on the number of pulses and repetition of the session, the ordinate indices the proliferative activity of irradiated cells relative to the sham group. The shaded region refers to the 95% confidence interval of the average proliferative activity of sham cells taken as 100%, * means that the difference from the sham is significant ($p \leqslant 0.05$).

The cell kinetics obtained using the MTT test (shown above) was also confirmed by iCELLigence. Both when exposed to a double sequential exposure (1000 + 1000 pulses) and when repeated four times 1000 pulses, the proliferative activity of cells practically does not decrease relative to the sham group (Fig. 6). After 70 hours, the cell index of experimental samples begins to increase. The results obtained prove the safety of exposure to this type of ns RF pulses on normal cells, since the decrease in proliferative activity is minimal.

Thus, the results of the experiments indicate that exposure to nanosecond radio frequency pulses under certain conditions of exposure can affect the rate of cell proliferation. The most sensitive to this type of exposure are tumor cells. It was found that the maximum effect is achieved with a double exposure of 1000 pulses with a pulse repetition frequency of 13 Hz and an electric field strength of up to 30 kV/cm. In this case, the conditions for double exposure of 1000 pulses with an 80-second interval between them are effectively triggered, since during this time the mechanisms for inhibiting proliferation are initiated. At the same time, the mechanisms of repair of lesions do not have time to work, so we observe a decrease in proliferative activity.

However, the resulting reaction of tumor cells is not long-lasting, since after 7th days, cell proliferation begins to recover and approach the values of the sham group. It is assumed that such a short duration of the effect can be corrected by repeated irradiation sessions. Therefore, it is necessary to conduct another session on 5–6th days after the first irradiation to obtain a more stable effect.

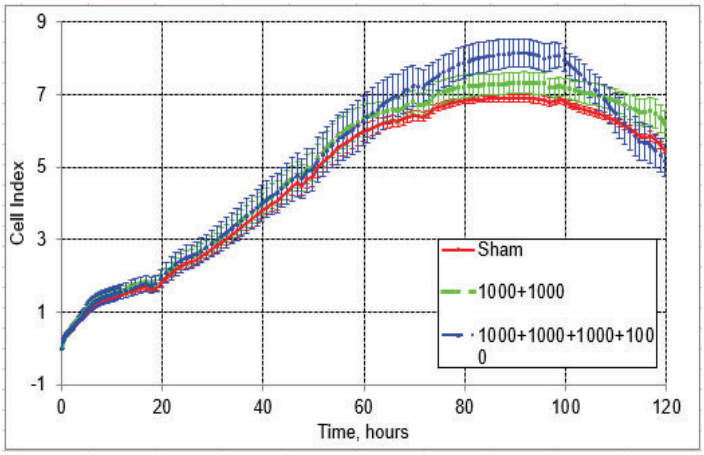

Fig. 6. The Cell Index kinetics of 3T3 cells were monitored for 120 hours after nanosecond radio frequency pulses exposure with a repetition rate of 13 Hz with iCELLigence system.

The obtained data illustrate the complex nature of the reaction of normal and tumor cells to nanosecond radiofrequency pulses. Therefore, it is necessary to identify common patterns of other types of tumor cell response and understand the mechanisms of ns RF pulses. The obtained knowledge of General regularities will allow one to choose the optimal conditions of exposure to achieve the desired result of inhibition of proliferation, since the studied factor can be combined with any physical or chemical factors to improve the effect of exposure. In addition, the minimal effect on normal cells indicates a high potential for using this type of radiation in solid tumors. Therefore, the mechanisms and ways to achieve the desired effect for biomedical purposes should be studied in more depth. The use of ns RF pulses is a promising direction when used in combination therapy, since nanosecond radiofrequency irradiation has a wide range of combinations of generation parameters, which opens up great opportunities for non-invasive manipulation.

IV. Discussion

The inhibition of proliferative activity of tumor cells shown by us can be caused by reversible electroporation. It can initiate the generation of reactive oxygen species, damage to the lipid and protein components of cells, disruption of mitochondrial function with a decrease in ATP synthesis, which together leads to the death of tumor cells [19, 20]. Shoenbach and co-authors also consider cellular and intracellular membranes, primarily the mitochondrial membrane and the endoplasmic reticulum as targets of antitumor action of nanosecond

electrical pulses [5]. In addition, many studies have shown various mechanisms of destruction of the membrane structures of tumor cells [21] and their genetic apparatus [22]. From this point of view, the study of the reactions of actively proliferating cells to nanosecond radio frequency pulses at various combinations of all the above parameters will allow to choose the optimal working conditions for fine-tuning and targeted control of the functional state of cells. The results of calculation of the heating of the irradiated medium and electric field strength inside it allow us to establish that the effect on cell cultures is non-thermal.

V. Conclusion

Consequently, our research on the sensitivity of normal and tumor cells to pulsed radiofrequency exposure allows us to draw the following conclusions. Exposure to nanosecond radiofrequency pulses under certain operating conditions affects the proliferation of both tumor and normal cells. Tumor cells are most exposed to radiation due to their metabolic characteristics. Inhibition of the proliferative activity of HeLa cells was maximal after double exposure to 1000 ns RF pulses with a pulse repetition rate of 13 Hz and an electric field strength of up to 30 kV/cm. It was most pronounced on the 5th day after exposure. However, this effect was short-lived and lasted only for the first 5-7 days after exposure.

Normal cells, on the other hand, are less susceptible to nanosecond radiofrequency pulses. The maximum value of inhibition of proliferative activity was observed on day 3rd, where it reached 10%. However, by 5th day, there is an increase in the cell growth curve relative to the sham group.

The complexity of the resulting cell response to nanosecond RF pulses requires further study with different types of tumor cells and allows for the development of methods for effective non-invasive inhibition of tumor growth *in vivo*.

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