ISSN 1063-780X, Plasma Physics Reports, 2023, Vol. 49, No. 5, pp. 595–601. © The Author(s), 2023. This article is an open access publication.
Russian Text © The Author(s), 2023, published in Fizika Plazmy, 2023, Vol. 49, No. 5, pp. 447–453.

LOW-TEMPERATURE PLASMA

Characteristics of Cold Atmospheric Plasma Jet when Excited by Sinusoidal and Positive Pulse Voltages for Medical Applications

I. V. Schweigert[a,*], Dm. E. Zakrevsky[a,b,c], E. V. Milakhina[a,b,c], P. P. Gugin[a,b], M. M. Biryukov[a,d], E. A. Patrakova[d], O. S. Troitskaya[a,d], and O. A. Koval[a,d]

[a] *Khristianovich Institute of Theoretical and Applied Mechanics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia*

[b] *Rzhanov Institute of Semiconductor Physic, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia*

[c] *Department of Radio Engineering and Electronics, Novosibirsk State Technical University, Novosibirsk, 630090 Russia*

[d] *Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia*

**e-mail: ivschweigert@gmail.com*

Received November 3, 2022; revised January 8, 2023; accepted January 9, 2023

Abstract—Low-temperature plasma jets at atmospheric pressure generated by sinusoidal and positive pulsed voltages interact differently with the treated surface. In the experiment and in numerical simulations, we compare the operating modes of helium plasma jets for these types of operating voltages. The discharge current on the treated surface over time and the surface heating are studied for different discharge parameters acceptable for anticancer therapy. The intensity of the emission spectrum is analyzed to improve the effectiveness of the plasma jet. Surface heating is controlled in order to meet the safety conditions of plasma exposure to biological objects. For the case of pulsed voltage the effect of voltage pulse duration on the intensity of plasma-surface interaction is discussed. The results on cancer cells A549 and MCF-7 demonstrate the high efficiency of the cold plasma jet generated at found optimal modes.

Keywords: cold plasma jet, positive pulsed voltage, pulse length, surface temperature, optimization

DOI: 10.1134/S1063780X2360010X

1. INTRODUCTION

Cold plasma jet generated by plasma sources in inert gas flow in the ambient air is currently actively used as an effective tool in multiple medical applications. Plasma jets can be initiated by a sinusoidal or positive pulsed voltage, and the excitation type determines the characteristics of a cold atmospheric plasma jet (CAPJ). Depending on the working voltage, its amplitude and frequency, a variety of streamer propagation modes occurs in the plasma jet. CAPJs are usually formed by sinusoidal voltage, but generation of plasma jet by positive pulsed voltage has such advantages as control of the thermal effect and the possibility of increasing the applied voltage. With the sinusoidal excitation of CAPJ a significantly increase of the temperature in the plasma-surface contact zone was observed when the applied voltage was increased (see, for example, [1, 2]). The surface temperature in [1] increased up to 35.2°C at a voltage amplitude of 2.5 kV and up to 39.8 °C at 3 kV at a sinusoidal voltage with a frequency of 25 kHz and helium flow rate of 4 L/min. At the same time, the measured radiation intensity of chemically active particles remained weak. In [2] at a higher helium flow rate (5 L/min), with a sinusoidal voltage amplitude of 3 kV and a frequency of 25 kHz, the mouse skin temperature increased to 96°C. Obviously, with the use of sinusoidal voltage to excite CAPJ the temperature should be monitored in parallel with the effect of plasma treatment on bio-tissues.

The original method of reducing the thermal effect of CAP generated by sinusoidal voltage was suggested in [3]. The novelty of the method [3] consists in reducing the surface temperature (confirmed by measurements) by clipping the front of the sinusoidal voltage when it reaches its maximum value. Thus, it was found that reducing the contact time of the plasma with the surface leads to a decrease in the surface temperature. In this work, we use the idea put forward in [3] and investigate the effect of pulse duration of positive pulsed voltage on the dynamics of streamer propagation and heating of the treated surface in comparison with similar studies of the CAPJ characteristics when excited by a sinusoidal voltage. In our experiments, we apply higher voltage amplitude (3–4.5 kV) that typi-

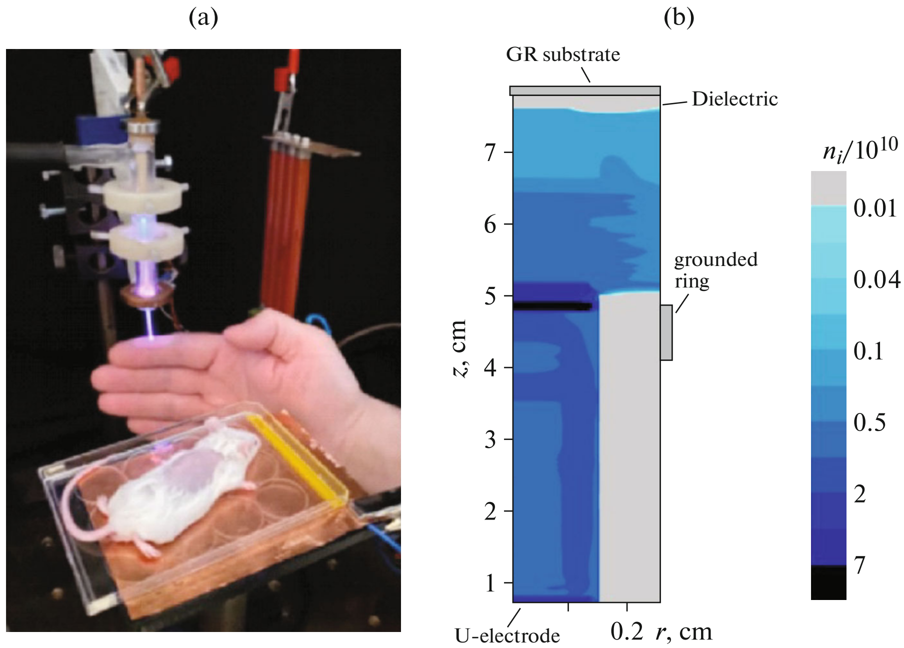

Fig. 1. (a) Low-temperature plasma jet device, (b) calculation domain with ion density distribution when the streamer propagates in the dielectric channel of the device.

cally used in CAP devices for plasma medicine. Our goal is to elevate the electric field strength in the streamer head when interacting with the surface for an increase of physical and chemical impacts on the bio tissue.

Previously, interesting results on the influence of the voltage pulse duration on the interaction of plasma with the surface were presented in [4, 5]. The interaction of CAPJ initiated by a pulsed voltage with a frequency of 5 kHz and a pulse duration of 230 and 960 ns with a dielectric surface under a floating potential was analyzed in [4]. The electric field distribution and the emission spectra were measured near the surface. The authors concluded that the pulse duration and the amplitude of the applied voltage determine the surface charge, the electric potential and, therefore, the time and intensity of plasma contact with the surface. Similar conclusions about the influence of the pulse duration were made in [5] for the case of electro-isolated surface when longer voltage pulses (1 and 10 μs) were applied. Thus, the duration of the voltage pulse is one of the main parameters determining the time and intensity of the plasma-surface interaction when CAPJ is generated by repetitive pulsed voltage.

2. EXPERIMENTAL SETUP, CALCULATION MODEL, AND METHODS FOR CELL RESPONSE STUDIES

2.1. Experiment

In the experiment, our plasma device with a cylindrical design was used to generate CAP in a helium flow in ambient air with sinusoidal and positive pulsed voltages [6, 7]. The potential electrode was coaxially inserted into a dielectric channel with an inner diameter of 8 mm. The capillary near the nozzle has a diameter of 2.3 mm. A ring grounded electrode is placed outside above the nozzle. The plasma source is shown in Fig. 1a. A target (dielectric Al_2O_3 plate or cancer cells in the medium) is placed on a grounded metal substrate at a distance of 2.5 cm from the nozzle. In our experiments, the voltage amplitude U ranges from 3 to 4.5 kV, and the frequency f = 13–50 kHz. Helium gas is used to generate the plasma jet, the gas flow rate varies from 3 to 12 L/min (gas flow rate >10 m/s). Both in the experiment and in the simulation, the positive pulsed voltage increases to its maximum value within 2.5 μs, has a plateau, and decreases within 2.5 μs. The pulse duration τ_p is regarded as a non-zero voltage time.

2.2. Simulations

The numerical simulations of the gas discharge and streamer dynamics for the experimental conditions were performed in the framework of fluid model (cylindrical symmetry) with author's code 2DPlasmaNovH [8]. In this study, we use a simplified model in which only helium gas without an admixture of nitrogen and oxygen is considered. The photoionization model is the same as in [8], and based on the following assumptions: (a) photons are emitted immediately after the excitation of the atoms and (b) the generation of electron/ion pairs by these photons takes place in the region around the streamer head. In the experiment [8], it was shown that the streamer images are luminous spots with a radius of about 2–2.5 mm around the streamer head. It follows that the characteristic time of emission of photons by excited atoms is less than the characteristic time of streamer propagation, and the photoionization length is less than 2.5 mm. Figure 1b shows the simulation domain with an example of the ion density distribution. The calculation region has cylindrical symmetry with $R = 6$ cm and $H = 7$ cm. As in the experiment, the potential electrode with a radius of 1 mm and a length of 7 mm is inserted coaxially in a dielectric channel. A grounded metal ring is placed near the nozzle. The target (dielectric surface) is on a grounded substrate at a distance of 2.5 cm from the nozzle. To ignite the discharge in the numerical simulation, a plasma spot with increased concentration was placed near the tip of the U-electrode inside the dielectric channel at the initial moment of time, and the rate of voltage rise was set higher than the experimental one. After the first streamer propagation the voltage $U(t)$ was set as in the experiment. The simulation showed that the streamer propagation is more intense during the first few voltage cycles, and the system relaxes to a quasi-stationary state after 10–15 calculated voltage cycles. To analyze various streamer propagation patterns, simulations are performed for 10–100 voltage cycles. For simulation of multipulse gas-discharge plasma dynamics and streamer propagation, a variable time step is used in the numerical calculation based on the temporal gradient and value of the plasma conductivity.

2.3. Materials and Methods for Analysis of Cell Response to Exposure to CAPJ

Cancer cells of human lung adenocarcinoma A549 in media were exposed to CAPJ generated by positive pulsed voltage with different pulse duration and sinusoidal voltage. The cells were treated for 1 min, the pulse amplitude voltage was $U_0 = 4.2$ kV, $f = 30$ kHz, and for a sinusoidal voltage, the parameters were $U_0 =$ 3.5 kV and $f = 50$ kHz. These sinusoidal voltage parameters were optimal for processing A549 cells, and the surface temperature did not exceed 39°C.

Before the treatment, cells were seeded (3×10^3 cells per well) in 96-well plates and grown in DMEM medium (DMEM, Sigma-Aldrich), supplemented with 10% fetal bovine serum (GIBCO, Thermo Fisher Scientific, Waltham, MA, USA), 2 mM L-glutamine, 250 mg/mL amphotericin B, and 100 units/mL penicillin/streptomycin [9]. When the cells reached 70–80% confluence, they were treated with CAPJ. MCF-7 breast adenocarcinoma cells were seeded at 5000/well, 100 μL IMDM, 10% FBS, a/a, Glu.

MTT assay (colorimetric assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to evaluate cytotoxicity and cell viability.

3. OPTIMIZATION OF CAPJ CHARACTERISTICS WITH SINUSOIDAL VOLTAGE

It was shown in [7] that the plasma jet initiated by a sinusoidal voltage consists of streamers of different lengths. Only at low amplitudes and frequencies of applied voltage, streamers regularly reach the surface, but the intensity of these streamers is weak. As U and f increase, the intensity of plasma jet interaction with the surface increases nonlinearly, since only part of the streamers reaches the surface.

In this work, the frequency and amplitude of the sinusoidal voltage were varied to determine the most effective modes of treatment of cancer cells with a sufficiently low thermal effect. The intensity of plasma interaction with the surface was evaluated by measuring the discharge current near the surface and integrating the ionization near the surface in numerical simulations. The study was done for the working voltage $U_0 < 4$ kV and frequency $f = 13$–50 kHz. The most intensive plasma-surface interaction at a reasonably low surface heating was found for modes with $f =$ 50 kHz and $U_0 = 3.5$–3.8 kV. In this case streamers reach the surface in every fourth voltage cycle (Fig. 2). The measured discharge current at the surface (Fig. 2a) reaches 6.8 mA that is 2–3 times higher than at $f = 13$ and 22 kHz and $U_0 = 3.5$–3.8 kV. Increasing the voltage to 4.8 kV at $f = 50$ kHz changes the streamer propagation pattern (every second streamer touches the surface), and the discharge current at the surface decreases (Fig. 2c). In the simulations, the total ionization was integrated over time in the region of 1 cm from the surface for different parameters of the CAPJ. The normalized total ionization is shown in Fig. 2c. It can be seen that the maximum interaction intensity is achieved at $f = 50$ kHz and $U_0 = 3.8$ kV compared to regimes with $f = 13$ kHz, $U_0 = 3.8$ kV and $f = 50$ kHz and $U_0 = 4.8$ kV.

3.1. Results of Exposure of Cancer Cells to CAPJ with Sinusoidal Excitation

The efficiency of different CAPJ regimes excited by sinusoidal voltage was tested in an experiment with cells of human lung adenocarcinoma A549 and breast adenocarcinoma MCF-7. Figure 3 shows the viability of A549 and MCF-7 cells obtained by MTT analysis 24 h after CAPJ treatment. The impact lasted 1 and 2 min. The cancer cell viability results confirmed that $f = 50$ kHz was the most effective mode compared to $f = 13$ kHz for both cell types.

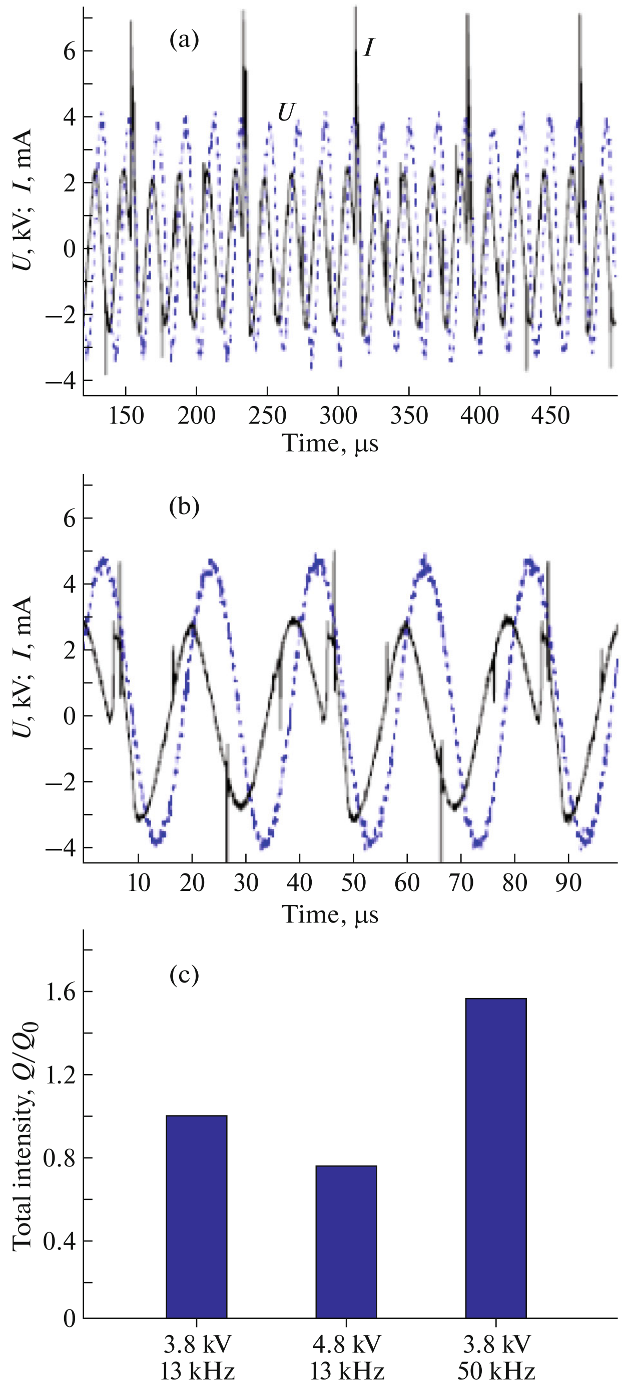

Fig. 2. Measured voltage (blue) and discharge current (black) over the dielectric surface at U_0 = 3.8 kV (a) and U_0 = 4.8 kV (b), f = 50 kHz and calculated total ionization near the surface for different discharge parameters (c).

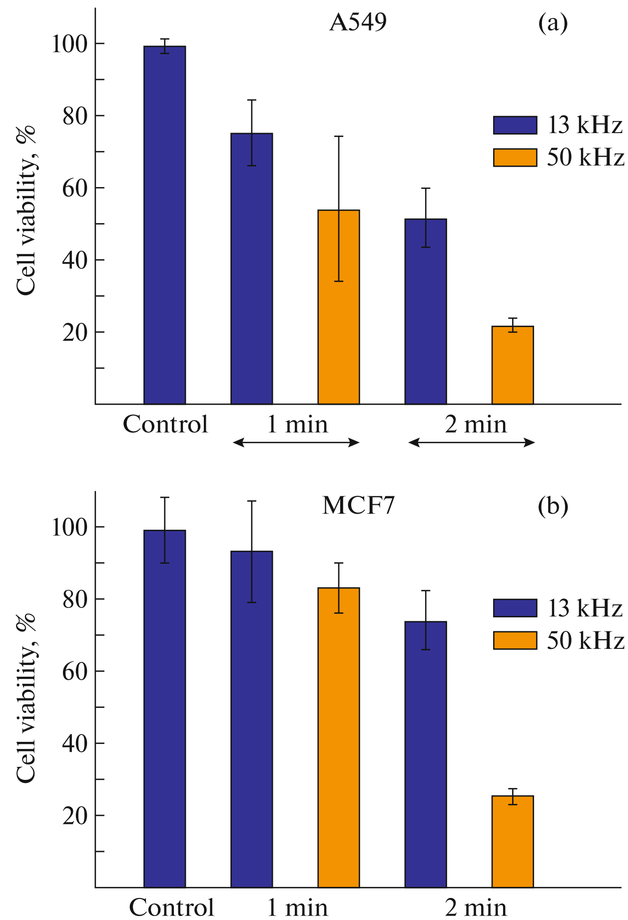

Fig. 3. Viability of cancer cell after exposure to CAPJ (a) A549, U_0 = 3.5 kV and (b) MCF-7, U_0 = 4 kV for f = 13 and 50 kHz.

4. OPTIMIZATION OF CAPJ PARAMETERS WITH POSITIVE PULSED VOLTAGE

The applied pulsed voltage and discharge current in the contact zone of the plasma jet with the dielectric surface are shown in Fig. 4 for voltage pulse lengths τ_p = 7 μs and 15 μs for U_0 = 4.2 kV and f = 30 kHz. It can be seen that with the shorter pulse length, the streamers touch the surface with each voltage pulse. At τ_p = 15 μs, the frequency of current near the target decreases by a factor of 2 since the streamers approach the target surface only every second voltage pulse, and the amplitude of the measured current decreases. This behavior typical for τ_p = 7 μs and 15 μs was observed for all cases within the range of U_0 from 3.5 kV to 4.2 kV and f from 25 kHz to 30 kHz.

The same features were found in the simulation for τ_p = 7 μs and 15 μs (see Fig. 5). The calculated z-coordinate of the streamer head associated with the maximum ionization rate indicates that the streamers touch the surface at every voltage pulse at τ_p = 7 μs and only at every second pulse at τ_p = 15 μs. Note that the ionization rate in the streamer head is significantly lower at τ_p = 15 μs. In the simulation to analyze the intensity of interaction of the plasma jet with the surface at different τ_P, the integration of the ionization rate near the surface over time was carried out. The maximum total

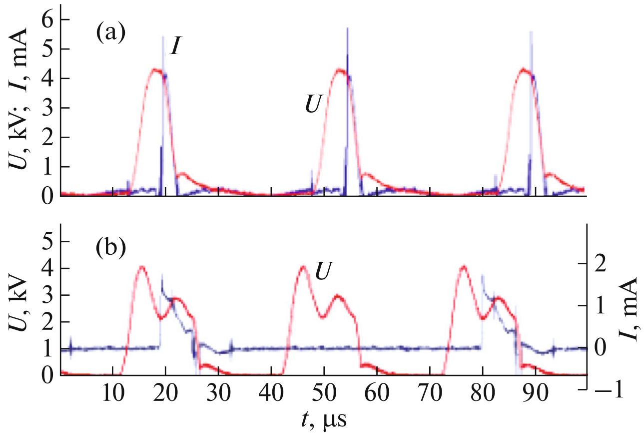

Fig. 4. Positive pulsed voltage and discharge current measured near the surface for $U_0 = 4.2$ kV, $f = 30$ kHz and voltage pulse duration τ_p = (a) 7 and (b) 15 μs.

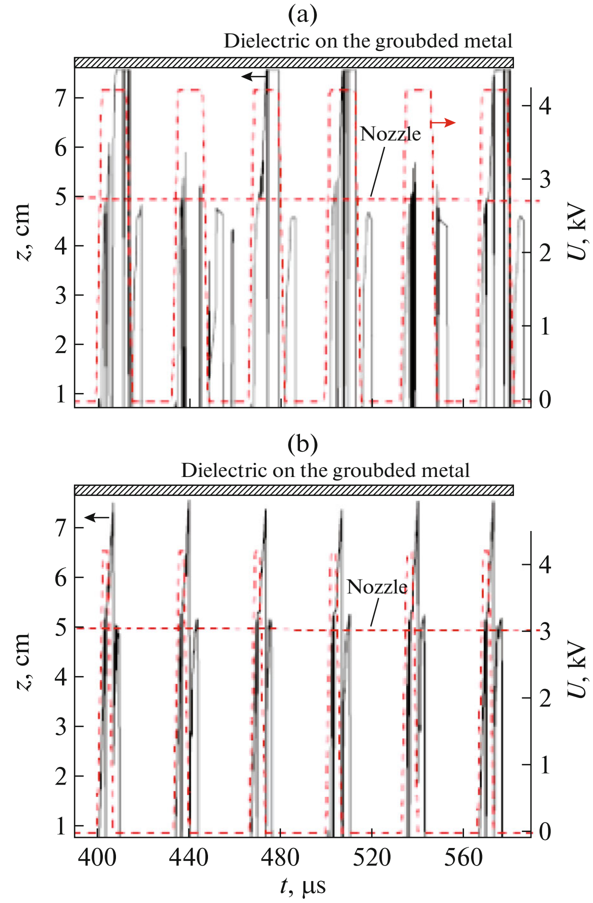

Fig. 5. Calculated z-coordinate of streamer head and discharge voltage as a function of time for U_0 = 4.2 kV, f = 30 kHz and voltage pulse duration τ_p = (a) 7 and (b) 15 μs.

ionization refers to a pulse duration of 7 μs for f = 30 kHz and U_0 = 4.2 kV.

5. COMPARISON OF HEATING AND EFFICIENCY OF CAP TREATMENT WITH SINUSOIDAL AND PULSE EXCITATION

The advantage of exposure to CAPJ generated by positive pulsed voltage is a significant reduction in target surface heating, which allows us to increase the working voltage and exposure time. Figure 6 shows the surface temperature for sinusoidal and pulsed voltages as a function of the voltage amplitude for different helium flow rates. Note that for sinusoidal voltage the dielectric plate temperature increases by 10°C with increasing U for every 0.5 kV and becomes above critical T at U_0 >3.5 kV. For the pulsed voltage case (Fig. 6b) the temperature remains low for U_0 < 5.4 kV at f = 30 kHz. The increase of the surface temperature less than 10°C is acceptable in experiments with animals. Note that usually the surface temperature increases rapidly for the first 60 s, and then increases relatively slowly.

The radiation spectra of the plasma jet measured near the surface of DMEM medium for culturing cells are shown in Fig. 7 for sinusoidal and pulsed voltages with different pulse duration. The OH line intensity at λ = 309 nm increases in 3 times when the pulse duration is reduced from 15 μs to 7 μs (U_0 = 4.2 kV and f = 30 kHz). The maximum OH line intensity corresponds to the case of sinusoidal voltage.

Lung adenocarcinoma A549 cells were treated for 1 or 2 min with plasma jet initiated by sinusoidal and positive pulsed voltage with pulse duration τ_p = 7 μs and 15 μs. After plasma exposure, cells were grown in DMEM media. In the case of CAPJ with sinusoidal voltage, the optimal generation mode with U_0 = 3.5 kV and f = 50 kHz, described above, was chosen. The results of MTT test made in 24 h after cell treatment with CAPJ are shown in Fig. 8. It is seen that the effect of exposure to CAPJ for 1 min is maximal at pulsed voltage with τ_p = 7 μs. For 2 min exposure to CAPJ the minimum cell viability corresponds to the case with sinusoidal voltage. For pulsed voltage with τ_p = 15 μs the plasma treatment of A549 cells was less effective, and the cell viability remains more than 86% even at 2 min treatment with CAPJ. Thus, we observed the high efficiency of the treatment for the optimal sinusoidal voltage mode and for the positive pulsed voltage with τ_p= 7 μs, but the temperature in the contact zone remains significantly lower with pulsed voltage.

6. CONCLUSIONS

We compared the characteristics of a low-temperature plasma jet generated by sinusoidal and positive pulsed voltages. Experimental measurements and simulation were used to analyze the patterns of the steady-

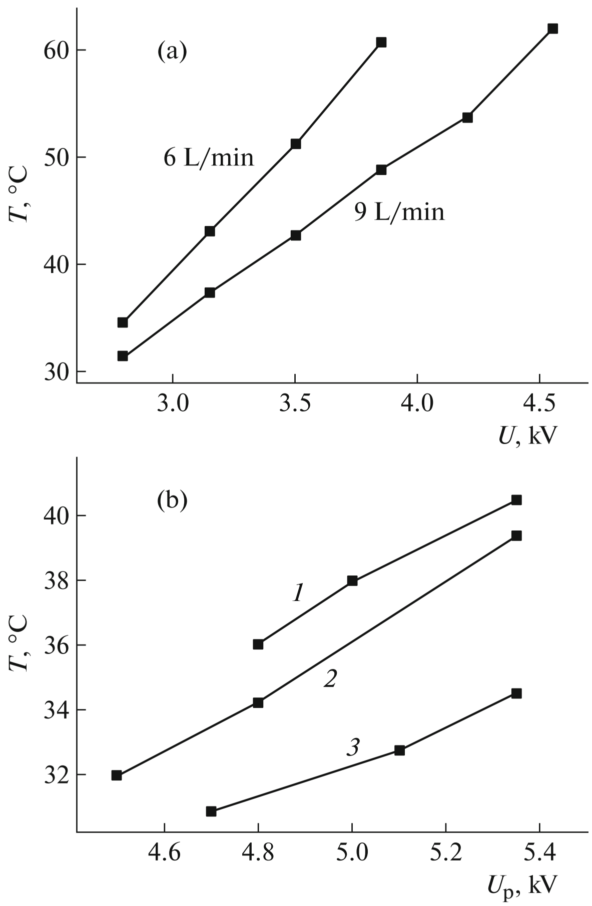

Fig. 6. Surface temperature of plate made from Al_2O_3 for (a) sinusoidal voltage as a function of voltage amplitude at $f = 22$ kHz and for (b) pulsed voltage with 7 μs duration, $f = 30$ kHz for ν = (*1*) 1.5, (*2*) 3, and (*3*) 6 L/min.

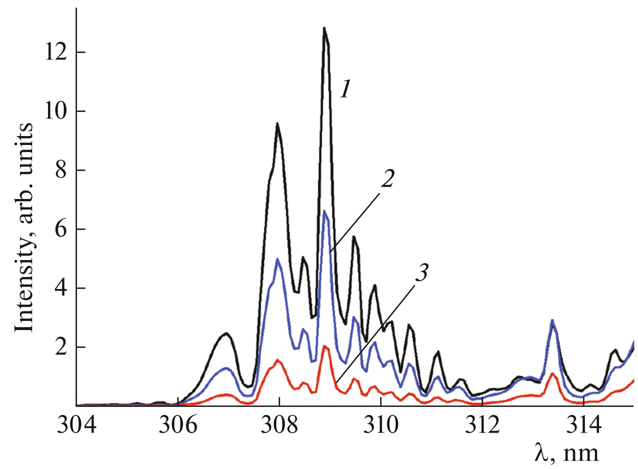

Fig. 7. Intensities of emission spectra measured near the surface for OH analysis at sinusoidal voltage ($U_0 = 3.5$ kV, $f = 50$ kHz) and pulsed voltage ($U_0 = 4.2$ kV, $f = 30$ kHz, $\tau_p = 7$ and 15 μs).

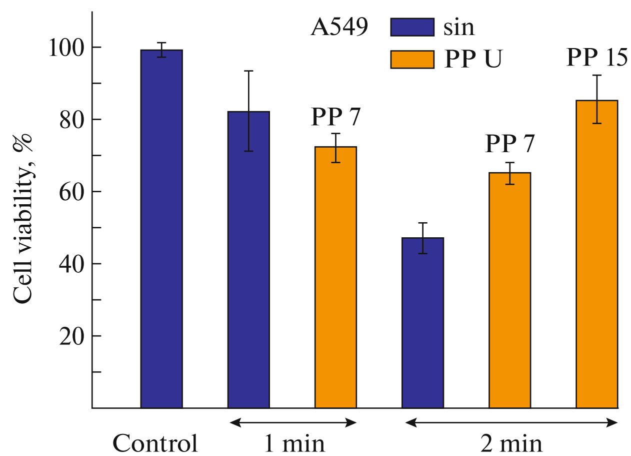

Fig. 8. Viability of A549 cells 24 h after treatment with CAPJ for 1 and 2 min, with sinusoidal voltage ($U_0 = 3.5$ kV and $f = 50$ kHz) and with pulsed voltage ($U_0 = 4.2$ kV, $f = 30$ kHz) with voltage pulse duration $\tau_p = 7$ μs and 15 μs.

state quasi-stationary propagation of streamers in the plasma jet based on the frequency and amplitude of the applied voltage. Optimal CAPJ parameters are found to suppress the viability of cancer cells. Under a sinusoidal operating voltage of $U = 3.5$ kV, voltage frequency ~50 kHz, and current frequency near the surface ~50/4 kHz, CAPJ suppresses the viability of cancer cells more effectively. However, this mode is implemented in a narrow voltage range and can switch to the mode with a current frequency equal to the voltage frequency, causing a sharp increase in heat release in the zone of plasma contact with the surface. CAPJ with pulsed voltage with a pulse duration of 7 μs also showed high efficiency in treating cancer cells. At a pulsed voltage with $\tau_p = 7$ μs, the streamers propagate regularly at each voltage cycle for voltage frequency $f <$ 30 kHz and voltage amplitude $U_0 < 4.5$ kV, and the heating of the impact zone is low. As the voltage pulse duration increases to 20 μs, the plasma jet becomes unstable, and the heating increases.

For a pulsed voltage, the intensity of the plasma - surface interaction has a maximum at $\tau_p = 7$ μs. The measured intensity of the OH line at λ = 309 nm near the medium with cells is the highest here. The discharge current measured near the surface has a maximum value at $\tau_p = 7$ μs and decreases with increasing pulse duration. The calculated total ionization level near the surface also has a maximum value at a 7-μs voltage pulse ($U_0 = 4.2$ kV, $f = 30$ kHz). An examination of the surface temperature for both sinusoidal and impulse voltage demonstrated a significant decrease in heating when using pulsed voltage.

Optimal modes of CAPJ with sinusoidal and pulsed voltages found in experiment and simulations were tested on A549 and MCF-7 A549 cancer cells. Cancer cell viability was measured by MTT-test 24 h after exposure to plasma jet. The results of the bio-experiment confirmed the high efficiency of optimal modes found for CAPJs generated with sinusoidal and pulsed voltages.

FUNDING

The authors are grateful for the financial support of the Russian Science Foundation, grant no. 19-19-00255-П. E.P. was supported by the Russian Foundation for Basic Research, grant no. 20-34-90021 for postdoctoral students.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

OPEN ACCESS

REFERENCES

1. M. Chatraie, G. Torkaman, M. Khani, H. Salehi, and B. Shokri, Sci. Rep. **8**, 5621 (2018).
2. S. Kos, T. Blagus, M. Cemazar, G. Filipic, G. Sersa, and U. Cvelbar, PLoS One **12**, e0174966 (2017). https://doi.org/10.1371/journal.pone.0174966
3. P. Gugin, D. Zakrevsky, and E. Milakhina, Pis'ma Zh. Tekh. Fiz. **47** (22), 41 (2021).
4. E. Slikboer, P. Viegas, Z. Bonaventura, E. Garcia-Caurel, A. Sobota, A. Bourdon, and O. Guaitella, Plasma Sources Sci. Technol. **28**, 095016 (2019).
5. P. Viegas, M. Hofmans, O. van Rooij, A. Obrusnik, B. L. M. Klarenaar, Z. Bonaventura, O. Guaitella, A. Sobota, and A. Bourdon, Plasma Sources Sci. Technol. **29**, 095011 (2020).
6. I. V. Schweigert, Dm. E. Zakrevsky, P. P. Gugin, E. V. Yelak, E. A. Golubitskaya, O. S. Troitskaya, and O. A. Koval, Appl. Sci. **9**, 4528 (2019).
7. I. V. Schweigert, A. L. Alexandrov, and Dm. E. Zakrevsky, Plasma Sources Sci. Technol. **29**, 12LT02 (2020).
8. I. Schweigert, S. Vagapov, L. Lin, and M. Keidar, J. Phys D: Appl. Phys. **52**, 295201 (2019).
9. O. Koval, G. Kochneva, A. Tkachenko, O. Troitskaya, G. Sivolobova, A. Grazhdantseva, A. Nushtaeva, E. Kuligina, and V. Richter, BioMed. Res. Int. **2017**, 3620510 (2017).