04

Investigation of electrophysical parameters of cold plasma jet in helium and argon

© E.V. Milakhina,[1,2] P.P. Gugin,[1] D.E. Zakrevsky,[1,2] I.V. Schweigert,[3] M.M. Biryukov,[4] E.A. Patrakova,[4] O.A. Koval[4]

[1] Rzhanov Institute of Semiconductor Physics, Siberian Branch, Russian Academy of Sciences,
630090 Novosibirsk, Russia
[2] Novosibirsk State Technical University,
630073 Novosibirsk, Russia
[3] Khristianovich Institute of Theoretical and Applied Mechanics, Siberian Branch, Russian Academy of Sciences,
630090 Novosibirsk, Russia
[4] Institute of Chemical Biology and Fundamental Medicine SB RAS,
630090 Novosibirsk, Russia
e-mail: lena.yelak@gmail.com

Received January 12, 2024
Revised March 6, 2024
Accepted March 10, 2024

Experimental studies of cold plasma jet generation parameters initiated by sinusoidal voltage in helium and argon in a single geometry of the discharge device and its interaction with model targets - dielectric plate and culture medium with cancer cells - have been carried out. Dependences of amplitude and frequency of current pulses reaching the target and its temperature under different conditions are investigated. The features and optimal parameters (amplitude and frequency of the initiating voltage, geometry of the interaction zone) and doses of helium and argon plasma jet irradiation for suppressing the viability of cancer cells of human lung adenocarcinoma A549 and breast adenocarcinoma MCF7 were determined.

Keywords: atmospheric pressure plasma jet, helium, argon

DOI: 10.61011/TP.2024.05.58517.4-24

Introduction

The low temperature plasma is an important tool in scientific and technological processes, and last time it is increasingly used in industry such as for surface treatment, ozone synthesis, exhaust gas purification, etc. [1–5] and other. Some types of [plasma due to its non-equilibrium nature (gas temperature is much below the temperature of electrons) ensure creation of active components without excessive heating of the gas and action on the targets, including living organisms that are sensitive to heat. So, last time the greater attention is paid to studies and application of plasma formations action on biological objects (see for example reviews [6–8] and literature cited in them).

Methods of cold plasma generation at atmospheric pressure are well developed, at that different geometry of the discharge gap, compositions of gas medium and methods of energy effect on gas are used. Among the many varieties of low-temperature atmospheric pressure plasma in biomedical applications the glowing high-frequency discharges, dielectric barrier discharges, and special attention is paid to so called cold plasma jets (CPJ). In this case the discharge zone is a dielectric channel, in which the work gas flow is organized, and contact (gas discharge between electrodes) or noncontact (UHF, HF, barrier discharge) excitation of medium is performed. Directly plasma jet is a type of gas discharge which under definite conditions exits beyond the discharge zone and is plasma formation spreading in the environment in the flow of the inert gas pumped through the discharge device (see for example [9–15] and other).

The result of the plasma formation action on biological object is generation in gas and water medium of ions, nitrogen and oxygen containing radical, which actively interact with the cells of biological tissue, damaging them and causing cell death. Of particular interest is the fact that active components, generated by plasma, suppress vital activity of malignancies, having selective effect on healthy and tumor cells. Antitumor effect was reliably demonstrated on more than twenty various tumor models *in vitro* and *in vivo* [6,13] etc.

Upon positive dynamics of number and quality of the performed studies there are many unsolved issues, mainly related the mechanisms of plasma-chemical and plasma-physical effects on tumor and healthy cells. The processes and mechanisms resulting in death of cancerous cells upon interaction with plasma formations are complex, and are currently studied intensively (see for example,[16]). The problem also lies in the difficulty of comparison and the non-reproducibility of study results as result of various geometry of the experimental set-ups[17,18]; various conditions and design of physical- biological experiments; absence of the standard methods of irradiation; differences in plasma parameters near the target surface and biological objects of various nature *in vivo* and *in vitro*; manifestation of physical features during plasma interaction with targets, absence of monitoring and comparison of the electrical characteristics of various objects, and effect of the external medium and other uncontrolled factors. During plasma effect on the biological objects for CPJ initiation various types of gas discharge are used in various frequency ranges, with significantly different initiating voltages and rates of plasma gas flow (air, helium, argon,their mixtures with oxygen, nitrogen). Variety of ways for generation and conditions of the plasma effect results in complexity of comparison of the study results, and hence, translation of developed methods for model and, especially, live objects with further transfer to clinical trials may be challenging.

Note that CPJ kinetics and dynamics of its interaction with various objects significantly depends on properties of irradiated target (see for example [19–23]).

Study of physical parameters of CPJ, generated in helium and argon, and of the effects of their action on the biological objects [24–30], systematization and summarization of the results show that plasma jet parameters (amplitude and frequency of initiating voltage; current reaching the target; flow rate and grade of plasma generating gas; irradiation dose), at which selectivity of action and maximal cytotoxic effect (effect of suppressing the vital activity of cancer cells) have significant differences. So, we assume as actual the „comparative" study of plasma jet parameters and limiting factors of plasma action on biological objects upon CPJ initiation by sinusoidal voltage in helium and argon in same geometry of the discharge device as applied to the biophysical studies. This is the objective of this paper.

1. Experimental set-up

The plasma jet source is a discharge cell in form of coaxial dielectric channel $L = 100$ mm long with internal diameter of 10 mm. In channel center a copper electrode in form of rod with length l and diameter d. The channel end comprises a nozzle — dielectric capillary with orifice diameter d_0 and length l_0. The discharge zone was formed by internal potential electrode and ring grounded electrode arranged outside the dielectric channel near the nozzle (Fig. 1).

For the plasma jet generation the sinusoidal voltage source with regulated frequency $f_U = 10-52$ kHz and voltage with amplitude U up to 7 kV was used. Voltage (current reaching the irradiation object) value was specifically limited to ensure safe CPJ treatment conditions for future experiments with animal models. Experiments were performed with helium He (volume fraction of helium 99.995%) and argon Ar (volume fraction of argon 99.99%). For voltage U measurement the ohmic high resistance divider was used. Current was measured by a sensor located at distance from the nozzle z at right angle to CPJ spreading axis, and it is a collector — flat metal electrode (copper plate). With the collector grounded via a low-inductance resistor, one could register amplitude and frequency of current pulse I reaching the collector.

As CPJ treatment object a target was used — ceramic plate of Al_2O_3 1 mm thick located on the collector. In all experiments the earthed metal collector was used as additional electrode to create a plasma streamer−grounded electrode for configuration of electric field with increased strength. This results in generation intensification of active radicals in contact zone of CPJ and target [23,26]. Distance from the nozzle to the target z is set such that the plasma jet touches the target, and is optimized by maximum suppression of vital activity of cancer cells in biophysical experiments [23–30]. Spectral measurements were performed by spectrometer „Kolibri-2" [31] set to wavelength band $\lambda = 190-360$ nm with optical resolution 0.17 nm. The optical irradiation of the contact area of plasma jet and target was registered at distance 3 mm at angle 30° from vertical. Then irradiation was transmitted to the spectrometer via multimode quartz optical fiber waveguide. All experiments were accompanied by temperature measurement of interaction area of plasma jet and object using thermal camera Testo 872 [32].

2. Experimental results and discussion

When work gas helium is supplied with flow rate $\upsilon \geq 1-2$ L/min, and sinusoidal voltage with frequency $f_U \geq 12$ kHz and amplitude $U \geq 1$ kV is applied to electrodes in the discharge zone potential electrode−earth electrode the discharge occurs. In helium CPJ development reduced to stages that are well separated by voltage: occurrence on potential electrode of luminous spots ($U \approx 1.1$ kV); discharge formation ($U > 1.4$ kV) followed by its spreading in the channel upon voltage increasing; occurrence of plasma jet outside the discharge channel and spreading in free space ($U > 2$ kV). In argon the discharge ignites at $\upsilon \geq 1$ L/min, at $U \geq 2.8$ kV and at frequency $f_U \geq 20$ kHz. In this case, the breakdown of the discharge gap and the formation of the plasma jet extending beyond the nozzle occur almost simultaneously.

In helium and argon visually a significant difference is observed between the nature of the discharge glow inside the dielectric channel and, respectively, by nature of current flow inside the channel. In helium the discharge is a stable unidirectional current cord (Fig. 1, a), in argon unstable (stochastic) nature of the discharge is observed (Fig. 1, b). Note that at decrease in inner diameter of the dielectric channel (below 5 mm) the discharge in argon stabilizes, and, as in helium, i becomes unidirectional. CPJ photographic registration demonstrates that the plasma jet in the gap nozzle−target is heterogeneous. In helium at the nozzle output the unidirectional current channel is observed, which in area before the target stratifies — in each pulse the formed streamer spreads via different spatial path. This phenomenon can be explained by charge accumulation on the dielectric target in the point where the streamer touches

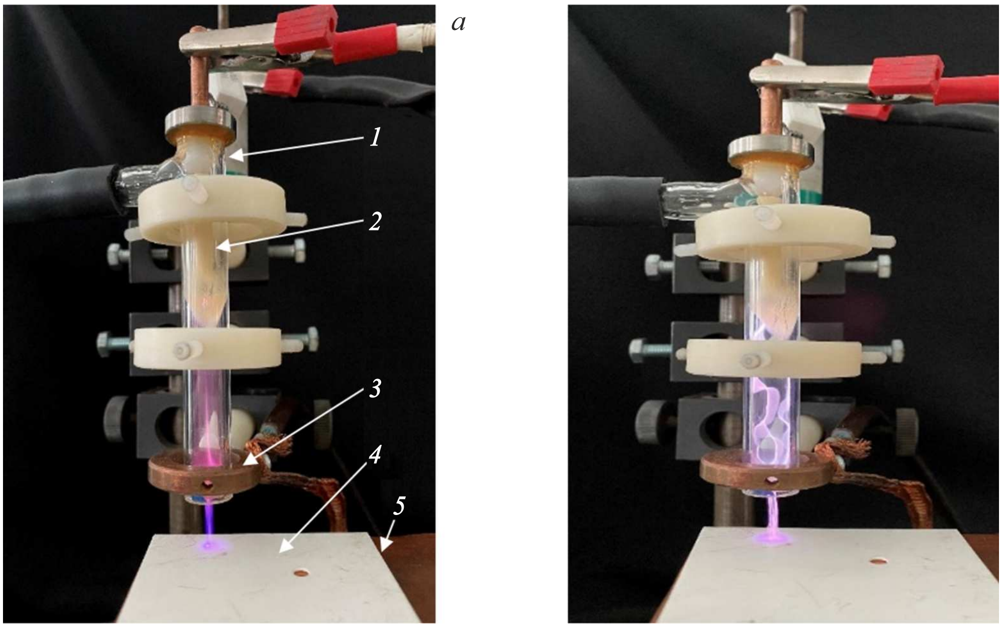

Figure 1. Design of device for CPJ generation and photography in helium (*a*) and in argon (*b*): *1* — dielectric channel, *2* — internal potential electrode, *3* — external grounded electrode, *4* — ceramic plate, *5* – additional grounded electrode.

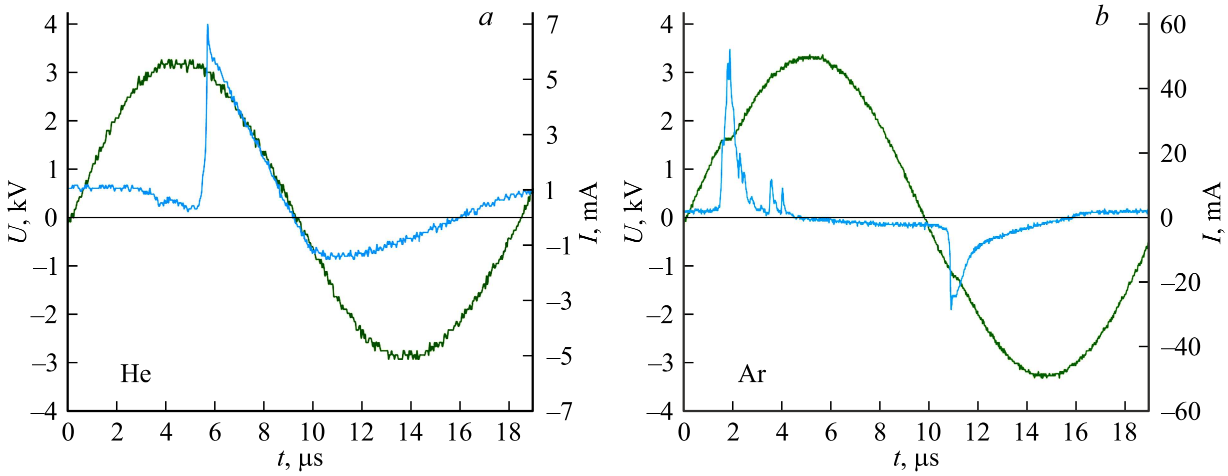

Figure 2. Fragments of oscillograms of CPJ in: *a* – helium ($\upsilon = 9$ L/min, $z = 20$ mm); *b* – argon ($\upsilon = 4$ L/min, $z = 15$ mm).

the surface. Due to heterogeneous distribution of the surface charge the radial electric field occurs, it ensures deviation of the next streamer head upon approaching the target surface. In argon this effect is stronger — CPJ stratification is observed along entire length of the jet.

The spatial localization of the plasma jet outside the source was governed by the channel geometry, the capillary size, the gas flow rate, and the voltage amplitude. Preliminary studies and optimization of the plasma jet by linear dimensions, required to manipulate and to perform biological experiments, as well as by glow intensity of hydroxyl radical OH ($\lambda \approx 309$ nm was registered, transition $A^2\Sigma - X^2\Pi$) ensure selection geometry of the discharge gap, nozzle, parameters of initiating voltage and work gas flow rate. At length of potential electrode $l = 50$ mm, its diameter $d = 2$ mm; dimensions of nozzle: orifice diameter $d_0 = 2.3-2.6$ mm and length $l_0 = 5$ mm, in helium at voltage amplitude $U \approx 3-5$ kV and flowrate $\upsilon = 6-9$ L/min the jet length was ~ 70 mm; in argon at $U \approx 4-5$ kV and $\upsilon = 4$ L/min the jet length was $\sim 25-30$ mm. Note that ratio of glow intensity of radical OH ($\lambda \approx 309$ nm) upon contact with the ceramic target/culture liquid upon presence/absence of grounded collector was ~ 10 and ~ 3 in helium and argon, respectively.

Fig. 2 shows oscillograms of one period of voltage and current of plasma jet reaching the target located on the grounded collector at distance $z = 20$ and 15 mm in helium and argon respectively. In helium at positive half-wave

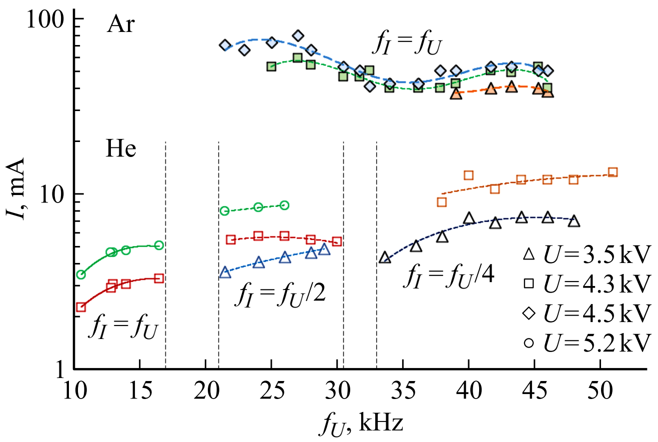

Figure 3. Dependences $I(f_U)$, $z = 15$ mm: He ($U = 3.5$, 4.3 and 5.2 kV ($\upsilon = 9$ L/min), Ar ($U = 3.5$, 4.3 and 4.5 kV, $\upsilon = 4$ L/min).

we observe positive current pulse (which is typical for the used scheme of current registration). In argon the current pulses are observed at positive and negative half-waves of voltage, at that they have different amplitudes. Note that in experiments in helium at small distances $z < 5$ mm current pulses are also observed at positive and negative half-waves of the initiating voltage.

In [33] it was shown that interaction of the plasma jet generated under sinusoidal excitation in the helium flow ($U = 2-5$ kV, $f_U = 25-50$ kHz), with target located on earthed metal collector, resulted to effect of self-stabilization of current frequency. Frequency of target touching by plasma jet f_I differs from frequency of applied initiating voltage f_U and can be a stable multiple of it — $f_I = f_U/2$, $f_U/3$, $f_U/4$. The effect depends on frequency, amplitude of applied voltage, and also on type of target, distance to it, and is determined by ratio of density of plasma formed in the streamer head to residual density of plasma above target surface. The streamer is formed and spreads inside the dielectric channel in each cycle of voltage, but after exit form the nozzle the streamer can reach the target or extinguish upon interaction with cloud of quasi-neutral plasma produced by previous streamers [26]. This results in that dependences of current amplitude reaching the target on frequency of initiating voltage $I(f_U)$ are irregular in nature, dependences of current amplitude on voltage amplitude $I(U)$ in wide range U for various f_U are ambiguous. As electromagnetic, chemical, thermal processes on the target surface depend on frequency of contacts of plasma and target, then ambiguity of CPJ parameters and respectively of the parameters of irradiation of biological object can result in uncertainty o the results of biophysical experiments.

Fig. 3 shows frequency characteristics — dependences $I(f_U)$ at $z = 15$ mm in helium for $U = 3.5$, 4.3 and 5.2 kV ($\upsilon = 9$ L/min) and in argon for $U = 3.5$, 4.2 and 4.5 kV ($\upsilon = 4$ L/min). In helium the common tendency is increase in amplitude of current reaching the target, upon increase in frequency of applied voltage for same voltages, and upon increase in voltage for same frequencies. Maximum achieved current amplitudes doe not exceed 15 mA. Upon f_U increasing the current frequency f_I is proportional to f_U, $f_U/2$ and $f_U/4$. Experiments performed for three fixed frequencies of the initiating voltage $f_U \approx 52$, 26 and 13 kHz at $\upsilon = 9$ L/min and $z = 20$ mm, demonstrated that on target the current pulses are registered with same frequency $f_I \approx f_U = 52/4$, 26/2 and 13 kHz respectively (Fig. 4).

Between the regions of one-to-one correspondence between the frequency of the initiating voltage and current reaching the target there are transition zones with irregular („floating“) frequency f_I, shown in Fig. 3 with vertical dashed straight lines. For all frequencies the distance increasing between the nozzle and target z results in current I decreasing. For example, at $U = 3.5$ kV and $f_U = 52$ kHz ($f_I = f_U/4 \approx 13$ kHz) at $z = 15$; 20 and 25 mm current reaching the substrate is $I \approx 6.8$, 6.2 and 5.1 mA, respectively.

In argon the dependences $I(f_U)$ have another nature. In entire studied range of amplitude and frequency of initiating voltage we observe the regular nature of current pulses $f_I = f_U$ is observed with tendency of current decreasing upon increase in f_U and accompanied $\sim 40\%$ modulation, and increase in current amplitude with voltage increasing for same frequencies. Upon U decreasing there is increase in frequency f_U, at which discharge ignites inside the dielectric channel. For example, at $U \leq 3.5$ kV the discharge ignites at $f_U > 38$ kHz, and at $U \approx 4.5$ kV the discharge ignites at $f_U \approx 20$ kHz. The current amplitude achieved ~ 80 mA at $U \geq 4.5$ kV. At comparable initiating voltages of the plasma jet currents of CPJ in argon are significantly larger than in helium, which can be uncomfortable and dangerous upon the living objects irradiation from the point of view of electrical safety.

Fig. 5 shows the dependences $I(U)$ for CPJ in helium and argon. For helium sets of curves $I(U)$ are given at $z = 20$ mm for the following conditions: $f_U \approx 13$ kHz, $\upsilon = 6$ and 9 L/min (conditions under which the one-to-one correspondence $f_I \approx f_U$ is observed, curves *1,2*); $f_U \approx 26$ kHz, $\upsilon = 6$ and 9 L/min (conditions under which $f_I \approx f_U/2$, curves *3,4*); $f_U \approx 52$ kHz, $\upsilon = 9$ L/min (conditions under which $f_I \approx f_U/4$, curve *5*). For argon the dependences $I(U)$ are given at $z = 15$ mm for: $f_U \approx 50$ kHz and $\upsilon = 3$; 4 and 6 L/min (curves *6-8* respectively), $f_U \approx 24$ kHz and $\upsilon = 4$ L/min (curve *9*) and $f_U = 13$ kHz and $\upsilon = 4$ L/min (curve *10*). In helium the dependences have same nature: with voltage U increasing the current I sublinearly increases; in the studied range of flow rates υ the current weakly depends on υ. Opposite to [34], where dependences $I(U)$ were studied upon CPJ contact with metal collector, here, upon CPJ contact with dielectric target the achieved current is below $I \sim 3-4$ mA. In argon upon regular nature of $I(f_U)$ with voltage increasing the amplitude of current pulses increases for all f_U and all υ.

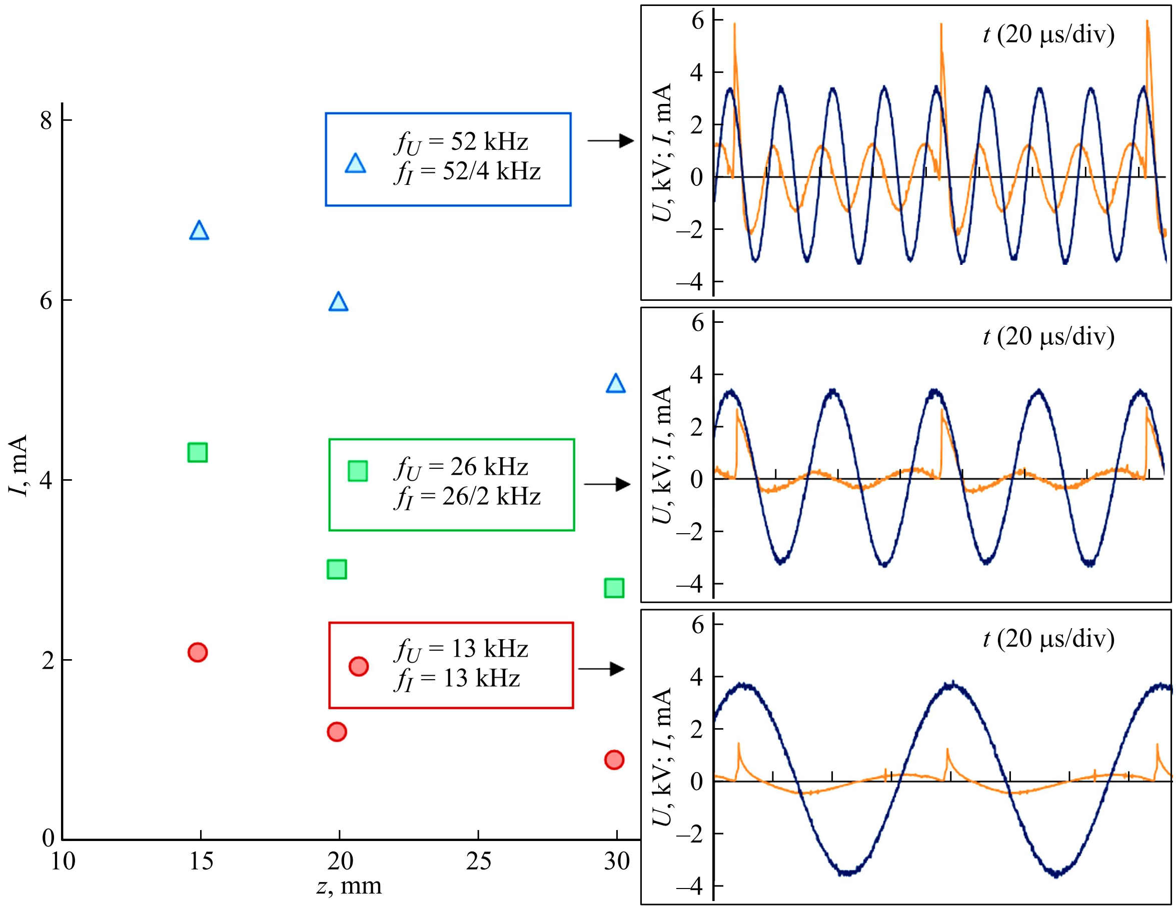

Figure 4. Dependences $I(z)$ and oscillograms of CPJ at different f_U; He, 9 L/min, $z = 20$ mm.

At same voltages with increase in flow rate $\upsilon = 1-5$ L/min the larger current are achieved. For lower $f_U = 13$ and 24 kHz the nature of dependences $I(f_U)$ does not change. In helium at $\upsilon > 12$ L/min, in argon at $\upsilon > 5$ L/min in such geometry of nozzle and discharge cell the gas flow exiting the nozzle becomes unstable in space, and irregularly reaches the collector, this is linked with the transition from laminar to turbulent mode of gas flow.

One of factors of CPJ action on the biological objects is the thermal effect. The therapeutic use of plasma action limits the allowable heating of the object thus decreasing the possible range of parameters of plasma jet. Set of biological experimental results of authors [25–30] demonstrates that suppression of the vital activity of malignant cells are more effective with more intense interaction of plasma with the object. With increase in voltage (current) and frequency of touch of streamer and object the intensity of CPJ interaction with target increases. It is obvious that temperature of the biological object $T = T_0 + \Delta T$ (where T_0 — own temperature, ΔT — temperature increasing due to irradiation by CPJ) shall not exceed the maximum allowable $T = 42°$C, as this can result in burn and destabilization of protein tissues.

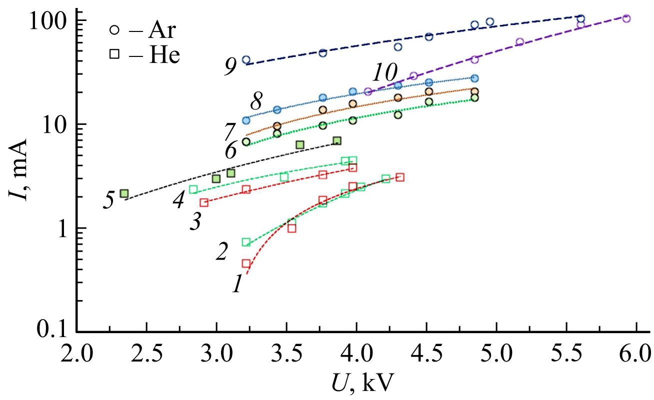

Figure 5. Dependences $I(U)$: He ($f_U \approx 13$ (*1,2*); 26 (*3,4*) and 52 kHz (*5*), $\upsilon = 6$ (*1,3*) and 9 (*2,4,5*) L/min, $z = 20$ mm); Ar ($f_U \approx 50$ (*6–8*); 24 (*9*) and 13 kHz (*10*), $\upsilon = 3$ (*6*); 4 (*7,9,10*) and 5 (*5*) L/min, $z = 15$ mm).

Fig. 6 shows temperature increase of target vs. time of exposure $\Delta T(t)$ upon irradiation by the plasma jet generated in helium (Fig. 6, *a*), at $U = 3.5$ and 3.9 kV, $f_U = 13$ kHz, $z = 20$ mm, $\upsilon = 3$ and 9 L/min (mode $f_I \approx f_U$) and $U = 2.9$ and 3.3 kV, $f_U = 52$ kHz, $z = 20$ mm, $\upsilon = 6$ and 9 L/min (mode $f_I \approx f_U/4$) and in argon (Fig. 6, *b*) at $U = 3.5$ and 3.9 kV, $f_U = 13$ and 50 kHz, $z = 15$ mm and $\upsilon = 4$ L/min.

In helium the characteristic time for target temperature setting does not exceed $t \approx 60$ s and further the dependence $\Delta T(t)$ has practically stationary view. In operation modes CPJ with frequency of the initiating voltage $f_U = 13$ kHz (mode $f_I \approx f_U$) at fixed distance nozzle — target z with increase in voltage amplitude U at constant flow rates υ (Fig. 6, *a*, curves *1,2*) and upon decrease in υ at constant U (Fig. 6, *a*, curves *2,3*) the target temperature T increases. At specified parameters the target temperature increase is in range $\Delta T = 4.5{-}15°$C. Similarly at $f_U = 52$ kHz (mode $f_I \approx f_U/4$) the target temperature increases upon increase in U at constant υ (Fig. 6, *a*, curves *4,6*) and decrease in υ at constant U (Fig. 6, *a*, curves *5,6*) with $\Delta T = 10{-}20°$C. At larger frequencies f_U at $U > 4.5$ kV ($f_I \approx f_U/2$) increase in ΔT can exceed 30°C [35]. For ΔT decreasing it is necessary either to decrease the work voltage or to increase distance between the nozzle and irradiated object, which is not always acceptable, as it leads to decrease in current reaching the target, and which value is linked to the intensity of generation of active radicals on its surface. Another method is flow rate increasing of the work gas, which, in

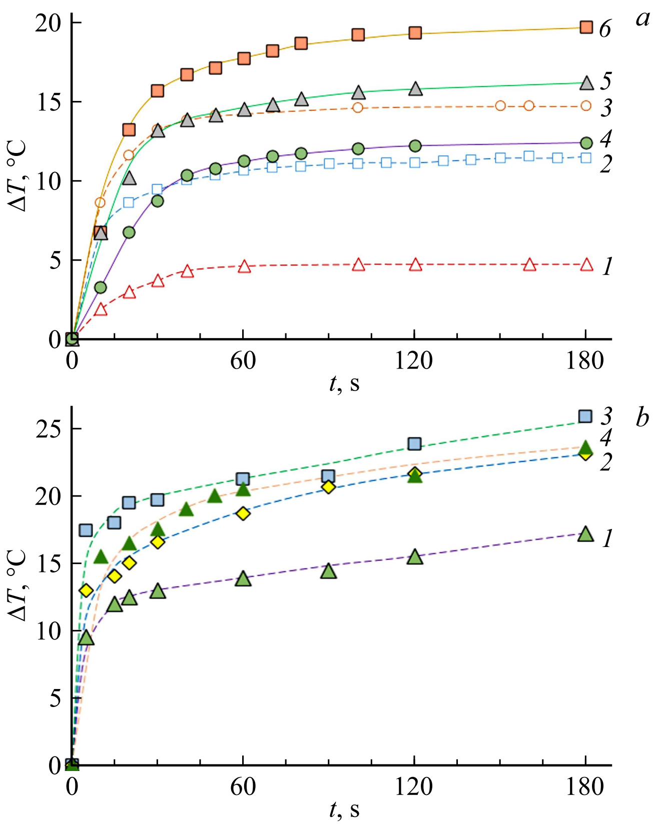

Figure 6. Dependences $\Delta T(t)$: *a* — He, $\upsilon = 9$ (*1,2,5*); 6 (*4*); 3 (*3*) l/min, $f_U = 13$ (*1–3*); 52 (*4–6*) kHz; $U = 3.9$ (*2,3*); 3.5 (*1*); 3.3 (*5,6*) and 2.9 (*4*) kV; $z = 20$ mm; *b* — Ar, $\upsilon = 4$ (*1,3,4*); 3 (*2*) l/min, $f_U = 52$ (*1–3*); 13 (*4*) kHz; $U = 3.5$ (*1*); 3.9 (*2–4*) kV; $z = 15$ mm.

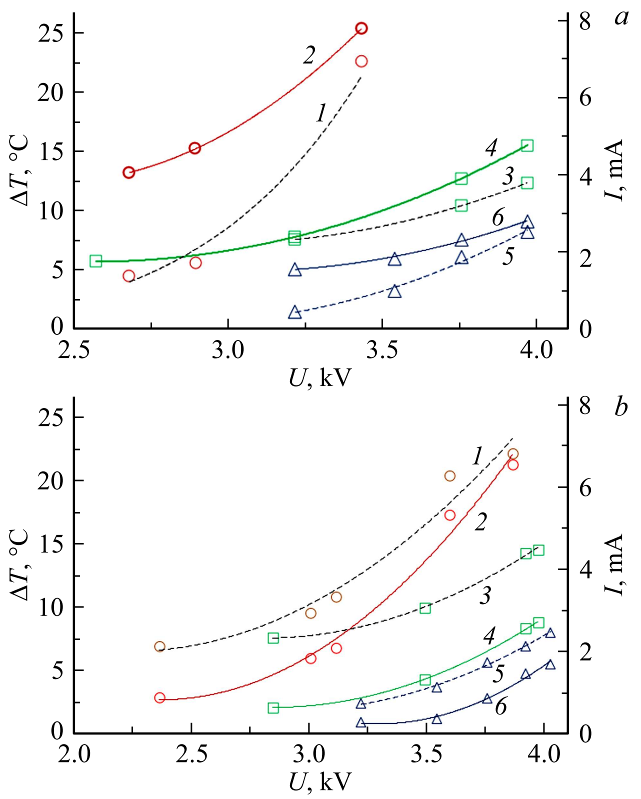

Figure 7. Dependences: *1,3,5* — $I(U)$, *2,4,6* — $\Delta T(U)$ for $\upsilon = 6$ (*a*) and 9 (*b*) L/min at $f_U = 52$ (*1,2*); 26 (*3,4*) and 13 (*5,6*) kHz.

its turn, is associated with deviation from the conditions of optimal concentration of the active radicals being the extreme function of flow rate [25]. If the temperature of surface (skin) of the vital object is $T \approx 34{-}35°$C then the allowable value is $\Delta T \approx 6{-}8°$C, which predetermines the choice of the necessary CPJ parameters, dose and distance to the irradiated object. So, in experiments of animals — mouses (8–12 week males of the line BALB/C with average weight 25–30 g breeding of vivarium of ICBFM of Siberian Branch of RAS) irradiation with helium plasma jet with parameters $U = 4.5$ kV, $f_U \approx 13$ kHz, $z = 20$ mm, $\upsilon = 9$ L/min, irradiation time $t = 1{-}2$ min average skin temperature achieved $T \approx 37.8 \pm 0.7°$C [26]. Under similar conditions with $f_U \approx 52$ kHz c $f_I = fU/4 \approx 13$ kHz the average skin temperature can exceed $T \approx 41°$C.

In argon the characteristic time for setting the target temperature is $t \approx 30$ s with further tendency of slow setting of stationary state. The target temperature increases with U increasing at constant υ (Fig. 6, *b*, curves *1,3*); upon f_U increasing at constant υ and U (Fig. 6, *b*, curves *3,4*); upon υ increasing at constant U. Under similar nature of dependences and patterns of target heating $\Delta T(t)$ and $\Delta T(U)$, typical for CPJ in helium, the temperature increase can achieve significant values $\Delta T \approx 15{-}30°$C and over. The same temperature increase is acceptable during study of the plasma action on cells *in vitro* with own temperature T_0,

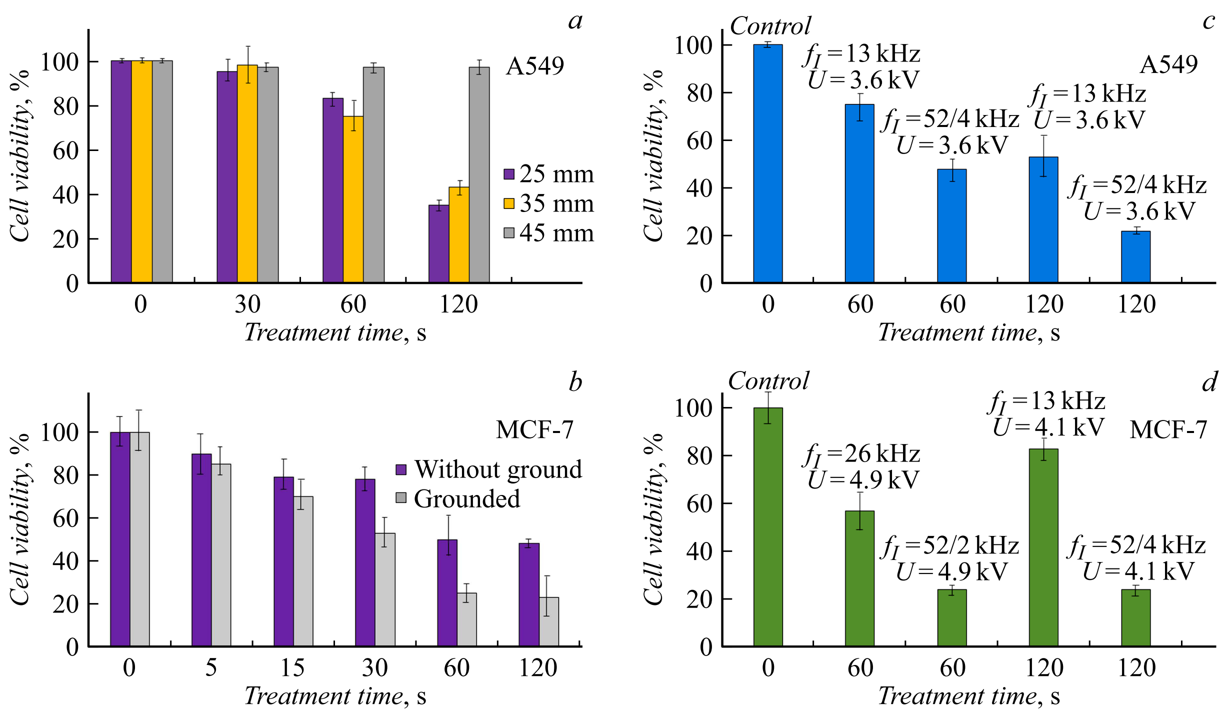

Figure 8. Viability of cancer cells A549 (*a,c*), MCF7 (*b,d*); He (*a,c,d*); Ar (*b*): a — $U = 4.5$ kV, $f_U \approx 23$ kHz, $\upsilon = 9$ L/min, $z = 25$; 35; 45 mm; b — $U = 4.9$ kV, $f_U \approx 40$ kHz, $\upsilon = 4$ L/min, $z = 25$ mm; $c - U = 3.9$ kV, $f_U \approx 13$ and 52 kHz, $\upsilon = 9$ L/min, $z = 25$ mm; $d - U = 4.1$ and 4.9 kV, $f_U \approx 13$; 23 and 52 kHz, $\upsilon = 9$ L/min, $z = 25$ mm, $t = 1$ and 2 min.

close to room temperature, but is unacceptable for action on vital organisms. Besides the above factors of ΔT decreasing the dose — irradiation time shall be limited. For example in [29] at CPJ with parameters $U = 4.9$ kV, $f_U \approx 40$ kHz, $z = 25$ mm, $\upsilon = 4$ L/min typical irradiation time $t = 30-120$ s depending on volume of holes of culture plate and irradiated biological material. Upon action on the vital objects the irradiation time shall be more limited. Results of experiments show that the argon plasma jet has more narrower range of allowable modes for action on the biological, especially vital objects.

Fig. 7 shows dependences of current amplitude I (curves *1,3,5*) and target temperature increase ΔT (curves *2,4,6*) on voltage amplitude U for flow rates $\upsilon = 6$ (Fig. 7, *a*) and 9 (Fig. 7, *b*) L/min at $f_U = 52$ (curves *1,2*); 26 (curves *3,4*) and 13 kHz (curves *5,6*), $z = 25$ mm. Figures show that at similar voltages the current amplitude with $f_I \approx f_U = 52/4$ kHz exceeds the current amplitude with $f_U = 26/2$ kHz and current amplitude with $f_U = 13$ kHz. In its turn the target heating is greater the greater current reaching the target is.

As illustration of the above mentioned we provide further the experimental results of action of plasma jet generated in helium and in argon, on cells of adenocarcinomas of the lung A549 and human breast adenocarcinoma cells MCF7 (Fig. 8). After irradiation by CPJ in all experiments the cells were grown up in nutrient media. The cells were cultured in the medium DMEM (A549) and IMDM (MCF7) in presence of 2 mmol L-glutamine, 10% fetal bovine serum (FBS), 1x antibiotic-antimycotic (100 u/mL penicillin, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin) at temperature $T = 37.0 \pm 1.0$°C and atmosphere CO_2 5.0 ± 0.5%.

Cell viability was assessed using the MTT assay (a colorimetric test of cell metabolic activity) performed in 24 h after irradiation. All results are presented as average value of fraction of living cells in three independent experiments±standard deviation.

Fig. 8, *a* presents the results of study of viability of human lung adenocarcinoma cells A549 upon treatment by plasma jet initiated in helium, with $U = 4.5$ kV, $f_U = 23$ kHz and $\upsilon = 9$ L/min at different distances from nozzle to level of culture liquid with cells in plate with $z = 25$, 35 and 45 mm at irradiation time $t = 30$, 60 and 120 s. The Figure shows that increase in dose−CPJ irradiation time; decrease in z, accompanied by decrease in current reaching the biologic object, definitely leads to decrease in the survival of cancer cells, but the effectiveness will be limited by thermal factors of influence (Fig. 6). Fig. 8, *b* shows the effect of treatment by argon cold plasma with $U = 4.9$ kV, $f_U = 40$ kHz and $\upsilon = 4$ L/min on viability of breast adenocarcinoma cells MCF7 using the grounded collector with installed studied objects and without it. Irradiation doses are determined, and, as in case of helium plasma jet [33], strengthening of the electric field in zone of interaction of argon plasma jet and cells, accompanied by generation intensification of hydroxyl radical OH, results in strengthening of the effect of suppression of vital activity of cancer cells. Fig. 8, c provides results on interaction of plasma het generated in helium, initiated by sinusoidal voltage, with frequency $f_U = 13$ kHz ($f_I = f_U$) and 52 kHz ($f_I = f_U/4 \approx 13$ kHz) with $U = 3.9$ kV, $\upsilon = 9$ L/min, $z = 25$ mm on lung adenocarcinoma cells A549 during $t = 1$ min. It is evident that CPJ treatment is maximum if initiated by voltage with $f_U = 52$ kHz, corresponding to large current reaching the biological object, as compared with conditions $f_U = 13$ kHz.

Fig. 8, d shows the similar results on treatment by plasma jet generated in helium,initiated by sinusoidal voltage with $U = 4.1$ and 4.9 kV with frequency $f_U = 13$ kHz ($f_I = f_U$), 23 kHz ($f_I = f_U$), 52 Hz ($f_I = f_U/2$ for $U = 4.9$ kV and $f_I = fU/4$ for $U = 4.1$ kV), $\upsilon = 9$ L/min, $z = 25$ mm of human breast adenocarcinoma cells MCF7 with $t = 1$ and 2 min. It is evident that in this case CPJ treatment is maximum when initiated by voltage with higher frequency, corresponding to lower contact frequency of current and higher current.

Conclusion

Study of the regeneration parameters of cold plasma jet in different modes and their optimization to achieve maximum cytotoxic effect when acting on cancer cells is necessary for the development of original methods of plasma medicine. In paper we perform comparative experimental studies of generation parameters of CPJ initiated by sinusoidal voltage in helium and argon in same geometry of discharge device and its interaction with model dielectric plate and culture medium comprising cancer cells. Effects of CPJ treatment are characterized based on amplitude and touch frequency of current pulses reaching the target, intensity of spectra of hydroxyl radical OH, and temperature on surface of the irradiated object. We determine the optimal parameters (amplitude and frequency of initiating voltage, geometry of interaction zone) and dose of irradiation by helium and argon plasma jet to suppress viability of human lung adenocarcinoma cancer cells A549 and adenocarcinomas of the mammary gland MCF7 under various conditions. It is shown that presence of the grounded collector under the culture plate with the irradiated cells strengthens the cytotoxic effect. Under other comparable parameters of excitation CPJ in argon is characterized by smaller spatial length, as compared to CPJ in helium, which determines the necessary closer location of the irradiated object to the source of plasma formation and achievability of much larger (practically by order of magnitude) currents on biological object. This in its turn is accompanied by increase in heating of the irradiated object, and this can be dangerous for the vital objects and limits the possible range of CPJ parameters in argon as per irradiation dose. In its turn the particularity of interaction of helium plasma jet and target is mismatch of touch frequency of jet and object and frequency of applied initiating voltage. This results in that the frequency dependences $I(f_U)$ have unregular nature, and dependences $I(U)$ in wide range of voltages and frequencies are ambiguous. Such situation results to uncertainty and unpredictability of the results of biophysical experiments. In this regard, based on the set of parameters, the pulsed initiation of CPJ with controllable pulse width at higher voltage seems more promising, as compared to the sinusoidal voltage which ensures larger current on the irradiated surface at limited heating, and generation of higher electric fields on surface [36].

Funding

This study was supported financially by State Assignment № FWGW 2021-0012 (relating to physical experiments) and Russian Scientific Fund № 22-49-08003 (https://rscf.ru/project/22-49-08003/).

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] U. Kogelschatz. Plasma Chem. Plasma Proc., **23**, 1 (2003). DOI: 10.1023/A:1022470901385
[2] F. Fanelli, F. Fracassi. Surf. Coatings Technol., **322**, 174 (2017). DOI: 10.1016/j.surfcoat.2017.05.027
[3] M. Kambara, S. Kawaguchi, H.J. Lee, K. Ikuse, S. Hamaguchi, T. Ohmori, K. Ishikawa. Jpn. J. Appl. Phys., **62**, SA0803 (2023). DOI: 10.35848/1347-4065/ac9189
[4] M. Kogoma, K. Tanaka. Rev. Modern Plasma Phys., **5**, 3 (2021). DOI: 10.1007/s41614-021-00050-4
[5] K. Takaki, K. Takahashi, N. Hayashi, D. Wang, T. Ohshima. Rev. Modern Plasma Phys., **5**, 12 (2021). DOI: 10.1007/s41614-021-00059-9
[6] M. Laroussi, X. Lu, M. Keidar. J. Appl. Phys., **122**, 020901 (2017). DOI: 10.1063/1.4993710
[7] Th. von Woedtke, S. Emmert, H.-R. Metelmann, S. Rupf, K.-D. Weltmann. Phys. Plasmas, **27**, 070601 (2020). DOI: 10.1088/1361-6595/ac604f
[8] J.C. Harley, N. Suchowerska, D.R. McKenzie. Biophys. Rev., **12**, 989 (2020). DOI: 10.1007/s12551-020-00743-z
[9] S. Reuter, Th. von Woedtke, K.-D. Weltmann. J. Phys. D: Appl. Phys., **51**, 233001 (2018). DOI: 10.1088/1361-6463/aab3ad
[10] M. Laroussi, T. Akan. Plasma Processes Polymers, **4**, 777 (2007). DOI: 10.1002/ppap.200700066
[11] W. Van Gaens, A. Bogaerts. J. Phys. D: Appl. Phys., **46**, 275201 (2013). DOI: 10.1088/0022-3727/46/27/275201
[12] A. Shashurin, M. Keidar. Phys. Plasmas, **22**, 122002 (2015). DOI: 10.1063/1.4933365
[13] M. Laroussi, S. Bekeschus, M. Keidar, A. Bogaerts, A. Fridman, X. Lu, K. Ostrikov, M. Hori, K. Stapelmann, V. Miller, S. Reuter, Ch. Laux, A. Mesbah, J. Walsh, Ch. Jiang, S.M. Thagard, H. Tanaka, D. Liu, D. Yan, M. Yusupov. IEEE Transactions Radiation Plasma Medical Sci., **6**, 127 (2022). DOI: 10.1109/TRPMS.2021.3135118
[14] G.V. Naidis. J. Phys. D: Appl. Phys., **44**, 215203 (2011). DOI: 10.1088/0022-3727/44/21/215203

[15] V.I. Arkhipenko, A.A. Kirillov, Y.A. Safronau, L.V. Europ. Phys. J. D, **60**, 455 (2010). DOI: 10.1140/epjd/e2010-00266-5

[16] *Book of Abstracts, 8th International Workshop on Plasma for Cancer Treatment* (Raleigh, NC, USA, 2023)

[17] M. Biryukov, D. Semenov, N. Kryachkova, A. Polyakova, E. Patrakova, O. Troitskaya, E. Milakhina, J. Poletaeva, P. Gugin, E. Ryachikova, D. Zakrevsky, I. Schweigert, O. Koval. Biomolecules, **13**, 1672 (2023). DOI: 10.3390/biom13111672

[18] V. Perrotti, V.C.-A. Caponio, L.L. Muzio, E.H. Choi, M.C.-D. Marcantonio, M. Mazzone, N.K. Kaushik, G. Mincione. Intern. J. Molecular Sci., **23**, 10238 (2022). DOI: 10.3390/ijms231810238

[19] S.A. Norberg, E. Johnsen, M.J. Kushner. J. Appl. Phys., **118**, 013301 (2015). DOI: 10.1063/1.4923345

[20] S.A. Norberg, W. Tian, E. Johnsen, M.J. Kushner. J. Phys. D: Appl. Phys., **47**, 475203 (2014). DOI: 10.1088/0022-3727/47/47/475203

[21] P. Viegas, M. Hofmans, O. van Rooij, A. Obrusník, B. Klarenaar, Z. Bonaventura, O. Guaitella, A. Sobota, A. Bourdon. Plasma Sources Sci. Technol., **29**, 095011 (2020). DOI: 10.1088/1361-6595/ac381d

[22] I. Schweigert, S. Vagapov, L. Lin, M. Keidar, IOP J. Phys.: Conf. Series, **1112**, 012004 (2018). DOI: 10.1088/1742-6596/1112/1/012004

[23] I. Schweigert, D. Zakrevsky, E. Milakhina, P. Gugin, M. Biryukov, E. Patrakova, O. Koval. Plasma Phys. Controlled Fusion, **64**, 044015 (2022). DOI: 10.1088/1361-6587/ac53f1

[24] Li Lin, M. Keidar. Appl. Phys. Rev., **8**, 011306 (2021). DOI: 10.1063/5.0022534

[25] I. Schweigert, Dm. Zakrevsky, P. Gugin, E. Yelak, E. Golubitskaya, O. Troitskaya, O. Koval. Appl. Sci., **9**, 4528 (2019). DOI: 10.3390/app9214528

[26] O. Troitskaya, E. Golubitskaya, M. Biryukov, M. Varlamov, P. Gugin, E. Milakhina, V. Richter, I. Schweigert, Dm. Zakrevsky, O. Koval. Intern. J. Molecular Sci., **21**, 2158 (2020). DOI: 10.3390/ijms21145128

[27] I.V. Schweigert, Dm.E. Zakrevsky, P.P. Gugin, E.V. Milakhina, M.M. Biryukov, M. Keidar, O.A. Koval. Plasma Sources Sci. Technol., **31**, 114004 (2022). DOI: 10.1088/1361-6595/aca120

[28] E. Patrakova, M. Biryukov, O. Troitskaya, P. Gugin, E. Milakhina, D. Semenov, J. Poletaeva, E. Ryabchikova, D. Novak, N. Kryachkova, A. Polyakova, M. Zhilnikova, D. Zakrevsky, I. Schweigert, O. Koval. Cells, **12**, 290 (2023). DOI: 10.3390/cells12020290

[29] E. Patrakova, M. Birukov, O. Troitskaya, D. Novak, E. Milakhina, P. Gugin, D. Zakrevsky, I. Schweigert. Cytology, **65**, 39 (2023). DOI: 10.31857/S004137712301008X

[30] I.V. Shvejgert, D.E. Zakrevsky, E.V. Milakhina, P.P. Gugin, M.M. Biryukov, O.S. Troitskaya, O.A. Koval. Fizika plazmy, **49**, 447 (2023). (in Russian). DOI: 10.31857/S0367292122601400

[31] I.A. Zarubin, V.A. Labusov, S.A. Babin. Zavodskaya laboratoriya. Diagnostika materialov, **85**, 117 (2019). (in Russian). DOI: 10.26896/1028-6861-2019-85-1-II-117-121

[32] Testo: Thermal imaging camera testo 872. URL: https://www.testo.ru/ru-RU/tieplovizor-testo-872/p/0560-8721

[33] I.V. Schweigert, A.L. Alexandrov, D.E. Zakrevsky. Plasma Sources Sci. Technol., **29**, 12LT02 (2020). DOI: 10.1088/1361-6595/abc93f

[34] P.P. Gugin, D.E. Zakrevsky, E.V. Milakhina. Pisma v ZhTF (in Russian) **48**, 74 (2022). DOI: 10.21883/PJTF.2021.22.51726.18977

[35] S. Hashimoto, H. Fukuhara, E.J. Szili, C. Kawada, S.-H. Hong, Y. Matsumoto, T. Shirafuji, M. Tsuda, A. Kurabayashi, M. Furihata, H. Furuta, A. Hatta, K. Inoue, J.-S. Oh. Plasma, **6**, 103 (2023). DOI: 10.3390/plasma6010009

[36] I.V. Shvejgert, D.E. Zakrevsky, E.V. Milakhina, A.L. Aleksandrov, M.M. Biryukov, O.A. Koval. Fizika plazmy, **49**, 1178 (2023). (in Russian). DOI: 10.31857/S0367292123601042

Translated by I.Mazurov