Mismatch of frequencies of ac voltage and streamers propagation in cold atmospheric plasma jet for typical regimes of cancer cell treatment

I Schweigert¹, A Alexandrov¹, D Zakrevsky², E Milakhina³, E Patrakova⁴, O Troitskaya⁴, M Birykov⁴ and O Koval⁴,⁵

¹Khristianovich Institute of Theoretical and Applied Mechanics, Novosibirsk, Russia
²A.V. Rzhanov Institute of Semiconductor Physics, 630090 Novosibirsk, Russia
³Novosibirsk State Technical University, 630090 Novosibirsk, Russia
⁴Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia
⁵Department of Molecular Biology, Novosibirsk State University, Novosibirsk, Russia

ivschweigert@gmail.com

Abstract. Cold atmospheric plasma (CAP) jet generated by the plasma source at 2-6 kV ac voltages with frequencies of 10-50 kHz demonstrate the different modes of operation. Depending on the voltage frequency and amplitude, some streamers in the plasma jet are short and decay before they approach the treated surface. In this case, the effect on the viability of cancer cells exposed to CAP jet strongly depends on the mode of operation of the discharge or, in other words, how many times the streamers hit the bio-target during the treatment. The effect on different modes on cancer cells A549 viability is reported.

1. Introduction
Plasma sources generating the cold atmospheric plasma jets for medical applications are electrophysical devices which can operate in different regimes, depending on their design and shape of voltage pulses. Even the CAP jet plasma sources with a similar geometry and a sinusoidal working voltage exhibit the different modes of the streamer generation, depending on the voltage frequency and amplitude, the working gas type and its pumping rate, the gap from the nozzle to the target and the type of the treated target. For an example, in CAP jet produced by the cylindrical design plasma source [1], for some range of discharge parameters, not every streamer reaches the target during a voltage ac cycle. Some streamers are short and decay before they reach the treated surface. In this case, the effect on the cancer cell viability exposed to CAP jet strongly depends on the discharge operation mode or, in other words, how many times the streamers touch the bio-target during the treatment time.

The results of the biotarget treatment (cancer cells in media, animal models or patients) by CAP jet can be also different, depending on where the grounded substrate is placed. The plasma device with the powered electrode inside of the dielectric tube is electrically connected to the target and to the grounded metal surface. Note that the animal models, for example, mice are on the floating potential during the CAP treatment. The streamers periodically generated by ac voltage follow the working gas flow and a
gradient of the potential distribution between the powered and grounded surfaces. Therefore, the results of the CAP treatment are not repeatable in different experimental rooms where the grounded surface can be at various distances from the exposed bio-target. A way to avoid these ‘uncertainties is to place a grounded substrate under the bio object. It will help to keep the same conditions in the various plasma treatment environment and increase the intensity of the plasma – target interaction. The grounded substrate under the target enhances the electric field and consequently the ionization rate, plasma density, electron energy and chemical reactions rates over the zone of contact of plasma with bio-target [2]. Previously it was shown that the effect of exposure to CAP jet of a certain cancer cell in vitro depends on several parameters, for an example, from the nozzle-target gap [3]. A variation of the nozzle-target gap gives a visible effect on the number of surviving cancer cells.

2. Experimental and numerical study of mismatch of streamer propagation and voltage frequencies

2.1 Experimental setup and simulation details
In our experiments, the source of the plasma jet was a coaxial dielectric channel with an inner diameter of 8 mm with a capillary nozzle with a diameter of 2.3 mm [1, 2]. A scheme of the device is shown in Figure 1(a). The discharge zone inside of the dielectric tube is formed by two electrodes. An ac voltage was applied to the inner electrode relative to the outer ring grounded electrode. At a distance from the nozzle \( z = 25 \text{ mm} \) perpendicular to the axis of propagation of the plasma jet, a dielectric plate or cells in media were placed on the metal grounded substrate. The \( z \) value was chosen from the typical conditions of biophysical experiments on the effect of CAP jet treatment of culture media with cancer cells and experimental animals. Grounding the substrate through a low-inductance resistance made it possible to record the current pulses hitting the substrate. In Figure 1(b), the voltage and discharge currents measured near the nozzle and at the dielectric surface are show as a function of time. In our study, the voltage amplitude \( U_0 \) ranges from 2 kV to 6 kV and the frequency \( f_U=10 \cdots 50 \text{ kHz} \). The working gas is helium, and the pumping rate from 1 to 10 L/min.

![Figure 1](image_url)

**Figure 1.** (a) Scheme of experimental setup, (b) measured voltage and discharge currents near the nozzle (black) and over the dielectric target (blue), \( U_0=3.1 \text{ kV}, f_U=30 \text{ kHz} \), (c) calculation domain with the ion density distribution when a streamer touches the target, \( U_0=4.2 \text{ kV}, f_U=30 \text{ kHz} \).
For theoretical study of the discharge current-voltage self-organization in CAP jet, the streamers dynamics during 10–100 ac voltage cycles was calculated in 2D fluid model simulations with PlasmaNovH code [5]. The verification of the calculation results at the first stage of modeling was carried out with a change in the time step and the steps along the spatial grid. It was shown that the calculation results do not depend on these parameters. In Figure 1(c), the cylindrical calculation domain of \( R = 6 \) cm, \( H = 7 \) cm is shown with the ion concentration distribution. The dielectric plate is placed 2.6 cm apart from the nozzle over the grounded substrate.

2.2. Experimental and simulation results on plasma dynamics

In our experiments and calculations we found that for some discharge regimes the streamers do not touch the target every ac voltage cycle. The frequency of the streamers touching the surface, \( f_j \) can be 2-10 times less than voltage frequency \( f_U \). The mismatch of frequencies of the streamer propagation and ac voltage was discussed for the first time in [2].

In Figure 2, the measured voltage and discharge current over the dielectric target placed with 25 mm from the discharge nozzle are shown for \( U_0=4.9 \) kV (left) and \( U_0=5.2 \) kV (right), \( f_U=22 \) kHz. It is seen that a small increase of the voltage amplitude changes the current pattern behavior, \( f_j = f_U /2 \) for \( U_0=4.9 \) kV and \( f_j = f_U \) for \( U_0=5.2 \) kV.

![Figure 2. Measured voltage (yellow) and discharge current (pink) over the dielectric target with time, \( U_0=4.9 \) kV (left) and 5.2 kV (right), \( f_U=22 \) kHz. Time axis has a division of 25 μs.](image)

In simulations, we also found that an increase of the voltage amplitude causes a change of regime of how the streamers hit the target. In Figure 3(a), the calculated ionization rate in the streamer head and z-coordinate of the streamer head with time are shown. It is seen that every second streamer stops near the nozzle at \( z=5 \) cm, and the rest approach the target at \( z=7.6 \) cm. In Figure 3(b), the electric field strength (in V/cm) is shown at the moment when a streamer touches the dielectric substrate.

A change of plasma parameters with time in a zone of plasma-target contact is shown in Figure 4 for five ac voltage cycles for two voltage amplitudes \( U_0 = 4.2 \) kV and 5.6 kV. The calculated electron density and energy over the surface as well as the surface charge and potential reflect the streamer propagation dynamics. For \( U_0 = 4.2 \) kV, surface plasma parameters are almost zero every second voltage ac cycle when the streamers are short, whereas for \( U_0 = 5.6 \) kV the plasma parameters oscillate every \( U \) cycle. With the streamer approaching the target during positive voltage cycles the electron density and energy quickly rise and drops after 2-5 μs of plasma-target contact. During the negative phase of the voltage, the electrons move to the targets and \( n_e \) increases near the target surface, but the \( \varepsilon_e \) drops to 1 eV. The surface charge \( q_s(r,t) \) is positive when the streamer approaching the target and during negative voltage cycle it becomes slightly a) negative for \( U_0 = 4.2 \) kV (\( f_j = f/2 \) case) and b) positive if \( U_0 = 5.6 \) kV (\( f_j = f \) case). In simulations, the accumulation of surface charge, \( q_s(r,t) \) was calculated with a summation of the time-dependent ion and electron fluxes on the surface, starting from
qs(r)=0 at t=0. So the streamers hit the target with the frequencies of 15 kHz for \( U_0 = 4.2 \) kV and of 30 kHz for \( U_0 = 5.6 \) kV for ac voltage frequency of 30 kHz.

**Figure 3.** (a) Ionization rate in streamer head and z-coordinate of streamer head with time, (b) Electric field strength in V/cm \( U_0 = 4.2 \) kV, \( f_j = 30 \) kHz, \( t_1 = 102.1 \) \( \mu \)s, \( t_2 = 104.2 \) \( \mu \)s (long streamer) and \( t_3 = 136.4 \) \( \mu \)s, \( t_4 = 138.5 \) \( \mu \)s (short streamer). (b) Electric field strength in V/cm at the moment of streamer touching the dielectric substrate.

**Figure 4.** Calculated plasma parameters near the dielectric surface in a zone of plasma touching the target, (a) electron density, (b) electron energy, (c) surface charge, \( q=(n_i-n_e) \) and (c) applied voltage and surface potential with time for \( U_0=4.2 \) kV (black) and \( U_0=5.6 \) kV (blue), \( f_u=30 \) kHz.
All our measurement and calculation results are summarized on the map of different streamer propagation regimes as a function of the voltage frequency and amplitude shown in Figure 5.

![Figure 5. Map of frequency of touching-target current for the dielectric target on the metal grounded substrate for different voltage frequencies and amplitudes. The measured voltage (red) and currents near the nozzle (black) and target (blue) on the top are examples of different regimes, d=25 mm, v=9 L/min.](image)

The measured voltage and discharge currents are also presented in Figure 5 for an illustration of different regimes. These results were obtained for the dielectric plate (target) on the grounded substrate with the 2.5 cm gap from the nozzle to the target. The helium gas pumping rate is 9 L/min.

3. Efficiency of cancer cell treatment with the different CAP regimes
To check the efficiency of CAP different regimes on the cancer cells, human lung adenocarcinoma cells A549 were grown in DMEM with 10% fetal bovine serum. We found that CAP jet induces the death of A549 cells. Cell viability (MTT) assay was performed 24 h after the CAP treatment Figure 6(a). CAP treatment was performed for 1 and 2 min, f_U=13, 22 and 50 kHz, U_0 = 3.5 kV.

The data are presented as a percent of the non-treated cells (100%). It is seen that one-minute treatment suppressed the cell proliferation, but the viability is high (>55%) for all voltage frequencies. Two- minute CAP treatment reduces cell viability up to 25% for 50 kHz voltage frequency and with the presence of the grounded substrate. Figure 6(b) shows the relative intensity of the plasma-target interaction for 1 min calculated by integration of the ionization rate over the target over 5 ac voltage cycles. We found that averaged over time ionization rate is 3.7 times higher for the case of 50 kHz compared to 13 kHz. It means that the electron density and energy, the electrical field and inelastic collisions are more pronounced during CAP treatment with 50 kHz voltage.
4. Conclusion
In our experiment and simulations, we studied the different regimes of helium CAP jet generated by the plasma source at 2-6 kV ac voltages with frequencies ranging from 10 kHz to 50 kHz. It was shown that not every streamer generated by ac voltage cycle successfully reaches the target surface. The system, consisting of the plasma device and the dielectric target on the grounded substrate, itself chooses the frequency of plasma delivery to the target depending on the frequency and amplitude of the voltage. This self-organization determines the energy input over the target and consequently the electromagnetic and chemical interaction of plasma with the target. The results of simulations showed that the plasma cloud from the device nozzle to the target, produced by previous streamers and decreased by the recombination, can be large enough to stop the streamer propagation or let it go through the plasma cloud for different repetitive modes. Previously it was discussed in [6], that a single streamer could not overpass the plasma cloud if its plasma density is too large. The results of our experimental and theoretical studies of ratio of voltage-current frequencies have been summarized on the map of different regimes. It was shown that different regimes of the CAP jet operation give various effects on the viability of the cancer cells during treatment.

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