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Cold plasma jet optimization for cancer treatment

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Abstract. Nowadays the cold atmospheric pressure plasma jets are widely used for the suppression of the malignant tumor growth. In this work, to maximize the effect of the CAPJ treatment on the different cancer cell lines, the optimization of the plasma jet device is reported for the sinusoidal initiation voltage. In experiments and in 2D fluid model simulations, we vary the discharge voltage amplitude from 3 kV to 5 kV with the frequency 13–50 kHz. The efficacy of plasma treatment is confirmed in our bio experiments with various cancer cell lines. A strong cytotoxic effect and selectivity of cells treated with the optimal CAP regimes are demonstrated on the A549 and MCF7 cancer cells and Wi-38 normal cells.

Keywords: cold atmospheric plasma jet, streamer, plasma medicine.

1. Introduction

Recently cold atmospheric pressure plasma jets (CAPJs) are considered as an advanced tool in medical oncology (see, for example, [1]). To find the optimal conditions for the CAPJ treatment of the different types of tumors is an urgent task for interdisciplinary research. A variation of regimes of streamer propagation governed by a change of the voltage amplitude and frequency [2] gives nonlinear effect of CAPJ treatment. For the smaller frequency and amplitude of sinusoidal voltage, for instance, for $f = 13$ kHz and $U_0 < 2.5$ kV, the streamer propagation is regular with a weak intensity. An increase of f or U_0 breaks this regularity, since a streamer overpasses the quasineutral plasma generated by previous streamers [2].

In this work, the discharge operation regimes in the typical cylindrical plasma device [3] with 2 mm powered electrode inside of the dielectric channel (see, Fig.1) were analyzed in the experiment and fluid model numerical simulations. Previously it was shown in [2], that regimes of the streamer propagation depend on the parameters U_0, f. A result of the change of the streamer propagation pattern is a non-linear variation of the intensity of the CAPJ-target interaction. The number of streamers touching the target during the CAPJ treatment, varies considerably with changing U_0 and f and the effect of the treatment can differ significantly.

Besides, the streamer dynamics differs for (a) an electro-isolated bio target and a target placed on the grounded metal substrate, (b) different distances between the plasma device nozzle and (c) different helium gas velocity v. We also monitored the temperature increase and the selectivity of CAPJ treatment for healthy and cancer cells. The efficacy of optimal regimes was confirmed in our bio experiments with cancer cell lines (A549, MCF7 etc). The viability of the cancer cells was obtained with MTT assay 24 hours after the CAPJ exposure. It was shown that the viability of cancer cell depends on the choice of the mode of the discharge operation.

2. Experimental Setup and Simulation Model

In our experiments and simulations, we took the voltage amplitude U_0 = 3–5 kV, that is higher than typically used for the CAPJ treatment, since we wish to increase the electric field in a zone of the streamer-target contact for cell activation. The voltage frequency f varies from 13 kHz to 50 kHz. In our experiment, the distance between the plasma device nozzle and bio target is d = 2.5 cm. Note that for a smaller gap, the cell media can evaporate during the treatment and affect the discharge in the dielectric channel. The helium gas velocity is v = 9 l/min in order to cool down the target surface during the treatment. The temperature of the zone of CAPJ contact was monitored to fulfil the safety conditions for experiments with animals

In 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. The device is shown in Fig.1. The discharge zone inside of the dielectric tube is formed by two electrodes. The ac voltage was applied to the inner electrode relative to the outer ring grounded electrode. At a distance from the nozzle, perpendicular to the propagation of the plasma jet, a dielectric plate or bio-target (cells in media or alive mouse) were placed on the metal grounded substrate (GR+).

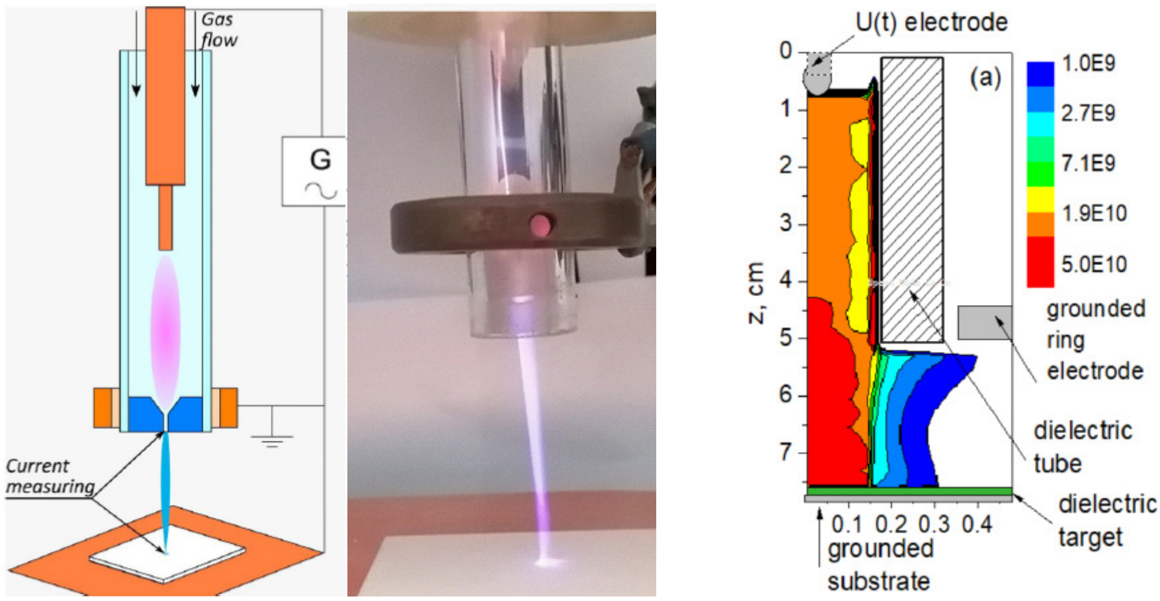


Fig.1. Scheme and photo of the experimental device (left) and calculation domain with the ion density distribution when a streamer touches the dielectric target, U_0 = 3.5 kV, f = 22 kHz (right).

For measurement of the current spreading over the surface during plasma-target contact, we used a sensor consisting of 16 symmetrically placed resistors (10 kΩ nominal). The surface temperature in the plasma jet interaction zone was carried out with a Testo 872 thermal imager with a measurement accuracy of 0.06°C. In all our bio experiments, the temperature in contact zone did not exceed 40°C.

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 code described in [5]. In Fig.1, the cylindrical calculation domain of R = 6 cm, H = 7 cm is shown with the ion concentration distribution.

The details of cell preparation and growth for CAPJ exposure can be found in [6].

3. Experimental and Simulation Results

In the experiments, streamers appear near the powered electrode and begin to propagate at each positive half-cycle of the alternating voltage. However a distance passed by each streamer outside of the dielectric channel is set by the discharge input parameters. For the voltage frequency f = 13 kHz all streamers touch the target surface for U_0 = 3.5–5.5 kV. For this case, the ionization in the streamer head is comparably low. With an increase of the voltage frequency a part of streamers decay before they approach the target. The measured discharge current over the dielectric plate/GR+ (placed on the grounded substrate) and the voltage with time are shown in Fig.2 for

$U_0 = 4$ kV and 5 kV, $f = 44$ kHz. It is seen that for $U_0 = 4$ kV only every forth streamer hits the target, so the effective frequency of the touching-target current is $f_j = 44/4$ kHz. With an increase of U_0 up to 5 kV, already every second streamer reaches the surface ($f_j = 44/2$ kHz) and the efficiency of the plasma-target interaction increases. This mismatch of frequencies of the ac voltage and streamer propagations [2] appears for higher voltages and voltage frequency.

The plasma jet looks continuous, but it is formed by the streamers propagating every 20–50 µs. The ionization, excitation and dissociation of atoms and molecules mainly take place around the streamer head during its propagation. For optimization of the treatment conditions we calculate and compare the total intensities Q of the CAP-target interaction for different U_0 and f. The calculated streamer dynamics for the experimental conditions is shown in Fig.3a. The Q is calculated by integrating over time the ionization rate near the target surface within a 1 cm zone over the z axis, shown in Fig.3a with a red square.

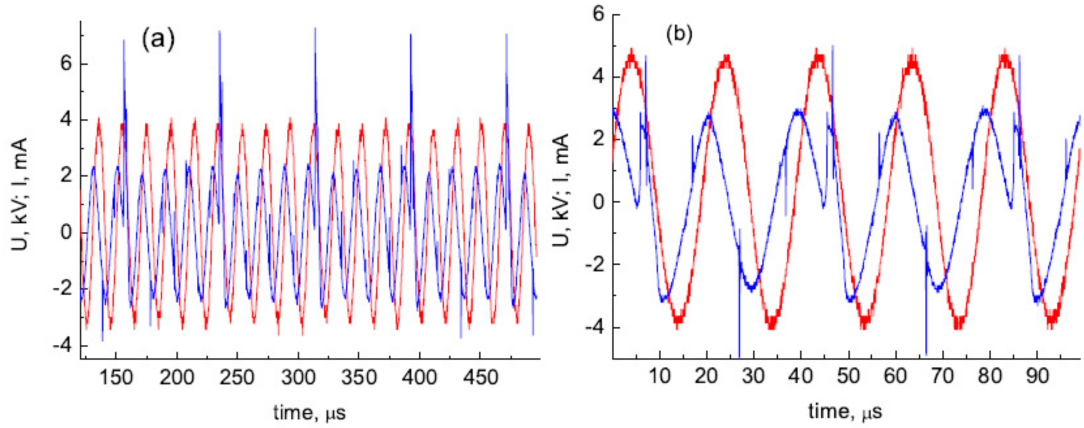
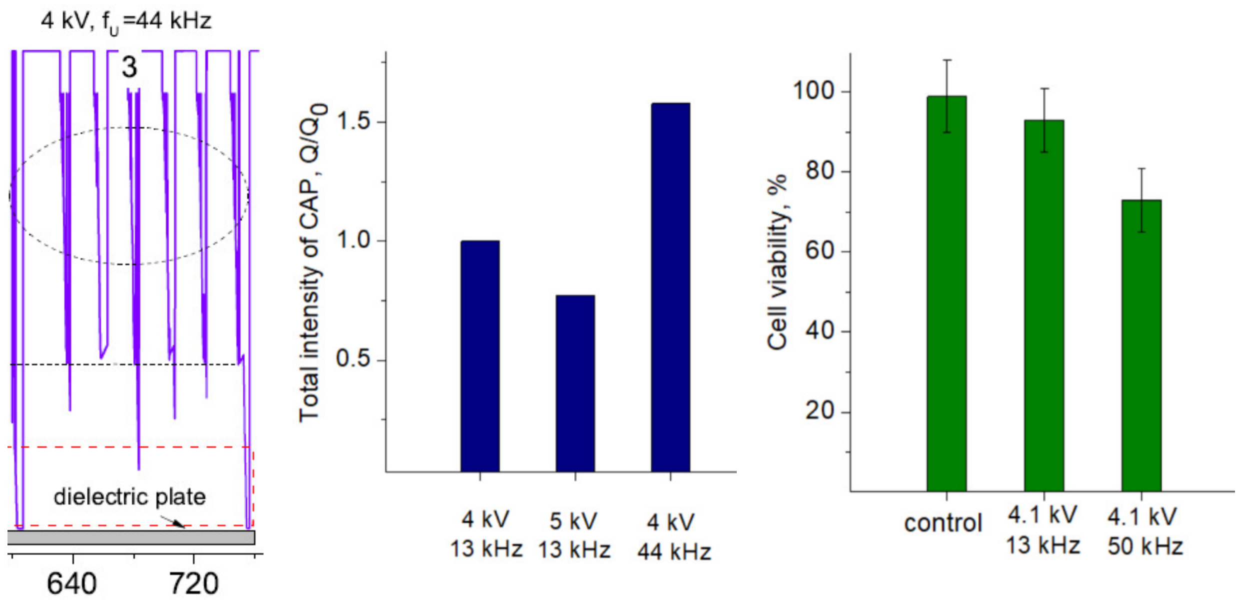


Fig.2. Measured voltage (red) and discharge current (blue) over the dielectric target/GR+ for $U_0 = 3.6$ kV (a) and $U_0 = 4.2$ kV (b), $f = 50$ kHz.



(a) (b) (c)

Fig.3. (a) z-coordinate of the streamer head with time for $f = 44$ kHz and $U_0 = 4$ kV, (b) total intensity of plasma treatment Q and (c) viability of human breast adenocarcinoma cells MCF7 (results of MTT assay) after CAP treatment during 1 min for $U_0 = 4.1$ kV, $f = 13$ kHz and 50 kHz. Cell viability was analyzed 24 h after the treatment. The median of three independent experiments. Student's t-test was used for statistical analysis. The differences with control were significant with $p < 0.05$.

The trajectories of streamer head (with the maximum ionization) constitute the same propagation pattern as observed in the experiment. Streamers appear at the U-electrode (at $z = 0.7$ cm) and move to the target (at $z = 7.5$ mm) at every voltage cycle, but not every streamer approaches the target surface. Fig.3b shows the total intensity Q for $f = 13$ kHz, $U_0 = 4$ kV and 5 kV, and $f = 44$ kHz, $U_0 = 4$ kV.

In our bio experiments, MCF7 human breast adenocarcinoma cells were exposed to CAPJ during 1 min 24 hours after CAP treatment. The viability of MCF7 cells shown in Fig.3c was analyzed and compared to the calculated total intensity Q of the CAP treatment (Fig.3b). It is seen that the CAPJ regime with the highest Q has the stronger cytotoxic effect. In Fig.4, the viability of A549 and MCF7 cancer cells is presented, that was measured 24 hours after the CAP treatment for 1 and 2 min for different voltage frequencies. The results show that A549 cells are more sensitive to the CAP exposure, and the optimal regime of 44 kHz provides maximum efficiency for both cell lines. As expected an increase of the time of the exposure elevated the effect of the treatment.

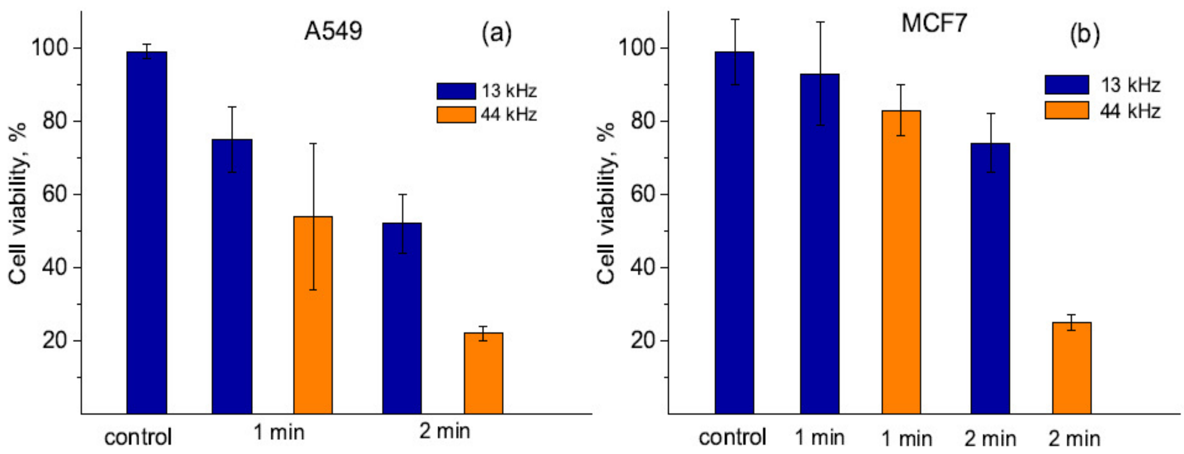


Fig.4. Viability of (a) A549 cells, $U_0 = 3.5$ kV and (b) MCF7 cells, $U_0 = 4$ kV; $f = 13$ and 44 kHz. Analysis was done with MTT assay 24 hours after CAP treatment for 1 and 2 min. The median of three independent experiments. Student's t-test was used for statistical analysis.

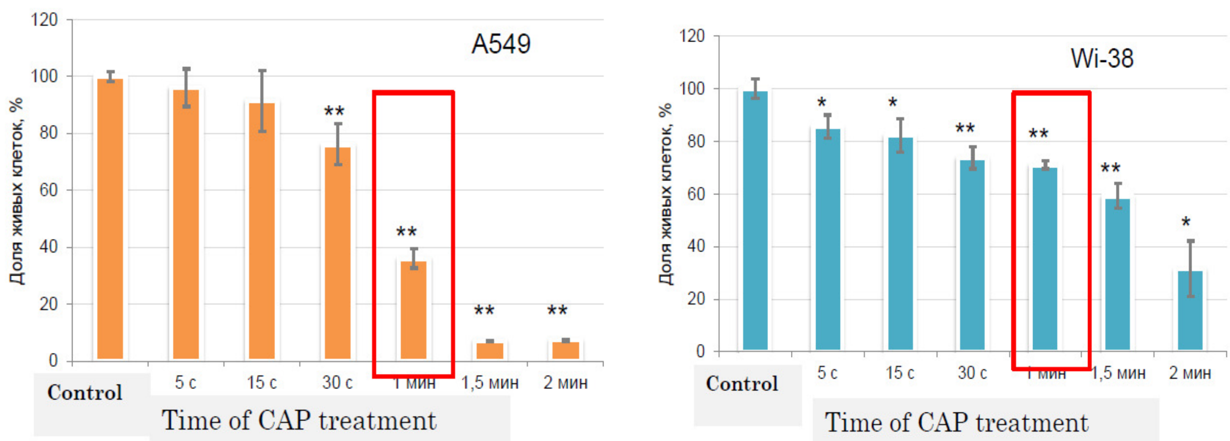


Fig.5. Viability of cancer A 549 and healthy cells Wi-38. Data of MTT assay 24 h after CAP treatment, $f = 50$ kHz, $U_0 = 3.5$ kV. A549 – human lung adenocarcinoma; Wi-38 – human lung fibroblasts.

Previously, in [6], the authors evaluated the viability of 33 cancer and healthy cell lines of the same hystological origins after CAPJ treatment. It was shown that 26 cell lines have strong CAPJ selectivity, 5 has a weak CAP selectivity, 2 cell lines have the negative selectivity. We analyze the CAPJ treatment conditions for the maximum positive selectivity effect with cancer and healthy cells with the same histological origins (A549 – human lung adenocarcinoma; Wi-38 – human lung fibroblasts). The viability of the cells after CAPJ treatment for different time is shown in Fig.5. It is seen that CAPJ exposure for 1 min gives significant difference, only 38% of A549 cells survive compared to 70% of Wi-38 cells.

4. Conclusion

In physical and bio experiments and in 2D fluid model simulations the different CAPJ generation regimes for the cancer cell treatment were analyzed. It was shown that the induced cancer cell death when exposed to CAPJ depends on the discharge operation conditions. We tested the discharge regimes, varying the voltage amplitude from 3 kV to 5 kV at the frequency 13–15 kHz. The optimal discharge regimes were reported. The maximum cytotoxic effect and positive selectivity of the CAPJ treatment with recommended regimes were demonstrated on the A549 and MCF7 cancer cells and Wi-38 normal cells.

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