Analysis of Grounded Substrate Effects on Cold Atmospheric Plasma Jet Irradiation of Cellular and Animal models

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Abstract. Plasma devices with cylindrical and plane geometries generating the nonthermal atmospheric plasma jets are used for the treatment of the cancer cells in vitro and in vivo. It is shown in the simulations and experiment that the grounded substrate placed under the bio target essentially increases the plasma treatment efficiency. Nevertheless the temperature control is needed to avoid the burns in a spot of contact of plasma jet with the mice skin. The measured and calculated local temperature increase with different regimes of plasma device operation is presented. The MTT and iCELLigence assays of the viability of cancer cells in vitro show the efficiency of plasma jet treatment and mice models in vivo demonstrate the safety regime for cold atmospheric pressure plasma irradiation.

Introduction
The cold atmospheric pressure plasma (CAP) jets are recently widely studied for applications in plasma medicine. The malignant tumor growth is efficiently suppressed after exposure to CAP jets (see for example, [1]). Another promising idea of anticancer therapy is a combination of CAP treatment with chemotherapy.

A variation of CAP jets parameters in experimental and theoretical study is performed to enhance the effect of plasma treatment. One of our findings [2] is the use of the grounded substrate under the bio target to increase the plasma enhanced chemistry and the electric field near the treated surface.

Here, the results of experimental and theoretical study of plasma interaction with cancer cell in vitro and in vivo are presented with grounded substrate and without it. The theoretical results are obtained in 2D fluid model simulations and bio experiment results are analyzed with MTT and iCELLigence viability assays of CAP-irradiated cancer cells.

The cancer cells were exposed to CAP jet from plasma device at atmospheric gas pressure with the voltage amplitude ranged from 3 kV to 5 kV with the frequency of 13 - 50 kHz. The streamers propagate...
over the jet of inert gas (helium or argon) which is pumped through the dielectric tube with the rate of 1-10 L/min.

Figure 1 shows the plasma devices with a cylindrical and plane design plasma channels. The additional flexible plastic tube helps to delivery the plasma jet to the target with some angle (Figure 1 (a)). The plane design of plasma device allows us to increase essentially the treated area (Figure 1 (b)).

![Figure 1](image1.png)

**Figure 1.** Plasma devices with a cylindrical (a) and plane (b) designs. Plasma device with flexible plastic tube adjuncted to the nozzle (working gas helium) and CAP interacting with metal substrate (argon). Plane discharge glows in helium (b).

![Figure 2](image2.png)

**Figure 2.** Experimental (a) and model (b) plasma device with a cylindrical design. The ion density distribution, $U=4$ kV.

Previously our results of simulations and measurements demonstrated that the plasma jet can be considerably intensified with placing grounded substrate (GS) under the treated object [3]. In Figure 3,
the measured plasma spectrum (a) and ionization rate (b) are shown for the cases with and without GS. The spectra were measured 1 mm above the target. It is seen that the measured intensity of OH-line and calculated ionization rate visibly increase with the presence of the GS. A range of optimal voltages and gas flow velocities for the enhanced OH production was found from the measurements of plasma spectra [3].

![Figure 3](image1.png)

**Figure 3.** Part of spectrum with of OH peaks measured with the presence of grounded substrate (1) and without it (2). An arrow indicates the OH peak at $\lambda = 309$ nm, helium, $U = 4.9$ kV, $v = 3$ L/min (a) and ionization rate profile over z for the cases without grounded substrate (1) and with it (2) (b).

The series of experiments with various cancer cells lines and mice were conducted to clarify the effect of CAP jet treatment with and without grounded substrate. In Figure 4, Cell Index (CI) curves, that reflect cell proliferation in real-time mode, are shown for the cases with and no GS. A549 cells were irradiated during 1 min for $U=4.2$ kV and $v=5$ L/min (working gas He) or 3.6 kV and $v=3$ L/min (Ar).

![Figure 4](image2.png)

**Figure 4.** iCELLigence data for Cell Index curves of A549 cancer cells with time. Irradiation by CAP jet without the grounded substrate (a) and with it (b) for $U=4.2$ kV and $v=5$ L/min (He) or 3.6 kV and $v=3$ L/min (Ar).
helium) or 3.6 kV and \( v=3 \) L/min (working gas argon). For these conditions without GS, the proliferation of A549 cells almost coincides with the control CI curve for helium jet and is terminated for argon jet. For GS case, the proliferation of A549 cells slows down with time compared with the control one.

The plasma jet treatment in vivo was performed to optimize the safety condition such as a control of temperature increase. The series of experiments were conducted to optimize the parameters of CAP jet treatment with and without GS for biosafety. Healthy female C3H/He mice were used as a model. For the visual and thermal control of treated spots on mice skin, these spots were shaven.

![Image](image_url)

**Figure 5.** Mouse under anesthesia is shown during CAP treatment (a) and the skin effects (b-d). (b) irradiation burn; (c) - whitish spot; (d) – non-damaged irradiated skin.

In the experiment and simulations, the temperature increase in the spot of plasma touching the bio target was studied for the different combinations of discharge plasma parameters (the discharge frequency and voltage amplitude, time of treatment, gas pumping rate). CAP irradiation of mice with- or no GS produced skin burn or whitish spot on the next day of irradiation (Fig. 5b) when treatment parameters were as follow: discharge frequency of 25 kHz, voltage amplitude \( U=5 \) kV, 1 min and 3 L/min gas pumping rate. For these parameters there was no difference in damaging effects of CAP irradiation with or no grounded substrate. For mice groups 1 and 2, the voltage amplitude was decreases to 3.5 kV and gas pumping rate to 3 L/min that obviated damaging effects with and without GS (Fig. 5c). The mice group 2 and 3 were treated with CAP with discharge frequency of 13 kHz, at \( U=5 \) kV and \( v=9 \) L/min with and without GS and no damage of the mice skin was observed for both cases.

In simulations of plasma jets, the initial conditions are typically chosen to mimic the plasma parameters between AC voltage pulses. Nevertheless it is more accurate to calculate a several voltage periods. During this time the dynamics of plasma jet reaches the quasistationary regime. In our
simulations, we start from the initial conditions with the plasma density of $n_0 = 10^8 \text{cm}^{-3}$ and calculated 5-10 voltage pulses allowing the system to reach quasisteady state. The maximum ionization rate $\nu_i$ and $z$-coordinate of $\nu_i$ with time are shown in Figure 6 (a) for 5 voltage cycles. The peak of $\nu_i$ corresponds to the streamer position near the target at $z=7 \text{cm}$. The temperature increase during 5 voltage cycles during 100 microseconds are shown in Figure 6(b). The temperature increase of dielectric in a spot of plasma touching is considered due to a) the plasma flux to the target and b) Joule heating of the gas near the surface reduced by the working gas pumping. Measured and calculated local temperature after 1 min CAP jet treatment with grounded substrate and without it is shown in Figure 6 (c) for different voltage amplitudes. For this case, the gap between nozzle and target is 2 cm and in simulation the coefficient of ion-electron emission was 0.25. An increase of the voltage elevates the temperature even for high speed of working gas pumping.

Figure 6. Maximum of ionization rate and position of ionization rate maximum (a), voltage and temperature dependence (b) and measured and calculated temperature of surface exposed to CAP during 1 min with grounded substrate and without it, 4 kV, 6 L/min, 2 cm gap, 9 L/min.

In conclusion, in our experiment and simulations, a considerably effect of the grounded substrate beneath the bio target has been observed. The proliferation of cancer cell in vitro was suppressed more efficiently with the presence of grounded substrate. The temperature increase for different discharge parameters was measured and calculated to determine the area of safety conditions.

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Addition of Editorial board
Following the reviewers recommendation the editorial board considers it necessary to supplement reference 2 with the following paper:
Yu S Akishev, V B Karalnik, M A Medvedev, A V Petryakov, N I Trushkin and A G Shafikov. How ionization waves (plasma bullets) in helium plasma jet interact with a dielectric and metallic substrate. IOP Conf. Series: Journal of Physics: Conf. Series 927 (2017) 012040 doi:10.1088/1742-6596/927/1/012040