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Living tissue under treatment of cold plasma atmospheric jet

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The interaction of the cold atmospheric plasma jet with fibroblast cells was studied. Plasma jet was initiated in the helium flow blowing through the syringe by application of high ac voltage to the discharge electrodes. The plasma jet had a length of 5 cm and a diameter of 1.5–2 mm in ambient air. Treatment of cells with plasma jet resulted in decreasing of cell migration rate, cell detachment, and appearance of “frozen” cells, while treatment with helium flow (no plasma) resulted in appearance of frozen cells only. A variety of cellular responses was explained by different intensities of treatment. © 2008 American Institute of Physics. [DOI: 10.1063/1.3020223]

Recent progress in atmospheric plasmas led to creation of cold plasmas with ion temperatures close to room temperature.1 Cold nonthermal atmospheric plasmas can have tremendous applications in nanotechnology2 and biomedicine.3 In particular, plasma treatment can potentially offer a minimum-invasive surgery that allows specific cell removal without influencing the whole tissue. Conventional laser surgery is based on thermal interaction and leads to accidental cell death and necrosis and can cause permanent tissue damage. In contrast, nonthermal plasma interaction with tissue may allow specific cell removal without necrosis.4 Overall plasma treatment offers the advantages superior to those seen in advanced laser surgery.4 Due to its vast potential, in the past several years cold plasma interaction with tissues has become an active research topic.

Stoffels et al.4,5 studied the plasma needle to demonstrate the nontraumatic nature of the plasma. Detachment of the cells from the extracellular matrix was observed and amount of the detached cells increased with increasing of treatment time. It was concluded that plasma can interact with organic materials without causing thermal/electric damage to the surface, although this conclusion was not supported by the direct measurements. In recent years several devices have been presented using the cold plasma jets.6–10 Studies investigating the interaction of plasma jets with living cells have shown eradication of yeast grown on agar,11 blood coagulation and tissue sterilization,12 and ablation of cultured liver cancer cells.13 This paper reports about two effects found regarding interaction of the cold plasma jet with living tissue.

The plasma gun is presented schematically in Fig. 1. It is a Pyrex syringe through which the helium flow is supplied (He feeding was varied by adjusting the tank regulator). The gun is equipped with pair of high voltage (HV) electrodes—central electrode (which is isolated from the direct contact with plasma by ceramics) and outer ring electrode as shown schematically in Fig. 1(a). Electrodes are connected to a secondary of HV resonant transformer (voltage U up to 10 kV, frequency ~30 kHz). A typical photograph of the plasma jet is shown in Fig. 1(b) for U~5 kV and helium feeding corresponding to the flow rate \( \nu_{H_2} = 17 \text{l/min} \) without plasma (measured at \( U = 0 \)). The visible plasma jet had a length of approximately 5 cm and was well collimated along the entire length. The length of the plasma jet varied with gas flow. In particular, the increasing of the helium feeding results in jet elongation, while further increase in the flow rate caused jet shortening and appearance of a turbulent tail at the jet’s end. Maximal plasma jet length was observed at conditions presented in Fig. 1(a). The increase in the HV amplitude applied to the electrodes resulted in increase in plasma jet intensity, but did not affect the plasma jet diameter. At the same time, the diameter of the jet \( (D_j) \) changed with the changing of outlet diameter of the syringe \( (D_o) \). Jet diameter was limited by syringe outlet diameter for small outlets \( (e.g., D_j = 1 \text{mm} \) for \( D_o = 1 \text{mm} \) ), while for larger outlets jet diameter increased \( [D_j = 1.5–2 \text{ mm} \) for \( D_o = 5 \text{ mm} \) as shown in Fig. 1(b)]. The results presented below are for a syringe outlet diameter of 5 mm. The oscillogram of the electrical current in the plasma jet measured using current transformer (diameter of jaw is 5 mm) is shown in Fig. 2. Analogous to previous studies6–8 the plasma jet is discontinuous and represents a series of propagating plasma bundles (two bundles per HV period) with peak current up to few hundred milliamperes. Short-time (up to 10 s) exposition of the finger to the jet results in a mild sensation of heat in the treated area.

![Fig. 1. (Color online) (a) Schematic view of the plasma gun and (b) typical photograph of plasma jet at \( U \approx 5 \text{kV} \) and \( \nu_{H_2} = 17 \text{l/min} \).](image-url)
FIG. 2. Oscillogram of electrical current in the plasma jet for $U=5$ kV and $v_0=17$ l/min.

The interaction of the plasma jet with a living tissue was examined on primary mouse dermal fibroblast cells. Fibroblasts are cells that synthesize and maintain the extracellular matrix of animal tissues. Dense and uniform cell culture was grown at the bottom of six-well dishes using methodology described in Ref. 14 [see Fig. 1(a)]. Experiments were conducted with (i) two different volumes of media covering the cells; depths $d$ were 1.5 and 3 mm, (ii) two different He feedings; flow rates $v_0=17$ and 31 l/min (measured at $U=0$), and (iii) with ($U=5$ kV) and without (no HV applied, He flow only) the plasma jet. Experiments consisted of treating the cells in culture from a distance of approximately 2 cm [see Fig. 1(a)]. Immediately following the plasma jet treatment, plates were used in time lapse cell migration analysis (several locations per each well). Imaging was performed on an Olympus IX81 research microscope equipped with a Proscan motorized stage and a temperature- and CO2-controlled chamber. Cells were maintained in normal cell culture media containing serum at 36 °C and 5% CO2.

Using relief-contrast optics, 10X images were taken of each well every 10 min (for 16 h, 40 min) until 100 images are captured. Images were then transferred to a workstation equipped with METAMORPH image analysis software, where temporal evolution of cell coordinates was obtained. Cell velocity distributions were built and analyzed using SAS software.15

Treatment of the cells with the plasma jet caused the media to be displaced away from the point of contact of jet with cells and for the duration of treatment, the cells were not covered by media [see area with radius $r_d$ as schematically shown in Fig. 1(a)]. Immediately after jet interruption the media flowed over the cells. Three distinct regions were observed in wells treated with $U=5$ kV, $d=1.5$ mm, and $v_0=17$ l/min. Region 1 is the zone which stayed uncovered during the treatment where the cells were subject to desiccation and after the treatment was occupied by immovable (“frozen”) dead cells. Second region is the area which stayed covered with a media at all times. This region was filled up with migrating alive cells. A third region, which was at the interface between the two areas (with alive and dead cells) contained voids with no cells. Images taken in the center of region with frozen cells at $r=0$ and at the edge $r=r_d \sim 3$ mm [see Fig. 1(a)] immediately after 30 s treatment with plasma jet demonstrating all three regions are presented in Figs. 3(a) and 3(b) for $d=1.5$ mm, $v_0=17$ l/min, $U=5$ kV.

No voids or frozen cells were found after treatment of the cell culture with He flow only (no plasma) at the same conditions as that on the Figs. 3(a) and 3(b) (30 s treatment at one point, $d=1.5$ mm, $v_0=17$ l/min, $U=0$). However an increase in the $v_0$ to 31 l/min did result in the appearance of frozen cells [see Figs. 3(c) and 3(d)]. The region occupied by the frozen cells (similarly to the previous case) was assigned by the shape of the uncovered region. Note, that after treatment with He flow (without plasma) no voids were found in the cell culture as shown in Fig. 3(d).

In order to reduce the intensity of treatment, the depth of protecting media covering the cells was increased from 1.5 to 3 mm layer and so that well bottom stayed covered by the thin layer of media at all times during the treatment. In those experiments no voids or frozen cells were found and cells remained migrating. The velocity of cells treated with these permissive conditions was compared to that of untreated cells and cells treated with He only (80 cells were tracked in each case). The velocity distributions are presented in Fig. 4. It is seen that cell velocity distributions after the treatment with helium (no plasma) coincide well with a distribution of untreated cells. Applying the plasma jet de-
increased the average cell migration rate and standard deviation by a factor of about 2 in comparison with both untreated cells and cells treated with helium (no plasma).

Experimental results indicate that the response of live cells to plasma jet can be classified in accordance with intensity of treatment (intense, medium, and mild). An intense treatment results media to be displaced away from the point of contact of jet with cells and observed with cells in this uncovered zone. Such treatment results in direct contact of the plasma jet with cells and causes cell death most likely by desiccation. Medium level treatment may be always found at the interface between the region that is covered and the region uncovered by the media. The minimal amount of media in this region protects cells from desiccation and provides effective interaction with plasma jet. Detachment of cells from the extracellular matrix is observed at this level of treatment. Decreasing of the treatment level to mild intensity causes even more modest cellular response—with no detachment, but cell migration rates are slowed.

In summary, this work considers the interaction of living cells with cold plasma jet. The cellular responses are governed by the level of treatment: at mild level the migration rate of cells is decreased, at medium level cells are detached from the extracellular matrix, and at intense treatment the protecting media is pressing out and cells dies most likely by desiccation in the uncovered by media region. It should be noted that similar effect of desiccation may be reached by helium flow only (no plasma), however, it requires increase in the gas feeding by a factor of 2 in comparison with the case with plasma.

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15See: http://www.sas.com/.