Importance of oxidative processes induced in normal and tumoral cell monolayers exposed to the action of cold plasma jets

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Résumé

We present the results of a study regarding the effects of atmospheric pressure cold plasma jets on normal (V79-4) and tumoral (HeLa) cell lines. Predominance of apoptotic or necrotic processes is described in relation to plasma contents and cell treatment times for two experimental setups: direct cell exposure and indirect diffused treatment.

Introduction

Atmospheric pressure non-thermal plasmas are nowadays extensively studied for their potential biomedical applications: treatment of certain types of skin cancers and dermatologic infections, burns, ulcers, blood coagulation inducement during surgery, sterilization of medical instrumentation, bacterial decontamination and others [1].

The conceptual possibility to adjust plasma and treatment parameters to ensure its non-invasivity, effectiveness and (ideally) selectivity regarding its action on different types of living organisms and cells has become a subject of interest and active research during the last several years [2]. Cold plasmas may possess chemical oxidative activity exposing biological material to oxidation processes mediated by the presence of reactive oxygen species (ROS) such as: superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen. These oxygen radicals are generated either within the plasma itself or as a consequence of the interaction between the plasma and the surrounding air. Based on our previous experience regarding the inducement of cell apoptosis under the action of chemically activated cold plasma jets (CPJ) [3,4], we investigate the presence of apoptotic and necrotic effects within cell populations exposed to a helium-oxygen non-thermal plasma. The experimental parameters that we have independently varied during the present study are the oxygen content of the plasma and the cell exposure times to the CPJ.

Experiment

The cold plasma jets used in our experiments was generated using pulsed high voltages with amplitudes in the range 20 – 30 kV, durations of 100 – 500 ns at half-maximum and frequencies of tens to hundreds of pulses per second. Plasma contained ROS derived from the molecular oxygen found in the initial composition of the helium-oxygen gas mixture.

We have used two cell lines: normal V79-4 (lung fibroblasts from Chinese hamster) cells and the tumoral line HeLa (human cervix cancer). The cells have been cultured with a number density of $1x10^6$ cells/ml in DMEM-F12 culture medium and allowed to become confluent. The plasma has been afterwards applied on cells in two experimental situations: directly, without the presence of the culture medium and indirectly by diffusion in the medium. For each case, the exposure times varied within the range 30-150s with a timestep of 30s. The helium-oxygen gas mixtures had the compositions He: 2.5l/min + O_2: X ml/min, the amount X of oxygen (O_2 ml/min) taking the values X=12.5, 25, 37.5 corresponding to 0.5%, 1%, 1.5% percents of oxygen in the plasma contents. We have determined the cell viability by means of the MTT technique and the ADP/ATP ratio using a dedicated commercial kit.

Results and discussions

In data analysis and interpretation we have considered the following aspects: a significant decrease of cell viability within the first two hours after the plasma treatment indicates the presence of necrosis. A relative reduced change in viability with respect to control within the first two hours following the exposure to the plasma jet reveals a small percentage of necrotic cells. Cell death detected afterwards is

attributed to apoptosis which, being an induced cell mechanism, implies a period of latency necessary for the specific cell modifications to take place.

Taking into account that the cell viability does not vary significantly, an increased ADP/ATP ratio is atributed to the inducement of apoptosis in a larger number of cells instead of necrosis which would affect a smaller number of cells producing a violent death and leading to the release of an ADP amount similar to that produced in the first case.

Since the ROS density within the cell environment depends on the oxygen percentage in the plasma gas mixture and on the treatment duration, we are justified to assume that the effect on the cell viability follows as a consequence of the action of the ROS produced within or by the plasma jet.

For the V79-4 cells necrosis was induced for all three helium-oxygen combinations used in the first experimental setup (direct action on cells). When culture medium was present (second experimental setup), the ROS had a more homogeneous action on the cells due to their diffusion in the medium before reaching the cell monolayer. In this case the apoptotic mechanism was induced for an oxygen amount of 12.5ml/min and necrosis was predominant for the other two cases.

HeLa cells exposed to plasma with an oxygen content of 12.5 ml/min became necrotic after 150s and 120s of treatment while for the other values of the treatment time (30, 60, 90s) an extended apoptosis was observed. Regarding the other two plasma compositions (O_2: 25ml/min and O_2: 37.5ml/min), based on the correlation of the ADP/ATP ratio and viability data, an extended apoptosis was diagnosticated for all plasma exposure times used in our experiments.

The ideal clinical use of atmospheric pressure plasma jets, in tumor treatment, would require the fine tuning of the device parameters aiming ensure a more aggressive exposure (leading to necrosis) inside the tumoral tissue and a less invasive treatment at the edges of the tumor in order to avoid the destruction of the adjacent healthy cells. Regarding these aspects, the plasma gas combinations He: 2.5l/min + O_2 12.5ml/min and He: 2.5l/min + O_2 25ml/min are of special interest. However, these ideas are difficult to implement in practice because of a poor delimitation between the tumoral and normal tissues.

A more realistic and safe alternative would be to use plasma jets at specific parameters in order to ensure a maximum percentage of apoptosis relative to that of necrosis. Our studies revealed such a situation for the He: 2.5l/min + O_2 37.5ml/min plasma gas contents, when apoptosis was induced for normal cells and, very important, for the tumoral cells too.

It is essential that the surface of the region about to be treated to be covered with a liquid/gel to ensure the diffusion of reactive species and the homogeneity of the treatment for the whole region of interest.

It is a matter of prime importance to establish a general procedure for finding those parameters, characteristic for each particular type of cell, for which a maximum percentage of apoptosis is obtained. In choosing those parameters one should take into account the types of cells involved in the considered disease and the fact that a reduced partial destruction of the normal cells at the treated site would be acceptable relative to the benefits brought in treating the cancer disease.

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