Plasma application for cancer therapy

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Plasma medicine is a relatively new field that outgrew from research in application of low-temperature (or cold) atmospheric plasmas in bioengineering. One of the most promising applications of cold atmospheric plasma (CAP) is the cancer therapy. Convincing evidence of CAP selectivity towards the cancel cells has been accumulated. This paper summarizes the state of the art of this emerging field presenting various aspects of CAP application in cancer such as role of reactive species (reactive oxygen and nitrogen), cell cycle modification, in vivo application, CAP interaction with cancer cells in conjunction with nanoparticles, computational oncology applied to CAP.

1. Introduction

Plasma medicine is a relatively new field that outgrew from research in application of low-temperature (or cold) atmospheric plasmas in bioengineering. One of the most promising applications of cold atmospheric plasma (CAP) is the cancer therapy.

The efficacy of cold plasma in a pre-clinical model of various cancer types such as lung, bladder, breast, head, neck, brain and skin has been demonstrated. Both *in-vitro* and *in-vivo* studies revealed that cold plasmas selectively kill cancer cells [1,2]. It was shown that: (a) cold plasma application selectively eradicates cancer cells in vitro without damaging normal cells. (b) Significantly reduced tumor size *in vivo*. The two best known cold plasma effects, plasma-induced apoptosis and the decrease of cell migration velocity can have important implications in cancer treatment by localizing the affected area of the tissue and by decreasing metastasic development. In addition, cold plasma treatment has affected the cell cycle of cancer cells. In particular, cold plasma induces a 2-fold increase in cells at the G2/M-checkpoint in both papilloma and carcinoma cells at ~24 hours after treatment, while normal epithelial cells did not show significant differences [3]. It was shown that reactive oxygen species metabolism and oxidative stress responsive genes are deregulated.

CAP contains variety of charged particles, reactive oxygen species (ROS), reactive nitrogen species (RNS), UV etc. It is known that both ROS and RNS can promote oxidative stress and trigger different signaling pathways in cells.

Effects related to plasma-activated media were observed [4]. In particular, recent study shows that by altering the concentration of fetal bovine serum in Dulbecco's modified Eagle's medium and the temperature to store CAP stimulated media, controllable strategies to harness the stimulated media can be developed [4].

2. Cold Atmospheric Plasmas

Sources producing CAP can be broadly classified into three major groups chiefly by the way biological objects are treated. This includes direct, indirect and hybrid approaches. In direct CAP source living tissue serves as one of the electrodes becoming active part of the discharge. In the indirect CAP source the active plasma species are transported by a gas flow from the main discharge are. In the hybrid configuration plasma generation of the direct CAP source is combined with that of indirect CAP source by creating current-free condition in the tissue.

Time resolved evolution of discharge electrical and plasma parameters as well as ICCD camera images for typical 20-30 kHz CAP jet is shown in Fig.1. One can see that the breakdown occurs once per period of the AC high voltage during a positive half wave at the central electrode. The breakdown of the interelectrode gap is indicated by the peak of the discharge current (I_d). Discharge current increases to about 6-8 mA at about 1 micro-second after the breakdown and then decays with characteristic times of about 3 μs. This stage of the discharge is characterized by the presence of the discharge in the interelectrode gap only with no ionization wave outside the discharge tube. The next stage of discharge starts at about 3 μs after the breakdown when ionization front (streamer) propagates out the discharge tube into an open air. The streamer propagates axially about 4-5 cm with speed of about 2×10^6 cm/s along the helium flow

until it decays at $t \approx 5$ μs as can be seen from series of instant photographs. Measurements of plasma density in the streamer channel yield averaged value of the electron density along the streamer channel of about $3\text{-}4\times10^{12}$ cm^{-3}. Recall that the plasma ionization degree in the jet is very low ~ 10^{-6}-10^{-7} (gas density at 1 atmosphere and 300 K is around 2×10^{19} cm^{-3}). Electrical potential of the streamer tip (U_h) measured during streamer development is indicated as well. It can be seen that the streamer tip carries potential close to the potential of the central electrode.

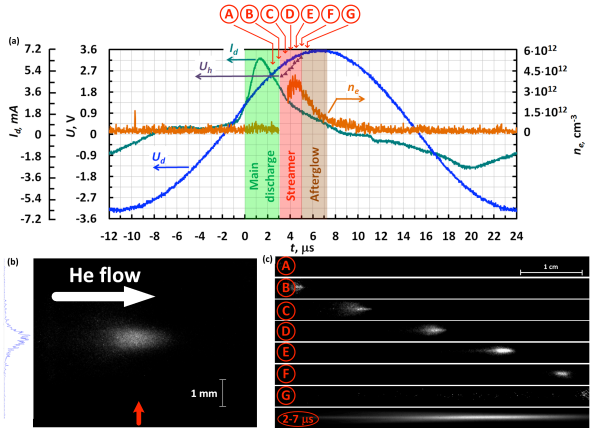

Figure 1. (a) Temporal evolution of discharge current, voltage and plasma density in the streamer. Three stages of the streamer development namely main discharge in the interelectrode gap (green bar), streamer growth (red bar) and afterglow of the streamer channel (blue bar) are shown. (b) Series of instant photographs demonstrating the streamer growth (100 ns exposure time). The photographs were taken at the moments of time indicated by the letters A-G taken at the moments of time indicated. The last image in the series was taken with exposure time of 5 μs covering temporal interval 2-7 μs. (c) Typical high-magnification of the streamer tip. The diagram on the left shows distribution of the intensity along the direction indicated by the red arrow.

3. On the mechanism of CAP action

Current understanding of the primary mechanism of CAP anti-treatment is related to ROS and RNS production. Both ROS and RNS play a central role is 'redox' or oxidation–reduction biology [5]. These species are known as agents associated with various diseases including cancer. In the context of CAP treatment we will differentiate between intracellular and extracellular ROS and RNS.

The overall possible process of CAP interaction with cell and consequent cell response is shown in Fig. 2. One can see that CAP treatment might lead to intracellular ONOO- and ROS increase. Both processes are correlated with apoptotic pathways [6]. Note that this model presents possible picture of CAP triggered processes in vitro. There is a still open question of how CAP interacts with tumor in vivo. One possibility is that ROS and RNS interact with the surface layer of cells and products of such interactions trigger cell-cell "communication" which is in radiation oncology known as "bystander effect". [7]

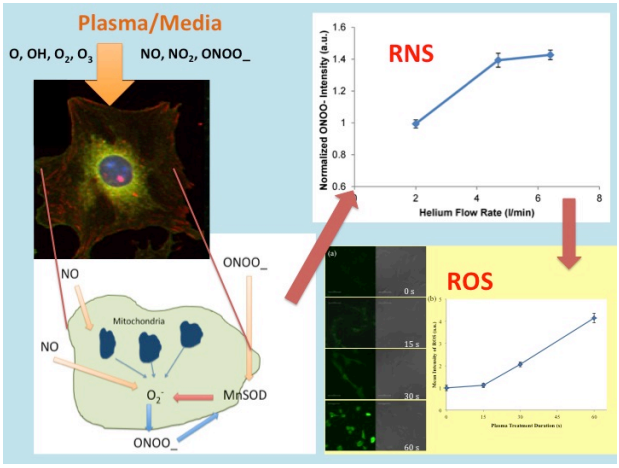

Fig. 2. Schematics of CAP interaction with cells. NO and ONOO- can penetrate the membranes of cells and organelles. The electron transport chain in mitochondria might be blocked by NO. As a result, ROS production in mitochondria rises and ultimately damages mitochondria and trigger apoptosis. On the other hand, NO reacts with O_2^- generated in mitochondria to form peroxynitrite, which will attack nearly all important macromolecules in cells altering the antioxidant activity. Inserts show intracellular measurements of RNS and ROS.

4. Simulation of plasma interaction with tumor

Recently computational model of CAP interaction with tumor was proposed [8]. The idea is to simulate a tumor area that has been exposed to the CAP treatment. This includes simulating the apoptotic cell death that occurs from plasma treatment, normal cell growth and normal cell death. Tumor modeling entails use of the mathematical and physical equations to describe biological disease, most importantly the uncontrolled cell growth and tumor life cycle [9]. The model utilized a 3D hybrid discrete-continuum formulation. It is shown that CAP has a critical dosage, in which it will induce apoptosis selectively for cancerous cells while

leaving healthy cells relatively unharmed, by using two separately calculated equations for the cancerous and healthy phenotype cells. Some results are shown in Fig. 3.

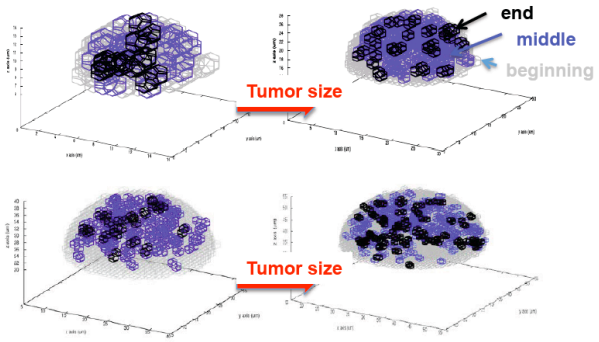

Figure 3. *3D model of tumor evolution under CAP treatment. Going from the left to right and top to bottom we see four graphs of overlaid tumor morphology. Each simulation represents three time delineations from the initial tumor (grey), the midway treatment tumor (blue) and near the end tumor (black).*

One can see from Fig. 3 that in the initial and halfway mark, the plasma-stimulated species have not fully diffused so the highest concentration of species is on the outer edge of the tumor. The probability of cell death is thus heavily dependent on both the species fluxes and a stochastic death function. This creates a more uniform cell death on the outer edges. The diffusion of RONS becomes more and more uniform with time increase. As result it is apparent that comparing the mid-treatment distribution and the final distribution, the cell death is more sporadic. This is indicative of the shift of the cell death probability from a mixed continuous/stochastic function to just a stochastic function.

5. Summary

Convincing evidence of CAP selectivity towards the cancel cells has been accumulated. Various aspects of CAP application in cancer were studied worldwide including role of reactive species (reactive oxygen and nitrogen), cell cycle modification, in vivo application, CAP interaction with cancer cells in conjunction with nanoparticles [10]. The two best known cold plasma effects namely plasma-induced apoptosis and the decrease of cell migration velocity have important implications in cancer therapy. CAP treatment can lead to localizing the cancer-affected area of the tissue and by decreasing the metastatic development. While mechanism of CAP action remains elusive such promising results warrant further research in this exciting field.

6. References

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