The selective anti-cancer mechanism of cold plasma

Maedeh Chatraie
Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

University of Tehran

Abstract

Introduction: Nowadays cancer is considered the second mortality reason worldwide. Surgery, chemotherapy and radiotherapy are expensive common therapies that have many side effects. Therefore it's so important to present new, efficient therapies. During recent decades, many studies have shown the effective potential of cold plasma in selective destruction of cancer cells.
In physics plasma is a neutral ionized gas composed of electrons, ions, radicals, electromagnetic fields and so on. Plasmas are generally divided into thermal or non-thermal and can be generated by different methods.

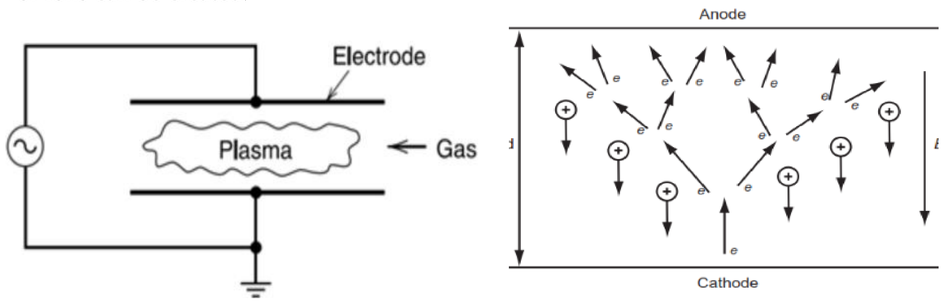
Methods: Optical emission spectroscopy is one of the most common methods to determine reactive species produced in the plasma as well as the temperature. Cold plasma irradiation to cultured cells or tissues can be done in a direct or indirect manner. Complementary to experiments, simulations, help us to understand the anti-cancer mechanism of cold plasma.

Results and discussion: The interaction of reactive oxygen and nitrogen species produced in plasma, with cell membrane lead to lipid oxidation and increase of cell membrane permeability. Subsequently, an increase in oxidative stress induces DNA damage and cell apoptosis. Furthermore, plasma induces an increase of intracellular calcium that can be considered as an anti-cancer mechanism of plasma. Tissue oxygenation is another result of plasma that open new opportunities for treatments in combination with radiotherapy.

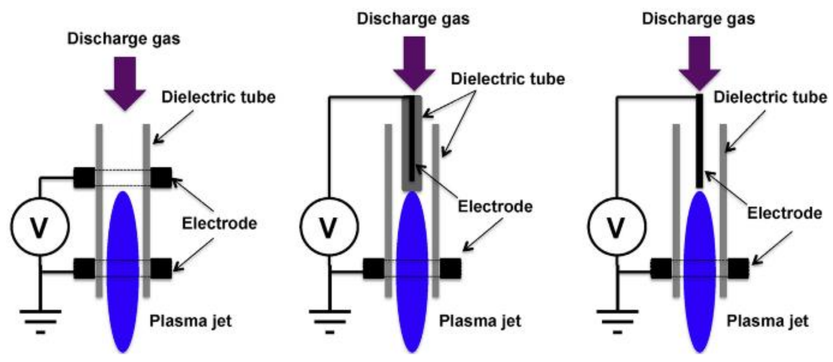
Conclusion: According to the positive results, it appears that cold plasma can be considered as a promising treatment option in cancer therapy.

I. Plasma generation

Plasma is created by applying energy to a gas in order to produce excited species and ions. This energy can be thermal or carried by an electric current or electromagnetic radiations.
Atmospheric cold plasmas are generally generated by electrical energy. The electric field transmits energy to the gas. This electronic energy is then transmitted to the neutral species by collisions and if the collisions were energetic enough, excited species or ions can be created.

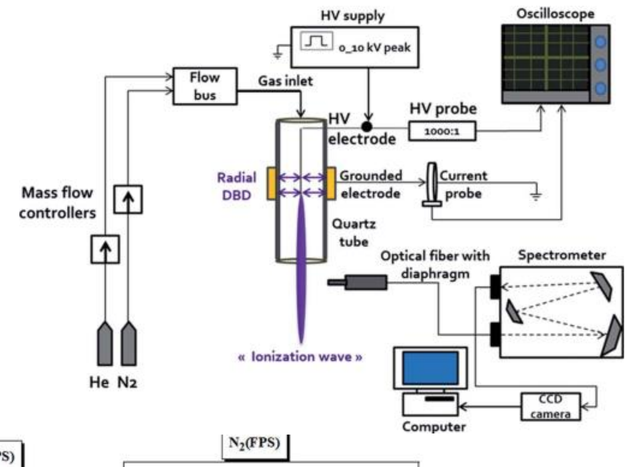


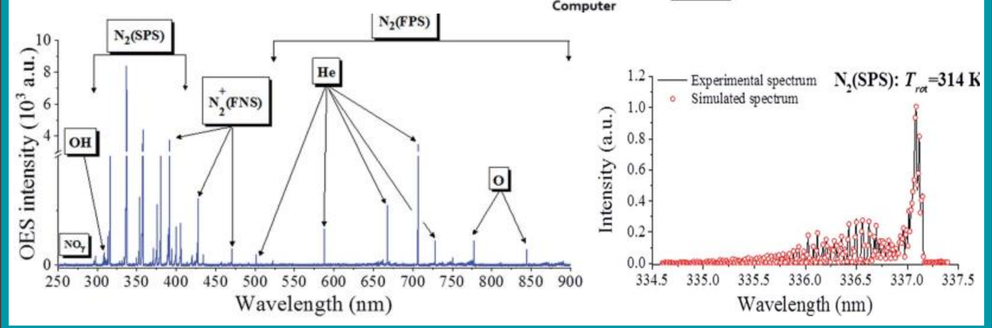
Different cold plasma sources can be categorized based on the electrode configuration. Plasma jets are one of the common sources in biomedical applications. A plasma jet generally composed of a cylindrical dielectric tube and pin or ring shape electrodes.



II. Plasma characterization

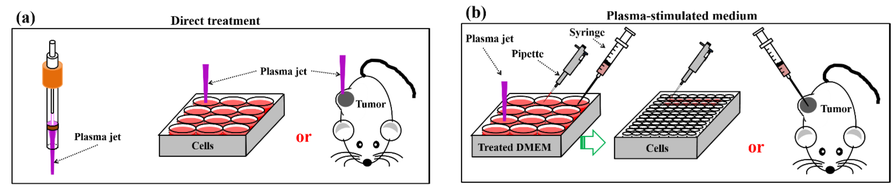
Plasma characterization is an important step that must be done before applying plasma on cell/tissue. Optical emission spectroscopy is one of the most common methods to determine reactive species produced in the plasma as well as the temperature.





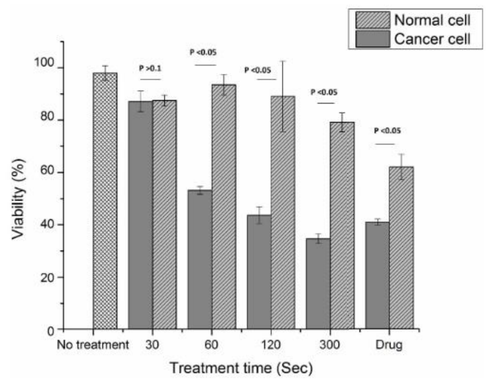
III. Cell/tissue treatment

Cold plasma irradiation to cultured cells or tissues can be done in a direct or indirect manner (using a plasma-stimulated medium) as shown in a and b figures respectively.



IV. Selective cell viability reduction

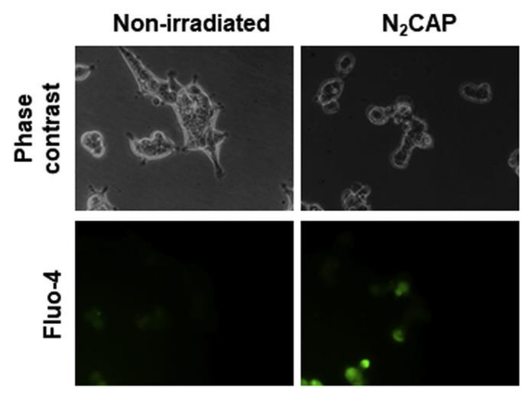
Normal cells in comparison with cancer cells show a different response to the cold plasma.
As the plot shows, the plasma induces a 37% viability reduction in cancer cells after 300 s treatment, while no drastic destruction was applied to normal cells (viability reduction difference is 43%). This may indicate the selective nature of the plasma treatment between cancerous and normal cells. Interestingly after 300s plasma treatment, the normal cells were less damaged than drug-treated cells with a difference of 20% in viability.



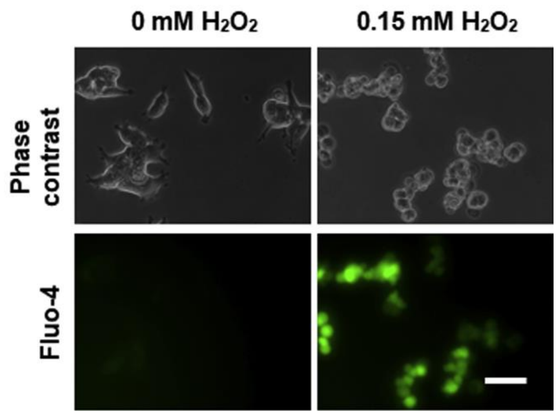
"Cell viability of cancer and normal cells after treatment by cold plasma in different duration times"

V. Increase of intracellular calcium

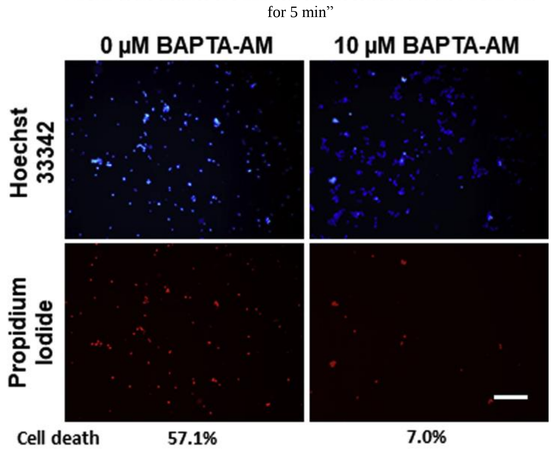
Assessment of intracellular calcium level using a Calcium Kit-Fluo 4 has been done. Based on the changes in Fluo-4 fluorescence signals observed by a fluorescence microscope, nitrogen plasma (N2CAP) treatment increases the intracellular calcium.
H2O2 also induces an increase in the intracellular calcium level in HEK293T cells.
Interestingly, the N2CAP-induced cell death is suppressed by BAPTA-AM as a cell-permeable calcium chelator. In contrast, EGTA, which is a calcium chelator and can reduce calcium influx from the medium, doesn't suppress the N2CAP-induced cell death.
These results indicate that the increase in intracellular calcium by N2CAP depends on the calcium efflux from intracellular calcium storage organelles such as the endoplasmic reticulum (ER) and mitochondria and can be considered as an anti-cancer mechanism of cold plasma.



"Increase of intracellular calcium level in HEK293T cells treated with N2CAP at the distance of 5 mm between the solution and the N2CAP exit for 5 min"



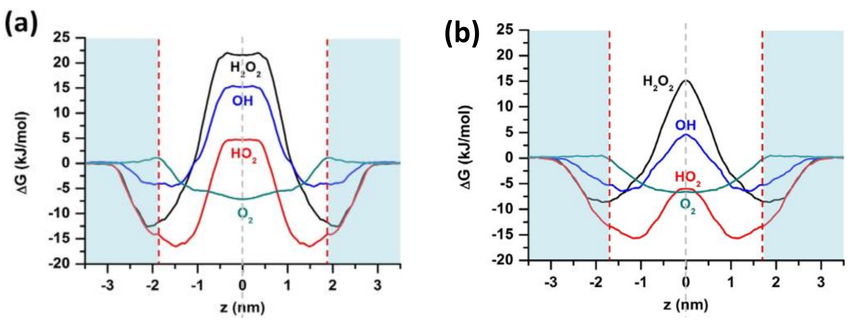
"Increase of intracellular calcium level in HEK293T cells treated with 0.15 mM H2O2 for 15 min at 37 °C with 5% CO2"



"Cell death suppression in HEK293T cells pre-treated with or without 10 μM BAPTA-AM for 1 h, and then treated with N2CAP at the distance of 5 mm for 5 min"

VI. Increase of cell membrane permeability

Based on in vitro studies, the cold plasma causes a noticeable increase of intracellular reactive oxygen in cancer cells compared to normal cells. Furthermore, it's shown that cold plasma leads to oxidation of the lipid tails in cell membrane models.
In order to understand the underlying mechanisms, the effect of lipid oxidation on membrane permeability was investigated in a Molecular Dynamics (MD) simulation study.
According to the free energy profiles for the translocation of reactive species across native and oxidized phospholipid bilayers (PLBs), lipid oxidation results in a decrease of the free energy barriers. However, the energy barriers still remain relatively high (order of tens of kJ/mol), so the ROS will not easily penetrate through the lipid bilayer. This indicates that specific protein channels, like AQPs, or pores are required in order to deliver the ROS into the cell interior.



"Free energy profiles for the translocation of reactive species across native (a), and 50 mol% aldehyde oxidized (b) PLBs"

References:
1. Iuchi, K. *et al.* Cold atmospheric-pressure nitrogen plasma induces the production of reactive nitrogen species and cell death by increasing intracellular calcium in HEK293T cells. *Arch. Biochem. Biophys.* **654**, 136–145 (2018).
2. Setsuhara, Y. Low-temperature atmospheric-pressure plasma sources for plasma medicine. *Arch. Biochem. Biophys.* **605**, 3–10 (2016).
3. Yusupov, M., Van der Paal, J., Neyts, E. C. & Bogaerts, A. Synergistic effect of electric field and lipid oxidation on the permeability of cell membranes. *Biochim. Biophys. Acta (BBA)-General Subj.* **1861**, 839–847 (2017).
4. Lazović, S. *et al.* Plasma induced DNA damage: Comparison with the effects of ionizing radiation. *Appl. Phys. Lett.* **105**, 124101 (2014).
5. Collet, G. *et al.* Plasma jet-induced tissue oxygenation: potentialities for new therapeutic strategies. *Plasma Sources Sci. Technol.* **23**, 12005 (2014).