# COLD ATMOSPHERIC PLASMA JET EFFECTS ON V79-4 CELLS

**Andreea-Roxana Lupu[1], N Georgescu[2]**

[1)] National Institute for Research/Development in Microbiology and Immunology "Cantacuzino", Immunomodulation Laboratory, Bucharest, Romania
[2)] National Institute for Laser, Plasma and Radiation Physics, Bucharest, Romania

ABSTRACT

The effects of cold plasmas are due to charged particles, reactive oxygen species (ROS), reactive nitrogen species (RNS), UV photons, and intense electric field. In order to obtain a more efficient action on mammalian cells (useful for cancer therapy), we used in our studies chemically activated cold plasma (He and $O_2$ gas mixture).

V79-4 cells were exposed to plasma jet for different time periods (30, 60, 90, 120 and 150s), using different combinations of helium and oxygen inputs (He:2.5l/min + $O_2$:12.5ml/min; He:2.5l/min + $O_2$:25ml/min; He:2.5l/min + $O_2$:37.5ml/min). Using MTT test we demonstrated that plasma jet induce cell viability decrease in all cases. The effect of chemically activated cold plasma –apoptosis or necrosis - depends on gas mixture an treatment period. Taking into account that ROS density in cell microenvironment is related to $O_2$ percent in the gas mixture and treatment period, we can presume that cell death is due to ROS produced in plasma jet.

**Key words:** cell treatment, cold atmospheric plasma jets

## INTRODUCTION

Cold plasma undergoes multiple interactions with bacterial and mammalian cells, generally by means of charged particles, reactive oxygen species (ROS), reactive nitrogen species (RNS), UV photons, and intense electric field [1]. Depending on plasma characteristics, its therapeutic applications point towards the enhancement of regenerative/degradative processes: (wound healing for lesions non-responsive to standard treatment procedures-for example diabetic lesions)/ (surgery, oral cavity sterilization in stomatology, antitumor therapy). A maximum degradative effect is obtained following plasma chemical activation by the presence of oxygen in the plasma content [2,3]

Low energy plasma particles (ions, radicals etc) have a weak interaction with the surrounding air molecules. Energetic electrons however produce molecular dissociations by means of collision events leading to the formation of reactive oxygen species. Following these interaction processes, the electrons energy decreases [4].

ROS generation processes starts with the dissociation of molecular oxygen ($O_2$) and generation of atomic oxygen:

$$e + O_2 \rightarrow O^- + O$$

Little is known about the way in which atomic oxygen acts on bacteria and animal cells. It has been shown that the presence of atomic oxygen in plasma, together with other reactive oxygen species, leads to an increase in the destruction rate of *Bacillus globigii* spores (surrogate for *Bacillus anthracis* spores) al lower input energies [5]. F. Sohbatzadeh et al (2009) have shown the efficiency of atomic oxygen in destroying three species with high clinical significance: *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus* [6]. Atomic oxygen plays an important role in many other bacterial strains inactivation proving its applicability in sterilization of culture media, material surfaces and air [7-11]. This makes it ideal for cleaning surgical implants, which are often contaminated with fragments of bacteria

cells even after sterilization. Consequently, patients can become subjects for pain and inflammation and even implant failure [12].

The ROS generation process based on the fact that atomic oxygen intensively reacts with the bioorganic molecules RH, producing R radicals and OH radicals:

$$O + RH \rightarrow R\cdot + {}^{-}OH$$

Hydroxyl radical, a very reactive oxygen species, will continue the oxidative chain reactions.

Nevertheless, by the reactive oxygen species that it produces, atomic oxygen is indirectly involved in the destruction of pathogens and induction of apoptosis or necrosis in mammalian cells, including cancer cells [8,9,13].

Free radicals are very reactive and unstable chemical species that react with different organic or inorganic components, especially with molecules that have a key role in the structure of cell membranes and nucleic acids. They also generate self-catalytic reactions following which the molecules that they interact to become free radicals continuing the chain of reactions.

First, a superoxide anion is generated from hydrated electrons either brought into the liquid medium by the plasma or formed at the plasma-medium interface:

$$e_{(H2O)} + O_{2(H2O)} \rightarrow O_2^{-}{}_{(H2O).}$$

Mammalians immediately convert superoxide into less harmful hydrogen peroxide through a reaction termed “superoxide dismutation”, which is catalyzed by an enzyme appropriately termed superoxide dismutase (SOD):

$$2\ O_2^{-} + 2\ H^{+} \rightarrow H_2O_2 + O_2$$

$H_2O_2$ is more stable than $O_2^{-}$, this stability making its diffusion in biological membranes possible (contrary to the case of $O_2^{-}$). $H_2O_2$ is however a weaker oxidant agent when compared to $O_2^{-}$.

Hydrogen peroxide is then converted to hydroxyl radicals through Fenton mechanism:

$H_2O_2 + Fe^{2+} \rightarrow OH + OH^- + Fe^{3+}$

$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$ [11]

Hydroxyl radicals can then react with nearby organics leading to chain oxidation and thus destruction of DNA molecules as well as cellular membranes and other cell components (here R is any organic molecule):

$^-OH + RH \rightarrow R\cdot + H_2O$

$R\cdot + O_2 \rightarrow RO_2$

$RO_2 + RH \rightarrow RO_2H + R\cdot$ [9]

Superoxide can react in water with nitric oxide (NO) producing another reactive oxidizing agent, peroxinitrite ($OONO^-$); $ONO_2^-$ is also produced by the reaction of hydrogen peroxide with nitrite.

$O_2^- + \cdot NO \rightarrow ONO_2^-$

$H_2O_2 + NO_2^- \rightarrow ONOO^- + H_2O$

Important molecular species such as DNA and proteins suffer peroxynitrite induced oxidative damages, the peroxynitrite generally acting as both oxidant and nitrating agent.

Neutral reactive species produce important oxidation effects within the cell membrane structures. Although extremely reactive, some of the neutral species such as O, OH and $O_2$ can only be effective inside or within the plasma proximity because of their very short lifetimes. Contrary, NO and $NO_2$ have a lower reactivity but longer lifetimes and they can be effective in regions separated from that where they have been produced [11].

Cells exposed to large amounts of ROS respond by either stopping their proliferation or by cell death (apoptosis or necrosis), depending on the phase of the cell cycle they undergo at the moment of cell lysis. Although at low concentrations of $H_2O_2$ and NO enhance cell proliferation, high concentrations of $H_2O_2$ and NO contributes to cell and tissue injury inducing apoptosis. In a concentration dependent manner, NO can either inhibit or enhance the hydrogen peroxide-mediated apoptosis in different cell types. ROS represent the major

cause for the spontaneous injury of DNA, proteins, lipids and carbohydrates. The two general mechanisms of ROS signaling are: alterations of the intracellular redox state and oxidative modification of proteins involved in signaling pathways [8].

$O_2^-$ and $H_2O_2$ promote loss of cell-cell adhesion and reorganization of actin cytoskeleton.

Studies using endothelial and epithelial cells have shown that exogenous ROS act especially on intercellular junctions; hence, the information is transmitted to the cytoskeleton components. The $H_2O_2$ treatment of epithelial cells is followed by cadherin internalization (glycoproteic adhesion molecule) and decrease of the occludin expression level (transmembrane protein involved in intercellular junction structure).

ROS alter actin filaments directly (alteration of –COOH and subsequent altered interactions between actin and other proteins) or indirectly (oxidative stress lead to rapid decrease of ATP and breakage of actin filaments). This process is followed by actin filaments reorganization and morphological changes of the cells (size and shape). Moreover, the ROS lead to the formation of intercellular gap.

All these factors contribute to alteration of intercellular adhesion and the subsequent increase of vascular permeability [14].

Exogenous ROS can activate membrane receptors and subsequent signaling cascades, while endogenous ROS are involved in transduction pathways only.

Activation of the "death receptors" – TNFR (tumor necrosis factor receptor) and Fas (CD95) induce apoptosis through activation of protein kinase and MAP kinase signaling pathways. PKC (protein kinase C), MAPK (mitogen activated protein kinase) and ERK 1/2 (extracellular signal-regulated kinases) are enzyme families involved in apoptosis. These proteins are activated by exogenous or/and endogenous ROS [12].

Transcription factors - NF-kB (nuclear factor kB), AP-1 (activator protein-1), STAT (signal transducers and activators of transcription) and HIF-1 alpha (hypoxia-inducible factor-

1 alpha) – are final points of the signaling pathways. Their activity is dependent on the redox state of cells [15]. Transcription factors binds to specific DNA sequences, thereby controlling the transfer (or transcription) of genetic information from DNA to mRNA and the synthesis of proteins which lead to a specific cell response.

ROS also act on the membrane ion transport systems: ion channels, ion pumps, ion exchangers, cotransporters. Either the inhibition or activation of these membrane transport mechanisms is followed by changes in the homeostasis of the main cell ions ($Na^+$, $Ca^{2+}$, $K^+$, $Cl^-$) leading to dramatic consequences for its functioning [16].

Genomic injuries produced by ROS through chromatin degradation, unavoidably lead to apoptosis through the generation of some signaling cascades, including poly(ADP-ribose) polymerase (PARP). This nuclear enzyme is activated by DNA strand breaks to catalyze the addition of poly(ADP-ribose) to a variety of nuclear proteins involved in DNA repair. In the case of massive DNA destruction, excessive PARP activity depletes its substrate NAD and, resynthesis of NAD depletes ATP. The eventual loss of energy (due to ATP depletion) leads to cell death [17]. Sublethal oxidative stress induces partial chromatin degradation only. This fact involves a high mutagenetic risk in surviving cells [18].

## MATERIAL AND METHODS

### Plasma jet generator

The cold atmospheric plasma is produced in a discharge chamber, by applying a voltage between high-voltage and ground electrodes. An inert gas (He) is the main atmospheric plasma forming gas. For bio-medical applications it is however necessary for the plasma to be chemically activated. That is why minute amounts of an additional gas as the source of reactive species are used. The reactive plasma is generated as a jet, which allows for the rapid transport of the excited species on the treated object (Fig. 1).

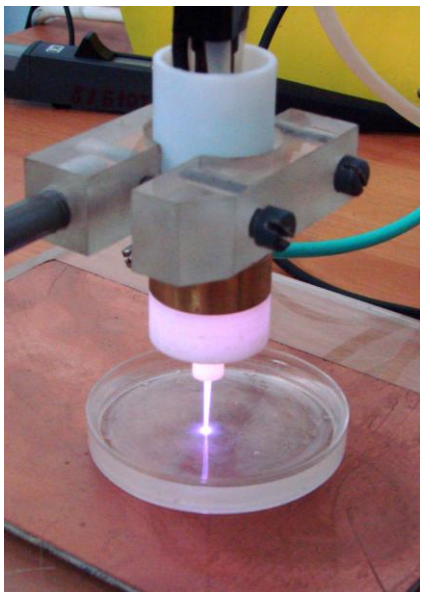

Fig. 1. The final device, generating high-voltage pulsed, cold atmospheric plasma jet.

In our cold atmospheric plasma jet generators, the inter-electrodes voltage applied for obtaining the plasma is pulsed high-voltage, with amplitudes of 20 – 30 kV, 100 – 500 ns width at half maximum, and frequencies of tens-hundreds of pulses per second [2,3,19,20]. Molecular oxygen is used as the source of reactive species in our research.

The chemical processes start with a generation of atomic oxygen, produced by electron impact dissociation, by dissociative attachment [21], and by Penning ionization [22].

In order to obtain higher quantities of atomic oxygen, it is necessary to increase the concentration of molecular oxygen ($O_2$) in plasma-forming gas (He, in our case). Unfortunately, this increase brings along the decrease of the plasma current. Consequently the quantity of atomic oxygen produced within the plasma has an initial increase along with the concentration of molecular oxygen, and then it drops as a result of the decrease of the plasma jet current. Therefore there is an optimal concentration of $O_2$ in He/Ar that maximizes the quantity of atomic oxygen.

Experimental works [2,3,23,24] have determined an optimal concentration of 0.5 – 0.6 % vol. $O_2$ / He. Using a model that considers 35 dominant chemical reactions in the RF He/$O_2$ plasma, an optimal theoretical value of 0.5 % vol. $O_2$ / He [25] has been obtained.

In all the papers mentioned above, a coaxial device with only one high-voltage electrode was used for generating the plasma jet. The ground electrode was mounted on the exterior, separated from the hig-voltage electrode by a dielectric barrier.

Our newest device [20] allows for the increase of the optimal concentration of molecular oxygen in He and, consequently, for the increase of the quantity of atomic oxygen in the plasma jet. Using the new device, the optimal value of the concentration of $O_2$ in He is 1.5 %. The obtained value is 3 times higher than in the case of experiments in [2,3,23,24] and of theoretical simulation in [25]. Accordingly, the quantity of atomic oxygen in the plasma jet increases and the plasma jet becomes more chemically active. The explanation for this increase of chemical activity is that our new device has in the discharge room three high voltage electrodes, which are placed in such a manner that three independent discharges take place. Each discharge has an optimal percentage of 0.5 % $O_2$ in He that maximizes the quantity of atomic oxygen in the plasma. The plasmas of the three discharges unite in a single jet, which contains a quantity of atomic oxygen that is a lot higher of that of the plasma of each discharge.

**Cell lines**

In our experiments we used V79-4 cell line (ECACC no 93010723, normal fibroblasts isolated from Hamster Chinese Lung).

**Plasma Jet treatment**

The V79-4 cells were cultured (1 x $10^6$) in DMEM-F12 culture medium (Sigma) supplemented with 1mM L-glutamine (Sigma), antibiotics and 10% fetal calf serum (Biochrom). When cells formed a monolayer, they were exposed to plasma jet (Fig. 2) for different time periods (30, 60, 90, 120 and 150s), using different combinations of helium and oxygen inputs (He:2.5l/min + $O_2$:12.5ml/min; He:2.5l/min + $O_2$:25ml/min; He:2.5l/min + $O_2$:37.5ml/min).

In order to exclude the pH decrease (data not shown), the culture medium was removed immediately before the treatment and re-added after plasma action time. Another reason for culture medium removal was the fact that the density of active oxygen species (produced by plasma and diffusing into medium) significantly decreased with increasing distance from the liquid interface) [Stoeffels et al, 2005]. In order to decrease, as much as possible, all the effects that the surrounding air might have upon the culture medium (pH changes, etc) and consequently upon the cells, all wells except for those being treated were covered (Fig.2).

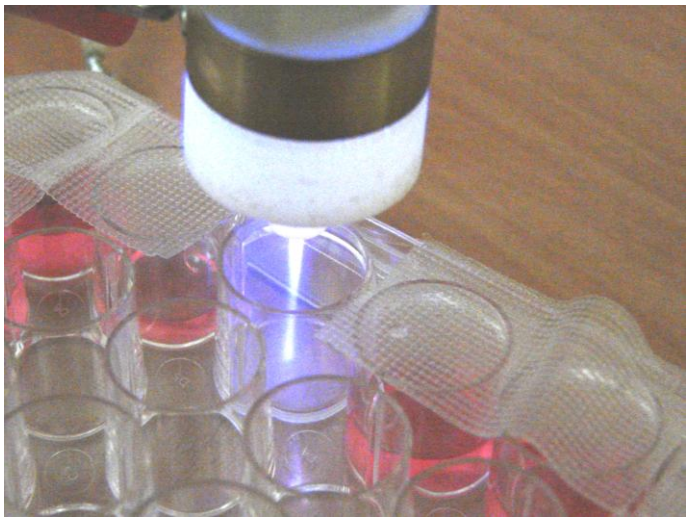

Fig. 2. Cell treatment with cold atmospheric plasma jets

**Microscopic analysis**

Shortly after plasma treatment, the culture plate was studied using a Krüss optical microscope (magnification 10X). The cells were photographed using a Canon PowerShot A510 digital camera. After the culture medium was removed, pictures of each well were taken (using the camera mentioned above) in order to emphasize possible modifications of cell distributions.

**MTT viability test**

The V79-4 cells (1 x $10^5$ cells/50 µl DMEM-F12 culture medium supplemented with 1mM L-glutamine, 10% fetal calf serum and antibiotics) were incubated with MTT (100µl) for 4 hours at 37°C, 5% $CO_2$ and about 90% relative humidity. Then 100µl DMSO (Aldrich) were added in each well in order to solubilize the formazan crystals. The optic density of purple formazan compound produced only in the presence of living cells has been measured using a Thermo Multiskan spectrophotometer at 540nm.

Taking into account the fact that proliferating cells are metabolically more active than non-proliferating cells, the MTT assay can be used not only for the determination of cell viability and factor mediated cytotoxicity but also for the determination of cell activation and proliferation. However, the MTT response may vary greatly in viable cells due to the metabolic state of the cells. For this reason the experimental results are analyzed by only taking into account the percentage values obtained by normalizing the optical densities of the samples to that of the control (Garn et al, 1994). Each sample was analyzed in triplicate.

**Apoptosis analysis**

The V79-4 cells were detached with trypsin and washed twice (2 min, 2000 rpm), the samples being afterward analyzed using the ApoGlow Assay Kit (Lonza). This assay is based on the fact that cell death is an energy driven process. Apoptosis is accompanied by ATP degradation and an increase in the cellular levels of ADP. Using an ADP:ATP ratio-based indicator, ApoGlow allows to distinguish between apoptosis, necrosis and cell proliferation. Apoptosis is characterized by a moderate increase of the ADP/ATP ratio vs control, while necrosis can be recognize for a significantly higher ADP/TP ratio than the control. Each sample was analyzed in triplicate.

In some cases, cells detached by plasma action keep their characteristics and become adherent again reentering the normal cell cycle if they are transferred to another culture plate

[26]. For this reason, in our study we used cells detached by the plasma jet as well as cells that remained adherent after the plasma exposure and were detached afterward using trypsin.

**Statistical analysis**

Data presented here has been calculated as the average value +/- s.e.m. for three identical samples in each experiment. Coefficient of variation (CV) for each sample did not exceed 25% in both MTT test and apoptosis assay.

## RESULTS AND DISCUSSION

**Microscopic analysis**

Following the microscopic analysis, cells detach from the plate was shown (Fig.3a), accordingly to other literature data [26]. In control sample a cell monolayer can be observed (Fig 3c).

After the removal of the culture medium, we have seen a large area which was not covered with cells at the center of the well, exposed to the direct action of the plasma jet (Fig. 3d). For the control sample, the cell distribution on the bottom of the well appeared to be homogeneous (Fig 3b).

**MTT viability test**

Concerning the effect of cold plasma jet on V79-4 cells viability, the results have shown that the number of viable cells was reduced in a treatment duration dependent manner only for the case of He:2.5l/min + $O_2$: 25ml/min gas mixture (Fig.4a). This type of behavior was not observed for the other two cases (He:2.5l/min + $O_2$: 12.5ml/min si He:2.5l/min + $O_2$: 37.5ml/min) (Fig. 4a and b). Moreover, the He:2.5l/min + $O_2$: 37.5ml/min gas combination drastically decreased the cells viability but however no significant treatment duration dependent variations could be observed.

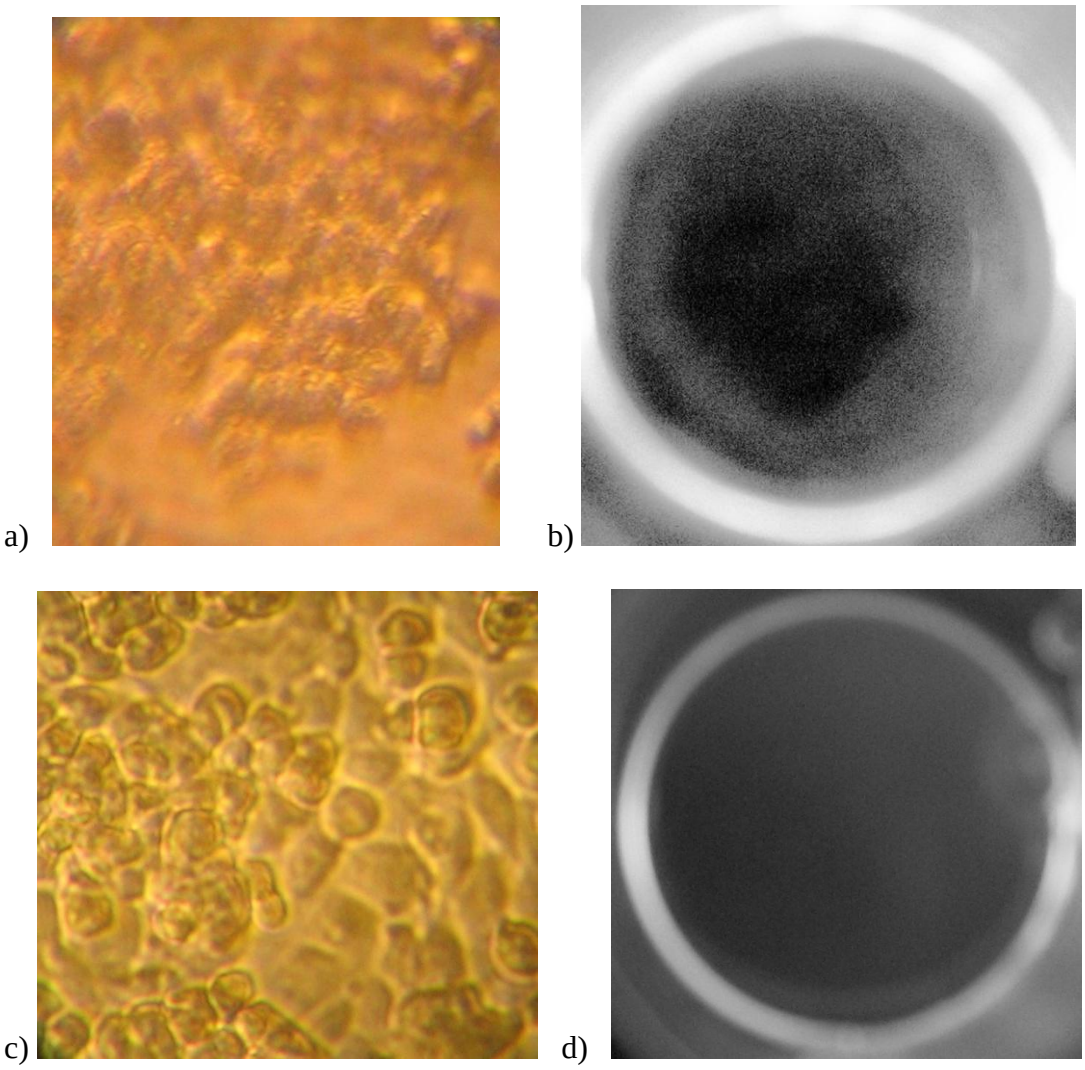


Fig.3. Cold plasma jet treatment (He: 2.5l/min + $O_2$: 25ml/min, 90s) is followed by V79-4 cells detachment from substrate (a, b) while control (untreated) cells remain adherent (c, d).

**Apoptosis analysis**

For the He: 2.5l/min + $O_2$ 12.5ml/min and He: 2.5l/min + $O_2$ 25ml/min cases, we observed an increase of ADP/ATP ratio depending on the treatment period. For the first gas combination we obtained a significant increase of ADP/ATP ratio (necrosis) after 60s of treatment; the second case shown a moderate increase of ADP/ATP ratio (apoptosis) after 30 and 60s of treatment and a significant increase for 90, 120 and 150s treatment durations. In the third case (He: 2.5l/min + $O_2$ 37.5ml/min) we observed a drastic increase of ATP/ADP ratio for 30, 60 and 90s and a small decrease vs control for 120 and 150s. The results obtained for 120 and 150s may be a consequence of the total destruction of cells after plasma treatment (Fig.5).

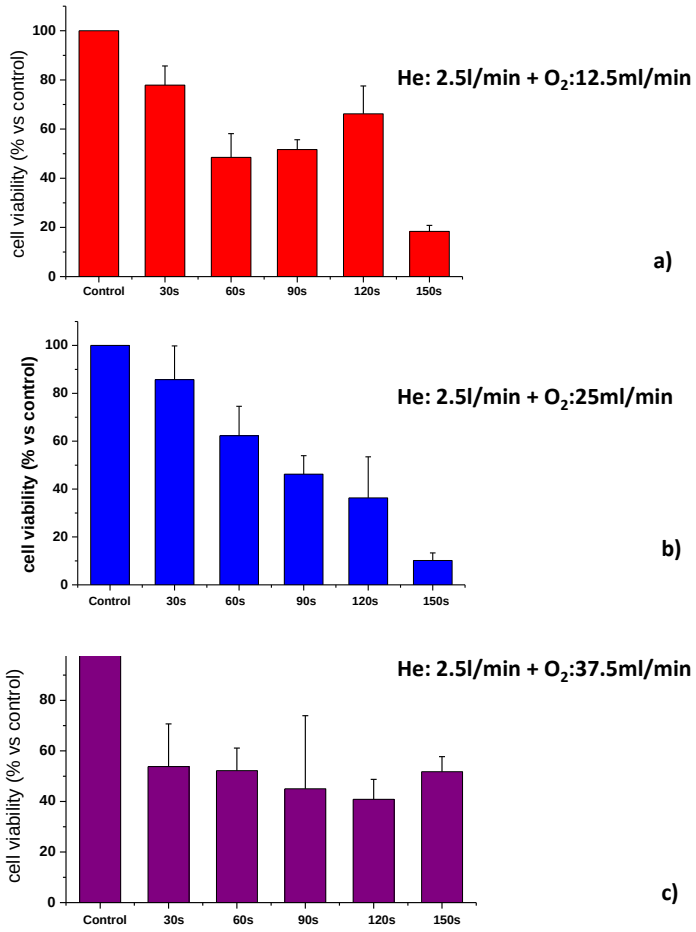


Fig. 4. The effect of cold plasma jet on V79-4 cells viability (MTT test)

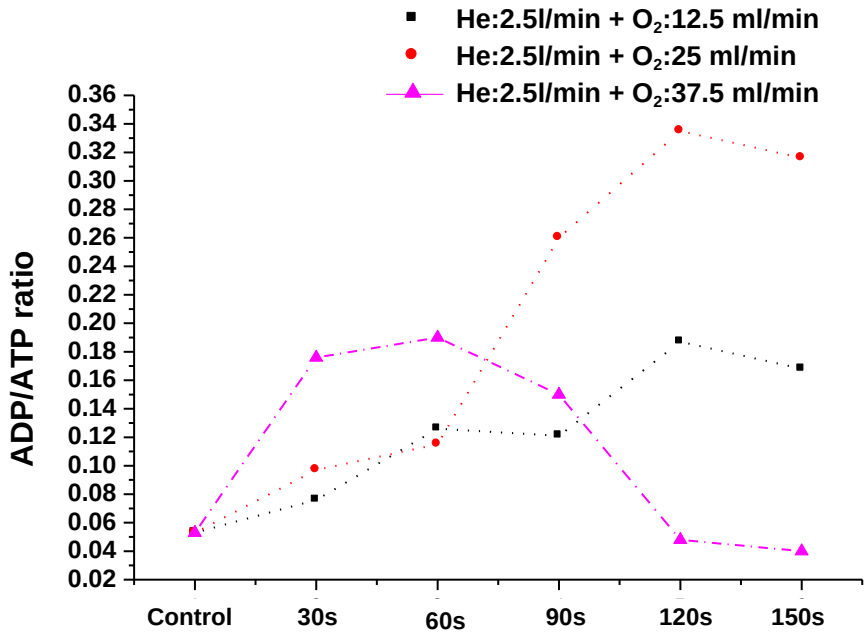


Fig. 5. Cold plasma jet induces V79-4 cell death; CV did not exceed 25%;

Taking into account that ROS density in cell microenvironment depends on $O_2$ percent in the gas mixture and treatment period, we can presume that cell death is due to ROS produced in plasma jet.

The clinical use of atmospheric pressure plasma jet in skin tumor treatment requires for fine plasma action control possibilities, a more invasive treatment inside the tumor mass and a less invasive treatment at its edges in order to avoid the unnecessary destruction of healthy cells. For this reason we focused our attention on the use of (He: 2.5l/min + $O_2$ 12.5ml/min and He: 2.5l/min + $O_2$ 25ml/min) gas combinations and we discarded the third alternative (He: 2.5l/min + $O_2$ 37.5ml/min) since for that case we obtained a sharp decrease (approx. 50%) in cell viability after only 30 seconds of treatment.

Since for therapeutic purposes the plasma jet should only produce cell apoptosis, the clinical implementation procedures should only consider short and repetitive treatment sessions instead of applying a single long time plasma exposure.

**CONCLUSIONS**

Our results showed that chemically activated cold plasma was able to induce death in the V79-4 cell line. This effect proved to be dependent of gas contents and treatment duration for our experimental settings. Apoptosis was obtained for: He2.5l/min + $O_2$ 12.5ml/min – 30s, He2.5l/min + $O_2$ 25ml/min – 30s, 60s and necrosis for He2.5l/min + $O_2$ 12.5ml/min – 60, 90, 120, 150s, He2.5l/min + $O_2$ 25ml/min – 90, 120, 150s and He2.5l/min + $O_2$ 12.5ml/min – 30s, He2.5l/min + $O_2$ 37.5ml/min – 30, 60, 90s. We consider the possibility that the ROS produced in the plasma jet are responsible for all cell death events. Tacking into account that ROS destroy the cells in a non-selective manner, the relevant parameters of the plasma jet device must be carefully set for each treatment case.

**ACKNOWLEDGEMENTS**

This work was supported by CNCSIS-UEFISCSU-Romania under Project IDEI_54 (contract no. 19/2007).

**REFERENCES**

1. **Yasuda H., Myura T., Kurita H., Takashima K. and Mizuno A.**, *Biological Evaluation of DNA Damage in Bacteriophages Inactivated by Atmospheric Pressure Cold Plasma.* Plasma Processes and Polymers. 7, pp. 301-308, 2010.
2. **Georgescu N., Lungu C. P., and Lupu A. R.,** *Chemical activation of the high voltage pulsed, cold atmospheric plasma jets.* Romanian Reports in Physics. Vol. 62, No. 1, pp. 142-151, 2010.
3. **Georgescu N., and Lupu A. R.,** *Tumoral and normal cells treatment with high voltage pulsed, cold atmospheric plasma jets*, IEEE Trans. Plasma Sci. Vol. 38, No. 8, Part 2, pp. 1949-1955, 2010.
4. **Laroussi M., Tendero C., Lu X., Alla S., and Hynes W.L.**, *Inactivation of Bacteria by the Plasma Pencil*, Plasma Process. Polym. 3, pp. 470-473, 2006.
5. **Hermann H.W., Henins I., Park J., and Selwyn G.S.**, *Decontamination of chemical and biological warfare (CBW) agents using an atmospheric pressure plasma jet (AAPJ).* Physics of Plasmas. Vol 6, No 5, 1999.
6. **Sohbatzadeh F., Colagar A.H., Mirzanejhad S., and Mahmodi S.,** , *E. coli, P. aeruginosa, and B. cereus Bacteria Sterilization Using Afterglow of Non-Thermal Plasma at Atmospheric Pressure*. Applied Biochemistry and Biotechnology. 2009.
7. **Lu X.P., Ye T., Cao Y.G., Sun Z.Y., Xiong Q., Tang Z.Y., Xiong Z.L., Hu J., Jiang Z.H., and Pan Y.,** *The roles of the various plasma agents in the inactivation of bacteria*, Journal of Applied Physics. 104, 053309, 2008.
8. **Kong M.G., Kroesen G., Morfill G., Nosenko T., Shimizu. T., van Dijk J., and Zimmermann J.L.,** *Plasma medicine: an introductory review*, New Journal of Physics. 11, 115012 (35pp), 2009.
9. **Dobrynin D., Fridman G., Friedman G., and Fridman A,** *Physical and biological mechanisms of direct plasma interaction with living tissue*. New Journal of Physics. 11, 115020 (26pp), 2009.
10. **Moreau M., Orange N., Feuilloley M.G.J.**, *Non-thermal plasma technologies: New tools for bio-decontamination*. Biotechnology Advances. 26, pp. 610-617, 2008.
11. **Fridman A.**, *Plasma Chemistry*. Cambridge University Press. 2008.
12. **NASA Glenn Research Center** http://www.nasa.gov/centers/glenn/moonandmars/med_topic_atomic_oxygen.html
13. **Srivastava R.**, *Cell Signalling and Human Diseases*. Humana Press. Cap. 2, 2007.
14. **Bruce A., et al.**, *Molecular Biology of the Cell*. 4th ed, New York, Garland Publishing. 2000.
15. **Surh Y.-J., Kundu J.K., Na H.-K., and Lee J.-S.,** *Redox-Sensitive Transcription Factors as Prime Targets for Chemoprevention with Anti-Inflammatory and Antioxidative Phytochemicals*, J. Nutr. 135, pp. 2993S-3001S, 2005.
16. **Bienert G.P., Schjoerring J.K., and Jahn T.P.,** *Membrane transport of hydrogen peroxide*. Biochimica et Biophysica Acta (BBA)-Biomembranes. Vol. 1758, Issue 8, pp. 994-1003, 2006.

17. **Fink S. L. and Cookson B.T.**, *Apoptosis, Pyroptosis, and Necrosis: Mechanistic Description of Dead and Dying Eukaryotic Cells*. Infection and Immunity. 73(4), pp. 1907-1916, 2005.
18. **Nanavaty U. B., Pawliczak R., Doniger J., Galdwin M. T., Cowan M. J., Logun C., and Shelhamer J. H.**, *Oxidant induced cell death in respiratory epithelial cells is due to DNA damage and loss of ATP*. Experimental Lung Research. 28(8), pp. 591-607, 2002.
19. **Georgescu N.**, *High voltage pulsed, cold atmospheric plasma jets: electrical characterization*. Romanian Reports in Physics. Vol. 60, No. 4, pp. 1025-1032, 2008.
20. **Georgescu N., Lungu C. P., Lupu A. R., and Osiac M.,** *Atomic oxigen maximization in high-voltage pulsed, cold atmospheric plasma jets*. In print, IEEE Trans. Plasma Sci. Vol. 38, 2010.
21. *NIST Handbook of Atomic Spectroscopic Data*. Available: http://physics.nist.gov/PhysRefData/Handbook
22. **Alvarez R., Quintero M. C., and Rodero A.,** *Radial description of excitation processes of molecular and atomic species in a high-pressure helium microwave plasma torch*. J. Phys. D: Appl. Phys. Vol. 38, No. 20, pp. 3768-3777, 2005.
23. **Knake N., Reuter S., Niemi K., von der Gathen V. S., and Winter J.,** *Absolute atomic oxygen density distributions in the effluent of a microscale atmospheric pressure plasma jet*. J. Phys. D: Appl. Phys. Vol. 41, No. 19, 194006, 2008.
24. **Walsh J. L., Liu D. X., Iza F., Rong M. Z., and Kong M. G.,** *Contrasting characteristics of sub-microsecond pulsed atmospheric air and atmospheric pressure helium–oxygen glow discharges*. J. Phys. D: Appl. Phys. Vol. 43, No. 3, 032001, 2010.
25. **Park G.Y., Hong Y. J., Lee H. W., Sim J. Y., and Lee J. K.,** *A global model for the identification of the dominant reactions for atomic oxygen in He/$O_2$ atmospheric-pressure plasmas*. Plasma Process. Polym. Vol. 7, No. 3 – 4, pp. 281–287, 2010.
26. **Stoffels E., Kieft I. E., Sladek R. E. J., van den Bedem L. J. M., van der Laan E. P., and Steinbuch M.,** *Plasma needle for in vivo medical treatment, recent developmentsd and perspectives*. Plasma Sources Sciences and Technology. pp. S169-S180, 2006.