The Selective Characterization of Nonthermal Atmospheric Pressure Plasma Jet on Treatment of Human Breast Cancer and Normal Cells

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***Abstract*— The aim of this paper is to investigate the effect of nonthermal atmospheric pressure plasma jet on human breast cancer and normal cells. High voltage, dc-pulsed power supply was used to generate low temperature helium plasma in atmospheric pressure. In this paper, cancer and normal cells were exposed to plasma during four time steps. For further exploration, Doxorubicin as common chemotherapy drug was employed to compare the efficacy of plasma treatment with convectional cancer therapy. Also, the Caspase-Glo 3/7 assay was obtained to reveal the death process of cancer and normal cells. The results of MTT and apoptosis assay showed that the plasma treatment has drastically reduced the viability of breast cancer cells, while it has no significant damage to the normal cells. In addition, after plasma treatment three different zones were formed in the plate, which will represent the detachment of cells from plate surface. Moreover, adding 5% oxygen to the helium plasma will lead to enhancement of cancer cells viability reduction. In addition, the outcome of this paper has verified that the plasma treatment successfully overcomes drug treatment in inhibition of the cancer cells viability, while decreases the adverse effect of drug treatment.**

***Index Terms*— Cancer, gas discharge, plasma applications.**

I. Introduction

DURING the last decades, nonthermal atmospheric pressure plasma (NAPP) has drawn a great deal of research interest because of its promising treatment potential as a new biomedical treatment modality. Various NAPP sources have been used for generating plasma to investigate its potential applications in different biomedical research areas such as bacterial and fungal sterilization [1], [2], blood coagulation [3], wound treatment [4], and surgical operation [5]. In the recent years, scientists have been successfully able to link the NAPP with cancer treatment by achievement in therapeutic treatment in various cancer cells [6]–[8].

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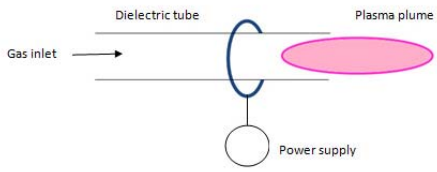
Breast cancer is the most prevalent cancer and the second leading cause of cancer-related death among women in the worldwide (22.9%). It affects one in eight women during their lives [9], [10]. There are several techniques for the treatment of breast cancer such as breast-tissue reduction operation, surgery, drug treatment, and chemotherapy. Although these methods provide possible cancer therapy solution, some significant disadvantages make these techniques unreliable. These techniques are time consuming, associated with normal tissue injury and don not provide a definitive heal. Therefore, it is necessary to develop an efficient alternative technique for the breast cancer treatment. Such a technique should have some properties including the capability of operating in low temperatures, imposing no adverse health to normal cells as well as no harmful environmental effects.

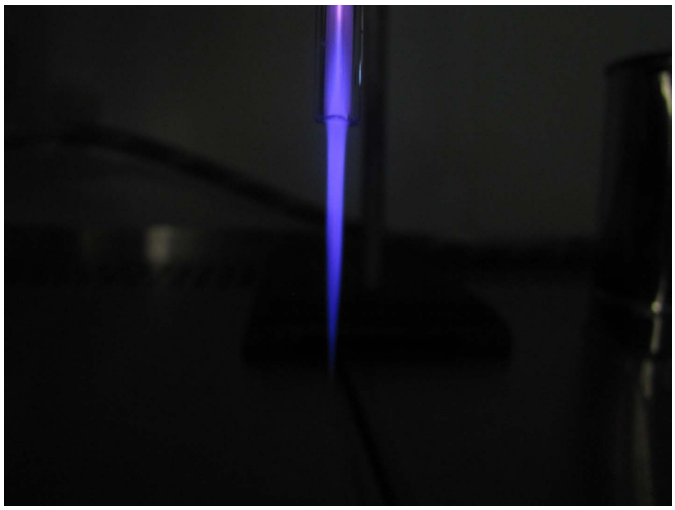
Plasma jet is one of the most widely used methods for generating cold atmospheric plasma. The ionized gas temperature reaching 30 °C–40 °C is very appropriate for heat sensitive treatment. The plasma jet configuration is made of a high voltage powered electrode wrapped around an insulating tube. The plasma generated by ionization of the gas fed into the tube. An ionized gas plume emitted out from the tube extending 4 cm into the open air. The plum consists of various groups of highly reactive chemical agents such as reactive oxygen species (ROS), reactive nitrogen species (RNS), nitric oxide (NO), and other short lived and metastable species. These species accompany in chemical reaction, ion bombardment, charge accumulation, and UV radiation processes, which play an important role in the treatment of biological samples [11]–[13].

Previous research has shown the therapeutic efficiency of NAPP jet on breast cancer cells [14]. The study proof that, plasma treatment has induced apoptotic effect on cancer cells. As regards, the lack of research on interaction of plasma with normal cell is obvious. In this paper, we further explore the effect of NAPP jet on breast normal cells, which represent the novel results in this paper for the first time.

Therefore, this paper aims to determine the different response of cancer and normal breast cell line to NAPP jet. In this line, the cancer and normal cells were exposed by plasma with various treatment times and the results compared with untreated and drug treated samples. Also, the optical emission spectroscopy was assisted to specify the comprised species in plasma and gas temperature.



(a)



(b)

Fig. 1. (a) Schematic of NAPP jet configuration. (b) Plasma discharge during an experiment.

II. Material and Methods

A. NAPP Jet Configuration

The plasma jet consisted of a Pyrex tube (ID: 2 mm and OD: 4 mm) as an insulating tube. Copper wire as power electrode was wrapped around the glass tube with 10-mm width as nozzle. The distance between the nozzle tip and the powered electrode is 10 mm. The power of electrode was driven by a 6-KHz pulsed dc (width of pulse $= 20\ \mu$s) high voltage with voltage-variable power supply. The applied voltage on the electrodes ranged from 0 to 10 KV. The feeding gases for this paper were 99.999% pure helium (He) with 2-L/min gas flow rate. Moreover, blends of helium with addition of 5% oxygen were employed to investigate the effect of oxygen on cancer cells treatment. Fig. 1(a) shows the plasma jet setup schematic meanwhile Fig. 1(b) shows the discharge photo during a typical experiment. To study the effect of plasma exposure time on the treatment efficiency, the prepared samples were treated with plasma jet for four different exposure times including 30, 60, 120, and 300 s. The feeding gas for this experiment was He and the distance between the samples and the nozzle tip was 1 cm that was kept unchanged. This process was repeated by He/O_2 admixture with the same condition. For determining the impact of the voltage and electric field of plasma on the cells, three different voltages were evaluated. All of three voltage treatments were done with 60 s exposure duration. The first voltage was chosen at 6 kV (beginning of discharge) and increased by increments of 2 kV at each step of experiment until it reached 10 kV. The experiments, repeated three times, were done under the same conditions.

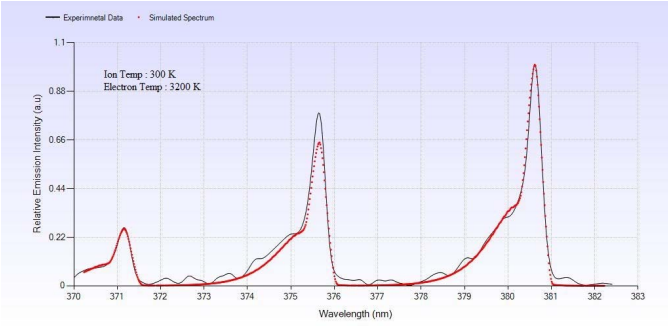


Fig. 2. Ion and electron temperature analyses of the nitrogen SPS.

B. Optical Emission Spectroscopy

Plasma spectroscopy was employed to study the plasma discharge characteristics because of its optical emission profile. Ocean Optics HR 2000 spectrometer was used to collect optical emission and subsequently to determine the plasma constituent species. The spectral range for this purpose was chosen from wavelength of 200–1000 nm with an optical resolution of 0.5 nm. The spectra were subtracted from dark baseline. Another characteristics obtained from the optical spectrum is analyzing the electron and ion temperatures. These parameters evaluated by second positive system (SPS) transition analysis of nitrogen can be observed at wavelengths between 370 and 382 nm. The optical emission was collected by high resolution echelle spectrometer (with resolution of 0.02 nm). The rotational and vibrational temperatures, referring to ion or gas and electron temperature respectively were calculated by processing collected spectrum with SpecAir software.

C. Human Breast Cancer and Normal Cell Culture

The anchorage human breast cancer cell line, MCF7 (ATCC-HTB22) and MCF10A (ATCC-CRL-10317) were kindly provided by Rahbarizadeh. MTT (M5655) was purchased from Sigma Corporation. MCF7 cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO) supplemented with 10% (v/v) fetal bovine serum (GIBCO) and 1% penicillin-streptomycin (Sigma) and also MCF10A cells were cultivated in DMEM/F12 growth medium (GIBCO) supplemented with 5% Horse Serum (GIBCO), 1% penicillin-streptomycin (Sigma), Hydrocortizone (1 mg/ml), Insulin (10 mg/ml) and EGF (100 mg/ml) (sigma). Cultures were maintained at 37 °C in a humidified atmosphere containing with 5% (v/v) CO_2. Cell morphologies were observed with an inverted microscope.

D. Drug Treatment

Adriamycin (Doxorubicin) drug (Sigma-Aldrich co.) were applied on cancer and normal cells. 20 μl of the 0.5-mg/ml concentration of the drug were added to each well. After 24-h incubation the drug treated cells were prepared for further analyses.

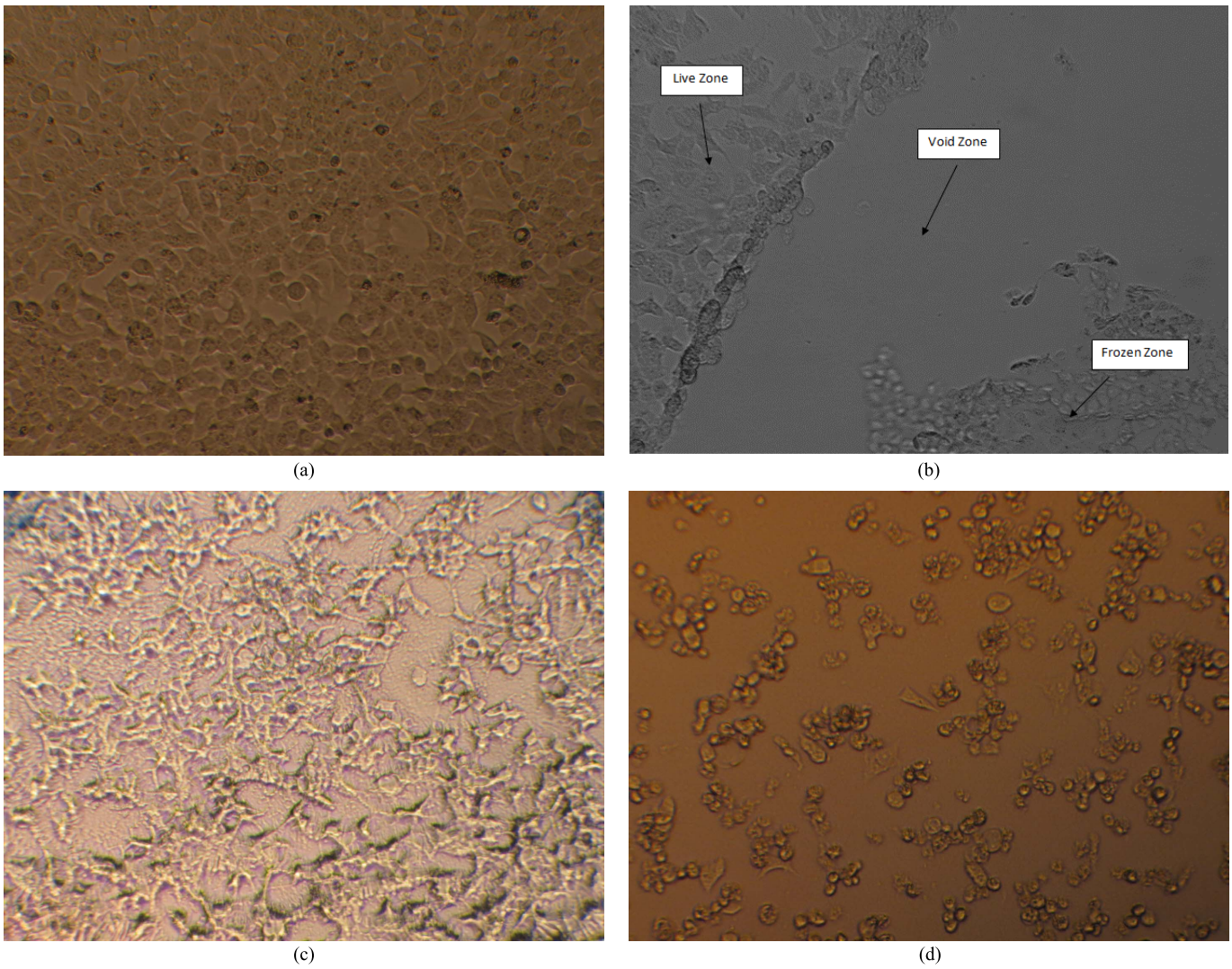


Fig. 3. (a) Untreated cancer cells. (b) Formation of three zones after 60 s of plasma treatment. (c) Morphology of the cancer cells after 300-s plasma treatment. (d) Morphology of the cancer cells after drug treatment. Magnification of pictures are 40x.

E. MTT Assay

Cell viability was performed indirectly by MTT assay, based on the enzymatic reduction of 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) to form formazan crystals through mitochondria and cellular dehydrogenase enzymes. 2×10^4 cells/well were seeded into 96-well flat bottomed microplates in 100-μl growth medium and incubated overnight until the cells adhered to the bottom of the plate. After 70%–75% cell confluency, the media of wells was removed and Phosphate buffered saline was added into each well, then the cells were treated with plasma. 24 h after the treatment, the media was aspirated from the wells and 10 μl MTT (5 mg/ml) along with fresh media was added to each well. The reaction was terminated after 4-h incubation by aspiration media. The triplicated wells were run for each group by adding 100–150 μl DMSO to wells to dissolve formazan crystals. The optical density of each well was measured with microplate reader at 570 nm. Before and after the treatment, the morphology of the cells were than observed with the inverted microscope.

F. Apoptosis Assay

Caspase activity was determined using a Caspase-Glo 3/7 assay kit (Promega, G811C), which uses luminogenic caspase substrates, according to the manufacturer's instructions. Briefly, the cancer and normal cells, respectively, MCF 7 and MCF 10 A (1×10^5) were cultured in 24 Well Microplate. After 24-h incubation, the cell lysates were prepared using cell culture lysis reagent buffer [100-mM potassium phosphate, pH 7.8, 1-mM EDTA, 7 mM 2-mercaptoethanol, 1% (v/v) Triton X-100, and 10% (v/v) glycerol]. Then, an equal amount of protein concentration (according Bradford assay) and luminescence caspase 3/7 substrate were mixed. The samples were incubated in the dark and luminescence was measured in tube luminometer (berthold, Germany). The experiments were performed in duplicate and repeated two separately.

III. Results and Discussion

The results of the temperature analysis were shown in Fig. 2. It proofs that the ion temperature is 300 K ($\pm$5 K) while the electron temperature reaches about 3000 K ($\pm$100 K),

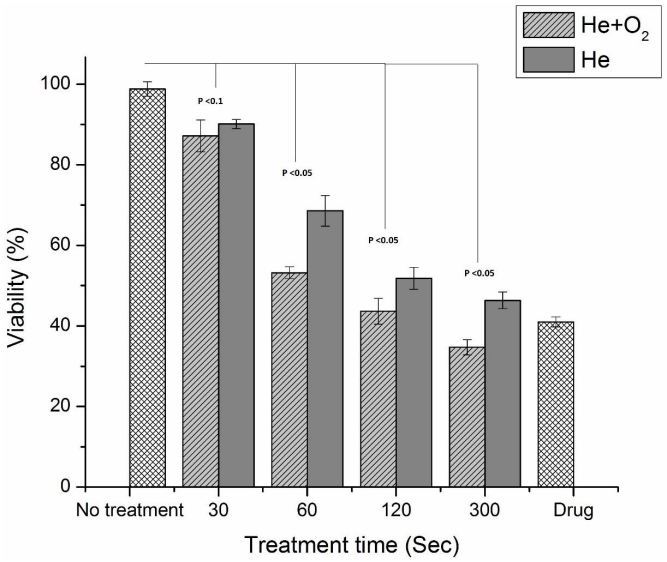


Fig. 4. Viability result of cancer cells treated by He and He/O_2 mixture plasma after four steps of treatment times. The results of plasma treated samples compared with untreated and drug treated samples result.

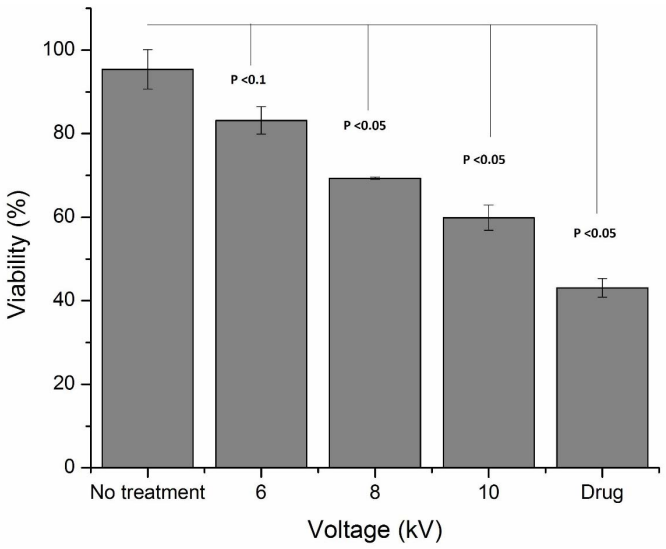


Fig. 5. Viability result of cancer cells treated by He plasma after three steps of power supply voltages. The results of plasma treated samples compared with untreated and drug treated samples result.

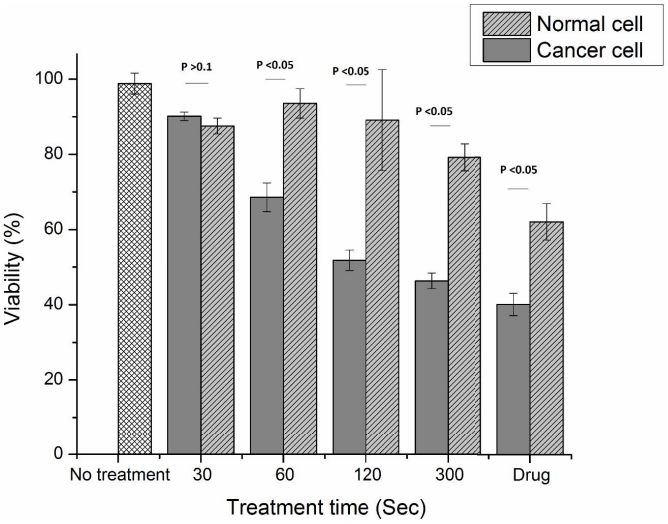


Fig. 6. Viability result of cancer and normal cells after He plasma treatment, which shows different response of cancer cells in comparison with normal cells after the treatment.

which shows that the atmospheric plasma jet operates at room temperature.

Fig. 3(a) and (b) shows the morphology of cancerous cells before and immediately after 60-s plasma treatment, respectively. As it appears in the figures, immediately after plasma treatment three regions were formed, which named, frozen zone appearing in plate center and containing dead cells, live zone formed alongside of the plate and is not affected by plasma exposure and void zone formed between the live and the frozen zones containing no cell. It is resulted that the cancer cells in void zone were detached from plate. It should be noted that no significant changes were observed in the formed zones after overnight cell incubation. Fig. 4 shows the plasma exposure effect on cancer cell viability 24 h after four different treatment time steps. The cell viability was compared with the nontreated samples as negative control and samples treated by Doxorubicin drug (common chemotherapy drug for cancer patients) as positive control. The experiments done by He and He/O_2 mixture are compared in Fig. 4. As the figure shows after 60 s it is possible to observe a significant difference between the cell viability of treated cells and negative control. Moreover, by adding oxygen to discharge feeding gas the cell viability reduction enhanced dramatically. It is clearly observed that after 300 s He/O_2 mixture treatment, the viability of cells treated by plasma reduced more than drug-treated cells population in the order of 35%. Fig. 3(c) and (d) shows the effect of He/O_2 mixture plasma and drug treatment in 300 s, respectively. The results showed that a major destruction was developed all over the plate. Fig. 5 shows the discharge voltage effect on cell viability. The initial voltage (6 kV), which is the breakdown threshold does not show any impressive difference with untreated samples. The results showed that by increasing the power supply voltage, cell viability reduced drastically. It should be noted that the void zone area became wider by increasing the power supply voltage. Thus, the plasma plum treatment area increased and affected a larger surface of the plate. As the final experiment, human breast normal cells were treated under NAPP. Fig. 6 shows the different responses of He plasma on normal cells in comparison with the cancer cells. This figure showed that plasma treatments for 30 s caused no significant difference between the response of cancerous and normal cells to plasma treatment in comparison with the control samples. By increasing treatment time the plasma affected to reduce the viability of cancer cells. The interesting point is that plasma did not induce any significant morphological change to the normal cells even at 300 s duration of treatment in comparison with the untreated sample (the difference of viability is 32%). This may indicate the selective nature of the plasma treatment between the cancer and normal cells. Comparing the plasma-treated normal cells

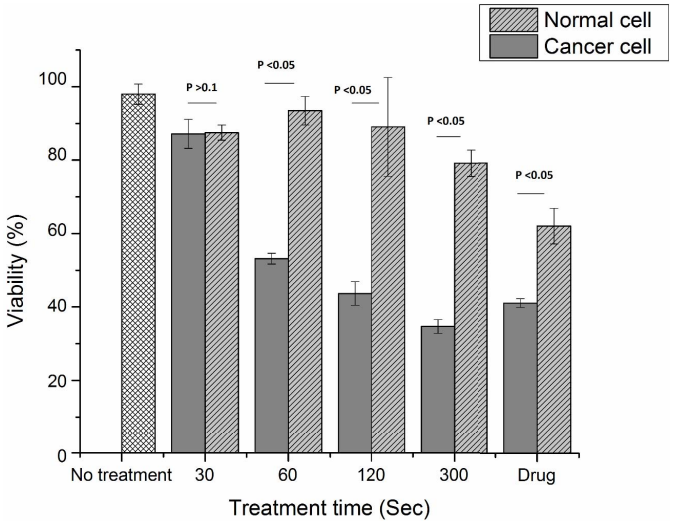
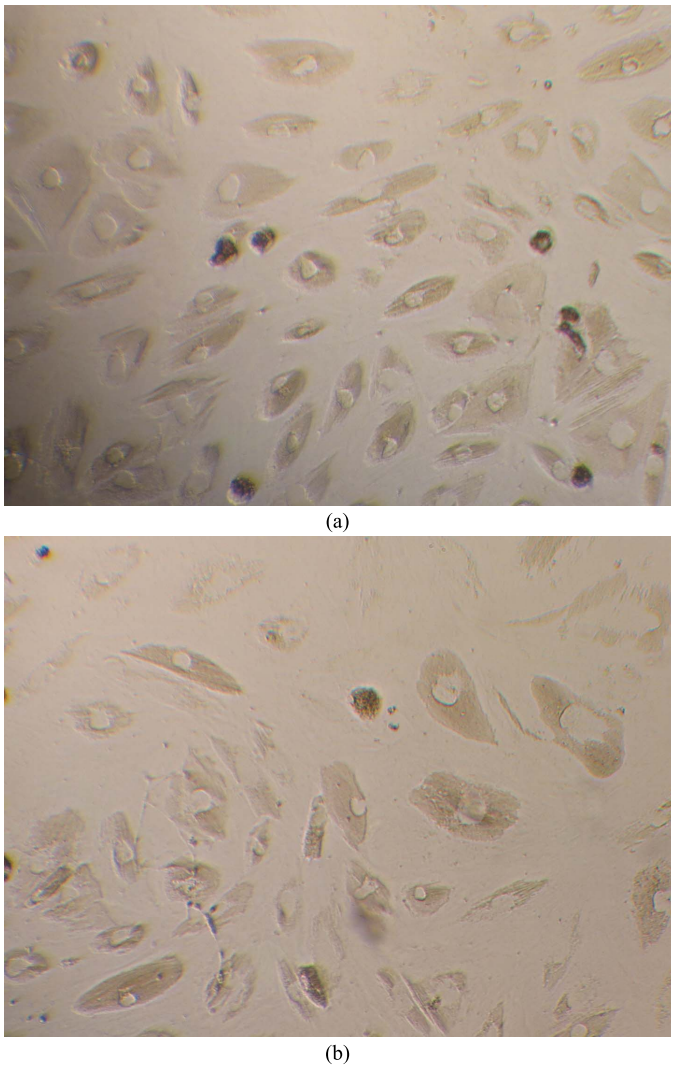


Fig. 7. Viability result of cancer and normal cells after He/O_2 plasma treatment.

at 300 s treatment and drug treated cells, it can be concluded that the normal cells were less damaged than drug-treated cells with difference of 20% in viability. Furthermore, the interesting result is the effect of He/O_2 plasma on normal cells, which is shown in Fig. 7. Despite the intensive damage of the plasma treatment to cancerous cell as the plot shows (viability reduction is 37% in 300 s), no drastic destruction was applied to normal cells during plasma treatment (viability reduction difference is 43%). The morphology of the 300-s plasma treated and untreated normal cells is shown in Fig. 8(a) and (b), respectively. As it can be observed, after plasma treatment no frozen or dead zone was formed on the plate. This would imply that plasma did not induce detachment process on normal cells. Another interesting analyses, which shows the selective effect of nonthermal atmospheric plasma on cancer cells is apoptosis assay, which perform by Caspase-Glo 3/7 assay. Fig. 9 shows the effect of nonthermal helium and Helium–oxygen mixture plasma on cancer cells apoptosis activity. It can be observed from the figure, plasma-induced apoptosis to the cancer cells. The results showed that after 30-s helium plasma treatment the apoptosis activity rate increased by a factor of 2.2 in comparison with the untreated cells. Moreover, the apoptosis activity rate reaches to its maximum at 60-s helium plasma treatment (by factor of 3.6). After this time, the apoptosis activity rate reduced drastically, which approximately equal to the drug treatment. The same process occurs for the helium–oxygen plasma treatment. The results showed that the maximum apoptosis was observed at 30-s plasma treatment (by factor of three) while by increasing the treatment time the apoptosis activity reduced and reached less than the drug treatment. It can be concluded from the MTT and Caspase-Glo assay at the initial treatment times (30 and 60 s) the cells death caused by apoptosis while by increasing the treatment time the process of cell death caused by other ways of apoptosis such as necrosis. More interestingly, is the results obtained from the effect of NAPP on normal cells, which shown in Fig. 10. It can be obtained from this figure that no significant change can be observe



(a)

(b)

Fig. 8. (a) Untreated normal cells. (b) Normal cells after 300 s plasma treatment, no major destruction is observed. Magnification of pictures are 40x.

in apoptosis activity. However, the maximum apoptotic cell death, which treated by helium plasma occur at 120 s by a factor of 1.4 while this value maximized for helium–oxygen plasma treatment at 60 s (by a factor of 1.5). Totally, it can be concluded from MTT and Caspase-Glo assay that plasma do not induce any significant damage to the cells. However, it should be noted that plasma induce less damage in initial treatment time. Kim *et al.* [14] reveal that oxygen plasma induce more apoptosis effect than helium and argon plasma to breast cancer cells. They found that the most of the apoptosis effect induced at low time step. Our results show a similar phenomenon too. However, we found that by increasing the treatment time after the maximum apoptosis the cell death occurred by necrosis. Also, the treatments has no effect on normal cells. Young *et al.* [15] represent the effect of nonthermal microplasma on apoptosis effect of cancer cells. They found that by increasing the treatment time the apoptotic effect of cancer cells increased, which is compatible with our results. It should be noted that the difference between the rate of apoptosis or treatment time may rise from different initial experiment conditions such as plasma jet probe, power

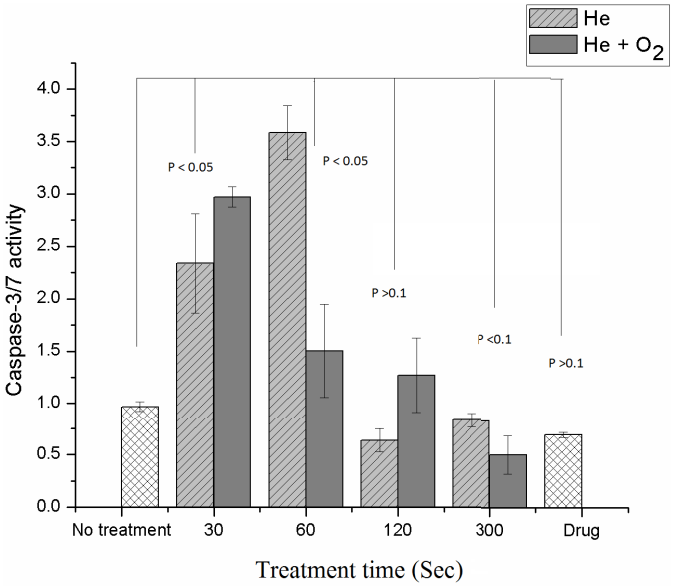


Fig. 9. Apoptosis activity of cancer cells treated by He and He/O_2 mixture plasma after four steps of treatment times. The results of plasma treated samples compared with untreated and drug treated samples result.

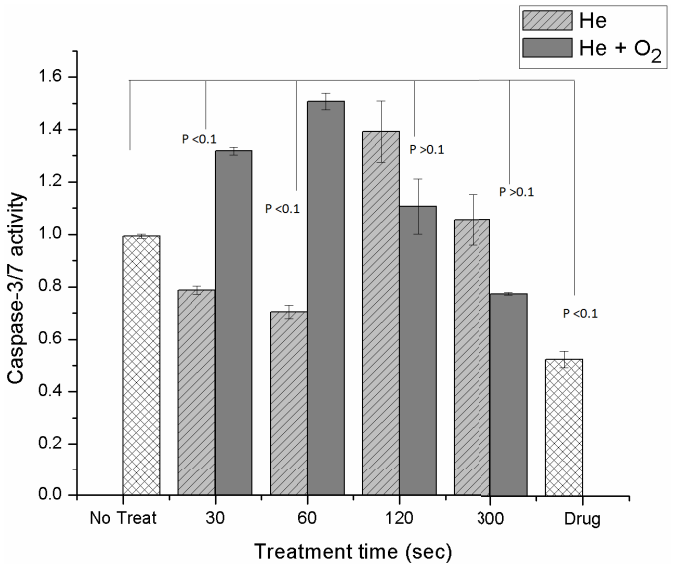


Fig. 10. Apoptosis activity of normal cells treated by He and He/O_2 mixture plasma after four steps of treatment times. The results of plasma treated samples compared with untreated and drug treated samples result.

supply, and so on. Fig. 11 shows the emission spectrum of NAPP (a for He and b for He/O_2 plasma). He plasma spectrum presents some important intense peaks such as first negative system of nitrogen (N_2^+) in 391 nm, atomic oxygen (O) in 777 nm and H_α in 656 nm. Kim *et al.* [14] have introduced these species as reactive agents for plasma therapeutic effect on cancer cells. By adding 5% oxygen to feeding gas, the cancer cell viability reduced in comparison with only He plasma treatment. Fig. 8(b) shows the spectrum of the He/O_2 plasma. It can be observed clearly, the peak intensity of atomic oxygen enhanced by adding small amount of oxygen to plasma. Gweon *et al.* [16] have introduced atomic oxygen as responsible factor to detachment of cancer

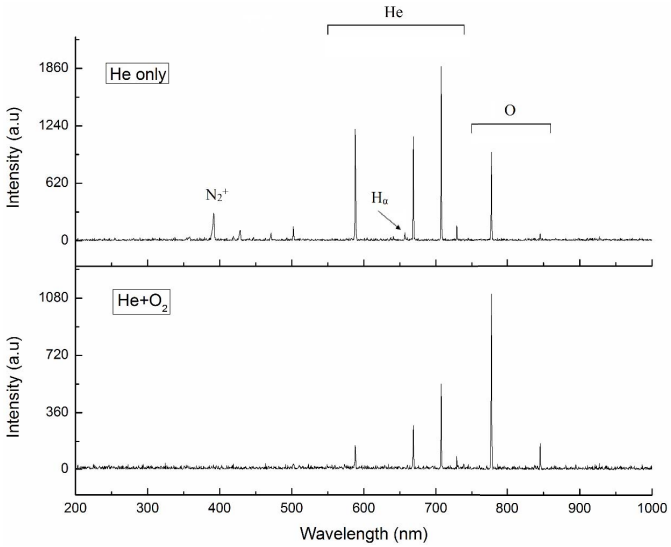


Fig. 11. Top: optical emission spectroscopy of He plasma jet. Bottom: optical emission spectroscopy of He/O_2 plasma in wavelength of 200–1000 nm.

cells from substrate for human liver cancer. The detachment could clearly be observed in void zone formed in cancer cells. The void area would become larger by adding oxygen to feeding gas in same treatment time in comparison with only He treatment. It seems that the diffused reactive species spreads over the cells by increasing treatment time that is resulted in a larger void zone. In addition, apoptosis results showed that by adding oxygen to helium plasma, the apoptosis death occur in less time than helium plasma. By comparison between the plasma optical emission spectrum and Caspase-Glo assay it can be concluded, the atomic oxygen can play an important role in apoptosis effect on cancer cells. Furthermore, the reactive charge accumulation may lead to cancerous cell damage by increasing treatment time. The ionization process production intention conducts to more treatment effect of reactive species by increasing discharge voltage. The notable result of this paper is the effect of plasma on breast normal cells. There are a few reports indicating the effect of NAPP jet on normal cells. Gweon *et al.* [16] have studied the effect of plasma on THLE-2 normal cells. They hypothesized that plasma would detach the cancer cells from plate substrate while it does not affect normal cells due to normal cell adhesion. Atomic oxygen decreases adhesion properties of cancer cells indicating cancer cell detachment in the void zone. Nikkhah *et al.* [17] have obtained that breast cancer adhesion is much lower than fibroblast normal cells. Our results confirm that the cancer cells detach more from substrate by increasing treatment time while it has no significant damage effect on normal cells. Moreover, He/O_2 admixture as feeding gas does not detach normal cells from substrate. The results of the normal cell MTT assay and He/O_2 plasma spectrum showed that atomic oxygen (O–777 nm) does not detach normal cell from substrate while it is main response of cancerous cell detachment. The apoptotic effect may be originated from various factors such as RNS, ROS and OH, and NO reactive agents. Another interesting point in

results is the difference effect of drug and plasma treatment. By comparison, the response of cancerous and normal cells treated by 300-s plasma exposure and drug treatment plasma is introduced as a therapeutic effect on cancerous cells while it has no significant damage on normal cells.

IV. Conclusion

The aim of this paper is the investigation of the effect of NAPP jet on cancerous and normal cell line. The results showed that longer treatment time had serious damage to cancer cells while it had no significant effect on normal cells. Moreover, He/O_2 admixture was employed to enhance plasma damage effect on cancer cells. Also, the results showed the viability of the cancer cells decrease as the discharge voltage increases. In addition, the results were compared with Doxorubicin, a common cancer chemotherapy drug. It can be obtained from the results the NAPP jet treatment successfully enhanced the reduction of the breast cancer cells viability more than the cells treated by chemotherapy drug, while it detracts the adverse effects on human breast normal cells. In addition, it can be obtained from the results that cells detach from surface of the plate, which lead to form void zone in plate area. Also, the Caspase-Glo 3/7 assay was reveal that plasma treatment enhance the apoptosis activity in cancer cells at initial treatment time while this rate do not change significantly at the same treatment time for normal cells. Moreover, adding oxygen to plasma will enhance the apoptosis effect in cancer cells. The results of this paper has confirmed and improved the previous outcomes of the cancer cells treated by NAPP jet.

References

[1] M. Laroussi, D. A. Mendis, and M. Rosenberg, "Plasma interaction with microbes," *J. Phys.*, vol. 5, no. 1, pp. 1–10, Apr. 2003.

[2] Z. Xiong, X. P. Lu, A. Feng, Y. Pan, and K. Ostrikov, "Highly effective fungal inactivation in He + O_2 atmospheric-pressure nonequilibrium plasmas," *Phys. Plasmas*, vol. 17, no. 12, pp. 123502-1–123502-6, Dec. 2010.

[3] S. U. Kalghatgi, G. Fridman, M. Cooper, G. Nagaraj, M. Peddinghaus, M. Balasubramanian, *et al.*, "Mechanism of blood coagulation by nonthermal atmospheric pressure dielectric barrier discharge plasma," *IEEE Trans. Plasma Sci.*, vol. 35, no. 5, pp. 1559–1566, Oct. 2007.

[4] N. Andrei Vasile, T. Ionut, G. Constantin, P. Valentin, and P. Gheorghe, "Stimulation of wound healing by helium atmospheric pressure plasma treatment," *J. Phys. D, Appl. Phys.*, vol. 44, no. 10, pp. 105204-1–105204-9, Mar. 2011.

[5] D. V. Palanker, J. M. Miller, M. F. Marmor, S. R. Sanislo, P. Huie, and M. S. Blumenkranz, "Pulsed electron avalanche knife (PEAK) for intraocular surgery," *Invest. Ophthalmol. Vis. Sci.*, vol. 42, no. 11, pp. 2673–2678, Oct. 2001.

[6] C.-H. Kim, J. H. Bahn, S.-H. Lee, G.-Y. Kim, S.-I. Jun, K. Lee, *et al.*, "Induction of cell growth arrest by atmospheric non-thermal plasma in colorectal cancer cells," *J. Biotechnol.*, vol. 150, no. 4, pp. 530–538, Dec. 2010.

[7] S. Iseki, K. Nakamura, M. Hayashi, H. Tanaka, H. Kondo, H. Kajiyama, *et al.*, "Selective killing of ovarian cancer cells through induction of apoptosis by nonequilibrium atmospheric pressure plasma," *Appl. Phys. Lett.*, vol. 100, no. 11, pp. 113702-1–113702-4, Mar. 2012.

[8] H. M. Joh, S. J. Kim, T. H. Chung, and S. H. Leem, "Reactive oxygen species-related plasma effects on the apoptosis of human bladder cancer cells in atmospheric pressure pulsed plasma jets," *Appl. Phys. Lett.*, vol. 101, no. 5, pp. 053703-1–053703-5, Aug. 2012.

[9] T. Petit, P. Dufour, and I. Tannock, "A critical evaluation of the role of aromatase inhibitors as adjuvant therapy for postmenopausal women with breast cancer," *Endocrine, Rel. Cancer*, vol. 18, no. 3, pp. R79–R89, Jun. 2011.

[10] J. S. Vaidya, D. J. Joseph, J. S. Tobias, M. Bulsara, F. Wenz, C. Saunders, *et al.*, "Targeted intraoperative radiotherapy versus whole breast radiotherapy for breast cancer (TARGIT-A trial): An international, prospective, randomised, non-inferiority phase 3 trial," *Lancet*, vol. 376, no. 9735, pp. 91–102, Jul. 2010.

[11] E. Stoffels, Y. Sakiyama, and D. B. Graves, "Cold atmospheric plasma: Charged species and their interactions with cells and tissues," *IEEE Trans. Plasma Sci.*, vol. 36, no. 4, pp. 1441–1457, Aug. 2008.

[12] D. Danil, F. Gregory, F. Gary, and F. Alexander, "Physical and biological mechanisms of direct plasma interaction with living tissue," *New J. Phys.*, vol. 11, no. 11, pp. 115020-1–115020-26, Nov. 2009.

[13] Y. Liang, Y. Wu, K. Sun, Q. Chen, F. Shen, J. Zhang, *et al.*, "Rapid inactivation of biological species in the air using atmospheric pressure nonthermal plasma," *Environ. Sci. Technol.*, vol. 46, no. 6, pp. 3360–3368, Mar. 2012.

[14] S. J. Kim, T. H. Chung, S. H. Bae, and S. H. Leem, "Induction of apoptosis in human breast cancer cells by a pulsed atmospheric pressure plasma jet," *Appl. Phys. Lett.*, vol. 97, no. 2, pp. 023702-1–023702-3, Jul. 2010.

[15] J. Y. Kim, J. Ballato, P. Foy, T. Hawkins, Y. Wei, J. Li, *et al.*, "Apoptosis of lung carcinoma cells induced by a flexible optical fiber-based cold microplasma," *Biosensors Bioelectron.*, vol. 28, no. 1, pp. 333–338, 2011.

[16] B. Gweon, M. Kim, D. B. Kim, D. Kim, H. Kim, H. Jung, *et al.*, "Differential responses of human liver cancer and normal cells to atmospheric pressure plasma," *Appl. Phys. Lett.*, vol. 99, no. 6, pp. 063701-1–063701-3, Aug. 2011.

[17] M. Nikkhah, J. Strobl, B. Peddi, and M. Agah, "Cytoskeletal role in differential adhesion patterns of normal fibroblasts and breast cancer cells inside silicon microenvironments," *Biomed. Microdevices*, vol. 11, no. 3, pp. 585–595, Jun. 2009.

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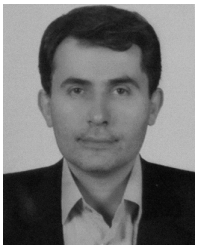

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